BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions[1]	Document identifier or version number
09/03/2015	Changes in CA 1.10.3 are highlighted in yellow	MCA Section 1 Version 1 BASF DocID 2013/1348637

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

BASF Agricultural Products de Puerto Rico
Road #2, KM 47.3
PO Box 243
Manati, PR 0067
Puerto Rico

Contact:

[redacted]
Phone No : [redacted]
Fax No: [redacted]
E-mail address: [redacted]

Alternative:

[redacted]
Phone No: [redacted]
Fax No: [redacted]
E-mail address: [redacted]

CA 1.2 Producer

Manufacturer of imazamox (legal entity):

[redacted]

Location of manufacturing plant:

CONFIDENTIAL information – data provided separately (Document J)

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

Imazamox

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC: (+/-)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-(methoxymethyl) nicotinic acid

CA: (+/-)-2-[4,5-dihydro-4-methyl-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridine carboxylic acid

CA 1.5 Producer's Development Code Numbers

BASF Number: BAS 720 H

BASF Registry Number: Reg.No. 4096483

BASF CL Number (old): CL 299263

CA 1.6 CAS, EC and CIPAC Numbers

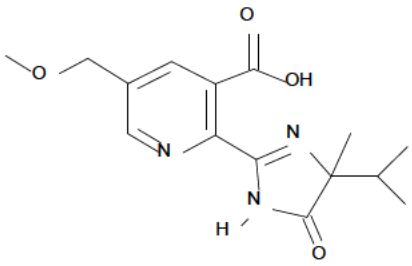
CAS No: 114311-32-9

EINECS No: Not allocated

CIPAC No: 619

CA 1.7 Molecular and Structural Formula, Molar Mass

- Structural formula



Molecular formula: $C_{15}H_{19}N_3O_4$

Molecular mass: 305.336 g/mol

CA 1.8 Method of Manufacture (synthesis pathway) of the active substance

CONFIDENTIAL information - data provided separately (Document J)

CA 1.9 Specification of Purity of the Active Substance in g/kg

Min. 950 g/kg, in accordance with 2003/23/EC (Annex I Inclusion Directive)

CA 1.10 Identity and Content of Additives (such as Stabilisers) and impurities

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.1 Additives

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.2 Significant impurities

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.3 Relevant impurities

Imazamox does not contain relevant impurities.

[redacted]

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J)

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DOCUMENT M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

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Date	Data points containing amendments or additions[1]	Document identifier or version number
10.02.2015	Additional information in column "Findings"	MCA Section 2 Version 1 BASF DocID 2013/1348638

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.1 Melting point and boiling point	OPPTS 830.7200, FP0091/002 (Differential scanning calorimetry/ thermo-gravimetry)	Pure Active Ingredient (99.8%) Batch No. L82-98	The melting point of imazamox PAI was determined to be 166 °C (onset); decomposition (by onset of mass loss) was observed to begin at a temperature of approximately 180 °C.	Y	[see 2013/1231744 Kroehl T. 2013 a]
CA 2.2 Vapour pressure, volatility	OPPTS 830.7950, EEC A.4, OECD 104, FP0073/007	Pure Active Ingredient (99.8%) Batch No. L82-98	The measurements were carried out at elevated temperatures (140 °C to 160 °C) to ensure a sufficient weight loss by evaporation during an appropriate time (1h). The vapour pressure at the required temperatures (20 °C and 25 °C) was then calculated by extrapolation (see table below). Temperature | Vapour Pressure p [°C] | [hPa, mbar] | [Pa] 20 | 6.3×10^{-13} | 6.3×10^{-11} 25 | 2.1×10^{-12} | 2.1×10^{-10} The Henry's Law Constant is calculated with the data for vapour pressure and for water solubility. $H = 4.9 \times 10^{-15}$ kPa m^3 / mol. * ***This report was mistakenly forgotten in BASF Application (July 2013), but is included since relevant for the requested information.***	Y	[see 2013/1231744 Kroehl T. 2013 a] [see 2013/1405168 Kroehl T. 2014 c]*

<table>
<tr><th>Test or Study & Data Point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CA 2.3 Appearance (Physical state, colour)</td><td>OPPTS 830.6302, FP0039/006,

OPPTS 830.6303, FP0039/006, FP0062/005</td><td>Pure Active Ingredient (99.8%) Batch No. L82-98</td><td>Imazamox PAI was solid at room temperature, present as a fine, white powder.

The data on appearance for the active substance as manufactured was already reported during the first submission (see DAR – Vol B.2, 1999).</td><td>Y</td><td>[see 2013/1231744 Kroehl T. 2013 a]</td></tr>
<tr><td>CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td>PFL0105/04</td><td>Pure Active Ingredient (99.8%) Batch No. L82-98</td><td>UV/VIS in Methanol:
pH of test solution: 4.7
<table>
<tr><th>Solution No.</th><th>Datapoint No.</th><th>Wavelength [nm]</th><th>Absorption [Units]</th><th>ε [L * mol^{-1} * cm^{-1}]</th></tr>
<tr><td>1</td><td>1</td><td>201</td><td>1.0671</td><td>26240</td></tr>
<tr><td>1</td><td>2</td><td>246</td><td>0.3937</td><td>9681</td></tr>
<tr><td>1</td><td>3</td><td>290</td><td>0.1130</td><td>2779</td></tr>
</table></td><td>Y</td><td>[see 2013/1231743 Kroehl T. 2013 b]</td></tr>
</table>

<table>
<tr><th>Test or Study & Data Point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
UV/VIS (watery conditions):
pH of test solution: 4.5
<table>
<tr><th>Solution No.</th><th>Datapoint No.</th><th>Wavelength [nm]</th><th>Absorption [Units]</th><th>ε [L * mol^{-1} * cm^{-1}]</th></tr>
<tr><td>2</td><td>1</td><td>191</td><td>1.1302</td><td>27791</td></tr>
<tr><td>2</td><td>2</td><td>267</td><td>0.2502</td><td>6152</td></tr>
<tr><td>2</td><td>3</td><td>290</td><td>0.1243</td><td>3057</td></tr>
</table>

UV/VIS (acidic conditions):
pH of test solution: 1.3
<table>
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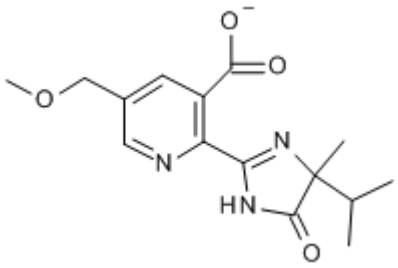
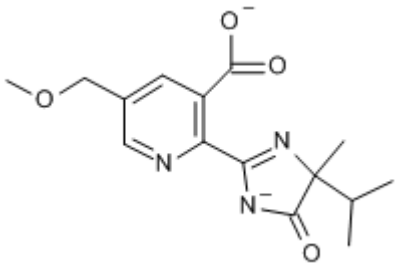
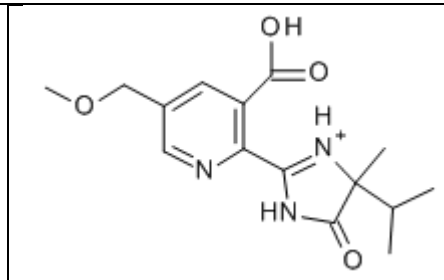
<tr><td>3</td><td>1</td><td>200</td><td>1.0054</td><td>24723</td></tr>
<tr><td>3</td><td>2</td><td>233</td><td>0.4314</td><td>10608</td></tr>
<tr><td>3</td><td>3</td><td>290</td><td>0.0798</td><td>1962</td></tr>
</table>
</td><td></td><td></td></tr>
</table>

<table>
<tr><th>Test or Study & Data Point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
UV/VIS (basic conditions):
pH of test solution: 12.2
<table>
<tr><th>Solution No.</th><th>Datapoint No.</th><th>Wavelength [nm]</th><th>Absorption [Units]</th><th>ε [L * mol^{-1} * cm^{-1}]</th></tr>
<tr><td>4</td><td>1</td><td>214</td><td>0.6024</td><td>14813</td></tr>
<tr><td>4</td><td>2</td><td>268</td><td>0.2708</td><td>6659</td></tr>
<tr><td>4</td><td>3</td><td>290</td><td>0.0814</td><td>2002</td></tr>
</table>
The IR, NMR, MS spectra for the pure active substance were already reported during the first submission (see DAR – Vol B.2, 1999).

Spectra for relevant impurity:
The inclusion directive 2003/23/EC does not contain information on the relevance of impurities. At the time of preparation of the supplemental dossier for the renewal, on a national level imazapic was considered being of relevance by German Authorities (maximum limit: 10 g/kg). This consideration is not valid any longer and BASF proposed to keep imazapic as significant impurity but not any longer as relevant impurity. Therefore, spectral data would not be required any longer.
</td><td></td><td></td></tr>
</table>

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.5 Solubility in water			Water: 3.89 g/l (20°C) pH 4: 21.53 g/l (20°C) pH 7: > 574 g/l (20 °C) pH 9: > 505 g/l (20 °C)		[see 2013/3006564 Campos L.F.P.,Silva C.M. da 2014 a]
CA 2.6 Solubility in organic solvents			Methanol: 71 g/l (20°C) Acetone: 28 g/l (20 °C) Information already peer-reviewed and reported previously: Hexane: 0.007 g/l (25 °C) Methanol: 67 g/l (25 °C) Toluene: 2.2 g/l (25 °C) Ethyl acetate: 10 g/l (25 °C)		[see 2013/3006563 Campos L.F.P.,Silva Mantovani C. da 2013 a] SANCO/4325/2000 – Final 29. November 2002
CA 2.7 Partition co-efficient n octanol/ water			Water: 0.2 (20°C) pH 4: -0.3 (20°C) pH 7: < -0.3 (20 °C) pH 9: < -0.3 (20 °C)		[see 2013/3006564 Campos L.F.P.,Silva C.M. da 2014 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.8 Dissociation in water • dissociation constant(s) (pKa values) • identity of dissociated species • dissociation constant(s) (pKa values) of the active principle			Information already reported previously (Registration Report SANCO/4325/2000 – Final 29. November 2002, also shown below) pKa: 2.3, 3.3, 10.8  Imazamox – Carboxylate Anion  Imazamox – Carboxylate and Imi Amide Anion		SANCO/4325/2000 – Final 29. November 2002

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			 Imazamox – Imi Nitrogen Protonation		
CA 2.9 Flammability and self-heating	EEC A.10 EEC A.16 Regulation (EC) 1272/2008, UN Transport Regulation Test N.4	Technical Grade Active Ingredient (98.0% nominal) Batch No. COD-001719	Technical imazamox is not considered highly flammable, as the attempt to ignite a pile of the substance in a preliminary test resulted only in brief burning and rapid extinction. The main test was therefore omitted. The test to determine the relative self-ignition temperature indicated no self-heating properties of technical imazamox. No temperature rise over the oven temperature (140 °C) within 24 h was observed in a 100 mm sample cube. Technical imazamox is therefore not classified as a self-heating substance in the sense of the regulation.	N	[see 2013/1065841 Achhammer 2013 a]
CA 2.10 Flash point			Not applicable, as imazamox has a melting point well above 40 °C.		

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.11 Explosive properties	OECD 113, Differential scanning calorimetry	Technical Grade Active Ingredient (98.0% nominal) Batch No. COD-001719	The test for explosive properties EEC A 14 has not been carried out because the exothermic decomposition energy, determined via DSC, is less than 500 J/g (UN Recommendations on the transport of dangerous goods, Manual of tests and criteria, Annex 6) <u>Results of differential scanning calorimetry measurements:</u> 1st reaction: Onset temperature: 180 °C Peak temperature: 228 °C Energy release: 60 J/g 2nd reaction: Onset temperature: 275 °C Peak temperature: 347 °C Energy release: 70 J/g 3rd reaction: Onset temperature: 380 °C Peak temperature: 408 °C Energy release: >130 J/g	N	[see 2013/1065841 Achhammer 2013 a]
CA 2.12 Surface Tension	OEDC 115, EEC A.5 1.6.1; FP0014/016	Pure Active Ingredient (99.8%) Batch No. L82-98	The surface tension was measured at 90% of the saturation solubility in pure water by the plate method (required force to withdraw a vertically suspended plate from the surface of the solution). It was determined to be 51.9 mN/m.	Y	[see 2013/1231744 Kroehl T. 2013 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.13 Oxidising properties	EEC A.17	Technical Grade Active Ingredient (98.0% nominal) Batch No. COD-001719	Technical imazamox is not considered an oxidizing substance because the highest burning rate of the mixtures tested (1.2 – 1.4 mm/s) is lower than that of the reference mixture (3.7 mm/s).	N	[see 2013/1065841 Achhammer 2013 a]
CA 2.14 Other studies			not required		

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Imazamox

DOCUMENT M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions[1]	Document identifier or version number
03.07.2014	Table 3.5-1, EPPO codes corrected.	2013/1348639 MCA Section 3 Version 1

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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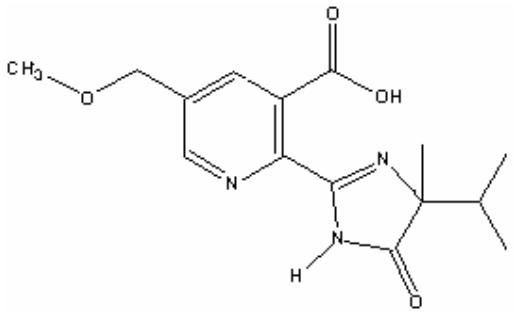
CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

The active substance Imazamox is belonging to the imidazolinones class of herbicides. The chemical name (IUPAC) is 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methoxymethylnicotinic acid. The active ingredient is used solo or in mixture with Bentazon, Metazachlor, Quinmerac and Pendimethalin for the control of dicot. and monocot. weeds in Sunflower, Oilseed rape and in various Legume crops. It is also used in Rice for weed control. The application is mainly done in post emergence of the crop but also it is used in pre emergence.

Table 3.1-1: Currently registered and under evaluation BASF Imazamox Products in the EU 28 (*Status 10/2013*).

BAS N°	Brandnames	Ai / Ratio per l	Max. Dose
BAS 720 06H	Pulsar 40, Beyond, Pivot	Imazamox 40 g/l	1,25 l/ha
BAS 721 03H	Nirvana, Oklohoma, Escort, Mutual	Imazamox 16,7 g/l Pendimethalin 250 g/l	4,5 l/ha
BAS 762 01H	Corum, Barox, Varisto	Imazamox 22,4 g/l Bentazon 480 g/l	1,875 l/ha
BAS 797 00H	Cleranda, Clamox, Cleratop	Imazamox 17,5 g/l Metazachlor 375 g/l	2,0 l/ha
BAS 798 00H	Cleravis, Clesura, Clesima	Imazamox 17,5 g/l Metazachlor 375 g/l Quinmerac 125 g/l	2,0 l/ha
BAS 798 01H	Vantiga D	Imazamox 6,25 g/l Metazachlor 375 g/l Quinmerac 125 g/l	2,0 l/ha
BAS 831 00H	Cleravo	Imazamox 35,0 g/l Quinmerac 250 g/l	1,0 l/ha
BAS 831 01H	Clentiga	Imazamox 12,5 g/l Quinmerac 250 g/l	1,0 l/ha



Molecular formula: $C_{15}H_{19}N_3O_4$

Figure 3.1-1: Structural and molecular Formula of Imazamox

CA 3.2 Function

Imazamox is used as an herbicide

CA 3.3 Effects on Harmful Organisms

Imazamox is an organonitrogenous heterocyclic molecule belonging to the imidazolinones class and according to the HRAC classification is in Group B (inhibit the activity of the enzyme acetohydroxyacid synthase ; AHAS) also known as acetolactate synthase (ALS). This enzyme is found in bacteria and plants, but not in animals and humans.

ALS is the first enzyme in the pathway for the biosynthesis of the essential branched-chain amino acids valine, leucine and isoleucine. The inhibition of ALS activity leads to amino acid starvation and the accumulation of toxic precursors. The primary effect following treatment of susceptible weeds with the herbicide is the restraint of new growth and cell development.

Imazamox can be absorbed by roots and foliage, and then translocated throughout the plant to the meristematic tissues. Growth of susceptible plants is inhibited soon after application while visual symptoms appear two to three weeks after application. Imazamox causes an almost immediate growth block followed by a gradual decolouration mainly on the youngest leafs and subsequent death of weeds. Imazamox shows moderate acropetal and basipetal translocation.

CA 3.4 Field of Use Envisaged

The Active Ingredient Imazamox is used in mixture or as solo Formulation in a range of various crops as a spray treatment with water carrier rates between 100-600 l/ha. The highest rate of Imazamox applied is 75 g/ha active substance (in Rice). Imazamox products are used for the control of monocotyledonous and dicotyledonous weeds, and give a high efficacy under many environmental conditions. Imazamox is applied in fall and aswell in spring, depending on the crop.

CA 3.5 Harmful Organisms Controlled and Crops or Products Protected or Treated

Imazamox herbicides have been used commercially for many years in crops that are inherently resistant to this class of herbicide compounds, such as Alfalfa, Peas, Beans, Soybeans and clovers. By breeding tolerance in to crops that were not previously selective to imidazolinone herbicides, BASF offers growers innovative solutions to traditional problem weeds within winter and summer oilseed rape and Sunflower as a part of the so called Clearfield Production System®. All Clearfield crops are non genetically modified organisms (GMOs), developed by using conventional breeding methods.

Table 3.5-1: Overview on major crop uses for Imazamox solo and Imazamox in combination with Pendimethalin, Metazachlor, Quinmerac and Bentazon

Imazamox solo	Imazamox in mixture
Rice* (ORYSA)	Alfalfa (MEDSA)
Alfalfa (MEDSA)	Peas (PIBSA, PIBSX, PIBSM, PIBSZ)
Soybeans (GLXMA)	Soybeans (GLXMA)
Sunflower* (HELAN)	Beans (PHSVX, PHSLU, PHSAF, PHSCO, PHSVN, VICFE, VICFX, VICFM)
	Clovers (TRFPR, TRFRE, TRFIN
	Oilseed Rape* (BRSNW, BRSNS, BRSSP)

**carrying tolerance against Clearfield Herbicide*

All crop codes can be refered under: http://eppt.eppo.org/search.php

As already mentioned, Imazamox controls solo or in product combination, monocotyledonous weeds such as SETVI, ECHCG, PANMI, SORHA, DIGSA, APESV and ALOMY. Also it is able to control volunteers of HORVW, HORVS, TRZAW, TRZAS and other cereal crop volunteers in broad-leafed crops. Also a broad range of dicotyledonous weeds such as AMARE, AMBEL,CHEAL, DATST, MATCH, MATIN, ANTAR, GALAP, STEME, LAMPU, RAPRA, SSYOF, DESSO,SINAR, XANST, PAPRH, GERRT, GERPU, GERRTI, CAPBP, THLAR, VERPE, VERAR are controlled.

The complete list of weeds controlled can be found in the respective registration material that has been submitted in the product registration process in the concerned countries.

CA 3.6 Mode of Action

Imazamox is classified within the HRAC as a Group B Herbicide. The mode of action is described as Inhibition of acetolactate synthase (branched chain amino acid synth.). In total the Group B consist out of 50 herbicidal active ingredients. The Group B is again divided in 5 subgroups. The group of the sulfonylureas is containing most of the active ingredients. Imazamox belongs to the subgroup of the Imidazolinones (Table 3.6-1).

Table 3.6-1: HRAC Group B including all active ingredients divided in subgroups

HRAC Group	Mode of Action	Subgroup	Active Ingredient
B	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS)	Sulfonylurea	amidosulfuron azimsulfuron bensulfuron-methyl chlorimuron-ethyl chlorsulfuron cinosulfuron cyclosulfamuron ethametsulfuron-methyl ethoxysulfuron flazasulfuron flupyrsulfuron-methyl-Na foramsulfuron halosulfuron-methyl *imazosulfuron* iodosulfuron mesosulfuron metsulfuron-methyl nicosulfuron *oxasulfuron* primisulfuron-methyl prosulfuron pyrazosulfuron-ethyl rimsulfuron sulfometuron-methyl sulfosulfuron thifensulfuron-methyl triasulfuron tribenuron-methyl trifloxysulfuron triflusulfuron-methyl *tritosulfuron*
		Imidazolinone	imazapic imazamethabenz-methyl imazamox imazapyr imazaquin imazethapyr
		Triazolopyrimidine	cloransulam-methyl diclosulam florasulam flumetsulam *metosulam* *penoxsulam*
		Pyrimidinyl(thio)benzoate	bispyribac-Na pyribenzoxim *pyriftalid* pyrithiobac-Na *pyriminobac-methyl*
		Sulfonylaminocarbonyl-triazolinone	flucarbazone-Na propoxycarbazone-Na

Source: http://www.hracglobal.com/Education/ClassificationofHerbicideSiteofAction.aspx, Status 12/11/2013

CA 3.7 Information on Occurrence or Possible Occurrence of the Development of Resistance and Appropriate Management Strategies

Like for every active ingredient, there is for Imazamox a risk that target weeds develop a resistance against the compound as well and can therefore not be controlled any more as effective as before.

Imazamox was first registered in the European Union on 1st January 1997 in Slovakia as a component of the herbicide "Pulsar". Table 3.7-1 shows reported resistance to Imazamox worldwide according to the weedscience.org website (http://www.weedscience.org/Summary/MOA.aspx?MOAID=3, BASF DOC ID 2013/1086850).

The vast majority of cases are from the USA, Canada, South America, & South Africa. Within Europe reported resistance to Imazamox appears to be limited to one single case of resistance in Barnyard (ECHCG) grass in Italy reported in 2007.

Table 3.7-1: Reported resistance against Imazamox by species and countrie (Source www.weedscience.org)

Common name	*Latin name*	Reported resistance to Imazamox	Crop
Smooth Pigweed	*Amaranthus hybridus*	2002 - USA (Michigan)	Soy
Pigweed (quitensis)	*Amaranthus quitensis*	2002 – Bolivia	Soy
Redroot Pigweed	*Amaranthus retroflexus*	1998 - USA (Pennsylvania) *Multiple - 2 MOA's 2003- Italy *Multiple – 2 MOA´s	Soy
Common Waterhemp	*Amaranthus rudis*	1994 - USA (Missouri) 2002 - USA (Illinois) *Multiple - 3 MOA's 2005 - USA (Missouri) *Multiple - 3 MOA's	Soy Corn, Soy Corn, Soy
Tall Waterhemp	*Amaranthus tuberculatus*	2000 - USA (Michigan)	Soy
Common Ragweed	*Ambrosia artemisiifolia*	1998 - USA (Ohio) 2005 - USA (Delaware) *Multiple - 2 MOA's	Soy Soy
Giant Ragweed	*Ambrosia trifida*	1998 - USA (Ohio)	Soy
Wild Oat	*Avena fatua*	1986 - South Africa *Multiple - 2 MOA's	Wheat
Downy Brome	*Bromus tectorum*	1997 - USA (Oregon)	Bluegrass (cultivated)
Japanese Brome	*Bromus japonicas*	2007 – USA (Kansas)	Wheat
Rye Brome	*Bromus sacalinus*	2007 – USA (Kansas) 2009 – USA (Oklahoma)	Wheat Wheat
Shepherd's-purse	*Capsella bursa-pastoris*	2008 – Canada	Wheat
Small Flowered catchfly	*Silen gallica*	2012 – Chile	Wheat
Lambsquarters	*Chenopodium album*	2001 - USA (Michigan)	Soy
Rice Flatsedge	*Cyperus iria*	2010 – USA (Kansas)	Rice
White Wallrocket	*Diplotaxis erucides*	2012 – Israel	Wheat
Barnyardgrass	*Echinochloa crus-galli*	2007 – Italy	Corn, Rice
Wild Poinsettia	*Euphorbia heterophylla*	2006 - Brazil *Multiple - 2 MOA's	Soy
Marshelder	*Iva xanthifolia*	2003 - USA (North Dakota)	Soy
Kochia	*Kochia scoparia*	2005 - USA (Michigan)	Sugar beets
Italian Ryegrass	*Lolium multuflorum*	2008 – USA (Arkansas)	Wheat
Raddish	*Raphanus sativus*	2010 – Chile	Wheat
Giant Foxtail	*Setaria faberi*	2004 - USA (Pennsylvania)	Corn
Green Foxtail	*Setaria viridis*	1999 - USA (Wisconsin)	Corn, Soy
Eastern Black Nightshade	*Solanum ptycanthum*	1999 - USA (Illinois) 1999 - USA (North Dakota) 1999 - USA (Wisconsin) 2000 - Canada (Ontario)	Soy Soy Soy Soy
Spiny Sowthistle	*Sonchus asper*	2000 - USA (Washington)	Wheat, Lentils
Shattercane	*Sorghum bicolor*	2001 - USA (Pennsylvania)	Corn, Soy

Reported resistance to other active ingredients in the B group of herbicides

Resistance to ALS-inhibitors is well documented. ALS-products were only introduced in the mid 1980s and since then a rapid increase in ALS-inhibitor resistance has been observed, especially relative to other herbicides that have been used for much longer. The ALS-inhibitors are now responsible for the largest number of weed species showing an herbicide resistance. The ALS-inhibitors include the sulfonylureas which are often the sole herbicide used in the USA & Australia, where yield potential is low, and for successive years.

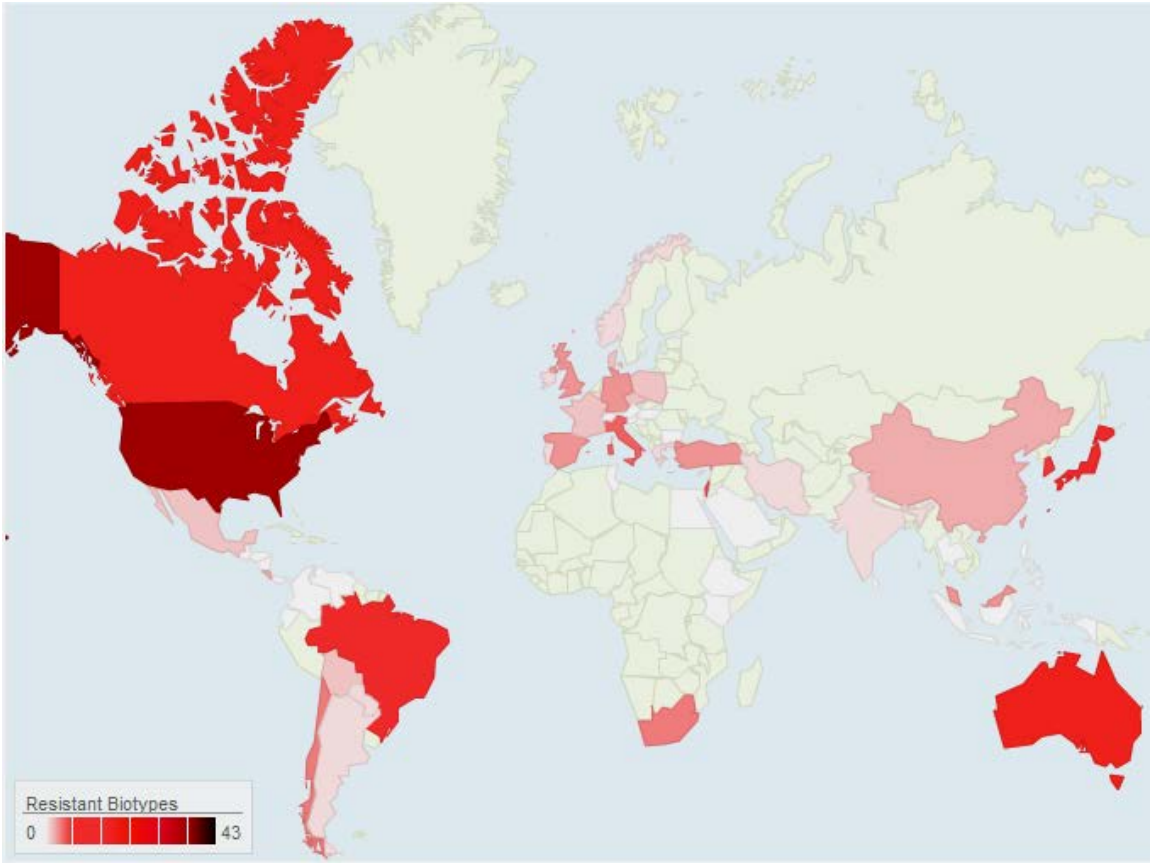


Figure 3.7-1: Distribution of ALS-resistant weed species in the world; number of cases per country (Source: http://www.weedscience.org/Graphs/Graphs.aspx)

Cross-resistance for products within the B group

Out of the plants showing resistance against Group B herbicides several species also show resistance against at least one other mode of action. The highest number of cross or multiple resistance is found between Group B herbicides and Group A herbicides (ACCase inhibitors) before cross or multiple resistance between Group B herbicides and Group C2 (Inhibition of photosynthesis at PSII). Other cases of a cross or multiple resistance with Group B herbicides are found only for a limited range of weeds (http://www.weedscience.org/Summary /MOA.aspx?MOAID=3, [see 2013/1086850 Anonymous 2013 b] ***This Document was not listed in BASF Application (July 2013). It was finally considered relevant for the requested information)***

Sensitivity Data

It is assumed that weeds vary in their sensitivity to herbicides both among and within populations. As a consequence, weed populations in different geographical regions may have different levels of sensitivity to Imazamox. This variation is well documented in the product registration dossiers. These dossiers contain data concerning the efficacy of Imazamox on all important weed species in different regions of Europe. This data set is a 'baseline' and gives an understanding of the variation in sensitivity of weeds to Imazamox.

If in the future, a population of a sensitive weed species is not controlled sufficiently by Imazamox, a shift of sensitivity to Imazamox can be investigated as a reason for the reduced control in this population. Efficacy data on the insensitive population can be compared with those of relevant weed species in the same geographical region; thus providing a measure for the assessment of shifts or changes of sensitivity to Imazamox.

On top of that BASF is also monitoring the resistance development within Europe actively with internal and external stakeholders, to prevent fast development of herbicide resistance.

Product risk

All natural weed populations regardless of the application of any weed control agent probably contain individual plants (biotypes), which are resistant to herbicides. Repeated use of a herbicide will expose the weed population to a selection pressure which may lead to an increase in the number of surviving resistant individuals in the population. As a consequence, the resistant weed population may increase to the point that adequate weed control cannot be achieved by the application of that herbicide.

Table 3.7-2 describes the resistance risk associated with a range of herbicide classes or modes of action as defined by CRD in the UK (Source: CRD Guideline 606 : Resistance risk analysis and use of resistance management strategies [see 2008/1100857 Anonymous 2008 a] ***This Document was not listed in BASF Application (July 2013). It was finally considered relevant for the requested information)***

Table 3.7-2: Examples of risk associated with a range of herbicide classes/modes of action

	Herbicide class
Low risk	Benzamides (e.g. propyzamide)
Moderate risk	Ureas, Dinitroanilines
High risk	ALS and ACCase Inhibitors

Pathogen risk

Table 3.7-2 describes the resistance risk associated with a range of common target weeds as defined by CRD in the UK (Source: CRD Guideline 606: Resistance risk analysis and use of resistance management strategies)

Table 3.7-3: Examples of risk associated with a range of common target weeds

	Weeds
Minimal risk	Most broad leaf weeds
Some risk	Poa spp, Wild Oats, Chickweed, Poppy,
Serious risk	Black-grass, Ryegrass

Resistance Risk Assessment of Unrestricted Use Pattern and Acceptability of Resistance Risk

There is a measurable likelihood that all natural weed populations, regardless of the application of any herbicide, contain individual plants (biotypes) that are resistant to the active principle. Based on the current evidence of resistance in Europe, the resistance risk assessment for the herbicide Imazamox can be stated as medium to high. To prevent further development of herbicide resistance, BASF is actively monitoring the resistance situation. Also, combination products that contain several modes of actions have been developed and are used whenever possible. Further BASF is communicating stewardship measure to prevent the further development of herbicide resistance (see below)

As a result, it can be stated that the resistance risk assessment for Imazamox does not result in an unacceptable risk. However, information on key elements of the resistance management strategy should be provided esp. when the control of weeds which have already evolved resistance is targeted.

Implementation of Management Strategy

BASF has a strong interest to defend their active substances as good as possible against weeds resistance to make the product as long as possible used in practical farming, and making farmers able to protect their crops proper against weeds.

Therefore, BASF is founder member of the HRAC Working Group and has participated in forming the guidelines for management strategy.

The basic principles of resistance management are similar in both the prevention of resistance in a given population, as well as in the limitation of resistance after its first occurrence. Once problems have been detected, management strategies have to be adapted to the particular situation. As a rule, the following methods can be recommended:

- Mixtures or sequences of herbicides with different modes of action within the same crop and within the crop rotation
- Crop rotation
- Cultivation practices

BASF promotes further awareness of general aspects of herbicide resistance management in product and technical leaflets, training sessions to sales personnel, distributors and growers' associations. Additionally, specific aspects on the product use are provided for achieving best control results.

Following is an example on key elements of the resistance management strategy for Imazamox. Products including general and product specific aspects:

- Always follow HRAC guidelines for preventing and managing herbicide resistant weeds.
- Maximise the use of cultural control measures wherever possible (e.g. crop rotation, ploughing, stale seedbeds, delayed drilling, etc).
- Use tank mixes or sequences of effective herbicides with different modes of action within individual crops, or successive crops.
- For the control of herbicide resistant grass weeds, always use Imazamox in tank mix or sequence with other effective graminicides with different modes of action.
- Apply pre-emergence of weeds wherever possible. If applications are delayed, apply post-emergence products/mixtures to small, actively growing weeds, especially where high levels of resistance are suspected and to reduce the risk of resistance development.
- Monitor fields regularly and investigate the reasons for any poor control

Monitoring, Reporting, and Reaction to Changes in Performance

In the case that BASF obtains information about a loss of field performance after application of Imazamox, and that resistance to Imazamox is identified as the cause, BASF will inform HRAC and the registration authorities. Furthermore, BASF will advise the respective farmer to apply the appropriate resistance management strategies as developed and recommended by HRAC. Once resistant biotypes have been detected, management strategies must be customized to the particular situation under consideration of points of view published by HRAC.

CA 3.8 Methods and Precautions Concerning Handling, Storage, Transport or Fire

Report: CA 3.8/1
Anonymous, 2013a
Safety data sheet - Imazamox tech
2013/1027310
Guidelines: EEC 1907/2006
GLP: no

The safety data sheet contains detailed information and advice on methods and precautions concerning handling, storage, transport or fire, based on scientific tests.

CA 3.9 Procedures for Destruction or Decontamination

Report: CA 3.9/1
Anonymous, 2013a
Safety data sheet - Imazamox tech
2013/1027310
Guidelines: EEC 1907/2006
GLP: no

The safety data sheet contains detailed information and advice on procedures for destruction or decontamination, based on scientific tests.

CA 3.10 Emergency Measures in Case of an Accident

Report: CA 3.10/1
Anonymous, 2013a
Safety data sheet - Imazamox tech
2013/1027310
Guidelines: EEC 1907/2006
GLP: no

The safety data sheet contains detailed information and advice on emergency measures in case of an accident, based on scientific tests.

BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section4

ANALYTICAL METHODS

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions[1]	Document identifier or version number
10.02.2015	Additional study CA 4.1.1 BASF DocID 2014/3013973 and paragraph on page 5	MCA Section 4 Version 1 BASF DocID 2013/1348640

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 4 ANALYTICAL METHODS

CA 4.1 Methods used for the generation of pre-approval data

CA 4.1.1 Methods for the analysis of the active substance as manufactured

(a) Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

Report:	CA 4.1.1/1 Rocha. C.C da, 2012a Short validation of the method M-2178.01 to determine the assay of Imazamox Technical (TGAI) by HPLC 2012/3004928
Guidelines:	<none>
GLP:	Yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Report:	CA 4.1.1/7
	Rocha C.C. da, 2014a
	Blank run interferance investigation in analytical method M-2178.01 - Imazamox Technical (TGAI) assay determination by HPLC
	2014/3013973
Guidelines:	<none>
GLP:	no

Principle of the method

The imazamox content is determined by reverse phase high performance liquid chromatography (HPLC) with UV detection at 220 nm and internal calibration with dimethyl phthalate as internal standard. Separation is achieved using isocratic flow conditions and quantification of the active ingredient by comparison with reference material.

Applicability of CIPAC methods

No CIPAC method is available for the analysis of the content of imazamox in technical grade imazamox.

Identity

The identity of the active substance was confirmed by comparison of the retention time of the reference compound with the retention time in the test item and by their UV absorbance spectra.

Linearity

Three standard solutions were used for the determination of linearity. The active ingredient imazamox was evaluated in the range of 290.8 to 484.6 mg/L and a correlation coefficient of 0.99955 was found. The results prove the linearity of the response in the investigated range.

y-axis intercept (b) :	- 0.01767
slope (m):	0.00289
correlarion factor:	0.99955
concentration range:	290.8 – 484.6.0 mg/L

Accuracy (Recovery)

The accuracy of the method was determined by analysis of three solutions fortified with imazamox at three concentration levels, respectively, expressed as the recovery percentage of the added active ingredient. The average recovery was 98.1% ± 0.9%.

Precision (Repeatability)

Repeatability was assessed by five independent replicate sample determinations of BAS 720 H (TGAI). The results were evaluated by the RSD% (Relative Standard Deviation) limit corresponding to the concentration and did not show higher value than the upper RSD% limit.

Component Reg.No.	Concentration found [%]	% RSD limit	% RSD analyzed	%RSD accepted
BAS 720 H	98.0	2.0	0.2	yes

Specifity

Applying the analytical method M-2178.01, no interference was observed between blank, sample and reference item preparations. The result is proven by comparison of spectra and retention times of the prepared solutions. Chromatograms and the UV spectra are reported in the attachment of study report BAS DocID 2014/3013973.

Conclusion

The analytical method M-2178-01 is suitable for the determination of imazamox in the technical grade BAS 720 H.

Report:	CA 4.1.1/2 Luz L.A. da, 2012a Validation of the analytical method for determination of resolved or racemic Imazamox in Tecnical Grade Active Ingredient (TGAI) Imazamox (BAS 720 H) 2012/3005122
Guidelines:	SOP-PA.2020
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Report:	CA 4.1.1/3 Luz L.A. da, 2013a Amendment 01 - Validation of the analytical method for determination of resolved or racemic Imazamox in Tecnical Grade Active Ingredient (TGAI) Imazamox (BAS 720 H) 2013/3000361
Guidelines:	SOP-PA.2020
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Principle of the method

The content of resolved or racemic Imazamox in technical grade BAS 720 H is determined by high performance liquid chromatography using isocratic separation on a chiral column, UV-DAD detection and external calibration with authentic, GLP-certified reference substances.

Applicability of CIPAC methods

No CIPAC method is available for the analysis of the content of resolved or racemic imazamox in technical grade imazamox.

Identity

The identity of the active substance was confirmed by comparison of the retention time of the reference compound with the retention time in the test item and by their UV absorbance spectra.

Selectivity

The selectivity was determined by comparison of a reference standard, a sample of technical product (TGAI) and a solvent solution (acetonitrile / water) injection. Two solutions from the reference standards R-imazamox (Reg.No. 4518133) and S-imazamox (Reg.No. 4535952) were prepared and also analysed. No co-elution or interference was observed in the results. Selectivity between both enantiomers was demonstrated.

Linearity

Seven standard preparations over a concentration range of 250 – 1100 mg/L were prepared from the reference substances R-imazamox (Reg.No. 4518133) and S-imazamox (Reg.No. 4535952) and used for the determination of the linearity. A correlation coefficient over 0.99 was found to the reference substances. The results prove the linearity of the responses in the investigated range.

R-Imazamox:

y-axis intercept (b) :	- 274.55304
slope (m):	37.65428
correlarion factor:	0.99993
concentration range:	253.97 – 1091.75 mg/L

S-Imazamox:

y-axis intercept (b) :	- 286.02028
slope (m):	36.95866
correlarion factor:	0.99985
concentration range:	259.02 – 1105.51 mg/L

Accuracy (Recovery)

The accuracy of the method was determined by analysis of three levels of the reference substance. The accuracy was expressed as the recovery percentage of the added active ingredient. The average recovery were 100.1% for R-imazamox and 99.9% for S-imazamox.

Precision (Repeatability)

Repeatability was assessed by five independent replicate sample determinations of each reference substance. The results were evaluated by the RSD% (Relative Standard Deviation) limit correspondent to the concentration and did not show higher value than the upper RSD% limit.

Component Reg.No.	Concentration found [%]	% RSD limit	% RSD analyzed	%RSD accepted
R-Imazamox [1)]	49.54	2.0	0.991	yes
R-Imazamox [2)]	49.32	2.0	0.094	yes
S-Imazamox [1)]	50.51	2.0	0.999	yes
S-Imazamox [2)]	50.28	2.0	0.094	yes

[1)] Statistics with sample RP-01 – RP-05, n=5

[2)] Sample RP-03 was rejected according to Grubbs test, n=4

Conclusion

The investigation showed that the analytical conditions used in the CAV-PA.2067 method are suitable for quantification of resolved and racemic imazamox in the technical grade BAS 720 H by chiral HPLC.

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

The analytical method for the determination of the relevant impurity impazapic and for the significant impurities contains CONFIDENTIAL information. Please refer to confidential part for further information (Document J).

CA 4.1.2 Methods for risk assessment

(a) Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Report:	CA 4.1.2/1 Gooding R., 2013b Validation of BASF analytical method D1102: Method for determination of BAS 720 H and Its metabolites CL 312622 and CL 354825 residues in soil using LC-MS/MS 2012/7004251
Guidelines:	EPA 835.6100, EPA 860.1340, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method

BAS 720 H –imazamox- and its metabolites CL 312622 and CL 354825 were extracted from soil samples (5 g each) by mechanical shaking with 0.5 N sodium hydroxide (twice, 10 mL each). The extracts were centrifuged, the supernatant decanted, and an aliquot of the combined extracts diluted with acidified water. The acidified extract was applied to a reverse phase (C18) solid phase extraction (SPE) column, and parent imazamox residues were eluted with dichloromethane and evaporated to dryness. The SPE column was connected to a strong cation exchange (SCX) column, and the combined columns were washed with methanol to remove co-extractives. The SPE column was then discarded and the SCX column, which retains the analytes (CL 312622 and CL 354825), was eluted using water: methanol (20:80, v/v) into the vessel with the imazamox residues, described above. The combined residues were evaporated to dryness, re-dissolved in acidified water: methanol (20:80, v/v).

Residues were determined by LC/MS/MS using the positive ionization mode monitoring two ion transitions m/z 306 → 261 for imazamox and CL 312622, and m/z 278 -→ 233 for CL 354825 as the primary transitions for quantitation for most soils. Other secondary or "alternate" transitions were used for confirmatory purposes. Limit of quantification (LOQ) of all test items was 0.001 ppm.

Recovery findings

The BASF method D1102 was proved to be suitable to determine imazamox and its metabolites CL 312622 and CL 354825 in soil, with LOQ of 0.001 ppm and a limit of detection (LOD) of 0.0002 ppm. Validation experiments were conducted in three different soils. All mean recovery values (mean of 5 replicates per fortification level and analyte) were within the acceptable range of 70-120% with RSD values less than 20%.The detailed results were given in Table 4.1.2-1 to Table 4.1.2-3.

Table 4.1.2-1: Method validation results for the determination of imazamox - in soil

Soil	Analyte	No. of replicates	Fortification level [ppm]	m/z 306.0 → 261.2		m/z 306.0 → 86.1	
				Mean Recovery [%]	RSD [%]	Mean Recovery [%]	RSD [%]
Clay loam soil	imazamox	5	0.001	100	6	97	7
		5	0.01	83	5	82	6
Sandy loam	imazamox	5	0.001	99	7	1002	7
		5	0.01	91	5	92	8
Sandy loam soil (German Lufa 2.2 soil)	imazamox	5	0.001	108	4	107	5
		5	0.01	96	5	95	5

Table 4.1.2-2: Method validation results for the determination of metabolite CL 312622 in soil

Soil	Analyte	No. of replicates	Fortification level [ppm]	m/z 306.0 → 261.2		m/z 306.0 → 264.1	
				Mean Recovery [%]	RSD [%]	Mean Recovery [%]	RSD [%]
Clay loam soil	CL 312622	5	0.001	85	9	84	5
		5	0.01	76	9	77	11
Sandy loam	CL 312622	5	0.001	76	10	75	7
		5	0.01	81	6	80	7
Sandy loam soil (German Lufa 2.2 soil)	CL 312622	5	0.001	86	13	87	11
		5	0.01	85	6	88	7

Table 4.1.2-3: Method validation results for the determination of metabolite CL 354825 in soil

Soil	Analyte	No. of replicates	Fortification level [ppm]	m/z 278.1 → 2332		m/z 278.1 → 165.3	
				Mean Recovery [%]	RSD [%]	Mean Recovery [%]	RSD [%]
Clay loam soil	CL 354825	5	0.001	72	1	72	9
		5	0.01	71	3	75	6
Sandy loam	CL 354825	5	0.001	79	2	81	4
		5	0.01	84	6	85	9
Sandy loam soil (German Lufa 2.2 soil)	CL 354825	5	0.001	89	7	88	9
		5	0.01	92	7	87	12

Linearity	Good linearity was observed in the range of 0.05 to 1 ng/mL for the test item.
Specificity	The method allowed for the specific determination of imazamox and its metabolites CL 312622 and CL 354825 in three different soil types by using LC/MS-MS and monitoring two ion transitions for each analyte. Apparent residues of imazamox and its metabolites were below the method limit of detection in all of the control soil samples (< 0.0002 ppm). No interferences from soil components or from reagents, solvents and glassware were observed.
Limit of Quantification	The limit of quantification was defined as the lowest fortification level successfully tested, which was 0.001 ppm for all analytes.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values for each test item are shown Table 4.1.2-1 to 4.1.2-3
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	The method for the analysis of imazamox and its metabolites CL 312622 and CL 354825 in soil uses LC-MS/MS for final determination, which is a highly selective detection technique. For every compound the quantitation is possible at two different ion transitions. Therefore, no additional confirmatory technique was required. It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of imazamox and its metabolites CL 312622 and CL 354825 in soil.

Report: CA 4.1.2/2
Bacher R., 2013b
Development and validation of an analytical method for the determination of Imazamox in air
2013/1134980

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Principle of the method

An analytical method was developed for the determination of BAS 720 H - imazamox residues in air. The analyte is spiked to XAD adsorption tubes at fortification levels of 0.16 mg/adsorption tube and 1.6 mg/adsorption tube, corresponding to approx. 0.4 mg/m3 (limit of quantification, LOQ) and approximately 4 mg/m3 (1OxLOQ). Warm, humid air (approx. 92% relative humidity) was sucked through XAD adsorption tubes at about 1.0 L/min for 6 hours (total air sampling volume of 0.36 m³). Subsequently, the adsorption material was extracted with acetonitrile. The extract was diluted and analysed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

Recovery findings

Mean recoveries (5 replicates) for both fortification levels and MS/MS transitions after air sampling ranged between 97 % and 102 %. For each fortification level, the relative standard deviations (RSD) were always ≤ 3%.Detailed results of recoveries for each mass transition and soil are given in Table 4.1.2-4.

No breakthrough (< 1 %) into the back layer of adsorption tubes was observed. The LC-MS/MS chromatograms of the blank control specimens showed no signals at the retention time of imazamox.

Table 4.1.2-4: Results of method validation of imazamox in air

Test System	Analyte	Replicates	Fortification level (µg)	Average C_{Air} (µg/m³)	306 m/z → 193 m/z		306 m/z → 261 m/z	
					mean [%]	RSD [%]	mean [%]	RSD [%]
air	imazamox	5	160	444	97	1	97	3
		5	1600	4452	101	2	102	1

Linearity	Good linearity (r > 0.99) was observed in the range of 1 ng/mL to 250 ng/mL for both mass transitions of imazamox.
Specificity	Under the described conditions the analytical method is specific for the determination of imazamox in air. Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of the test item. LC/MS-MS employed electron spray ionization and protonated molecular ions [M + H] + with m/z 306 as parent ion for MS/MS, monitoring the additional daughter ions m/z 193 and m/z 261. LC/MS-MS is thus considered selective and highly specific and does not require an additional confirmatory method.
Limit of Quantification	Limit of quantification should take into account relevant human and ecotoxicological-based limit values or exposure levels. Based on the equation described in SANCO/825/00 rev. 8.1 the limit of quantification should comply with the concentration C (mass of test item per volume air) calculated from the AOELinhalative. The AOELinhalative value at time of method development was proposed to be 0.015 mg/kg body weight/day. Considering a body weight of 60 kg, a safety factor of 0.1 and an average respiratory volume of 20 m3 per day, the limit of quantification (C) for the air method should be 4.5 ng/L. Based on a collected air volume of 360 L and a tenfold lower C, the amount to be spiked on the adsorber was calculated to be 1.6 µg. The lowest fortification level used for validation was 4.44 ng/L air and is therefore defined as limit of quantification.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 3%
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	The analytical method for the determination of imazamox in air was validated at spiking levels of C and 10 fold C corresponding to concentrations of 4.44 ng/L air (C) and 44.4 ng/L air (10 C). This method fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of imazamox in air.

Report:	CA 4.1.2/3 Toledo F., 2013a Method development and validation of an analytical method for the determination of BAS 720 H and its 2 metabolites Reg.No 4110603 and Reg.No 4110542 in water (analytical method L0209) 2013/1224024
Guidelines:	OECD-DOC ENV/MC/CHEM(98)17 Paris 1998, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 4.1.2/4***** Holzer S., 2013a Method development and validation of an analytical method for the determination of BAS 720 H and its 2 metabolites Reg.No 4110603 and Reg.No 4110542 in water (analytical method L0209) 2013/1327750
Guidelines:	OECD-DOC ENV/MC/CHEM(98)17 Paris 1998, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Principle of the method

BAS 720 H - imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 were isolated from a prepared test systems (ground water and surface water) by solid phase extraction (SPE). Residues were extracted from a 10 g water sample acidified with 0.5 mL of 2 N HCl. The water sample was loaded to a previously conditioned C-18 SPE column and eluted with 3 mL of dichloromethane (DCM) into a 15 mL glass centrifuge vial. The DCM eluent was evaporated at 40°C with a gentle nitrogen stream and the glass vial saved. The C-18 column was coupled with SCX SPE column and eluted with additional 3 mL of methanol. The SCX SPE column was detached prewashed and then eluted with 6 mL of water/methanol (20:80, v/v) into the same glass vial used to collect the eluent from the C-18 SPE column. The resultant solvent was mixed with a vortex, evaporated until dryness and reconstituted in 0.01 % formic acid in water/methanol (20:80, v/v).

(*No awareness of need of Report Amendment at time of Application)

Final determination of imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 was performed by LC-MS/MS method by monitoring two parent daughter ion transitions. The limit of quantification (LOQ) of the method was 0.025 µg/L.

The analytical method L0209 was successfully validated for the residue determination of BAS 720 H, Reg. No. 4110603 and Reg. No. 4110542 in water.

Recovery findings

The method was suitable to determine BAS 720 H and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in water. Validation experiments were conducted in surface and ground water. Mean recovery values (mean of 5 replicates per fortification level and analyte) ranged from 70 to 120 % with a relative standard deviation (RSD) < 20 %. The limit of quantification (LOQ) of the method was 0.025 µg/L for ground water and surface water. The detailed results are given in Table 4.1.2-5.

Table 4.1.2-5: Results of the method validation for the determination of imazamox and its metabolites in groundwater and surface water

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
BAS 720 H	306→261	Groundwater	5	0.025	93	2.5
				0.25	96	3.3
		Surface water	5	0.025	94	5.5
				0.25	96	2.2
	306→86	Groundwater	5	0.025	95	3.0
				0.25	93	5.9
		Surface water	5	0.025	95	5.2
				0.25	96	3.2
Reg. No. 4110603	278→233	Groundwater	5	0.025	85	3.9
				0.25	87	4.1
		Surface water	5	0.025	82	3.2
				0.25	86	3.0
	278→165	Groundwater	5	0.025	84	11.2
				0.25	89	4.3
		Surface water	5	0.025	80	3.3
				0.25	86	4.4
Reg. No. 4110542	306→261	Groundwater	5	0.025	84	4.3
				0.25	82	5.9
		Surface water	5	0.025	90	6.0
				0.25	87	7.2
	306→264	Groundwater	5	0.025	76	6.2
				0.25	83	8.2
		Surface water	5	0.025	78	5.0
				0.25	78	4.1

Linearity	Good linearity ($r \geq 0.999$) was observed in the imazamox concentrations ranging from 0.05 ng/mL to 2.0 ng/mL, and from 0.05 ng/mL to 2.5 ng/mL for the two ion transitions (306→261 m/z and 306→86 m/z) in the two different water types (groundwater and surface water), respectively. Similarly, a linear calibration curve ($r \geq 0.999$) was observed for each of the two ion transitions of the metabolites Reg. No. 4110603 and Reg. No. 4110542 in both water systems (groundwater and surface water).
Specificity	The method successfully determines imazamox -BAS 720 H- and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in the two water systems tested (groundwater and surface water) by using LC/MS-MS and monitoring two ion transitions for each analyte. No interferences (> 30% of the limit of quantification) in the tested untreated samples from water components or from reagents, solvents and glassware were observed at the retention times and ion transitions of each analyte.
Limit of Quantification	The method had a limit of quantification of 0.025 µg/L for BAS 720 H and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in groundwater and surface water.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-5.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	The method for the analysis of imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in groundwater and surface water uses LC-MS/MS for final determination is a highly specific technique with a limit of quantification of 0.025 µg/L. It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in groundwater and surface water samples.

Report:	CA 4.1.2/5 Schmitt J.L.,Patel D., 2013a Independent laboratory validation of BASF analytical method L0209/01: Method for the determination of BAS 720 H and its 2 metabolites reg. no. 4110603 and Reg. No. 4110542 in water 2013/7002701
Guidelines:	EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Objective

The objective of this independent laboratory validation (ILV) study was to demonstrate that BASF Analytical Method L0209/01 [*Toledo (2013), BASF DocID 2013/1224024*] could be performed successfully at an outside facility with no prior experience with the method.

Principle of the method

BAS 720 H - imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 were isolated from prepared test systems (ground water and drinking water) by solid phase extraction (SPE). Residues were extracted from a 10 g water sample acidified with 0.5 mL of 2 N HCl. The water sample was loaded to a previously conditioned C-18 SPE column and eluted with 3 mL of dichloromethane (DCM) into a 15 mL glass centrifuge vial. The DCM eluent was evaporated at 40°C with a gentle nitrogen stream and the glass vial saved. The C-18 column was coupled with SCX SPE column and eluted with additional 3 mL of methanol. The SCX SPE column was detached prewashed and then eluted with 6 mL of water/methanol (20:80, v/v) into the same glass vial used to collect the eluent from the C-18 SPE column. The resultant solvent was mixed with a vortex, evaporated until dryness and reconstituted in 0.01 % formic acid in water/methanol (20:80, v/v).

Final determination of imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 was performed by LC-MS/MS method in positive ion mode. The limit of quantification (LOQ) of the method was 0.025 µg/L.

In ground and drinking water, the ILV was tested at the LOQ (0.025 µg/L) and at 10× the LOQ (0.25 µg/L). For each fortification level and matrix, five replicates were analysed. Additionally, a method blank and at least two replicates of unfortified samples were examined.

The analytical method L0209/01 was successfully validated for the residue determination of BAS 720 H, Reg. No. 4110603 and Reg. No. 4110542 in water.

Recovery findings

The BASF method L0209/01 was suitable to determine BAS 720 H and Its metabolites Reg. No. 4110603 and Reg. No. 4110542 in water. Validation experiments were conducted in surface and drinking water. Mean recovery values of imazamox were found to be 78–88% and 79–83% for ground and drinking water, respectively. The mean recovery values of Reg. No. 4110542 were found to be 79–82% and 72–75% for ground and drinking water, respectively. The mean recovery values of Reg. No. 4110603 were found to be 77–80% and 85–86% for ground and drinking water, respectively. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The limit of quantification (LOQ) of the method was 0.025 µg/L in ground water and drinking water. The detailed results are given in Table 4.1.2-6.

Table 4.1.2-6: Independent laboratory validation results of the BAS method L0209/01 for the determination of imazamox and its metabolites in groundwater and drinking water

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	SD [%]	RSD [%]
BAS 720 H	**306→261**	Groundwater	5	0.025	82	11.9	14.5
				0.25	93	9.3	10.0
		Drinking water	5	0.025	73	7.5	10.3
				0.25	85	4.4	5.2
	306→86	Groundwater	5	0.025	70	11.5	16.5
				0.25	86	9.2	10.8
		Drinking water	5	0.025	75	10.0	13.4
				0.25	92	7.7	8.4
Reg. No. 4110603	**278→233**	Groundwater	5	0.025	78	5.4	6.9
				0.25	82	6.8	8.3
		Drinking water	5	0.025	84	3.0	3.6
				0.25	89	5.3	5.9
	278→165	Groundwater	5	0.025	77	8.6	11.2
				0.25	78	10.8	13.7
		Drinking water	5	0.025	83	1.3	1.6
				0.25	88	5.4	6.2
Reg. No. 4110542	**306→261**	Groundwater	5	0.025	77	12.8	16.6
				0.25	81	8.4	10.4
		Drinking water	5	0.025	75	11.9	15.8
				0.25	70	11.9	17.0
	306→264	Groundwater	5	0.025	85	20.5	24.1
				0.25	79	12.4	15.7
		Drinking water	5	0.025	76	15.0	19.8
				0.25	75	14.2	18.9

Linearity	Good linearity (r > 0.99) was observed in the range of 0.01 ng/mL to 2.5 ng/mL in mixed standard solutions for all analytes and mass transitions analysed.
Specificity	The method successfully determines imazamox -BAS 720 H- and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in the two water systems tested (groundwater and drinking water) by using LC/MS-MS and monitoring two ion transitions for each analyte. No interferences (> 30% of the limit of quantification) in the tested untreated samples from water components or from reagents, solvents and glassware were observed at the retention times and ion transitions of each analyte. The use of matrix-matched standards was not necessary.
Limit of Quantification	The LOQ was defined by the lowest fortification level successfully tested. The method had a limit of quantification of 0.025 µg/L for BAS 720 H and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in groundwater and drinking water.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-6.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	The method for the analysis of imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in water uses LC-MS/MS for final determination is a highly specific technique with a limit of quantification of 0.025 µg/L. It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine imazamox and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in groundwater and surface water samples.

(b) Methods in soil, water and any additional matrices used in support of efficacy studies

Not Relevant

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Since imazamox was not classified as toxic or very toxic according to the current requirements, methods of analysis for parent or metabolites in human body tissues or fluids were not investigated.

Methods for concentration control in feed or other matrices are reported, where necessary, along with the respective toxicological studies.

(d)Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

Since no exposure studies were conducted with imazamox, such methods of analysis were not developed.

(e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

The methods for which validation data are provided below were used for data generation in the submitted residue trials. Other methods used for data generation are either reported in chapter 4.2 since they are proposed for monitoring purposes (method L0188/01) or have been part of the last evaluation (e.g. method M3515 and CEM-236), but are however summarized in the table below for the reviewers' convenience.

Table 4.1.2-7: Summary table of analytical methods used for the quantitation of imazamox and its metabolites CL 312622, CL 263284 and CL 189215 in plant matrices

Group	Crop / Matrix	Analyte(s)	BASF Method No.	LOQ (mg/kg)	Reference
Dry commodities (high protein/high starch content)	Lentil (seed) Lentil (forage)	Imazamox CL 263284 CL 189215 CL 312622	LC-MS **M 3519** **Validation**	0.05	2004/5000274
Dry commodities (high protein/high starch content)	Lentil (seed) Lentil (forage)	Imazamox CL 263284 CL 189215 CL 312622	LC-MS LC-MS/MS **M 3519** **ILV**	0.05	2002/5004302
Commodites with high water content	Alfalfa (forage + hay) Alfalfa (seed)	Imazamox CL 263284 CL 189215 CL 312622	HPLC-MS HPLC-MS/MS **M 3178** **ILV**	0.1	ID-244-022
Commodities with high acid content Dry commodities (high protein/high starch content)	Grapes Oranges Peas Maize grain Wheat grain	Imazamox CL 263284	LC-MS/MS **M 3515** **Validation**	0.05 (0.01 for wheat grain)	ID-244-029 (included in previous evaluation, not part of this dossier)
Commodities with high acid content Dry commodities (high protein/high starch content)	Grapes Maize grain Wheat grain	Imazamox CL 263284	LC-MS LC-MS/MS **M3515** **ILV**	0.05 (0.01 for wheat grain)	2002/5002749 (included in previous evaluation, not part of this dossier)

Table 4.1.2-7: Summary table of analytical methods used for the quantitation of imazamox and its metabolites CL 312622, CL 263284 and CL 189215 in plant matrices

Group	Crop / Matrix	Analyte(s)	BASF Method No.	LOQ (mg/kg)	Reference
Dry commodities (high protein/high starch content)	Bean	Imazamox	HPLC-UV	0.05	ID-244-018
	Peas	CL 263284	**CEM 236**		ID-244-017
	Maize grain				ID-244-016
Commodites with high water content	Maize ears				(included in previous evaluation, not part of this dossier)
	Maize immature plant				

Validation of method M 3519

The following method was used as data generation method in some of the oilseed rape trials.

Report:	CA 4.1.2/6 Nejad H., 2004a Validation of BASF analytical method M 3519 (2002) entitled BAS 720 H and BAS 685 H: LC/MS determinative and LC/MS/MS confirmatory method for BAS 720 H, CL 263284, CL 189215, CL 312622, BAS 685 H, CL 288511 CL 182704 in lentils seed, forage 2004/5000274
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, PMRA 98-02 (June 1998), SANCO/825/00 rev. 6 (20 June 2000)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method: In Method M 3519 residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 189215 and CL 312622 are extracted from lentil seed and forage samples using an acidic methanol-water solution. An aliquot of the seed or forage sample extract is diluted with methanol and then passed through an SCX cartridge, which retains the analytes. The SCX cartridge is washed with methanol to remove co-extractives; then the residues are selectively eluted with a water-methanol solution. The eluate is evaporated to dryness and the residues are dissolved in acidic water for analysis. Measurement of the residues is accomplished by liquid chromatography with mass spectrometric detection (LC-MS or LC-MS/MS).

Recovery findings: In both matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.1.2-8: Validation results of method M 3519: imazamox and metabolites CL 263284, CL 189215 and CL 312622 in lentils

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Imazamox (BAS 720 H)	Lentil (seed)	0.05	5	93	4
		0.50	5	95	2
	Lentil (forage)	0.05	5	94	2
		0.50	5	92	1
CL 263284	Lentil (seed)	0.05	5	98	4
		0.50	5	95	2
	Lentil (forage)	0.05	5	94	2
		0.50	5	93	1
CL 189215	Lentil (seed)	0.05	5	89	3
		0.50	5	99	2
	Lentil (forage)	0.05	5	92	3
		0.50	5	96	3
CL 312622	Lentil (seed)	0.05	5	86	4
		0.50	5	75	2
	Lentil (forage)	0.05	5	95	4
		0.50	5	87	3

Linearity: The linearity was tested using standard solutions at concentrations between 0.25 and 2.0 ng/mL. Linear correlations with coefficients >0.999 were obtained for imazamox and CL 263284.

Specificity: The results for the residue findings by use of LC-MS were confirmed by HPLC-MS/MS (see BASF DocID 2002/5004302 below). No interferences from plant components or from reagents, solvents and glassware were reported.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.05 mg/kg in lentils seeds and forage.

Repeatability: The relative standard deviations (RSD, %) for both commodities and fortification levels were <10%. The detailed values are shown in Table 4.1.2-8.

Reproducibility: An independent laboratory validation has been conducted and is reported below.

Conclusion: Analytical method M 3519 is considered suitable for the analysis of imazamox and metabolites CL 263284, CL 189215 and CL 312622 in lentils seeds and forage.

Independent laboratory validation of method M 3519

Report: CA 4.1.2/7
Jordan J.M., 2003a
Independent method validation of BASF analytical method M 3519 entitled BAS 720 H and BAS 685 H: Determination and confirmation of BAS 720 H, CL 263284, CL 189215, CL 312622, BAS 685 H, CL 288511 and CL 182704 residues in lentils
2002/5004302
Guidelines: EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000)
GLP: Yes
(certified by United States Environmental Protection Agency)

Principle of the method: In Method M 3519 residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 189215 and CL 312622 are extracted from lentil seed and forage samples using an acidic methanol-water solution. An aliquot of the seed or forage sample extract is diluted with methanol and then passed through an SCX cartridge, which retains the analytes. The SCX cartridge is washed with methanol to remove co-extractives; then the residues are selectively eluted with a water-methanol solution. The eluate is evaporated to dryness and the residues are dissolved in acidic water for analysis. Measurement of the residues is accomplished by liquid chromatography with mass spectrometric detection (LC-MS or LC-MS/MS).

Recovery findings: In both matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.1.2-9: Independent laboratory validation results of method M 3519: imazamox and its metabolites CL 263284, CL 189215 and CL 312622 in lentils (LC-MS)

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Imazamox (BAS 720 H)	Lentil (seed)	0.05	5	95	16
		0.50	5	87	7
	Lentil (forage)	0.05	5	84	15
		0.50	5	88	5
CL 263284	Lentil (seed)	0.05	5	109	19
		0.50	5	99	4
	Lentil (forage)	0.05	5	100	9
		0.50	5	87	14
CL 189215	Lentil (seed)	0.05	5	86	7
		0.50	5	93	7
	Lentil (forage)	0.05	5	84	2
		0.50	5	89	6
CL 312622	Lentil (seed)	0.05	5	86	10
		0.50	5	88	6
	Lentil (forage)	0.05	5	85	11
		0.50	5	87	13

Table 4.1.2-10: Independent laboratory validation results of method M 3519: imazamox and its metabolites CL 263284, CL 189215 and CL 312622 in lentils - confirmatory method results (LC-MS/MS)

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Imazamox (BAS 720 H)	Lentil (seed)	0.05	5	84	4
		0.50	5	85	3
	Lentil (forage)	0.05	5	85	2
		0.50	5	82	7
CL 263284	Lentil (seed)	0.05	5	92	11
		0.50	5	90	3
	Lentil (forage)	0.05	5	92	6
		0.50	5	89	9
CL 189215	Lentil (seed)	0.05	5	91	15
		0.50	5	84	11
	Lentil (forage)	0.05	5	83	18
		0.50	5	80	9
CL 312622	Lentil (seed)	0.05	5	94	5
		0.50	5	88	6
	Lentil (forage)	0.05	5	87	5
		0.50	5	87	4

Linearity: The linearity was tested using standard solutions ranging from 0.25 to 2.0 ng/mL covering the working range of the sample concentrations. Linear correlations with coefficients >0.99 were obtained for all investigated analytes.

Specificity: The results for the residue findings by use of LC-MS were confirmed by LC-MS/MS. Results are shown in Table 4.1.2-10.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.05 mg/kg in lentils seeds and forage.

Repeatability: The relative standard deviations (RSD, %) for both commodities were below 20% when fortified at 0.05 mg/kg and below 15% when fortified at 0.50 mg/kg for each analyte. The detailed values are shown in Table 4.1.2-9.

Reproducibility: This report represents an independent laboratory validation.

Conclusion: The independent laboratory validation confirmed Method M 3519 to be suitable for the analysis of imazamox (BAS 720 H) and metabolites CL 263284, CL 189215 and CL 312622 in lentils seeds and forage.

Validation of method M 3178

This method has been used for data generation in some soybean and alfalfa trials.

Report:	CA 4.1.2/8 Wickremesinhe E.,Safarpour H., 1998a CL 299263 (Imazamox): Independent laboratory validation of HPLC/MSD method M 3178 for the determination of residues of CL 299263, CL 263284, CL 189215 and CL 312622 in alfalfa seed, forage (whole green plant) and hay (whole dried plant) ID-244-022
Guidelines:	EPA 40 CFR 158.240, EPA 860.1340, EPA 171-4
GLP:	Yes (certified by United States Environmental Protection Agency)

Principle of the method: In Method M 3178 residues of imazamox, CL 263284, CL 189215 and CL 312622 are extracted from the samples with acidic water-methanol followed by filtration. For imazamox, CL 263284 and CL 189215, the extracts are cleaned up with precipitation, centrifugation and Solid-Phase Extraction techniques using RP-102 and SCX columns. For CL 312622, the extracts are cleaned up by filtration with an ISOLUTE filtration column and Solid-Phase Extraction techniques (C18, QMA and SCX columns). Measurements of the residues are accomplished by reverse-phase HPLC with mass spectrometric detection (LC-MS) and monitoring product ions (LC-MS/MS). LC-MS/MS is used for the analysis of imazamox, CL 263284, CL 189215 and CL 312622 in alfalfa seed samples.

Recovery findings: In all matrices tested, the mean recovery values were between 70% and 110%, except for CL 189215 in hay where the mean recovery was 68%. However, the mean recovery over all fortification levels was acceptable with 76%. The detailed results are given in the table below.

Table 4.1.2-11: Independent laboratory validation results of method M 3178: imazamox and metabolites CL 263284, CL 189215 and CL 312622 in alfalfa

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Imazamox (BAS 720 H)	Alfalfa (forage)	0.1	2	75	N/A
		0.2	2	83	N/A
		0.5	2	80	N/A
		10	2	101	N/A
		overall	*8*	*85*	*12*
	Alfalfa (hay)	0.1	2	101	N/A
		0.2	2	83	N/A
		0.5	2	93	N/A
		10	2	93	N/A
		overall	*8*	*92*	*7.5*
	Alfalfa (seeds)	0.1	2	85	N/A
		0.2	2	87	N/A
		0.5	2	87	N/A
		10	2	72	N/A
		overall	*8*	*83*	*12*
CL 263284	Alfalfa (forage)	0.1	2	82	N/A
		0.2	2	83	N/A
		0.5	2	81	N/A
		10	2	102	N/A
		overall	*8*	*87*	*11*
	Alfalfa (hay)	0.1	2	85	N/A
		0.2	2	78	N/A
		0.5	2	86	N/A
		10	2	86.5	N/A
		overall	*8*	*84*	*4.9*
	Alfalfa (seeds)	0.1	2	88	N/A
		0.2	2	92	N/A
		0.5	2	96	N/A
		10	2	89	N/A
		overall	*8*	*91*	*13*

Table 4.1.2-11: Independent laboratory validation results of method M 3178: imazamox and metabolites CL 263284, CL 189215 and CL 312622 in alfalfa

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
CL 189215	Alfalfa (forage)	0.1	2	76	N/A
		0.2	2	78	N/A
		0.5	2	79	N/A
		10	2	97	N/A
		overall	*8*	*82*	*11*
	Alfalfa (hay)	0.1	2	76	N/A
		0.2	2	68	N/A
		0.5	2	79	N/A
		10	2	82	N/A
		overall	*8*	*76*	*7.9*
	Alfalfa (seeds)	0.1	2	83	N/A
		0.2	2	73	N/A
		0.5	2	74	N/A
		10	2	72	N/A
		overall	*8*	*75*	*13*
CL 312622	Alfalfa (forage)	0.1	2	80	N/A
		0.2	2	88	N/A
		0.5	2	89	N/A
		10	2	87	N/A
		overall	*8*	*86*	*5.6*
	Alfalfa (hay)	0.1	2	83	N/A
		0.2	2	74	N/A
		0.5	2	81	N/A
		10	2	83	N/A
		overall	*8*	*80*	*5.0*
	Alfalfa (seeds)	0.1	2	77	N/A
		0.2	2	76	N/A
		0.5	2	79	N/A
		10	2	81	N/A
		overall	*8*	*78*	*4.0*

Linearity: Not reported.

Specificity: No interferences from plant components or from reagents, solvents and glassware were reported.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.1 mg/kg for alfalfa commodities.

Repeatability: The relative standard deviations (RSD, %) for all commodities over all fortification levels were <15%. The detailed values are shown in Table 4.1.2-11.

Stability in solution: Within the validation study, also the stability of the analytes imazamox, CL 263284, CL 189215 and CL 312622 in solution was investigated. The test materials were found to be stable up to one month in 10% methanol/water at a temperature of 4°C ± 2°C with no more than 8% difference between the response of the one month old and the new fortification solution.

Conclusion: The independent laboratory validation showed that Method M 3178 is suitable for the analysis of imazamox, CL 263284, CL 189215 and CL 312622 in alfalfa commodities.

(f) in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies;

Methods for concentration control are reported, where necessary, along with the respective ecotoxicological studies.

(g) in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests.

Where necessary, these methods are reported along with the respective studies.

CA 4.2 Methods used for post-approval control and monitoring purposes

In the course of the MRL re-evaluation according to Article 12 of Regulation (EC) No 396/2005, a validated method of analysis for the determination of imazamox residues in high oil content crops and a confirmatory method for the determination of imazamox residues in high oil content, high water content, acidic and dry crops were indicated as necessary data supplements supporting the existing analytical methods during the renewal process of the active substance imazamox (see EFSA Journal 2013;11(6):3282, 34 pp. doi:10.2903/j.efsa.2013.3282).

Regarding food of animal origin, no analytical method of analysis was proposed during the peer review under Directive 91/414/EEC as the estimated feed intake for livestock was below 0.1 mg/kg feed and it was not considered that residues would occur in food of animal origin under approved conditions of use (France, 1999). Considering a wider range of crops in the MRL re-evaluation as well as in this submission, the estimated intake of residues by livestock is above the trigger for significance for some species. Although still no detectable residues are expected in animal products with an intake at the level of the maximum dietary burden, a validated method of analysis for the determination of imazamox residues in commodities of animal origin, supported by independent laboratory validation data and a confirmatory method were required during the EU MRL re-evaluation (see Reasoned opinion on the review of the existing maximum residue levels (MRLs) for imazamox according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(6):3282, 34 pp. doi:10.2903/j.efsa.2013.3282).

The following residue method data requirements were listed in the EFSA Reasoned Opinion

- A validated method of analysis for the determination of imazamox residues in high oil content crops
- a confirmatory method for imazamox determination in high oil content, high water content, acidic and dry commodities
- a validated analytical method for the determination of imazamox residues in commodities of animal origin (supported by independent laboratory validation data and a confirmatory method)

The requested method validation data are provided with this dossier. None of the below listed studies have been peer reviewed before.

Table 4.2-1: Summary table of analytical methods used for the quantitation of imazamox and its metabolites CL 312622, CL 263284 and CL 189215 in plant and animal matrices

Group	Crop / Matrix	Analyte(s)	Method BASF Method No.	LOQ (mg/kg)	Reference
Dry commodities (high protein/high starch content)	Peas (dry) Rice (grain) Rice (straw)	Imazamox CL 263284 CL 189215 CL 312622	LC-MS/MS L0188/01 Validation	0.01	2012/1294678 2013/1177533
Commodites with high water content	Rice (whole plant) Green beans				
Commodities with high acid content	Grapes				
Commodities with high oil content	Sunflower (seed)				
Dry commodities (high protein/high starch content)	Peas (dry) Rice (grain) Rice (straw)	Imazamox CL 263284 CL 189215 CL 312622	LC-MS/MS L0188/01 ILV	0.01	2013/1249356
Commodites with high water content	Rice (whole plant) Green beans				
Commodities with high acid content	Grapes				
Commodities with high oil content	Sunflower (seed)				
Dry commodities (high protein/high starch content)	Peas (dry) Rice (grain)	Imazamox CL 263284	LC-MS/MS QuEChERS	0.01	2013/1249355
Commodites with high water content	Green beans	CL 189215* CL 312622*	Validation		
Commodities with high acid content	Grapes				
Commodities with high oil content	Sunflower (seed)				
Commodities of animal origin	Bovine kidney Bovine liver Bovine muscle Bovine fat Bovine milk Poultry eggs				

Table 4.2-1: Summary table of analytical methods used for the quantitation of imazamox and its metabolites CL 312622, CL 263284 and CL 189215 in plant and animal matrices

Group	Crop / Matrix	Analyte(s)	Method BASF Method No.	LOQ (mg/kg)	Reference
Commodities of animal origin	Bovine kidney Bovine liver Bovine muscle Bovine fat Bovine milk	Imazamox CL 263284	LC-MS/MS Validation D0303	0.01	2003/5000116
Commodities of animal origin	Bovine kidney Bovine liver Bovine muscle Bovine fat Bovine milk Poultry eggs	Imazamox CL 263284	LC-MS/MS Validation D0303	0.01	2013/7002842
Commodities of animal origin	Bovine kidney Bovine liver Bovine muscle Bovine fat Bovine milk Poultry eggs	Imazamox CL 263284	LC-MS/MS ILV D0303	0.01	2013/7002962

* No successful validation for this compound

Validation of method L0188/01

The validation of the following method (including its independent lab validation) provides the required data for enforcing imazamox residues in all relevant commodity groups as specified in SANCO 825/00 rev. 8.1: high acid content (→grapes), high oil content (→sunflower seeds), high water content (→rice forage, green beans) and dry commodities (→rice grain, rice straw and dry peas).

Report:	CA 4.2/1 Lehmann A., 2013a Validation of BASF method no. L0188/01: Method for the determination of Imazamox (BAS 720 H, Reg.No. 4096483) and its metabolites Reg.No. 4110542 (CL312622), Reg.No. 4110773 (CL263284) and Reg.No. 4110445 (CL189215) in plant matrices 2012/1294678
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 6 (20 June 2000), EEC 96/46 (16.07.1996), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 4.2/2 Lehmann A., 2013b Amendment No. 1: Validation BASF No. L0188/01 - Method for determination of Imazamox (BAS 720 H, Reg.No. 4096483) and its metabolites Reg.No. 4110542 (CL312622), Reg.No. 4110773 (CL263284) and Reg.No. 4110445 (CL189215) in plant matrices 2013/1177533
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 6 (20 June 2000), EEC 96/46 (16.07.1996), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method: BASF Method No. L0188/01 allows the determination of imazamox and its metabolites as CL 312622, CL 263284 and CL 189215. Imazamox and its metabolites are extracted from plant matrices with methanol/water/1 N HCl (60:39:1, v/v/v). In case of high protein samples (e.g. peas and beans), ammonium sulfate is added to the extract. A portion of the extract is centrifuged for 5 min at 4000 rpm, filtered through a disposable syringe filter and an aliquot of the filtrate is diluted to obtain the final volume for LC-MS/MS determination against matrix matched standards.

Recovery findings: In all matrices tested, the mean recovery values were between 60% and 120% when fortified at 0.01 mg/kg and between 70% and 120% when fortified at 0.1 mg/kg. The detailed results are given in the table below.

Table 4.2-2: Validation results of method L0188/01: imazamox and metabolites CL 312622, CL 263284 and CL 189215 in plant matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**306 m/z → 261* m/z**	**306 m/z → 193 m/z**	**306 m/z → 261* m/z**	**306 m/z → 193 m/z**
Imazamox (BAS 720 H)	Rice (whole plant)	0.01	5	101.3	107.4	9.1	6.3
		0.1	5	92.3	91.0	2.5	2.7
	Rice (grain)	0.01	5	95.4	94.4	1.5	2.5
		0.1	5	94.4	95.0	1.9	0.9
	Rice (straw)	0.01	5	102.5	100.3	6.5	7.8
		0.1	5	87.2	86.9	4.0	4.2
	Green beans	0.01	5	96.9	95.0	1.8	1.6
		0.1	5	92.8	92.6	0.5	1.3
	Sunflower (seeds)	0.01	5	95.4	94.8	2.5	4.3
		0.1	5	91.1	92.3	2.8	3.4
	Peas (dry)	0.01	5	89.1	89.5	11.7	12.0
		0.1	5	86.8	90.5	4.2	7.4
	Grapes	0.01	5	94.1	93.6	3.1	6.7
		0.1	5	85.7	85.5	4.4	4.3
Transition				**306 m/z → 261* m/z**	**306 m/z → 69 m/z**	**306 m/z → 261* m/z**	**306 m/z → 69 m/z**
CL 312622	Rice (whole plant)	0.01	5	98.7	105.5	9.0	9.1
		0.1	5	92.2	90.8	1.4	1.7
	Rice (grain)	0.01	5	96.7	93.9	1.6	4.9
		0.1	5	94.8	95.1	2.2	1.6
	Rice (straw)	0.01	5	96.0	100.8	5.3	7.6
		0.1	5	85.7	84.4	3.1	3.8
	Green beans	0.01	5	93.6	91.0	2.1	4.6
		0.1	5	91.9	92.5	1.5	1.5
	Sunflower (seeds)	0.01	5	92.6	92.0	6.9	4.4
		0.1	5	89.0	87.7	3.0	3.8
	Peas (dry)	0.01	5	92.0	92.0	4.5	10.2
		0.1	5	89.7	92.2	4.9	1.5
	Grapes	0.01	5	94.7	101.9	9.1	6.8
		0.1	5	86.6	88.9	3.9	4.5

Table 4.2-2: Validation results of method L0188/01: imazamox and metabolites CL 312622, CL 263284 and CL 189215 in plant matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**292 m/z → 232 m/z**	**292 m/z → 247* m/z**	**292 m/z → 232 m/z**	**292 m/z → 247* m/z**
CL 263284	Rice (whole plant)	0.01	5	95.1	98.8	9.4	8.8
		0.1	5	94.1	93.8	2.5	2.5
	Rice (grain)	0.01	5	99.8	99.5	4.5	3.9
		0.1	5	97.9	96.9	2.9	2.6
	Rice (straw)	0.01	5	101.4	103.2	8.6	9.2
		0.1	5	89.4	89.2	3.2	1.9
	Green beans	0.01	5	94.1	92.4	4.1	1.8
		0.1	5	91.3	91.2	1.6	1.4
	Sunflower (seeds)	0.01	5	89.4	88.4	2.8	3.7
		0.1	5	85.0	84.7	2.5	3.0
	Peas (dry)	0.01	5	93.2	94.8	11.8	4.6
		0.1	5	97.3	95.3	2.2	1.9
	Grapes	0.01	5	97.1	93.4	4.4	3.8
		0.1	5	87.8	87.3	3.2	2.9
Transition				**454 m/z → 292* m/z**	**454 m/z → 86 m/z**	**454 m/z → 292* m/z**	**454 m/z → 86 m/z**
CL 189215	Rice (whole plant)	0.01	5	98.6	98.1	8.5	10.0
		0.1	5	94.7	93.7	1.1	2.6
	Rice (grain)	0.01	5	103.1	107.7	3.3	7.3
		0.1	5	100.0	98.9	2.8	2.2
	Rice (straw)	0.01	5	101.8	93.5	8.9	8.5
		0.1	5	91.8	90.3	1.4	2.9
	Green beans	0.01	5	89.0	90.6	2.2	5.4
		0.1	5	89.1	88.7	3.1	3.4
	Sunflower (seeds)	0.01	5	87.7	80.1	2.1	23.3
		0.1	5	88.3	88.0	1.8	3.6
	Peas (dry)	0.01	5	92.7	89.9	12.2	14.1
		0.1	5	95.8	95.8	4.7	3.0
	Grapes	0.01	5	102.0	94.3	8.2	8.2
		0.1	5	88.0	91.6	2.9	4.3

* proposed for quantitation

Linearity: Good linearity was observed in the range tested with correlation coefficients >0.99.

Specificity: LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg in green beans, died peas, grapes, sunflower seeds and the rice matrices whole plant, grain and straw.

Repeatability: The relative standard deviations (RSD, %) for all commodities were below 30% when fortified at 0.01 mg/kg and below 20% when fortified at 0.1 mg/kg for each analyte. The detailed values are shown in Table 4.2-2.

Reproducibility: An independent laboratory validation of this method is presented in the following (DocID 2013/1249356).

Conclusion: Analytical method L0188/01 is suitable for monitoring of imazamox and its metabolites CL 312622, CL 263284 and CL 189215 residues in plant matrices. Covering all relevant matrix groups (high acid, high oil, high water and dry matrices), it is proposed as enforcement method for imazamox residues.

Independent laboratory validation of method L0188/01

Report:	CA 4.2/3 Mewis A., 2013a Independent laboratory validation (ILV) of an analytical method L0188/01 for the determination of BAS 720 H and 3 metabolites in plant matrices 2013/1249356
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method: BASF Method No. L0188/01 allows the determination of imazamox and its metabolites CL 312622, CL 263284 and CL 189215. Imazamox and its metabolites are extracted from plant matrices with methanol/water/1 N HCl (60:39:1, v/v/v). In case of high protein samples (e.g. peas and beans), ammonium sulfate is added to the extract. A portion of the extract is centrifuged for 5 min at 4000 rpm, filtered through a syringe filter. Final determination is performed with LC-MS/MS against matrix matched standards.

Recovery findings: In all matrices tested, the mean recovery values were between 70% and 120%. The detailed results are given in the table below.

Table 4.2-3: Independent laboratory validation results of method L0188/01: imazamox and metabolites CL 312622, CL 263284 and CL 189215 in plant matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**306 m/z → 261* m/z**	**306 m/z → 193 m/z**	**306 m/z → 261* m/z**	**306 m/z → 193 m/z**
Imazamox (BAS 720 H)	Rice (whole plant)	0.01	5	106	102	8	4
		0.10	5	78	78	12	12
	Rice (grain)	0.01	5	101	97	3	2
		0.10	5	85	84	6	6
	Rice (straw)	0.01	5	99	102	3	1
		0.10	5	86	85	16	16
	Green beans	0.01	5	106	106	7	4
		0.10	5	86	86	8	8
	Sunflower (seeds)	0.01	5	97	93	5	6
		0.10	5	85	83	6	7
	Peas (dry)	0.01	5	100	99	3	5
		0.10	5	83	84	4	3
	Grapes	0.01	5	108	106	6	4
		0.10	5	92	90	7	7
Transition				**306 m/z → 261* m/z**	**306 m/z → 69 m/z**	**306 m/z → 261* m/z**	**306 m/z → 69 m/z**
CL 312622	Rice (whole plant)	0.01	5	106	96	6	15
		0.10	5	80	77	11	14
	Rice (grain)	0.01	5	103	101	4	3
		0.10	5	84	88	5	3
	Rice (straw)	0.01	5	103	101	4	3
		0.10	5	82	81	16	16
	Green beans	0.01	5	106	106	5	4
		0.10	5	84	87	7	7
	Sunflower (seeds)	0.01	5	98	94	6	11
		0.10	5	86	83	6	6
	Peas (dry)	0.01	5	102	106	6	3
		0.10	5	83	83	3	4
	Grapes	0.01	5	103	100	7	9
		0.10	5	92	92	6	8

Table 4.2-3: Independent laboratory validation results of method L0188/01: imazamox and metabolites CL 312622, CL 263284 and CL 189215 in plant matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**292 m/z → 232* m/z**	**292 m/z → 247 m/z**	**292 m/z → 232* m/z**	**292 m/z → 247 m/z**
CL 263284	Rice (whole plant)	0.01	5	109	108	11	6
		0.10	5	80	78	13	11
	Rice (grain)	0.01	5	104	107	3	4
		0.10	5	84	84	8	5
	Rice (straw)	0.01	5	104	101	6	3
		0.10	5	82	81	17	16
	Green beans	0.01	5	102	106	9	8
		0.10	5	83	82	8	8
	Sunflower (seeds)	0.01	5	96	100	11	7
		0.10	5	85	84	8	6
	Peas (dry)	0.01	5	99	106	2	3
		0.10	5	83	84	2	3
	Grapes	0.01	5	108	108	7	5
		0.10	5	94	92	6	7
Transition				**454 m/z → 292* m/z**	**454 m/z → 86 m/z**	**454 m/z → 292* m/z**	**454 m/z → 86 m/z**
CL 189215	Rice (whole plant)	0.01	5	108	94	10	11
		0.1	5	77	76	13	11
	Rice (grain)	0.01	5	104	100	6	9
		0.1	5	86	87	7	10
	Rice (straw)	0.01	5	106	101	8	12
		0.1	5	86	85	15	16
	Green beans	0.01	5	107	107	5	18
		0.1	5	82	82	7	7
	Sunflower (seeds)	0.01	5	99	100	9	11
		0.1	5	81	83	6	5
	Peas (dry)	0.01	5	105	100	2	9
		0.10	5	85	82	4	5
	Grapes	0.01	5	106	110	5	10
		0.1	5	90	89	6	7

* proposed for quantitation

Linearity: For analysis of imazamox and its metabolites in all matrices at the quantifier and qualifier mass transitions, the detector response was linear within the range from 0.1 to 10 ng/mL (0.002 mg/kg to 0.2 mg/kg) with r >0.99.

Specificity: LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Limit of Quantitation: The limit of quantitation was 0.01 mg/kg in green beans, dry peas, grapes, sunflower seeds and the rice matrices whole plant, grain and straw.

Repeatability: The relative standard deviations (RSD, %) for all commodities were below 20% for each analyte. The detailed values are shown in Table 4.2-3.

Reproducibility: This independent laboratory validation confirmed the results of the method validation.

Conclusion: The independent lab validation of analytical method L0188/01 proves its suitability for monitoring of residues of imazamox and its metabolites CL 312622, CL 263284 and CL 189215 in plant matrices.

Validation of QuEChERS method

Report: CA 4.2/4
Yozgatli H.P.,Breyer N., 2013a
Validation of the multi-residue method QuEChERS for the determination of Imazamox (BAS 720 H) and metabolites in different matrices of plant and animal origin
2013/1249355

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, SANCO/825/00 rev. 8.1 (16 November 2010), EPA 850.6100, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

GLP: yes
(certified by Freie und Hansestadt Hamburg, Behoerde fuer Soziales, Familie, Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method: The multi-residue method QuEChERS was validated for the analysis of imazamox and its metabolites. After addition of water (necessary for specimens with water content below 80%) and extraction with acetonitrile, a salt mixture of magnesium sulphate, sodium chloride and sodium citrate was added to the extracts followed by centrifugation. Especially for bovine fat purification was done by freeze-separation at ≤-18°C. Detection was performed with liquid chromatography tandem mass spectrometry (LC-MS/MS).

Recovery findings: Mean recovery values obtained for imazamox in matrices of animal and plant origin at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10x LOQ) comply with the standard acceptance criteria of SANCO Guideline 825/00 rev. 8.1., which demand that the mean recovery should be in the range of 70-120%.

Mean recovery values obtained for CL 263284 in matrices of animal and plant origin at fortification levels of 0.01 mg/kg and 0.1 mg/kg were in the range of 70-120% except for grapes and kidney. For grapes and kidney the mean recovery was 65-66%.

The mean recovery values obtained for metabolite CL 312622 in matrices of animal and plant origin were outside the standard acceptance criteria at both fortification levels (70-120%).

The mean recovery values obtained for the metabolite CL 189215 in matrices of plant origin were outside the standard acceptance criteria at both fortification levels (70-120%).

The detailed results are given in the tables below.

Table 4.2-4: Validation results of QuEChERS method for imazamox in various plant and animal matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Transition			**m/z 306 → 261***			**m/z 306 → 193****		
Imazamox (BAS 720 H)	Bean	0.01	5	93	8.8	5	98	2.3
		0.1	5	85	6.3	5	86	1.3
	Grape	0.01	5	94	11	5	87	4.9
		0.1	5	71	6.5	5	73	4.8
	Dry pea	0.01	5	97	2.7	5	90	4.9
		0.1	5	76	13	5	76	9.0
	Rice (grain)	0.01	5	86	5.5	5	90	3.4
		0.1	5	80	11	5	79	5.0
	Sunflower (seed)	0.01	5	76	6.3	5	77	2.4
		0.1	5	73	4.2	5	73	3.0
	Bovine (milk)	0.01	5	94	9.2	5	89	9.3
		0.1	5	90	7.4	5	88	5.6
	Bovine (fat)	0.01	5	103	8.4	5	94	6.7
		0.1	5	90	13	5	92	4.9
	Bovine (kidney)	0.01	5	83	10	5	81	5.5
		0.1	5	73	2.0	5	75	3.5
	Bovine (liver)	0.01	5	77	4.8	5	81	3.0
		0.1	5	75	2.0	5	75	3.2
	Bovine (meat)	0.01	5	109	13	5	105	6.5
		0.1	5	92	19	5	93	4.2
	Poultry (eggs)	0.01	5	86	19	5	86	1.3
		0.1	5	75	8.9	5	76	3.6

* Transition used for quantitation

** Transition used for confirmatory purposes

Table 4.2-5: Validation results of QuEChERS method for CL 263284 in various plant and animal matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Transition			**m/z 292 → 247***			**m/z 292 → 232****		
CL 263284	Bean	0.01	5	93	8.4	5	90	3.2
		0.1	5	90	5.4	5	85	4.1
	Grape	0.01	5	73	4.9	5	72	5.1
		0.1	5	66	7.8	5	65	7.4
	Dry pea	0.01	5	82	14	5	85	2.1
		0.1	5	71	5.5	5	70	7.4
	Rice (grain)	0.01	5	99	5.3	5	93	2.0
		0.1	5	78	5.7	5	81	2.0
	Sunflower (seed)	0.01	5	74	4.7	5	76	4.8
		0.1	5	73	4.1	5	72	1.6
	Bovine (milk)	0.01	5	92	9.2	5	91	3.9
		0.1	5	83	6.8	5	87	7.8
	Bovine (fat)	0.01	5	96	5.7	5	95	4.0
		0.1	5	98	6.7	5	93	7.1
	Bovine (kidney)	0.01	5	78	11	5	73	4.1
		0.1	5	65	6.2	5	66	3.0
	Bovine (liver)	0.01	5	82	7.7	5	74	4.6
		0.1	5	74	4.7	5	70	2.0
	Bovine (meat)	0.01	5	85	14	5	92	3.8
		0.1	5	73	11	5	81	4.9
	Poultry (eggs)	0.01	5	81	5.1	5	77	5.6
		0.1	5	73	7.4	5	74	2.2

* Transition used for quantitation
** Transition used for confirmatory purposes

Table 4.2-6: Validation results of QuEChERS method for CL 312622 in various plant and animal matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Transition			**m/z 306 → 261***			**m/z 306 → 69****		
CL 312622	Bean	0.01	5	49	8.9	5	46	10
		0.1	5	46	4.7	5	48	6.0
	Grape	0.01	5	60	11	5	46	17
		0.1	5	45	5.2	5	43	8.7
	Dry pea	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	15	26	5	15	33
	Rice (grain)	0.01	5	47	15	5	42	6.0
		0.1	5	35	5.1	5	36	5.4
	Sunflower (seed)	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	16	11	5	19	8.2
	Bovine (milk)	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	40	12	5	39	8.7
	Bovine (fat)	0.01	5	43	12	5	48	13
		0.1	5	51	11	5	52	3.5
	Bovine (kidney)	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	16	12	5	16	9.2
	Bovine (liver)	0.01	5	n.r.	n r.	5	63	9.4
		0.1	5	21	11	5	20	9.3
	Bovine (meat)	0.01	5	57	11	5	50	5.8
		0.1	5	45	15	5	38	7.0
	Poultry (eggs)	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	23	13	5	23	4.9

* Transition used for quantitation
** Transition used for confirmatory purposes
n r. not reported

Table 4.2-7: Validation results of QuEChERS method for CL 189215 in various plant matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Transition			**m/z 454 → 292***			**m/z 454 → 86****		
CL 189215	Bean	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	12	5.9	5	24	8.8
	Grape	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	4.6	12	5	8.8	20
	Dry pea	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	12	12	5	12	20
	Rice (grain)	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	26	2.7	5	28	7.1
	Sunflower (seed)	0.01	5	n.r.	n r.	5	n.r.	n r.
		0.1	5	11	7.8	5	13	6.3

* Transition used for quantitation
** Transition used for confirmatory purposes
n r. not reported

Linearity: Acceptable linearity was observed for the standard range and the two mass transitions tested for each analyte. The method-detector response was linear over the 0.03-10 ng/mL range ($r \geq 0.98$).

Specificity: LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions for each analyte. For imazamox the transition m/z 306 → 261 was used for quantitation and m/z 306 → 193 for confirmatory purposes. For the metabolite CL 263284 the transition m/z 292 → 247 was used for quantitation and m/z 292 → 232 for confirmatory purposes. For the metabolite CL 312622 the transition m/z 306 → 261 was used for quantitation and m/z 306 → 69 for confirmatory purposes. For the metabolite CL 189215 the transition m/z 454 → 292 was used for quantitation and m/z 454 → 86 for confirmatory purposes.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg for the all tested matrices and all analytes.

Repeatability: The relative standard deviation (RSD) for all commodities was below 20% for imazamox and CL 263284. The detailed values are shown in Table 4.2-4 and Table 4.2-5. For metabolites CL 312622 and CL 189215 the RSD was up to 33%.

Conclusion: From the results of the validation it can be concluded, that the QuEChERS method has proven its applicability for the determination of residues of imazamox in matrices of animal and plant origin with an LOQ of 0.01 mg/kg. Regarding the metabolite CL 263284, limited suitability of the QuEChERS multi-residue method could be demonstrated with sufficient recoveries for all matrices except for grapes (high acid commodity group) and kidney.

The multi-residue method QuEChERS is not suitable for the extraction and determination of metabolites CL 312622 and CL 189215.

Validation of method D0303

Report: CA 4.2/5
Stewart J., 2003a
Method validation of BASF Analytical Method D0303 entitled Method for the Determination of BAS 720 H (CL 299263) and its metabolite CL 263284 in bovine matrices using LC/MS/MS
2003/5000116
Guidelines: EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000)
GLP: Yes
(certified by United States Environmental Protection Agency)

Principle of the method: BASF Method No. D0303 was developed to identify residues of imazamox in animal matrices. Residues of BAS 720 H and CL 263284 are extracted from bovine tissues (liver, kidney and muscle) with acidic methanol water. Following centrifugation and dilution with methanol the extract is filtered through a SCX cartridge and the analytes are selectively eluted with a water-methanol solution. The eluate is evaporated to dryness and re-dissolved in water for analysis. Residues of BAS 720 H and CL 263284 are extracted from bovine milk and tissue fat with acidic acetonitrile in hexane. Following solvent partitioning, an aliquot of the acetonitrile extract is diluted with methanol and filtered through the SCX cartridge (see tissue extraction above). The analytes are detected by LC-MS/MS and the concentrations of BAS 720 H and CL 263284 are calculated by direct comparison of the sample peak responses to those of external standards. The limit of quantitation (LOQ) of the method is 0.01 mg/kg.

Recovery findings: In all matrices tested, the mean recovery values were between 60% and 120% when fortified at 0.01 mg/kg and between 70% and 120% when fortified at 0.1 mg/kg. The detailed results are given in the table below.

Table 4.2-8: Validation results of method D0303: imazamox and metabolite CL 263284 in bovine matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Imazamox* (BAS 720 H)	Liver	0.01	5	92	25
		0.1	5	93	9
	Kidney	0.01	4	82	7
		0.1	5	73	7
	Muscle	0.01	5	81	6
		0.1	5	87	7
	Fat	0.01	5	95	9
		0.1	4	69	7
	Milk	0.01	5	103	10
		0.1	5	86	8
CL 263284**	Liver	0.01	5	116	16
		0.1	5	102	10
	Kidney	0.01	4	106	4.5
		0.1	5	78	2.9
	Muscle	0.01	5	98	17
		0.1	5	83	17
	Fat	0.01	5	116	4
		0.1	4	79	19
	Milk	0.01	5	93	6
		0.1	5	93	4

* Imazamox detection: Transition 306 m/z → 261 m/z
** CL 263284 detection: Transition 292 m/z → 247 m/z

Linearity: Good linearity of detection was observed in the range tested with correlation coefficients ≥0.99.

Specificity: LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg for the bovine tissues (liver, kidney and muscle), milk and fat.

Repeatability: The relative standard deviation (RSD) for all commodities was below 30% when fortified at 0.01 mg/kg and below 20% when fortified at 0.1 mg/kg for each analyte. The detailed values are shown in Table 4.2-8.

Reproducibility: An independent laboratory validation of this method was successfully conducted and is summarized further below.

Conclusion: Analytical method D0303 is fully validated according to the guidance document SANCO 825/00 and is thus suitable for monitoring of imazamox and metabolite CL 263284 residues in animal matrices.

Since in the initial validation, no data was generated for poultry eggs, a new method validation study has been performed for the Annex I renewal of imazamox.

Report:	CA 4.2/6 Gooding R.F., 2013a Validation of BASF analytical method D0303: Method for the determination of BAS 720 H (CL 299263) and its metabolite CL 263284 in animal matrices using LC-MS/MS 2013/7002842
Guidelines:	EPA Residue Chemistry Test Guidelines US, EPA 860.1340, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method: Residues of imazamox and CL 263284 are extracted from tissues (liver, kidney, muscle) and egg with an acidified methanol/ water solution and from milk and fat with an acidified acetonitrile/ hexane solution. After sample extraction and clean-up by partitioning against hexane (fat and milk) and by solid phase extraction, residues are determined by HPLC-MS/MS.

Recovery findings: In all matrices tested, the mean recovery values were between 70% and 120%. The detailed results are given in the table below.

Table 4.2-9: Validation results of method D0303: imazamox and metabolite CL 263284 in bovine matrices (muscle, kidney, liver, fat and milk) and egg

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Transition			**m/z 306 → 261***			**m/z 306 → 86****		
Imazamox (BAS 720 H)	Muscle	0.01	5	79	9	5	72	10
		0.1	5	81	4	5	77	1
	Kidney	0.01	5	78	9	5	76	6
		0.1	5	79	4	5	77	9
	Liver	0.01	5	90	17	5	78	14
		0.1	5	89	15	5	85	13
	Fat	0.01	5	110	10	5	105	13
		0.1	5	89	5	5	89	5
	Milk	0.01	5	107	12	5	106	13
		0.1	5	93	17	5	93	9
	Egg	0.01	5	107	4	5	104	5
		0.1	5	89	6	5	87	9
Transition			**m/z 292 → 247***			**m/z 292 → 179****		
CL 263284	Muscle	0.01	5	77	9	5	80	7
		0.1	5	82	6	5	77	2
	Kidney	0.01	5	76	6	5	77	7
		0.1	5	81	2	5	81	3
	Liver	0.01	5	80	18	5	70	22
		0.1	5	92	13	5	90	14
	Fat	0.01	5	96	6	5	97	7
		0.1	5	87	3	5	86	3
	Milk	0.01	5	108	10	5	111	12
		0.1	5	82	8	5	86	8
	Egg	0.01	5	100	13	5	106	13
		0.1	5	99	9	5	99	10

* Transition used for quantitation

** Transition used for confirmatory purposes

Additionally, the extract stability was investigated. Each analyte was shown to be stable in methanol, the solvent used for preparation of stock and intermediate standard solutions, for at least 56 days, and in calibration solutions prepared by serial dilution of the intermediate solutions with acidified water (1% acetic acid in water), for up to 28 days, when stored under refrigeration. Further imazamox and CL 263284 were demonstrated to be stable in the extracts of livestock matrices (muscle, kidney, fat, milk and egg) for at least the time period tested 2 to 6 days.

Linearity: Acceptable linearity was observed for the standard range and the two mass transitions tested for each analyte. The method-detector response was linear over the 0.04-1.0 ng/mL range ($r \geq 0.9887$).

Specificity: LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions. For imazamox the transition m/z 306 → 261 was used for quantitation and m/z 306 → 86 for confirmatory purposes. For the metabolite CL 263284 the transition m/z 292 → 247 was used for quantitation and m/z 292 → 179 for confirmatory purposes.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg for the all tested matrices (liver, kidney, muscle, fat, egg and milk).

Repeatability: The relative standard deviation (RSD) for all commodities was below 30% when fortified at 0.01 mg/kg and below 20% when fortified at 0.1 mg/kg for each analyte. The detailed values are shown in Table 4.2-9.

Reproducibility: An independent laboratory validation of this method was successfully conducted and is summarized below.

Conclusion: The results of this method validation study demonstrate that BASF Method No. D0303 fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is, therefore, suitable to determine residues of imazamox and its metabolite CL 263284 in livestock commodities. It is proposed for enforcement purposes.

Independent Laboratory Validation of method D0303

Report:	CA 4.2/7 Sears K., 2013a Independent laboratory validation of analytical method number D0303: Method for determination of BAS 720 H (CL 299263) and its metabolite CL 263284 residues in animal matrices using LC-MS/MS 2013/7002962
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method: Residues of imazamox and CL 263284 are extracted from tissues (liver, kidney, muscle) and egg with an acidified methanol/ water solution and from milk and fat with an acidified acetonitrile/ hexane solution. After sample extraction and clean-up by partitioning against hexane (fat and milk) and by solid phase extraction, residues are determined by HPLC-MS/MS.

Recovery findings: In all matrices tested, the mean recovery values were between 70% and 120% except for CL 263284 in milk (122%) and in liver (126%) each fortified with 0.1 mg/kg. The detailed results are given in the table below.

Table 4.2-10: Independent laboratory validation results of method D0303: imazamox and metabolite CL 263284 in bovine matrices (muscle, kidney, liver, fat and milk) and egg

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)	No. of Tests	Average Recovery (%)	Relative Standard Deviation (%)
Transition			**m/z 306 → 261***			**m/z 306 → 86****		
Imazamox (BAS 720 H)	Muscle	0.01	5	92	11	5	105	19
		0.1	5	90	10	5	93	7
	Kidney	0.01	5	91	4	5	90	5
		0.1	5	79	9	5	79	9
	Liver	0.01	5	119	7	5	104	18
		0.1	5	116	3	5	70	14
	Fat	0.01	5	94	6	5	88	10
		0.1	5	94	6	5	87	10
	Milk	0.01	6	105	13	6	108	20
		0.1	5	115	17	5	120	15
	Egg	0.01	5	101	9	5	86	18
		0.1	5	93	6	5	90	6
Transition			**m/z 292 → 247***			**m/z 292 → 179****		
CL 263284	Muscle	0.01	5	93	11	5	97	9
		0.1	5	91	12	5	90	9
	Kidney	0.01	5	86	3	5	83	10
		0.1	5	87	7	5	86	10
	Liver	0.01	5	119	8	5	122	8
		0.1	5	126	2	5	123	2
	Fat	0.01	5	89	3	5	92	8
		0.1	5	96	7	5	97	7
	Milk	0.01	6	107	9	6	112	23
		0.1	5	122	18	5	121	16
	Egg	0.01	5	106	6	5	110	19
		0.1	5	104	4	5	110	4

* Transition used for quantitation

** Transition used for confirmatory purposes

Linearity:	Acceptable linearity was observed for the standard range and the two mass transitions tested for each analyte. The method-detector response was linear over the 0.04-1.0 ng/mL range ($r \geq 0.996$).
Specificity:	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions. For imazamox the transition m/z 306 → 261 was used for quantitation and m/z 306 → 86 for confirmatory purposes. For the metabolite CL 263284 the transition m/z 292 → 247 was used for quantitation and m/z 292 → 179 for confirmatory purposes.
Limit of Quantitation:	The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg for the all tested matrices (liver, kidney, muscle, fat, egg and milk).
Repeatability:	The relative standard deviation (RSD) for all commodities was at or below 30%. The detailed values are shown in Table 4.2-10.
Reproducibility:	The independent laboratory validation confirms the good results of the validation.
Conclusion:	The results of this independent laboratory validation study demonstrate that BASF Method No. D0303 fulfils the requirements with regard to specificity, repeatability, limit of quantitation, recoveries and reproducibility. This proves the suitability of this method for enforcement purposes of imazamox residues in products of animal origin.

(f)Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

(g)Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions[1]	Document identifier or version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

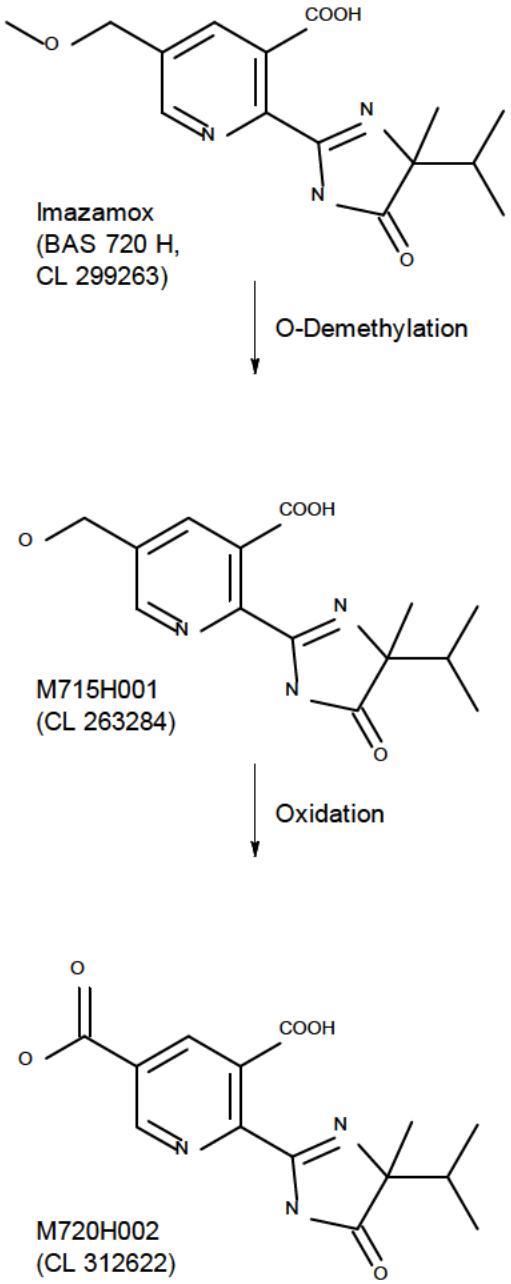
CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

Studies already peer-reviewed and presented in the original Annex II Dossier (1997):
For the determination of the toxicokinetic properties of Imazamox, two studies are available with the test substance radiolabelled in the pyridine ring. One study (DocID ID 440-003 or MET 95-009) investigated absorption, distribution, excretion and metabolism in rats after single and multiple oral low dose (10 mg/kg bw), single oral high dose (1000 mg/kg bw) and single intravenous dosing (10 mg/kg bw) while the other study (DocID ID-440-004 or MET 95-022) covers dose groups for blood pharmacokinetics, tissue distribution and biliary excretion after single oral low (10 mg/kg bw) or high doses (1000 mg/kg bw). Both studies have been part of the previous evaluation and are therefore not submitted again in this dossier. For reasons of convenience, a short summary of the main conclusions is given below.

Both studies show that Imazamox administered orally to rats at 10 mg/kg and 1000 mg/kg body weight is absorbed to approximately 75-80 % of the administered dose. The fact that a higher portion of dose was excreted via feces following oral gavage vs. intravenous dose indicates that oral absorption of the test substance is not complete. The blood pharmacokinetic parameters derived from the study showed a T_{max} of approx. 30 minutes and 60 minutes for low and high dose, respectively. The half-life for terminal elimination ($T_{1/2\ term}$) of the applied dose was approximately 20 minutes after reaching T_{max} and 60 minutes for the low and high dose group, respectively, showing that both the absorption and elimination of carbon-14 residues of imazamox in blood was rapid.
Levels of radioactivity in the tissues (7 days post last dose) were generally below the detection limit and accounted for 0.007% or less of the administered dose. The tissue distribution investigations at T_{max} in the second study showed highest residues in kidney which declined to non-detectable residues within 7 days post dosing.
The excretion of Imazamox occurs mainly as unchanged test compound via the urine (>75% of the dose). Elimination is very efficient and occurs rapidly within 6 hours post dose. Biliary excretion is not an important route of excretion.
Besides parent Imazamox, minor amounts of metabolites CL 263284 and CL 312622 were detected in urine and feces, but –like in the new study (see below CA 5.1.1)- seemed to be present already in the dosing solution as TLC analysis showed.
No differences between sexes were observed.

Based on these studies, the following metabolic pathway was proposed:

Proposed metabolic pathway of imazamox (BAS 720 H) in rats



Submission of new not yet peer-reviewed studies in this AIR3-Dossier.
The following studies (see CA 5.1.1) have been performed in addition to the previously submitted studies. With only one dose group (oral administration of a single high dose) it has only a limited scope, i.e. to provide information about the metabolic fate of the imidazolinone moiety in rats after oral administration. The results of this new study confirmed the findings of the previous full study (with the radiolabel in the pyridine ring) with regard to excretion patterns and kinetics. Since the new study also shows that the molecule is not cleaved in the rat it corroborates the previously submitted data package for point 5.1. (with the pyridine ring label) as giving a valid and complete description of the absorption, distribution, and excretion behavior of Imazamox in rats.

In summary, the absorption, distribution, elimination and metabolism of Imazamox have been studied in rats. Imazamox was rapidly absorbed and the oral absorption was estimated to be approximately 80% of the actual administered dose. Urine was the major route of excretion (> 74 %). Imazamox was mostly excreted as unchanged parent compound and the majority of the dose was eliminated within the first 24 hours after administration. Smaller amounts of the test substance were excreted through feces (< 24 %). Only trace amounts of tissue residues were detected. Imazamox was metabolized only to a very limited degree. Trace levels of metabolites were detected in the urine and feces.
Based on all available studies including the newly performed studies on the imidazolinone ring label, the ADME endpoints, which were fixed in the European Commission Review Report for the active substance Imazamox (SANCO/4325/2000 –Final, 29 November 2002) as indicated below, are still considered to be valid:

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of absorption:	Rapid (approx. 80%)
Distribution:	Widely distributed. Very low levels in tissues
Potential for accumulation:	No evidence of accumulation
Rate and extent of excretion:	Rapid (approx. 80-90% in urine and 10-20% in feces within 24 h)
Toxicologically significant compounds:	Parent compound
Metabolism in animals:	Limited (90% unchanged parent in excreta and tissues) via O-demethylation

In accordance with the new data requirements for active substances of Commission Regulation (EU) No 283/2013 of 1 March 2013, an *in vitro* metabolism study of Imazamox was conducted.
The purpose of this study was a comparison of the *in vitro* metabolism of Imazamox (BAS 720 H) in humans and those species which were used for the *in vivo* toxicological testing.
There is no guideline for this type of study available yet, therefore the study was conducted according to an internal protocol with dog, rabbit, rat, mouse and human liver microsomes..
As a result Imazamox (BAS 720 H) was found to be not metabolized by liver microsomes of dogs, rabbits, mice, rats and humans and no unique human metabolite was identified. A detailed study summary is given below in chapter CA 5.1.2.

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Report:	CA 5.1.1/1 [redacted], 2012a Metabolism of 14C-Imazamox (BAS 720 H) in rats 2011/1080475
Guidelines:	EPA 870.7485, EPA 860.1000: EPA Residue Chemistry Test Guidelines, MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan), OECD 417, EEC 87/302 B
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Report:	CA 5.1.1/2 [redacted] 2012a 14C-BAS 720 H - Study on the kinetics in rats 2012/1044751
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, OECD 417, EPA 870.7485, JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on In Vivo Fate in Animals (2001)
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The toxicokinetic properties and the metabolism of imazamox were investigated in male and female rats after application of a single oral dose of 542.6 mg/kg and 525.5 mg/kg bodyweight, respectively. To facilitate metabolite identification by mass spectroscopy and HPLC, a mixture of ^{14}C, ^{15}N-labeled (in the imidazolinone ring) and unlabeled test substance was administered (4 rats/sex). Urine and feces were collected at prescribed intervals. Animals were sacrificed 7 days after administration of the radiolabeled dose. Selected tissue samples were collected, weighed and analyzed for total radioactivity. Selected excreta samples were analyzed for parent compound and metabolites.
It can be concluded that imazamox, when orally administered to female or male rats, is not metabolized but rapidly excreted unchanged via urine and feces. However, via the application solution rats were also exposed to CL 263284, CL 312622 and SES15698 in amounts of 1.70 %, 1.20 % and 1.95 % of the administered dose, respectively. The study also investigated a possible change in the enantiomer ratio of imazamox, but results showed that the ratio of enantiomers does not change, demonstrating a similar absorption and excretion rate for the individual enantiomers of imazamox in the rat.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Imazamox (BAS 720 H)
Description: Beige solid
Batch # / purity: Radiolabeled (^{14}C, ^{15}N): 1003-1001 / 98.7%
Nonlabeled: AC12820-7, AC6935-63 / 99.5%, 97.1%
Stability of test compound: Stable during dosing period

2. Vehicle and/or positive control: water with addition of approx. 1% (w/w) of ammonium hydroxide solution (25%)

3. Test animals:
Species: Rat
Strain: Wistar (Crl: WI (HAN)) (Charles River Laboratories, Germany)
Age: About 8-13 weeks at start of acclimatization
Sex: Male and female
Number of animals: 16 (4 males+4 females for original and repeated experiment each)
Weight at dosing: About 200-300 g (prior to dosing)
Acclimation period: Not reported
Diet: Kliba lab diet for mouse and rat, *ad libitum*
Water: Tap water *ad libitum*
Housing: During acclimatization in groups in Macrolon Cages (2000P; H Temp (PSU), Tecniplast), then individually in all-glass metabolism cages type Metabowl (Jencons Leighton Buzzard, UK)
Husbandry:
Environmental conditions:
Temperature: 20-24°C
Humidity: 30-70%
Photoperiod: Alternating 12-hour light and dark cycles

4. Preparation of dosing solutions: Initially, the dosing solution was prepared in deonized water with 0.5% Carboxymethylcellulose (CMC). Since HPLC/UV analyses demonstrated non-homogenous results for the test substance preparation, the experiment was repeated and a new dosing solution was prepared as described below, for which homogeneity, stability and a correct concentration could be confirmed by analysis. The report and thus also this summary show only the results of the repeated experiment.
^{14}C-imazamox and unlabeled imazamox were mixed with a part of the required amount of highly deionized water. After the pH was adjusted to 7-8 with ammonium hydroxide solution (25%), the application solution was filled up to the needed amount with highly deionized water. The target concentration was 50 mg/mL and the target specific activity was about 11 MBq/mL (corresponding to a target dose of 40 MBq/animal). The preparation resulted in a solution that was dosed to the animals.

The application solution was analyzed by HPLC-MS in order to determine possible impurities. Besides the parent compound imazamox, MS analysis led to the identification of CL 263284, CL 312622 and SES15698, all of which are structurally related to the parent compound. For all three derivatives, the methoxymethyl side chain of the parent compound is either cleaved (CL 263284) and/or oxidized (CL 312622, SES 15698).
An aliquot of the application solution was also analyzed using another HPLC method, whereby the identified components could be assigned by comparison of the retention times and the chromatographic pattern with the chromatogram of the HPLC-MS run. The parent compound was the main component and represented > 95 % of the ROI (Region Of Interest: integrated peak area in radio-HPLC). The impurities CL 263284, CL 312622 and SES15698 represented about 1 to 2 % ROI each. CL 312622 and SES15698 were already present in the batch of the labeled test item, used for this study.
The application solution was also analyzed using the enantiomer-specific HPLC method. The two possible enantiomers of imazamox were present in a ratio of approximately 1:1.

B. STUDY DESIGN AND METHODS

1. Dates of work: October 12, 2010 – August 5, 2011

The treated rats consisted of an oral single dose group (4 rats/sex, 500 mg/kg bw). All animals received the oral dose administered via gavage.

Urine was collected 0-6, 6-12 and 12-24 hours after dosing and subsequently in time intervals of 24 hours up to 168 hours (7 days); feces were collected in intervals of 24 hours up to 168 hours or until 90% of the applied radioactivity was excreted. After 168 hours, animals were sacrificed and the following tissues were checked for remaining radioactivity: heart, liver, spleen, brain, skin, lung, bone, muscle, kidney, carcass, adipose tissue, testes, bloodcells and plasma, pancreas, thyroid gland, adrenal glands, gut and contents, stomach and contents. For balance estimates the cage wash was also checked for radioactivity. Samples were analyzed for total ^{14}C-radioactivity by samples combustion and/or liquid scintillation counting (LSC).

II. RESULTS AND DISCUSSION

Storage stability
The test substance preparation was realized as aqueous solution with the addition of about 1 weight % of ammonium hydroxide solution (25%). The analytical investigation performed demonstrated the stability, homogeneity and correctness of the concentrations of ^{14}C-imazamox in this aqueous solution.

Balance and Absorption
The mean total recovery of radioactivity was 97.20 and 97.51% of the administered dose for male and female rats, respectively.
The estimated absorbed dose (calculated as sum of % dose values in urine, cage wash, carcass and organs (with exception of GI tract)) showed comparable values for both sexes and was calculated to be 78.28% and 74.75% for males and females, respectively. Single animal data ranged from 76.69% to 80.81% for males and from 69.95% to 81.78% for females.

Distribution
The gut content, skin and carcass contained little of the total radioactive residue (0.01% for both males and females). In all other tissues examined, no radioactivity was found.
The cage wash contained 0.30% and 0.51% of the radioactivity administered for male and female rats, respectively.

Excretion
The excretion via urine over the observed period of 168 hours amounted to 77.96% of dose for males and 74.22% of dose for females. The major part of the urinary excretion (73.06 % of dose for males and 65.47% of dose for females) occurred within the first day after test substance administration. Smaller amounts of the test substance were excreted through feces. These amounts accounted for 18.93% of dose for males and 22.74% of dose for females.

Table 5.1.1-1: Route of excretion and total recovery of imazamox in rat (percent of radioactive dose)

Group	Target dose [mg/kg bw]	Route of administration	Sex of animal	Urine [%]	Feces [%]	Total [%]
Treated	500	Single oral	M	77.96	18.93	96.89
			F	74.22	22.74	96.96

Table 5.1.1-2: Excretion and retention of radioactivity via urine, feces and different organs after single oral administration of ^{14}C-BAS 720 H to male and female rats at a dose levels of nominal 500 mg/kg bw (group mean values, in percent of radioactive dose)

	[% of the administered radioactivity]	
	Male	**Female**
Urine (h)		
Urine 0-6	56.76	51.14
Urine 6-12	6.52	8.19
Urine 12-24	9.78	6.14
Urine 24-48	3.31	4.82
Urine 48-72	0.83	2.35
Urine 72-96	0.31	0.93
Urine 96-120	0.18	0.30
Urine 120-144	0.13	0.23
Urine 144-168	0.14	0.12
Subtotal Urine	**77.96**	**74.22**
Feces (h)		
Feces 0-24	13.03	15.88
Feces 24-48	4.94	3.16
Feces 48-72	0.65	0.61
Feces 72-96	0.10	0.30
Feces 96-120	0.04	0.10
Feces 120-144	0.03	0.04
Feces 144-168	0.12	2.67
Subtotal Feces	**18.90**	**22.74**
Other sources		
Cage wash	0.30	0.51
Bloodcells	0.00	0.00
Plasma	0.00	0.00
Lung	0.00	0.00
Heart	0.00	0.00
Spleen	0.00	0.00
Kidney	0.00	0.00
Adrenals	0.00	0.00
Testes/Ovaries	0.00	0.00
Uterus	---	0.00
Muscle	0.00	0.00
Brain	0.00	0.00
Adipose Tissue	0.00	0.00
Bone	0.00	0.00
Bone marrow	0.00	0.00
Thyroid	0.00	0.00
Pancreas	0.00	0.00
Stomach cont.	0.00	0.00
Stomach	0.00	0.00
Gut cont.	0.01	0.01
Gut	0.00	0.00
Liver	0.00	0.00
Skin	0.01	0.01
Carcass	0.01	0.01
Total	**97.20**	**97.51**
Estimated absorbed dose[1]	**78.28**	**74.75**

[1]: Calculated as sum of % dose values in urine, cage wash, carcass and organs (with exception of GI tract)

Metabolism

The extractability of feces with methanol was very high accounting for 96.1 to 97.3% of the total radioactive residues. The subsequent water extraction released additional 1.3 to 1.8% of the radioactivity. The residue after solvent extraction accounted for 1.3 to 2.2% of the TRR. Overall, there was no major difference in the extractability of feces with methanol and water between males and females. The extractability with methanol and water for the different time intervals was also similar.

For both sexes, imazamox was rapidly excreted via urine and feces as the unchanged parent compound. About 73.0% (females) and 76.9% (males) of the applied dose were excreted within 96 hours via urine as the parent compound. Additionally, about 21.1% (females, 0-72 h and 144-168 h) and 15.4% (males, 0-72 h) of the dose were excreted via feces as the parent compound. Therefore, a total of 94.1% (females) and 92.3% (males) of the dose were excreted as BAS 720 F via urine and feces.

The components CL 263284, CL 312622 and SES15698, which were identified in urine and feces of both sexes, were already present in the application solution in a comparable order of magnitude. No significant increase of these components was observed during metabolism. The fact that the isotope patterns of these components were different to the one of the administered test substance supports the assumption that the amounts of CL 263284, CL 312622 and SES15698, detected in urine and feces samples, may not have been formed by metabolization of imazamox in the rat. The same holds true for SES15996, which was identified in feces, since its isotope pattern also did not match with that of the parent compound. SES15996 is most probably a degradation product of CL 312622 (decarboxylation product).

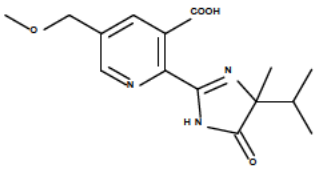
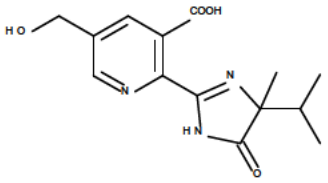
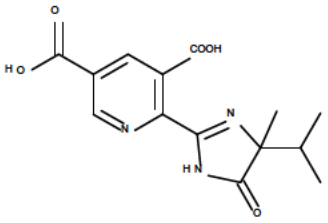
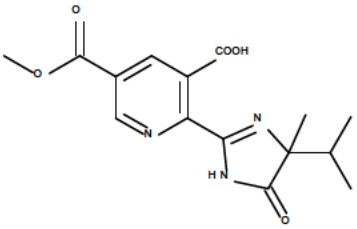
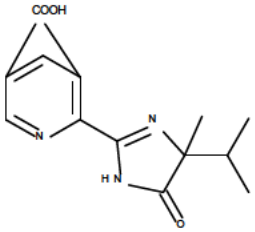
The application solution and imazamox isolated from urine were also analyzed using an enantiomer-specific HPLC method. The two possible enantiomers of imazamox were present in a ratio of approximately 1:1 both in the application solution as well as in the urine sample, demonstrating a similar absorption and excretion rate for the individual enantiomers of imazamox in the rat.

Table 5.1.1-3: Summary of metabolites identified in urine and feces and comparison with the application formulation

Designation	Females			Males			Content in Application Formulation [% ROI*]
	Urine [% Dose]	Feces [1)] [% Dose]	Sum [% Dose]	Urine [% Dose]	Feces [1)] [% Dose]	Sum [% Dose]	
BAS 720 H	73.05	21.07	**94.12**	76.94	15.39	**92.33**	**95.15**
M715H001 (CL263284)	0.20	1.27	**1.47**	0.14	1.07	**1.21**	**1.70**
Reg. No. 4110542 (CL312622) / (SES15996 [2)])	0.23	1.10	**1.33**	0.25	0.83	**1.08**	**1.20**
SES15698	0.09	1.07	**1.16**	0.18	0.96	**1.13**	**1.95**

1) Sum of methanol and water extract
2) SES15996 only identified in feces
* ROI: Region of Interest.

Table 5.1.1-4: Structures of metabolites identified in urine and feces

Metabolite Designation				Structure/Name
BASF Code/ Synonym (Mol. Weight)	Reg. No.	Metabolite Code	CAS-Nr.	
BAS 720 H CL 299263 (305)	4096483	-	114311-32-9	
CL 263284 (291)	4110773	M715H001	81335-78-6	
CL 312622 (305)	4110542	M720H002	146953-32-4	
SES15698* (319)	-	-	-	
SES15996* (260)	-	-	-	

* impurity of application solution

III. CONCLUSION

It can be concluded that imazamox, when orally administered to female or male rats, is not metabolized but rapidly excreted unchanged via urine and feces. However, via the application solution rats were also exposed to CL 263284, CL 312622 and SES15698 in amounts of 1.70 %, 1.20 % and 1.95 % of the administered dose, respectively. The study demonstrates that the molecule is not cleaved in the rat, supporting the validity and completeness of the previously submitted studies for Chapter 5.1. (with the radiolabel in the pyridine ring moiety). Furthermore, the new study also provides information about the stability of the enantiomer ratio of Imazamox in the rat showing a comparable absorption and excretion rate for both individual enantiomers.

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Report: CA 5.1.2/1
Funk D., Taraschewski I., 2013
Comparative in-vitro-metabolism with ^{14}C-BAS 720 H
2013/1233155
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The purpose of this study was the comparison of the *in vitro* metabolism of Imazamox (BAS 720 H) in humans and those species which were used for the *in vivo* toxicological testing in order to investigate whether a metabolite occurs in human samples that might not be sufficiently covered by the animal testing.
Therefore, the ^{14}C/^{15}N-labelled/non-labelled test item mix was incubated with dog, rabbit, rat, mouse and human liver microsomes in the presence of a NADPH-generating system to enable Cytochrome P-450-related metabolism. After incubation, the samples were analysed by HPLC with radio detection and HPLC-MS and the resulting human metabolite pattern (only peaks above 5% TRR) was compared to the animal ones. Negative and positive controls were included to prove the metabolic activity of the liver microsomes.
With a recovered radioactivity of 90% and above, only the parent molecule was detected in all test systems by HPLC analysis in fresh samples after the incubation.
In summary the study shows that Imazamox (BAS 720 H) is not metabolised by liver microsomes of dogs, rabbits, mice, rats and humans. No unique human metabolite was detected.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Non-labelled material:	BAS 720 H, Imazamox
Lot/Batch #:	AC12820-7
Purity:	99.5%
Stability of test compound:	Expiration date: 01-May-2021
Solvent used:	DMSO
Radio-labelled material:	$^{14}C/^{15}N$-labelled Imazamox (Pyridine-3-C14)
Lot/Batch #:	1004-1101
Purity:	97.2%
Radiochemical purity:	98.6% , specific activity 6.65 MBq/g
Stability of test compound:	The compound was tested on 18-Jul-2012.
Solvent used:	DMSO

2. Control Materials:

Negative and solvent control:	Dimethyl sulfoxide; DMSO (0.5% (v/v) in cell culture medium)
Positive control:	Testosterone; 10 µM

3. Activation:

Composition of the NADPH generating system:

Constituent	Concentration
β-D-Glucose-6-Phosphate monosodium salt	78.6 mg
NADPH	20.0 mg
Magnesium chloride ($MgCl_2$) (1M)	85.7 µL
Glucose - 6 - phosphate dehydrogenase (1U/µL)	120.0 µL
Water, double distilled	394.3 µL

4. Test organisms:

For the tests the liver microsomes of male and female animals of one species were pooled in a ratio of 1:1, relative to the protein content. The human liver microsomes were already pooled at the purchase. The species are detailed in the following table.

Liver microsomes from	Strain	Sex
rat	Wistar	female
		male
mouse	CD-1	female
		male
rabbit	New Zealand	female
		male
dog	Beagle	female
		male
human	(XTreme 200)	100 males/100 females

5. Test concentrations:

The tested concentrations were 1 µM, 10 µM and 100 µM Imazamox in DMSO with a relation between radiolabelled to unlabelled test item of 82%/18%, 8%/92% and 0.8%/99.2%, respectively. The test solutions are further detailed in the following table.

Dose group	Amount of radiolabelled test item [µL]	Amount of unlabelled test item [µL]	Amount of radiolabelled test item [µg]	Amount of unlabelled test item [µg]	Volume of DMSO [mL]	Concentration [mg/mL]
BAS 720 H 1 µM	34.2	7.8	37.14	30.33	6	0.012
BAS 720 H 10 µM	33.4	396.6	56.819	674.22	6	0.122
BAS 720 H 100 µM	33.4	4276.1	56.819	7269	6	1.221
Testosterone 10 µM	212	-	113.5	-	1.0	0.107

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 19-Oct-2012 to 02-Sep-2013

2. *In vitro* assays:

Treatment:

Each experimental setup was performed in triplicates. The test solutions were incubated at approximately 100 µM for high dose, 10 µM for medium dose or 1 µM for low dose conditions with rat, mouse, rabbit, dog or human liver microsomes. Each sample (2 mL total volume) comprised 1.0 mg of microsomal protein, a NADPH generating system and potassium phosphate buffer. The reactions were performed at 37 °C for 3 h in a shaker and stopped by adding 4.7 mL ethanol and sonicating for 15 minutes.

Controls:

For each species three negative controls and one positive control were performed in triplicates.

The negative controls comprised “heat denatured control”, a “t=0 control” and a “buffer control” and were performed only with the high dose of 100 µM. Under all these conditions no metabolisation or degradation should occur.

For the heat denatured control, the liver microsomes were inactivated by incubating in hot water (>95 °C) for 10 minutes before pipetting them into the mixture.

The buffer control contained only application solution and buffer.

For the “t=0” control, the enzymatic reaction was stopped immediately after addition of the NADPH generating system by adding 4.7 mL of cold ethanol.

In the positive control the liver microsomes from the different species were incubated with testosterone instead of the active substance to prove their metabolic activity. The following amounts of testosterone should be metabolized at least: rat 75%, human 30%, mouse 70%, dog 25% and rabbit 75%.

Sampling: The stopped incubation mixture was centrifuged and concentrated to a volume of approx. 2 mL by a centrifugal evaporator. Supernatant and pellet were frozen separately at -18 °C or below. After thawing, the total radioactivity of the supernatant samples was determined by LSC measurements. In cases where the recovery of the initial applied radioactivity in the supernatant was below 90%, the pellet was concentrated to dryness and resuspended in 50 µL of water. Subsequently 200 µL of methanol were added and the sample was sonicated, vortexed and centrifuged. Finally the radioactivity in the methanol supernatant was determined by LSC. The residue pellet was resuspended again with 150 µL of water and the radioactivity was determined by LSC.

Evaluation of MS data: For the qualitative evaluation of the masses of metabolites, which were formed during the incubation, at least one representative replicate of the three replicates of each experimental setup was analyzed by means of HPLC-MS, respectively. Based on the peak pattern and the peak intensity, the radio signals from the ^{14}C-chromatogram of the HPLC-MS analysis were assigned to the signals of the primary ^{14}C-chromatogram of the HPLC. Based on the retention time in the LC-MS measurements m/z-values were assigned to the radio signals. Only m/z-values for peaks that contain more than 5% of TRR were considered in the report.

II. RESULTS AND DISCUSSION

The triplicates were comparable in all investigated samples.
In the three negative controls only the active substance Imazamox occurred. Hence no metabolization or non-metabolic degradation of Imazamox occurred during the incubation.
The positive control with Testosterone showed that the metabolic activity of the microsomes was sufficiently high. The defined threshold values were reached in all cases.
In most of the samples the recovered radioactivity after stopping the microsomal reaction with ethanol compared to the applied amount of active substance was larger than 90%. Only the 10 µM human sample needed a further extraction with methanol.
In none of the test systems of the different species the active substance Imazamox was metabolized. In all chromatograms only the parent peak at Rt = 10.3 min appeared after the incubation. The m/z-value obtained by HPLC-MS analysis of these samples confirmed the presence of parent and no another m/z-value was obtained for this peak.

During HPLC-MS-analysis additional peaks occurred in the MS chromatograms, which were also confirmed by HPLC reanalysis of some of these samples. As the HPLC-MS-analysis was performed after some storage time of the samples, it was concluded that sample degradation occurred during storage. But as the main peak could still be confirmed to be the parent molecule and this was the only peak present in fresh samples, only this peak was considered relevant.

The comparison of peaks detected in human samples with peaks detected in animal samples based on retention time and m/z-values is summarized in the following table

Table 5.1.2-1: Comparison of the metabolites formed with human liver microsomes and metabolites formed with animal liver microsomes

RT	m/z	Tested concentrations		
		1 µM	10 µM	100 µM
		Human (Mean % TRR)		
10.2	306.145	100	99.9	100
		Rat (Mean % TRR)		
10.2	306.145	100	100	100
		Dog (Mean % TRR)		
10.2	306.145	100	100	100
		Rabbit (Mean % TRR)		
10.2	306.145	100	100	100
		Mouse (Mean % TRR)		
10.2	306.145	100	100	100

III. CONCLUSION

In summary, the study shows that Imazamox (BAS 720 H) is not metabolised by liver microsomes of dogs, rabbits, mice, rats and humans. No unique human metabolite was detected.

CA 5.2 Acute Toxicity

Studies already peer-reviewed and presented in the original Annex II Dossier (1997):
No new acute *in vivo* studies were performed. All information available is presented in the original Annex II Dossier (1997) and has been evaluated by European authorities and France as the Rapporteur member state and the endpoints were fixed in the European Commission Review Report for the active substance Imazamox (SANCO/4325/2000 –Final, 29 November 2002).
For convenience of the reviewer brief summaries of the acute toxicity studies as extracted from the Draft Monograph (1999) are provided under the respective chapters CA 5.2.1 – CA 5.2.6.

Imazamox has been tested in a battery of acute toxicity tests in various species and via different routes of administration. These GLP studies have been evaluated by European authorities and France as the Rapporteur Member State during the last Annex I inclusion and are still considered to be valid. Imazamox shows a low toxicity by the oral, dermal, and inhalation routes of exposure. In the rabbit, Imazamox was found to be non-irritating to the skin and to the eye. Skin sensitization studies in guinea pigs conducted according to the methods of Buehler and Magnusson and Kligman (maximization test) showed that the compound is not a sensitizer.

Submission of not yet peer-reviewed studies in this AIR3-Dossier:
In accordance with the data requirements for active substances of Commission Regulation (EU) No 283/2013 of 1 March 2013, an *in vitro* NRU photoxicity study was conducted with Imazamox in Balb/c 3T3 cells and a detailed summary is given in chapter CA 5.2.7. Imazamox was not phototoxic *in vitro* at the concentrations tested up to the limit dose.

An overview of all acute toxicity studies is given in Table 5.2-1. For the already peer-reviewed studies, the respective EU agreed endpoints are marked in bold. Based on the available studies and according to CLP Reg. (EC) 1272/2008 no classification is warranted as to acute toxicity for Imazamox

Table 5.2-1: Summary of acute toxicity studies with BAS 720 H

Study type	Species (Strain)/Test system	Result	Classification	Reference
Acute oral LD_{50}	Rat (Sprague-Dawley)	**> 5000 mg/kg bw**	None	[redacted] (1995a) ID-411-001
Acute oral LD_{50}	Mouse (CD-1)	> 5000 mg/kg bw	None	[redacted] (1995a) ID-411-003
Acute dermal LD_{50}	Rabbit (New Zealand White)	**> 4000 mg/kg bw**	None	[redacted] (1995b) ID-412-001
Acute inhalation LC_{50}	Rat (Sprague-Dawley)	**> 6.3 mg/L**	None	[redacted] (1994) ID-413-001
Skin irritation	Rabbit (New Zealand White)	**Not irritating**	None	[redacted] (1995c) ID-415-001
Eye irritation	Rabbit (New Zealand White)	**Not irritating**	None	[redacted] (1996a) ID-415-002
Skin Sensitization (Buehler)	Guinea pig (Haz: (DH) fBR)	Not sensitizing	None	[redacted] (1992) ID-416-001
Skin Sensitization (Maximization Test)	Guinea pig (Hartley)	**Not sensitizing**	None	[redacted] (1996) ID-416-002
In vitro 3T3 NRU phototoxicity test*****	Balb/c3T3 cells	Not phototoxic at concentrations up to 1000.0 µg/mL	None	Cetto, V.; Landsiedel, R.2012 2012/1264018 *

***** new study

CA 5.2.1 Oral

Rat

[redacted] **(1995a):** Oral LD_{50} study in Albino Rats with AC 299,263 Technical; BASF DocID ID-411-001

Groups of 5 male and 5 female Sprague-Dawley rats (Crl CD (SD) BR strain) were administered 5000 mg/kg bw imazamox (98.2% purity, batch AC 6935-63) as a 50% w/v dispersion in corn oil (dose volume of 10 ml/kg bw) by gavage and were observed for 14 days. There were no deaths. Clinical signs comprised decreased activity in female rats during the first 2 hours following dosing. Body weight gain was unaffected. At necropsy, no pathological changes were observed that could be attributed to administration of the test material.
The oral LD_{50} of the test substance was greater than 5000 mg/kg bw in male and female rats. Thus, according to CLP Reg. (EC) 1272/2008 no classification is warranted as to acute oral toxicity for Imazamox.

Mouse

[redacted] **(1995a)**: Oral LD_{50} Study in Albino Mice with AC 299,263 Technical; BASF DocID ID-411-003

Groups of 5 male and 5 female CD-1 mice (Crl: CD-1 (ICR) BR strain) were administered 5000 mg/kg bw imazamox (97.1% purity, batch AC 6935-63) as a 50% w/v dispersion in corn oil by gavage. The substance/carrier mixture was not assayed to determine concentration, homogeneity or stability in conjunction of the study, but it could be considered that the deviation from the GLP regulations did not adversely impact the quality or the integrity of the study. The animals were observed for 14 days. No deaths occurred and no clinical signs of toxicity were observed. All animals gained weight during the 14-day study period. There were no gross pathological changes at necropsy that were attributed to administration of the test material.
The oral LD_{50} of the test substance was greater than 5000 mg/kg bw in male and female mice. According to CLP Reg. (EC) 1272/2008 no classification is warranted as to acute oral toxicity for Imazamox.

CA 5.2.2 Dermal

[redacted] **(1995b)**: Dermal LD_{50} Study with AC 299263 Technical in the Albino Rabbit; BASF DocID ID-412-001

Groups of 5 male and 5 female New Zealand White (NZW) rabbits received a topical application of 4000 mg/kg bw of Imazamox (98.2% purity; batch AC 6935-63) to the shaved skin, on an area equivalent to approximately 10% of the body surface area. After the exposure period of 24 hours any remaining test material was wiped from the skin and the animals were observed for 14 days. No deaths occurred and there were no clinical signs of toxicity during the 14-day study period. All animals gained body weight. No gross pathological changes were observed at necropsy.
The dermal LD_{50} was greater than 4000 mg/kg bw for both sexes of NZW rabbits.
According to CLP Reg. (EC) 1272/2008 no classification is warranted as to acute dermal toxicity for Imazamox.

CA 5.2.3 Inhalation

[redacted] **(1994):** Acute Inhalation Toxicity Study with AC 299263 in Rats; BASF DocID ID-413-001

Five male and five female Sprague-Dawley rats were exposed via whole-body inhalation to imazamox (98.2% purity, batch AC8322-64), administered as a dust. An additional group of rats (5/sex) served as controls and received house-line air.
The mean analytical exposure concentration of the test substance was determined to be 6.3 mg/L with a gravimetric concentration of 5.9 mg/L and a nominal concentration of 31 mg/L.
The mass median aerodynamic diameter (MMAD) was 4.8 µm with a GSD of 2.2; 2.6% of the particles were less than one micron in size, 26% less than 3 microns, 51% less than 5 microns and 83% less than 10 microns. This concentration was considered to be the maximum attainable concentration with the test substance air-milled as finely as possible to yield the smallest size particles. No deaths occurred. Exposed rats were not observable for clinical signs during the exposure due to the dust obscuring the view into the chamber. Following exposure, labored breathing (females only), rales, red and mucoid nasal discharge, lacrimation, salivation, and yellow staining of the ano-genital fur was observed in the exposed animals for 2 hours post-exposure. A few of these responses (labored breathing, rales, nasal discharge) continued during the first week of the observation period before abating, and alopecia was also observed in 4/5 males and 5/5 females. Body weight gain was comparable between controls and exposed rats. Upon necropsy, brown/red areas in the lungs were observed for 1 of 5 control males and 3 of 5 males exposed to the test material. The toxicological significance of this finding was equivocal.
Thus, the inhalation LC_{50} was greater than 6.3 mg/L (analytical) for male and female rats.
According to CLP Reg. (EC) 1272/2008 no classification is warranted as to acute inhalation toxicity for Imazamox.

CA 5.2.4 Skin irritation

[redacted] **(1995c):** Skin Irritation Study in Albino Rabbits with AC 299263 Technical; BASF DocID ID-415-001

The dermal irritation potential of imazamox (98.2% purity; batch AC 6935-63) was investigated in 6 male New Zealand White rabbits. The clipped skin was exposed to 0.5 g of the solid test item, moistened with water, for 4 hours under semi-occlusive conditions. After exposure, the test sites were wiped to remove any remaining test material and the application sites were examined for irritation at the following intervals: 1 hour, 24 hours, 48 hours, and 72 hours. For each animal, a control site was selected on the opposite side of the dorsal midline. No deaths and no overt signs of toxicity occurred during the 3 day observation period. A barely perceptible erythema was observed in 2 of 6 animals at the 24-hour reading time point which had resolved at the 48-hour observation interval. The individual animal mean scores at the 24-, 48-, and 72- hour observations were 4x0.0 and 2x 0.3 for erythema, the individual mean scores for edema were zero at all intervals for each of the six animals.
Based on the findings of this study BAS 720 H is considered as non-irritating to rabbit skin and according to CLP Reg. (EC) 1272/2008 no classification is warranted.

CA 5.2.5 Eye irritation

[redacted] **(1996a)**: Eye Irritation Study in Albino Rabbits with AC 299263 Technical; BASF DocID ID-415-002

An amount of 0.1 g of the unchanged test substance (imazamox technical; 98.2% purity, batch AC 6935-63) was instilled into the conjunctival sac of the left eye of 6 female New Zealand White rabbits. After a 24-hour exposure period, treated eyes were rinsed with tap water and examined 1, 24, 48, 72, 96 and 168 hours after exposure for eye irritation. One hour following instillation of the test material, all animals exhibited slight redness of the conjunctivae, slight (4 of 6 animals) to moderate (2 of 6 animals) chemosis and moderate ocular discharge (all animals). At the 24-h observation, all animals exhibited slight to moderate redness of the conjunctivae, 4 of 6 animals exhibited scattered and diffuse areas of corneal opacity, slight chemosis and a mild to moderate ocular discharge, and 1 of 6 rabbits exhibited mild iritis. By 7 days following dosing, irritation had resolved in all animals. No overt signs of toxicity or mortalities occurred during the test period. The individual average animal scores from the 24-, 48- and 72-h observations were 0.0, 0.3, 0.3, 0.3, 0.0, 0.3 for corneal opacity and 5x 0.0 and 1x 0.3 for iris effects. For redness the individual mean average scores were 1.0, 1.0, 1.3, 1.7, 0.7, 1.7; and 0.0, 0.3, 0.3, 0.7, 0.0, 0.7 for chemosis.
Hence, the test substance is considered as non-irritating to the rabbit eye and according to CLP Reg. (EC) 1272/2008 no classification is required.

CA 5.2.6 Skin sensitisation

Buehler assay

[redacted] **(1992):** Dermal Sensitization Study with AC 299263 in Guinea Pigs; BASF DocID ID-416-001

The Buehler assay with imazamox (98.2% purity; batch AC 6935-63) was conducted on young adult male albino guinea pigs of the Haz:(DH) fBR strain. Protocol deviations comprised deviated humidity and temperature in the animal rooms from targeted values in the protocol. These were not considered to adversely impact the conclusions of the study. In the main study 10 animals were induced with a topical application of the undiluted test material (0.2 g moistened with deionized water) for a 6 h contact period once per week over totally three weeks. A topical challenge application of the undiluted test material was administered 2 weeks following the last induction application. No deaths and no clinical signs of toxicity were observed. All animals gained body weight during the study. There were no erythema or edema dermal reactions observed for any of the animals during either the induction or challenge phases of the study. In contrast, all 10 positive control (0.1% w/v DNCB) animals exhibited moderate to strong dermal reactions at challenge.
Since none of the test substance-treated animals exhibited a positive skin reaction, Imazamox is considered as a non-sensitizer under the conditions of this Buehler assay.

Guinea Pig Maximisation test (GPMT)

[redacted] **(1996):** Dermal Sensitization Study of AC 299263 in Guinea Pigs Maximization Test; BASF DocID ID-416-002

A dermal sensitization study with Imazamox (97.1% purity; batch AC 6935-63) was conducted in young adult male Hartley strain guinea pigs (Crl:(HA)BR strain) using the maximization method of Magnusson and Kligman. Analysis of the test material for stability and the test material mixtures for concentration, homogeneity/solubility and stability was not conducted and on day 8, induction sites were inadvertently not observed. However, these deviations were regarded to not adversely impact the outcome of the study. 20 test group animals were treated with the following concentrations of the test substance in mineral oil: 5% for intradermal induction and 25% for topical induction and challenge. 10 animals served as negative control group. All animals gained weight during the study, and no treatment-related clinical signs were observed. Mild-to-moderate erythema and edema reactions as well as scab formation were noted at the intradermal and topical induction application sites for the test and irritation control groups. None of the animals in the test or irritation control groups exhibited a dermal reaction to the challenge application of the test or control material at either the 24- or 48-hour observations.
A positive control group was not included in this study, but a dermal sensitization study with hexylcinnamaldehyde was conducted within 6 month of initiation of the study with the test substance, inducing a positive sensitization response in 100% of the tested animals, thereby ensuring the sensitivity of the test method.
Based on the 0% incidence of animals in the test group exhibiting sensitization reactions at challenge, Imazamox is considered as a non-sensitizer. Thus, according to CLP Reg. (EC) 1272/2008 no classification as to skin sensitization is required.

CA 5.2.7 Phototoxicity

Report:	CA 5.2.7/1 Cetto V.,Landsiedel R., 2012a BAS 720 H (Imazamox) - In vitro 3T3 NRU phototoxicity test 2012/1264018
Guidelines:	OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

BAS 720 H (Imazamox) (batch COD-001579; purity 98.9%) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells *in vitro*. The photo-cytotoxicity was estimated by the means of the Neutral Red Uptake (NRU) method. A single experiment was carried out, with and without irradiation with an UVA source. BAS 720 H was tested up to the limit concentration at dose levels of 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2, and 1000.0 µg/mL.
In this study no cytotoxicity in the absence and the presence of UVA irradiation was observed up to the highest required concentration indicated by Neutral Red Uptake method. The positive control chlorpromazine led to the expected cytotoxicity both with and without UVA irradiation (Photo-Irritancy Factor (PIF)=*1), thus, demonstrating the sensitivity of the test system.

Under the experimental conditions of this study, BAS 720 H is considered not to be a phototoxic substance in the *in vitro* 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Imazamox (BAS 720 H)
Description:	Solid, white
Lot/Batch #:	COD-001579
Purity:	98.9% (tolerance ± 1%)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Jul 2014. Homogeneity of the preparations was ensured by mixing.
Solvent used:	Dimethylsulfoxid; DMSO

2. Control Materials:

Negative and solvent control:	DMSO (1% (v/v) in culture medium)
Positive control:	Without irradiation: 1.9, 3.8, 7.5, 15.0, 30.0, 60.0, 90.0, and 180.0 µg/mL chlorpromazine With irradiation: 0.03, 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, and 3.20 µg/mL chlorpromazine

3. Test organisms: Balb/c 3T3, clone A31 cells.
The cell line was isolated from the muscle tissue of mouse embryo. This fibroblast cell line has a high proliferation rate and a high plating efficiency of untreated cells both necessary for the appropriate performance of the study. The Balb/c 3T3 cell line was obtained from the "European Collection of Cell Cultures" Salisbury, UK.

4. Culture media and reagents:

Culture medium:	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with - 10% (v/v) newborn calf serum (NCBS) - 4 mM L-glutamine - 100 IU penicillin - 100 µg/mL streptomycin
Neutral red solution:	- 0.4 g Neutral Red powder - 100 mL deionized water
Neutral red medium:	- 1 mL Neutral Red solution - 79 mL culture medium Incubated overnight at 37° C with 5% CO_2 and filtered with a 0.22 µm filter prior to use.
Other solutions and reagents:	- Phosphate buffered saline (PBS) without Ca/Mg - Trypsin/EDTA solution (0.05%; 0.02%) - Neutral Red desorb solution (1 mL acetic acid, 50 mL ethanol, 49 mL deionized water).
Irradiations source:	Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfeling, Germany) with the filter H1. The produced wavelength was > 320 nm. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

5. Test concentrations:

Pretest:	Based on the purity and the molecular weight of the test substance 1000 µg/mL (approx. 3.3 mM) was used as top concentration both with and without irradiation.
Main test:	With and without UVA irradiation: 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2, and 1000.0 µg/mL A single experiment was performed in 96 well plates with 6 replicates per concentration with and without irradiation.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 24-Sep-2012 - 28-Sep-2012

2. NRU phototoxicity test: Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.5×10^5 cells/well). After an attachment period of about 24 hours the cells were treated with the respective substance (8 concentrations each with 6 replicates) and the vehicle control in parallel for 1 h in the dark (5% (v/v) CO_2, 37°C). Then, one microtiterplate per substance was irradiated for 50 min with UVA at room temperature (UV intensity underneath the lid 1.5–2.1 mW/cm^2 (equal to 5 J/cm^2) whereas the respective reference plate was kept in the dark at room temperature for the same time period. After test substance removal the cells were washed with 100 µL PBS, incubated in culture medium overnight and thereafter in Neutral Red medium for additional 3 hours, each step under light protected conditions in the lab to prevent uncontrolled photo activation. Thereafter, the cells were washed, the dye was extracted by Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake using a microplate reader (Perkin Elmer, Waltham, Massachusetts, US) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.

3. Evaluation assessment: For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor Prediction model for substances which allow the comparison of two equi-effective concentrations (EC50) in concurrently performed experiments in the presence and absence of UV light. This model includes the special case of absence of cytotoxicity in the presence and absence of light for substances obviously showing no phototoxic potential.
- The Mean Photo Effect prediction model is used if no equi-effective concentrations (EC_{50}) are obtained in the absence and presence of UV light

Cytotoxicity: The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrarily set at 100 %.

$$Cytotoxicity\,[\%] = \frac{\text{Absorbance}_{\text{mean}}\ \text{of the test group}}{\text{Absorbance}_{\text{mean}}\ \text{of the vehicle control}} \times 100$$

In case of cytotoxicity, an EC_{50} value (Inhibition concentration 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve).

Photo-irritancy Factor: For substances which induce a 50% cytotoxicity (EC_{50}) in the presence and absence of light the Photo-Irritancy-Factor (PIF) is calculated based on comparison of the EC_{50} values in the absence (-UVA) and presence (+UVA) of UVA irradiation.

$$PIF = \frac{EC50\ (-UVA)}{EC50\ (+UVA)}$$ The resulting classification rules are:

PIF ≥ 5:	phototoxic potential predicted
2 < PIF < 5:	probable phototoxic potential predicted
PIF ≤ 2:	no phototoxic potential predicted

If cytotoxicity occurs only after irradiation a C PIF has to be calculated using the highest test concentration (Cmax) applied in the experimental part in the absence of UV light (-UVA):

$$C\ PIF = \frac{C\max\ (-UVA)}{EC50\ (+UVA)}$$ The resulting classification rules are:

C PIF > 1	probable phototoxic potential predicted
C PIF ≤ 1	no phototoxic potential predicted

If no cytotoxicity occurs in the concurrently performed experiments in the absence and presence of UV light up to the highest applied test concentration it has to be considered that the test substance has no phototoxic potential.

In this case, a formal PIF = *1 is used to characterize the result:

$$PIF = *1 = \frac{C\max(-UVA)}{C\max(+UVA)}$$ resulting in the following classification rule:

PIF = *1	no phototoxic potential predicted

Mean photo effect: The Mean Photo Effect is calculated based on a comparison of the +UVA and −UVA concentration response curves on a grid of concentrations ci (i=1,..., N) chosen from the common concentration range of the (−UVA) and (+UVA) experiments. The photo effect (PE_i) at concentration c_i is calculated as the product of the concentration effect (CE_i) and the response effect (RE_i). The mean photo effect (MPE) is defined as a weighted averaging across all PEi values, with a weighting factor defined by the highest response value.
The resulting classification rules are:

MPE ≥ 0.1	phototoxic potential predicted
MPE < 0.1	no phototoxic potential predicted

Further parameters: pH, osmolarity, solubility, and cell morphology

4. Statistics: No special statistical tests were performed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Jul 2014 as indicated by the sponsor.
Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UVA irradiation no precipitation in culture medium was observed up to the highest applied test substance concentration.

B. CYTOTOXICITY OF THE TEST SUBSTANCE

Treatment with BAS 720 H (Imazamox) did not induce cytotoxic effects in the absence or presence of UVA irradiation, i.e., Neutral Red absorbance values below 50% of the control were not observed. Therefore, no EC_{50} values could be calculated. The cell densities were not reduced. Based on these observations a formal PIF= *1 has to be used to characterize the result.
The results are summarized in the following table.

Table 5.2.7-1: Mean cytotoxicity of BAS 720 H

Test groups [µg/mL]	Mean $OD_{corr.}$*	Relative cytotoxicity	
		Mean [% of control]	SD [%]
Without UVA irradiation			
Vehicle[1]	0.594	100.0	2.6
4.6	0.594	100.0	4.9
10.0	0.629	105.9	4.1
21.5	0.643	108.2	2.4
46.4	0.643	108.2	3.7
100.0	0.617	103.7	1.9
215.4	0.629	105.7	3.0
464.2	0.615	103.5	2.2
1000.0	0.580	97.6	2.3
With UVA irradiation**			
Vehicle[1]	0.590	100.0	2.3
4.6	0.589	99.9	2.0
10.0	0.588	99.7	3.2
21.5	0.598	101.4	1.4
46.4	0.593	100.5	0.8
100.0	0.600	101.7	1.7
215.4	0.602	102.1	1.7
464.2	0.563	95.4	2.2
1000.0	0.566	96.0	1.3

* Mean OD-corrected: mean absorbance (test group) minus mean absorbance (blank)

** Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)

[1] DMSO 1% (v/v)

C. CYTOTOXICITY OF THE POSITIVE CONTROL

Treatment with chlorpromazine induced clear cytotoxic effects. Without UVA irradiation, a decrease in the cell number from 30 µg/mL (EC_{50}: 27.8 µg/mL) onward was observed. The cell densities were distinctly reduced. With UVA irradiation, a decrease in the cell number at 0.8 µg/mL (EC_{50}: 0.8 µg/mL) and above was observed. The cell densities were distinctly reduced.
Based on the EC_{50} values a PIF of 37 (a phototoxic potential) was obtained.
The results are summarized in the following table.

Table 5.2.7-2: Mean cytotoxicity of the positive control chlorpromazine

Test groups [µg/mL]	Mean $OD_{corr.}$*	Cytotoxicity	
		Mean [% of control]	SD
Without UVA irradiation			
Vehicle[1]	0.609	100.0	2.1
1.9	0.631	103.6	1.3
3.8	0.613	100.7	3.0
7.5	0.623	102.2	1.7
15.0	0.582	95.6	1.4
30.0	0.256	42.0	7.3
60.0	0.001	0.1	0.2
90.0	0.002	0.3	0.2
180.0	0.002	0.3	0.3
With UVA irradiation**			
Vehicle[1]	0.604	100.0	3.3
0.03	0.577	95.5	3.6
0.05	0.591	97.9	4.6
0.10	0.579	95.9	2.8
0.20	0.569	94.3	4.6
0.40	0.542	89.8	2.0
0.80	0.268	44.4	8.6
1.60	0.009	1.5	1.9
3.20	0.012	2.0	1.4

* Mean OD-corrected: mean absorbance (test group) − mean absorbance (blank)

** Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)

[1] DMSO 1% (v/v)

C. CELL MORPHOLOGY

Osmolarity and pH values were not influenced by treatment with the test substance. No precipitation in culture medium was observed up to the highest applied test substance concentration in the absence and the presence of UVA irradiation.

III. CONCLUSION

Under the experimental conditions of the present study, the test substance BAS 720 H (Imazamox) has **no phototoxic potential in the *in vitro* 3T3 NRU Phototoxicity** test after UVA irradiation.

CA 5.3 Short-Term Toxicity

No new short-term toxicity studies were conducted since the last Annex I evaluation.

Imazamox was of low toxicity following repeated dosing. Subchronic dietary toxicity studies in rats (28-day and 13-week) and dogs (90-day and 1-year) revealed no adverse effects that were attributed to treatment with Imazamox up to the limit dose and above. Moreover, results from a 28-day subchronic dermal toxicity study conducted in rats also revealed no treatment-related effects via dermal exposure. Imazamox is a non-volatile compound and therefore further repeated dose studies to examine other routes of exposure via inhalation are not considered necessary. Since the last submission there were no new short-term toxicity studies performed. The existing toxicity data (for summary see Table 5.3-1) are still acceptable and thus, the short-term toxicity endpoints fixed in the European Commission Review Report for the active substance Imazamox (SANCO/4325/2000 –Final, 29 November 2002) are considered to be still valid.

Table 5.3-1: Summary of peer-reviewed short-term toxicological data obtained with BAS 720 H

Study type (strain)	Dose levels	Findings	NOAEL [mg/kg bw/d]	Reference
28-day oral, Rat (Sprague Dawley)	5000, 10000, and 20000 ppm	No adverse effects	2438 [20000 ppm]	[redacted] (1996b) ID-420-002
13-week oral, Rat (Sprague Dawley)	1000, 10000, and 20000 ppm	No adverse effects	1661 [20000 ppm]	[redacted] (1995d) ID-425-001
90-day oral, Dog (Beagle)	1000, 10000, and 40000 ppm	No adverse effects	1368 [40000 ppm]	[redacted] (1994) ID-425-002
1-year oral, Dog (Beagle)*	1000, 10000, and 40000 ppm	No adverse effects	1174 (males) and 1156 (females) [40000 ppm]	[redacted] (1995a) ID-427-001
28-day dermal, Rat (Sprague Dawley)	250, 500, and 1000 mg/kg bw/day	No adverse effects	1000	[redacted] (1995) ID-420-001

*a brief summary of the chronic 1-year oral dog study is presented in chapter CA 5.5

No classification for repeated exposure, such as specific target organ toxicity is required for Imazamox. Based on the available studies, the lowest relevant endpoint for repeated dose exposure was identified from the 1-year oral dog study and determined as follows in the European Commission Review Report for the active substance Imazamox (SANCO/4325/2000 – Final, 29 November 2002):

Target / critical effect:	None
Lowest relevant oral NOAEL/NOEL:	> 1156mg/kg bw/d (12-month dog study): 40000 ppm
Lowest relevant dermal NOAEL/NOEL	>1000 mg/kg bw/d (28-day dermal rat study)
Lowest relevant inhalation NOAEL/NOEL	No data: not required

No detailed summaries are presented in this AIR3 Dossier. For the convenience of the reviewer brief summaries of the repeated dose studies as extracted from the monograph (1999) are provided under the respective chapters CA 5.3.1 – CA 5.3.3. For a short summary of the 1-year dog study, please refer to the chapter CA 5.5.

CA 5.3.1 Oral 28-day study

[redacted] **(1996b):** A 28-Day Dietary Toxicity Study in Albino Rats with AC299,263; BASF DocID ID-420-002

Imazamox (AC 299263 technical; 98.2% purity; batch 7963-33) was evaluated for its short-term toxicity in groups of 5 male and 5 female Sprague-Dawley rats (Crl CD® (SD) strain)–when administered via diet at concentrations of 0, 5000, 10000, and 20000 ppm for 28 days. No deaths occurred and no treatment-related clinical signs of toxicity were observed during the 28-d study period. There were no statistically significant changes observed in food consumption and values for treated males and females were generally comparable to those of control animals at all measurement intervals. Body weights and body weight gains were not adversely affected by treatment. Only female body weights were slightly decreased in all treated groups at most measurement intervals, resulting in a statistically significant decrease of 7% (compared to control) for both the 10000 and 20000 ppm levels during study week 3. Body weight gain was statistically significantly reduced for females at all treatment levels during study week 1, resulting in overall body weight gain depressions of 7%; 10%, and 8% at 5000, 10000, and 20000 ppm, respectively. These effects on body weights observed for treated females were not considered toxicologically significant, as no dose-response was evident. Furthermore, similar findings were not observed in males, nor in females in the 13-week rat dietary toxicity study (see below) or during the first four weeks of treatment in the 24-month rat chronic toxicity/oncogenicity study, at concentrations up to and including 20 000 ppm. Moreover, the decrease in overall body weight gain in the treated females likely reflected the decrease observed during the first study week. Absolute and relative (to body weight) liver weights were statistically significantly increased for males in the 10000 ppm group only. These increases were not considered treatment-related given the absence of a dose-response relationship, and there were no macroscopic or microscopic changes in the liver which would account for these increases. Furthermore, there were no treatment-related gross pathological or histopathological changes in any of the tissues evaluated. Hematological, clinical chemistry, ocular or urinary parameters were not evaluated in this study.

Based on these results, the NOAEL of Imazamox for this study was 20000 ppm (equivalent to 2438 mg/kg bw/day), the highest concentration tested.

CA 5.3.2 Oral 90-day study

Rat

[redacted] **(1995d)**: AC 299263: A 13-Week Dietary Toxicity Study in the Albino Rat; BASF DocID ID-425-001

Imazamox was evaluated for its subchronic toxicity in groups of 10 male and 10 female Sprague Dawley rats derived rats (Crl CD® (SD) strain), which were administered dietary concentrations of 0, 1000, 10000 and 20000 ppm of the test substance (AC 299263 technical; 98.2% purity; batch 6935-63) for 13 consecutive weeks. There were no mortalities and no signs of toxicity observed during the study period in all treatment groups. Food consumption values for treated males and females were not adversely affected when compared to controls. Although body weights for both male and female treated rats were generally comparable to those of controls during the study period, statistically significant increases were observed in females regarding body weight in the 1000 ppm dose group at weeks 3, 4, 6, and 7 of the study and for body weight gain at week 6 in the low and mid dose group. Overall gains for treated females over the whole study period were slightly increased (1-19%) compared to controls. Overall body weight gain for male rats was slightly decreased at 1000 ppm (-2%) and 20000 ppm (-6%), which likely resulted from slight, non-statistically significant decreases in body weight gains during weeks 12 and 13. This decrease in overall body weight gain noted for males in the 20 000 ppm group was not considered treatment-related because the magnitude of the decrease was so small, a decrease was noted in only one sex, and there was no effect on total body weight gain from weeks 1 to 13 for 20000 ppm males (compared to controls) in the 24-month chronic toxicity/oncogenicity study in Sprague-Dawley rats (see chapter CA 5.5). There were no statistically significant changes in any of the hematological, clinical chemistry or urinary parameters for either sex at any treatment level. There was no indication of treatment-related ocular abnormalities during ophthalmological examinations. Absolute liver, kidney, heart, and spleen weights were significantly increased in females at 1000 ppm vs. controls. These changes were considered to reflect the increased body weight observed for these animals at termination, as the relative weights for these organs were not statistically significantly different from controls. Likewise, significantly decreased brain-to-body weight ratios in females at 20000 ppm were considered to reflect increased terminal body weight observed for these animals as compared to controls. There were no treatment-related gross pathological or histopathological changes observed.

Based on these findings the NOAEL for this study was 20000 ppm, the highest concentration tested, which was equivalent to an average daily intake of 1661 mg/kg bw/day (based on food consumption).

Dog
[redacted] **(1994)**: 90-Day Dietary Toxicity Study with AC 299263 in Purebred Beagle Dogs; BASF DocID ID-425-002

Imazamox (AC 299263 technical, 97.1-98.2% purity; batch AC 6935-63) was evaluated for its subchronic toxicity in groups of 4 male and 4 female purebred beagle dogs when administered via diet at concentrations of 0, 1000, 10,000, and 40,000 ppm for 13 weeks. No mortalities or treatment-related clinical signs of toxicity occurred during the study period. Food consumption, body weight, and body weight gains were not affected by treatment. Treatment did not affect clinical chemistry, urinalysis and ophthalmologic parameters. Absolute and relative organ weights were similar between control and treated animals, and there were no gross pathological or histopathological findings attributed to treatment with Imazamox.
Thus, the oral NOAEL for this study in dogs was 40000 ppm, the highest concentration tested, which was equivalent to approximately 1368 mg/kg bw/day (1333 mg/kg bw/day in males and 1403 mg/kg bw/day in females).

CA 5.3.3 Other routes

28-day dermal toxicity
[redacted] **(1995)**: A 28-Day Dermal Toxicity Study with AC 299263 in Rats; BASF DocID ID-420-001

The unchanged, marginally moistened test substance Imazamox (AC 299263 technical; 98.2% purity; batch 6935-63) was dermally administered under an occlusive gauze-bandage to the clipped skin of the back and sides to groups of 5 male and 5 female Sprague-Dawley rats at dose levels of 0, 250, 500, and 1000 mg/kg bw/day (exposure period: 6h/day, 5 days/week) for a period of 28 days. Control animals received 0.5 mL of 0.9% saline only At the end of each exposure, the wrappings were removed and the test sites were cleansed with castile soap and water.
There were no mortalities or clinical signs of toxicity noted throughout the study period. In addition, there were no signs of dermal irritation observed. Food consumption and mean body weight and body weight gains for treated groups were comparable to controls. Hematology and clinical chemistry values were also similar among treated and control animals. There were no test material-related ocular changes observed at termination of the study. Absolute organ weights were unaffected by administration of Imazamox. The brain-to-body weight ratio for 250 mg/kg bw males was significantly greater than that for controls. This increase likely resulted from a very small decrease in mean terminal body weights observed for males at this dose level. In the absence of a dose-response, this finding was not considered to be biologically significant. There were no compound-related macroscopic or microscopic changes observed in any animal.
Based on the results of this study, the dermal NOAEL in rats was 1000 mg/kg bw/day, the highest dose tested.

90-day dermal toxicity

The results from the available 28-day dermal toxicity study in rats have shown that Imazamox has a low order of toxicity and thus, has not revealed any cause for concern. Therefore, no further repeated dose dermal toxicity study is considered to be necessary.

28-day and 90-day inhalation toxicity

Imazamox is a non-volatile compound and therefore, no 28-day or 90-day inhalation toxicity study is considered to be necessary. Moreover, the available acute inhalation toxicity study with BAS 720 H revealed low toxicity via the inhalation route of exposure and did not indicate any specific inhalation toxicity. Therefore, the absence of 28-day and 90-day inhalation toxicity studies in rats is not considered to constitute a data gap.

CA 5.4 Genotoxicity Testing

No new studies were conducted since the last Annex I evaluation.

Studies already peer-reviewed and presented in the original Annex II Dossier (1997):
In a series of *in vitro* and *in vivo* tests Imazamox did not show any genotoxic potential. The summary of the studies is provided in the following table.

Table 5.4-1: Summary of already peer-reviewed genotoxicity data *in vitro* and *in vivo* obtained with Imazamox (BAS 720 H)

Test system	Concentrations/ dose	Results	References
In vitro Bacterial/ Microsome Mutagenicity (Ames)	100, 500, 1000, 2500, and 5000 µg/plate	Negative	Mulligan, E. (1992) ID-435-001
In vitro CHO/HGPRT mutation assay	50, 100, 500, 1000, 2000, and 4000 µg/mL	Negative	Sharma, R.K. (1993a) ID-435-002
In vitro chromosome aberration test in CHO cells	417, 833, 1667, and 3333 µg/mL	Negative	Kumaroo, P.V. (1994) ID-435-004
In vivo micronucleus assay in bone marrow cells of mice	1250, 2500, and 5000 mg/kg bw	Negative	[redacted] (1993b) ID-435-003

Based on the available studies, which were all negative, BAS 720 H was evaluated to have **no genotoxic potential** in the European Commission Review Report for the active substance Imazamox (SANCO/4325/2000–Final, 29 November 2002) as indicated below. The studies are still considered to be valid and acceptable and to accurately address the genotoxic potential of the active ingredient. Therefore, no new studies on genotoxicity *in vitro* or *in vivo* have been performed since the last submission.
For convenience of the reviewer a short summary of each individual study as extracted from the Draft Monograph (1999) is presented below.

CA 5.4.1 In vitro studies

Mutagenicity in bacteria

Mulligan E. (1992): Evaluation of CL 299,263 in a bacterial/microsome mutagenicity assay; BASF DocID ID-435-001

Bacterial reverse mutation tests were performed on Imazamox technical (CL 299,263 technical; 98.2% purity; batch AC 6935-63) in test strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) and E. coli (WP2 *uvrA*). Experiments were carried out with and without metabolic activation (S9 mix) at dose levels of 100, 500, 1000, 2500, and 5000 µg/plate in dimethyl sulfoxide (DMSO), or with DMSO alone as the negative control for three replicates per dose. Imazamox did not induce toxicity, and the results obtained from both trials showed no significant or dose-related increases in revertant frequencies in the presence or absence of metabolic activation. The positive controls (5 µg/plate of 2-Aminoanthracene with metabolic activation administered to all 6 strains; 10 µg/plate N-methyl-N'-nitro-N-nitrosoguanidine without metabolic activation administered to WP2 *uvrA*, TA100 and TA1535; 20 µg/plate 2-Nitrofluorene without metabolic activation administered to TA98 and TA1538 as well as 50 µg/plate 9-Aminoacridine without metabolic activation administered to TA1537) elicited the expected increase in revertant frequencies, indicating that the test system was capable of detecting base-pair and frameshift mutations and that the metabolic activation system was functioning properly.
In conclusion, Imazamox did not show any genotoxic potential in 6 tester strains of bacteria at concentrations up to and including 5000 µg/plate in the presence and absence of S-9 metabolic activation.

Mutagenicity in mammalian cells

Sharma, R.K. (1993a): Evaluation of CL 299,263 in the mammalian cell CHO/HGPRT mutagenicity assay; BASF DocID ID-435-002

Imazamox technical (CL 299,263 technical; 98.2% purity; batch 6935-63) was tested for its potential to induce mutations at the HGPRT locus in Chinese Hamster Ovary (CHO) cells *in vitro*. Based on the results from a dose-range finding test, experiments in the definitive study were carried out at dose levels of 50, 100, 500, 1000, 2000, and 4000 µg/mL (limited by solubility) with and without metabolic activation (S9 mix) at concentrations in 2 independent trials and in duplicate. DMSO was used as the vehicle control. The test item did not induce any significant toxicity or mutations at the HGPRT locus in CHO cells. The concurrent positive controls (3 µg/mL 7,12-dimethylbenzanthracene with metabolic activation and 200 µg/mL ethylmethanesulfonate without metabolic activation) elicited the expected mutagenic responses indicating that the test system was valid and that the metabolic activation system was functioning properly. No significant toxicity was induced by Imazamox, and results obtained from both trials indicated that the test material did not induce mutations at the HGPRT locus in CHO cells.
In conclusion, Imazamox was found not to induce mutations at the HGPRT locus in CHO cells at doses up to and including 4000 µg/mL with and without metabolic activation.

Cytogenicity in mammalian cells

Kumarro P.V. (1994): AC 299,263: Test for chemical induction of chromosome aberration in cultured Chinese Hamster Ovary (CHO) cells with and without metabolic activation; BASF DocID ID-435-004

Imazamox technical (AC 299263 technical; 97.1% purity; batch 6935-63) was tested for its potential to induce chromosome aberrations in Chinese Hamster Ovary (CHO) cells *in vitro.* Based on the results of a pretest, main experiments were carried out at concentrations of 417, 833, 1667, and 3333 μg/mL test substance dissolved in DMSO with and without metabolic activation (S9 mix) in two independent assays (definitive and confirmatory). The high dose level tested represented the limit of solubility in this assay system. At 3333 μg/mL the test substance induced some toxicity in both the activated and non-activated systems, as evidenced by up to a 58% reduction in mitotic indices at the 19-hour harvest (activated), and a 38% reduction in mitotic indices at the 48-hour harvest (non-activated). A confirmatory chromosome aberration assay using the same dose levels and treatment procedures as the definitive study resulted in similar findings for both the 13 and 37 hour (one cell cycle time plus 24 hours) harvest intervals. No statistically significant increases in chromosomal abnormalities were found at any dose or time interval. The concurrent positive controls (mitomycin C (0.16 and 0.2 μg/mL) without metabolic activation and cyclophosphamide (10 and 12.5 μg/mL) with metabolic activation) elicited an adequate increase in chromosomal aberrations demonstrating the validity of the test system and that the S-9 metabolic activation system was functioning properly.
In conclusion, Imazamox was not genotoxic in the *in vitro* chromosome aberration assay in CHO cells in the presence and absence of metabolic activation.

CA 5.4.2 In vivo studies in somatic cells

Micronuclei in mouse bone marrow

[redacted] **(1993b):** Evaluation of CL 299,263 in the in vivo micronucleus assay in mouse bone marrow cells; BASF DocID ID-435-003

The test substance, Imazamox technical (CL 299,263 technical; 98.2% purity; batch 6935-63), was tested for its potential to induce chromosomal damage (Micronucleus assay) in groups of 5 male and 5 female CD-1 mice when administered single oral gavage doses at levels of 1250, 2500, and 5000 mg/kg bw. Dose levels were selected based on results from a range-finding test which showed no toxicity at a limit dose of 5000 mg/kg bw. The vehicle control group received an oral gavage administration of corn oil. Animals were sacrificed 24, 48, and 72 hours following dosing for collection of bone marrow cells. For each animal, 1000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei and the ratio of polychromatic to normochromatic erythrocytes (NCE) was calculated from 1000 erythrocytes per animal.
2 deaths occurred in males (1 in the 24-h harvest of the 5000 mg/kg group and 1 in the 48h harvest of the 2500 mg/kg group).
There was no significant increase (in %) of polychromatic erythrocytes or in the frequency of micronucleated polychromatic erythrocytes for any treatment group at any harvest time. The vehicle control values were within the expected range. For the positive control group (intraperitoneal injection of cyclophosphamide at 90 mg/kg bw; animals were sacrificed at 24 hours following dosing) a significant increase in the frequency of micronucleated polychromatic erythrocytes, as well as in percent PCEs was observed confirming the validity of the assay
In conclusion, Imazamox did not induce cytogenetic damage as measured by micronucleus induction in CD-1 mice.

CA 5.4.3 In vivo studies in germ cells

In the absence of any mutagenic activity, no *in vivo* mutagenicity test in germ cells was conducted or considered necessary.

CA 5.5 Long-Term Toxicity and Carcinogenicity

No new Long-term toxicity and carcinogenicity studies were conducted since Annex I evaluation.

Studies already peer-reviewed and presented in the original Annex II Dossier (1997):
The long-term oral toxicity of BAS 720 H was investigated in a combined chronic toxicity/carcinogenicity study in in rats and an 18-month carcinogenicity study in mice. Moreover, a 1-year oral chronic study in dogs is available.
In these long-term studies, Imazamox was administered in the diet at doses levels up to or exceeding the limit dose and results did not show increased mortalities or clinical signs of toxicity that could be attributed to treatment. Moreover, there were no consistent treatment-related effects on food consumption, body weight, absolute and relative organ weights, or hematology, clinical chemistry, urinalysis or ophthalmologic parameters. There was no gross or microscopic evidence of treatment-related lesions, and the NOAELs from the studies were the highest concentrations tested (see Table 5.5-1). There was no evidence of carcinogenicity from the 18-month mouse or 24-month rat studies.

Since the last submission no new long-term studies were performed and the existing studies have been evaluated by European authorities and France as the Rapporteur member state.

Table 5.5-1: Summary of long-term toxicity data obtained with BAS 720 H as provided in the monograph (1999)

Study	Dose levels	Findings	NOAEL [mg/kg bw/d]	Reference
24-month oral, Rat, Chronic toxicity/ Carcinogenicity	1000, 10000, and 20000 ppm	No adverse effects	Chronic toxicity and carcinogenicity: 1068 (male) and 1284 (female) [20000 ppm]	[redacted] (1995) ID-427-002
18-month oral, Mouse, Carcinogenicity	500, 3500 and 7000 ppm	No adverse effects	Chronic toxicity and carcinogenicity: 1053 (male) and 1348 (female) [7000 ppm]	[redacted] (1995b) ID-428-001
1-year oral, Dog (Beagle)*	1000, 10000, and 40000 ppm	No adverse effects	1174 (males) and 1156 (females) [40000 ppm]	[redacted] (1995a) ID-427-001

Based on the available studies the long-term and carcinogenicity endpoints were fixed in the European Commission Review Report for the active substance Imazamox (SANCO/4325/2000 – Final, 29 November 2002) as indicated below. In the absence of a carcinogenic potential no classification as to carcinogenicity is required for Imazamox:

Long-term toxicity and carcinogenicity	
Target/critical effect:	None
Lowest relevant NOAEL	1068 mg/kg bw/d (2-year rat study)
Carcinogenicity:	Not carcinogenic

For convenience of the reviewer, brief study summaries as extracted from the monograph are provided below.

Rat

[redacted] **(1995):** Chronic dietary toxicity and oncogenicity study with AC 299,263 in the albino rat; BASF DocID ID-427-002

The test substance Imazamox (98.2 % - 97.1% purity; batch AC 6935-63) was tested for its chronic toxicity potential in groups of 65 male and 65 female Sprague-Dawley rats when administered via diet at dietary concentrations of 0, 1000, 10000, and 20000 ppm for 24 months. Samples for hematological, clinical chemistry and urinalysis determinations were collected from 10 rats/sex/dose level at 6 month intervals during the study period.
Survival was not affected by the treatment and adjusted survivals rates were comparable between treated animals and controls in either sex. All observed clinical signs and/or ophthalmological lesions observed were considered as incidental changes or typical findings for rats of this strain and age which occurred at similar rates in all groups including controls and not to be treatment-related. Food consumption at all treatment levels was comparable or greater than that of controls in male and female rats. Body weights and weekly body weight gains of treated rats were generally comparable to that of controls at most measurement intervals, with minor body weight gain increases or decreases which were considered unrelated to treatment. No significant changes were noted in any of the hematological parameters at any sampling intervals. A few significant changes were noted among clinical chemistry parameters at some sampling intervals, but none occurred consistently over the study period or in both sexes nor in a dose-related manner and values were within historical range. Hence, these changes occurred sporadically without histopathological corroborate and therefore could not be attributed to treatment. The same was true for urinalyses parameters changes. No statistically significant changes were noted in either absolute or relative organ weights for either sex in any treatment group, except in the 10000 ppm male rats which exhibited increased absolute and relative kidney weights. No dose-response relationship was seen for this finding which was not seen in the corresponding females. There were no macroscopic or microscopic findings of toxicological significance at any treatment level associated with dietary administration of test substance.

The NOAEL for chronic toxic effects over 24 months of treatment with Imazamox was 20000 ppm, the highest concentration tested. No carcinogenic effect of test substance was demonstrated in this study. Therefore, the NOAEL for carcinogenic effects was also 20000 ppm, equivalent to an average daily intake of 1068 and 1284 mg/kg bw/day of the test substance in the males and females, respectively.

Mouse

[redacted] **(1995a):** An oncogenicity study with AC 299,263 in Mice; BASF DocID ID-428-001

The test substance, Imazamox technical (98.2 % - 97.1% purity; batch AC 6935-63), was tested for its chronic toxicity potential in groups of 55 male and 55 female CD-1 mice when administered via diet at dietary concentrations of 0, 500, 3500, and 7000 ppm for 18 months. The survival was not affected by treatment. No treatment-related clinical signs were observed. Mean body weights and body weight gain for treated mice of either sex were within 5% of the mean control values throughout the study period, although statistically significant differences occurred at several intervals in all treated groups. Since these changes did not exhibited time- or dose-related consistent trend towards increases or decreased in body weight, they were regarded as incidental. No treatment-related effects on food consumption or hematological parameters occurred at any time-point. Absolute and relative organ weights of treated groups were similar to that of controls at end of the study and there were no macroscopic findings or histopathological changes that could be attributed to treatment in any of the animals that died during the study period or that were sacrificed as scheduled per protocol.
No effect of toxicological significance or carcinogenic effects were demonstrated in mice after dietary administration of Imazamox at dietary levels up to and including 7000 ppm for 18 month. Thus, the NOEL was 7000 ppm which was equivalent to 1053 and 1348 mg/kg bw/day in males and females, respectively.

Dog – Chronic Toxicity

[redacted] **(1995b**): One-year dietary toxicity study with AC 299,263 in purebred Beagle dogs; BASF DocID ID-427-001

Imazamox (97.1% purity; batch AC 6935-63) was tested for its chronic toxicity potential in groups of 5 male and 5 female purebred Beagle dogs at dietary concentrations of 0, 1000, 10000, and 40000 ppm for a period of 1 year. No deaths occurred during the study period. There were no clinical signs of toxicity observed that were attributed to treatment and ophthalmological examinations did not reveal any treatment-related ocular change. Food consumption and feed efficiency values as well as body weight and body weight gain for treated and control animals were comparable. Hematology and urinalysis parameters evaluated at month 3 and 6 and at termination were also comparable between treatment groups. Although sporadic instances of statistically significant differences in clinical chemistry parameters were observed for treated and control animals, values for treated animals were considered to be within normal biological limits, were not consistently observed at different time intervals, and were not observed in both sexes. Thus, these differences were not attributed to the administration of the test substance. There were no treatment related effects in organ weights. Macroscopic and microscopic changes occurred sporadically among control and treated groups and were considered to be incidental findings and not treatment-related.

There were no significant toxicological findings in any of the animals of either sex at any dose levels. Therefore, the NOEL was 40000 ppm (equivalent to approximately 1165 mg/kg bw/day; 1174 mg/kg bw/day and 1156 mg/kg bw/day in males and females, respectively).

CA 5.6 Reproductive Toxicity

No new Reproductive toxicity studies were conducted since the last Annex I evaluation.

Studies already peer-reviewed and presented in the original Annex II Dossier (1997):
Imazamox did not affect reproductive performance in a 2-generation reproductive toxicity study, nor was there evidence of significant pre-or postnatal effects nor was there evidence of significant pre-or postnatal effects up to the highest dose tested, resulting in a NOAEL at 20000 ppm (1639 mg/kg bw/d) for parental/developmental and offspring toxicity.
Furthermore, no evidence of developmental toxicity or teratogenic effects was revealed for fetuses of either species in developmental toxicity tests conducted in rabbits and rats. Maternal toxicity in both rats and rabbits was manifested as reduced food consumption at 600 and 900 mg/kg bw/d) and reduced body weight gain at 900 mg/kg bw/d (rabbits) and decreased body weight, body weight gains and food consumption (rats). Respective maternal and fetal/developmental NOAELs for these studies were 300 and 900 mg/kg bw/d in rabbits and 500 and 1000 mg/kg bw/d in rats.

No new data was generated since the last submission. The available studies have been evaluated by European authorities and France as the Rapporteur member state (European Commission Peer Review Program). Though the studies were not conducted according to the current OECD guidelines (updated in 2001), they have been performed in compliance with the OECD guidelines, which were in place and standard at that time and are still considered to be acceptable and valid.
The 2- generation study was performed in 1995 according to the old OECD 416 guideline. The major deviations to the current OECD 416 comprise the following: no sperm parameters were assessed; no functional investigations of the F1 offspring were performed; no organ weights were reported, however histology was done on relevant reproductive organs as indicated below in chapter CA 5.6.1.
The developmental toxicity studies in rats and rabbits were performed in 1994 -1995 according to the OECD 414 guideline. The major deviation to the current OECD 414 guideline (updated in 2001) comprises that the treatment was done during organogenesis only (GD 6-15 in rats or GD 7-19 in rabbits).
However, for animal welfare reasons and in the absence of any specific effects on reproductive organs in the repeated dose studies with Imazamox (see chapters CA 5.3 and 5.5), and due to the lack of any reproduction toxicity in 2- generations in the rat, as well as in the absence of developmental toxicity or teratogenic effects in rats and rabbits at dose levels up to or exceeding the limit dose, it was agreed with the RMS that no additional data are required for Imazamox.

A summary of reproductive and developmental toxicity studies in rats and rabbits is given in Table 5.6-1.

Table 5.6-1: Summary of peer-reviewed reproductive and developmental toxicity data obtained with Imazamox

Study (strain)	Dose levels [mg/kg bw/d]	Findings	NOAEL [mg/kg bw/d]	References
2-generation study, rat (Sprague Dawley)	1000, 10000 and 20000 ppm	No adverse effects	Parental/reproduction /offspring toxicity: 1639 [20000 ppm]	[redacted] (1995) ID-430-001
Teratogenicity, rat (Sprague Dawley)	100, 500, and 1000	Decreased body weights, body weight gains and food consumption at 1000 mg/kg bw/d.	Maternal toxicity: 500 Fetal/developmental toxicity: 1000	[redacted] (1994) ID-432-001
Preliminary prenatal toxicity, rabbit (New Zealand White)	500, 750, 1000	Decreased food consumption and body weight during dosing period (GD7-19) at 1000 mg/kg bw/d; reduction of litter size and increased resorptions/litter at 1000 mg/kg bw/d	Maternal toxicity: 750 Fetal/developmental toxicity: 750	[redacted] (1995) ID-432-002
Teratogenicity, rabbit (New Zealand White)	300, 600, and 900	Decreased food consumption at 600 and 900 mg/kg bw/d	Maternal toxicity: 300 Fetal/developmental toxicity: 900	[redacted] (1995) ID-432-002

Based on the available studies the endpoints for reproductive toxicity were fixed in the European Commission Review Report for the active substance Imazamox (SANCO/4325/2000 –Final, 29 November 2002) as indicated below. In the absence of any effects on reproduction or developmental toxicity no classification is required for Imazamox:

Reproductive toxicity	
Target/critical effect-Reproduction:	None
Lowest relevant reproductive NOAEL/NOEL:	1639 mg/kg bw/d (20000 ppm)
Target/critical effect-Developmental toxicity:	Litter size reduction, increased resorptions at maternally toxic doses
Lowest relevant developmental NOAEL/NOEL:	900 mg/kg bw/d (rabbit)

For convenience of the reviewer, brief study summaries as extracted from the monograph are provided below.

CA 5.6.1 Generational studies

[redacted] **(1995):** A two generation reproduction study with AC 299,263 in rats; BASF DocID ID-430-001

Imazamox technical(98.2%-97.1% purity; batch AC 6935-63) was tested for its reproductive toxicity potential in groups of 60 (30/sex/generation) Sprague-Dawley rats when administered via diet at dietary concentrations of 0, 1000, 10,000 and 20,000 ppm through 2 generations. P1 and F1 generation rats were treated during a pre-mating period of 10 to 11 weeks, and treatment continued during both a 20-day mating period and post-mating period. Mated females continued to be treated during the ensuing gestation, lactation and post-weaning periods until sacrifice. Parental animals (P1, F1) were observed twice daily for mortality and unusual findings and each animal received a detailed physical examination weekly; vaginal smear samples were evaluated daily for parental females (P1, F1) to evaluate estrous cycling for a two week period prior to initiation of mating. Body weights and food consumption for the parental animals were recorded weekly during the pre-mating treatment periods and these parameters continued to be recorded weekly for males during the post-mating period until sacrifice. Body weights and food consumption were recorded for females at regular intervals during the gestation and lactation periods. Each parental generation produced a single litter and pups were weaned on lactation day 21. On lactation day 4, litters with greater than eight pups were culled to equalize sex distributions (four/sex) when possible; litters with fewer than eight pups at Day 4 were not adjusted. At sacrifice, parental animals were given a gross postmortem examination. Reproductive tissues (coagulating glands, prostate, seminal vesicles, testes with epididymes; cervix, ovaries, uterus, vagina) were evaluated histomorphologically for P1 and F1 control and high-dose animals and gross lesions were evaluated for all animals. The unselected F1 pups were sacrificed soon after the Day 28 weighing interval and evaluated for external irregularities; pups with external findings were also evaluated internally and abnormal tissues saved. Additionally, at Day 21 or soon thereafter, one pup/sex/litter/group for each litter interval was selected at random and given a detailed macroscopic evaluation and abnormal tissues were saved.
No treatment-related mortality occurred. Food consumption, body weight, body weight gain of the generations, sexes, or intervals/phases were considered to be not affected by treatment. Although P females in the 20000 ppm group exhibited mean weight gains that were similar to controls during the pre-mating period, F_1 females exhibited a statistically significant decrease (-11.3%) in mean weight gain over this period. Given the absence of similar decreases for P females or for P or F_1 males at 20000 ppm, this slight decrease in mean weight gain was not considered biologically significant. Treatment did not affect the reproductive performance (estrous cycle data, mating indices, pregnancy rates, male fertility indices, gestation indices and parturition indices). Treatment did not affect the mean litter size data (pre-cull– throughout the remainder of lactation), litter or pup survival indices, mean pup weights (at birth, during lactation, and on neonatal day 28), pup sex distribution, pup developmental landmarks (pinna detachment, upper incisor eruption, eye opening, fur growth, mean day to completion for vaginal opening and preputial separation for the selected F1 pups) or the number of dead pups at birth or during the 21-day lactation period. The mean pup live birth indices for the treated groups were comparable to controls for both litter intervals, except for the F1 litter high dose group, in which the mean number of live pups at birth was significantly lower than control values.

However, this decrease was within the range of the recent historical control data of the laboratory and the mean number of live pups in the control group during the same litter interval reached the upper range of this historical data. Therefore, there was no clear evidence of a treatment-related effect, the observed difference being most likely related to the low number of dead pups recovered in the control group. No gross macroscopic findings were observed for either parental or pup generations. Treatment did not affect the mean number of uterine implantation scars for each litter interval or mean total number of respective pups born. There were no microscopic compound-related changes observed.

Based on the results of this study, no adverse effects were indicated from the evaluation of parental or neonatal parameters and no treatment-related effects on reproductive performance were noted at dietary levels up to and including 10000 ppm. At 20000 ppm, reproductive performance was not affected by test substance and the only suggestion of parental toxicity was a slight reduction in mean body weight gain in the F1 females over the entire 11 week pre-mating treatment period, which was not seen in the P1 females or in the P1 and F1 males. As no clear dose-response was noted, this decrease is considered as incidental. The NOEL for reproductive toxicity was 20000 ppm, i.e. approximately 1639 mg/kg bw/day.

CA 5.6.2 Developmental toxicity studies

Rat

[redacted] **(1994):** An oral developmental toxicity (embryo-fetal toxicity/teratogenicity) study with AC 299,263 in rats; BASF DocID ID-432-001

Imazamox technical (AC 299263 technical 97.1 % purity; batch AC 6935-63), was tested for its developmental toxicity potential in groups of 25 presumed pregnant Sprague-Dawley rats when administered via gavage at doses of 0, 100, 500, and 1000 mg/kg bw/day in an aqueous suspension of 0.5% w/v carboxymethyl-cellulose (CMC), by oral gavage once daily on days 6 through 15 of gestation. No mortalities, abortions, or premature deliveries occurred during the study, and there were no clinical signs observed that were attributed to treatment. Absolute and relative feed consumption values for the entire dosage and post-dosage periods were reduced in the 1000 mg/kg dosage group; however, none of these reductions were statistically significant. The body weight gain value for the entire dosage period (day 6 to 16 of gestation) tended also to be reduced in the high dose group and this was related to an early significantly reduced body weight gain on day 6 to 12 of gestation. Body weight gains were comparable among all groups for the remainder of the dosing period (day 12 to 16 of gestation) and post-dosing period. Treatment did not affect gravid uterine weights neither were gross lesions identified at necropsy on day 20 of presumed gestation. Pregnancy occurred in 24 or 25 of the 25 presumed pregnant females of each group. No Caesarean-sectioning or litter parameters were affected by the test substance at any dosage level. Litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent male fetuses were comparable among the four dosage groups. Similarly, treatment did not induce fetal gross external, soft tissue, or skeletal malformations or variations. The only skeletal malformations occurred in the high dose group (short, borad, bent ribs) and were not considered to be treatment- related because they were seen in only one fetus.

Based on decreased body weight, body weight gains and absolute and relative feed consumption in the 1000 mg/kg dose group, the maternal NOAEL for this study was 500 mg/kg bw/day. However, this is debatable since the changes were very slight and not statistically significant. The fetal/developmental toxicity NOAEL was 1000 mg/kg bw/day, the highest dose tested.

Rabbit

[redacted] **(1995):** An oral developmental toxicity (embryo-fetal toxicity/teratogenicity) pilot study with AC 299,263 in rabbits; BASF DocID ID-432-002

Pilot Study (Appendix G in Study Report)

The purpose of this study was to determine the dosage levels to be used in the definitive developmental toxicity study conducted with Imazamox technical in rabbits. Three groups of 8 artificially inseminated New Zealand White rabbits were administered Imazamox technical (97.1 % purity; batch AC 6935-63) in an aqueous suspension of 0.5% w/v carboxymethylcellulose (CMC) by oral gavage at doses of 500, 750 and 1000 mg/kg bw/day on days 7 through 19 of gestation. The fourth group of 8 rabbits received CMC as the vehicle control.

One high dose group doe was found dead on day 22 of gestation. This death was considered an effect of the test material because it occurred in the high dosage group, and this doe exhibited test substance-related findings prior to death, such as abnormal feces (days 15 through 21 of gestation) and weight loss and reduced food consumption (from day 7 of gestation). Postmortem examination revealed ulcerations in the gallbladder, slightly hemorrhagic lungs, a gastric trichobezoar, parovarian cysts, perivaginal red substance, and a late resorption found in the vaginal canal and this doe had a litter of three early resorptions and five late resorptions. There were no other mortalities observed, and there were no abortions or premature deliveries. The only clinical sign observed was soft or liquid feces noted for 2 of 8 does dosed at 1000 mg/kg. Although commonly observed in rabbits, this finding was considered a possible effect of the test substance since it occurred in the high dosage group only. Absolute and relative feed consumption values and mean body weights and body weight gains were reduced in the 1000 mg/kg dosage group during the entire dosing period. However, during the postdosage period, body weight gains and feed consumption were comparable to those of controls. Gravid uterine weights were also reduced in the 1000 mg/kg dose group and this was related to a smaller live litter size. Gross necropsy findings were noted only for the doe in the 1000 mg/kg dosage group that was found dead (see above).

Absolute and group mean live litter size was reduced, and the percent resorbed conceptuses per litter was increased in the 1000 mg/kg dosage group. These findings were considered possibly treatment-related because they occurred at the highest dosage tested. Litter averages for corpora lutea, implantations, fetal body weights and percent male fetuses were comparable among the four dosage groups. Similarly, there were no gross external fetal malformations observed that were caused by treatment of the does with Imazamox at dosages as high as 1000 mg/kg bw/day.

Based on maternal toxicity (reduced body weight and body weight gains and feed consumption) as well as embryo-fetal mortality (increased resorptions) at 1000 mg/kg bw/day, dosages of 300, 600 and 900 mg/kg bw/day were selected for use in the definitive study.

[redacted] **(1995):** An oral developmental toxicity (embryo-fetal toxicity/teratogenicity) definitive study with AC 299,263 in rabbits; BASF DocID ID-432-002

Definitive Study

Groups of 20 presumed pregnant New Zealand White (Hra:(NZW)SPF) rabbits were administered orally (via stomach tube) once daily 0, 300, 600 and 900 mg/kg bw/day of the test substance (AC 299,263 technical; 97.1 % purity; batch AC 6935-63) in an aqueous suspension of 0.5% w/v carboxymethylcellulose (CMC) on day 7 to day 19 of presumed gestation.

No mortalities or abortions occurred during the study. One doe in the 900 mg/kg group prematurely delivered on day 29 of gestation, a litter of 10 conceptuses among which 8 were live pups that appeared normal for their developmental age and 2 were presumed cannibalized. This premature delivery was considered a possible effect of the test substance because this doe exhibited reduced body weight and food consumption after day 11 of gestation and abnormal feces on day 21 through day 29 gestation. There were no clinical signs observed that were considered related to test substance intake.

Treatment dose-dependently reduced absolute and relative feed consumption values for the entire dosage period in the 600 and 900 mg/kg dosage groups (12-13% and 15-16% for mid and high dose group, respectively). A biologically significant reduction in body weight gain was noted during the dosage period (19%) and post-dosage period (21%) at 900 mg/kg bw/day.

Gravid uterine weights and day 29 body weights corrected for gravid uterine weights were not affected in any dose group. Exclusion of the values for the 6 does with unilateral pregnancies, i.e. 4 control does and 2 mid-dose does, did not affect the results. There were 20, 18, 15 and 19 pregnant does Caesarean-sectioned on day 29 gestation in the control, low, mid and high dose groups respectively. Pregnancy in the mid dose group was significantly reduced. This effect was considered as incidental since it was not dose dependent. Gross necropsy findings for the does were considered unrelated to test substance intake. Litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weight and percent male fetuses were comparable among the 4 dosage groups. Similarly, there were no fetal gross external, soft tissue or skeletal malformations or variations observed that were considered effects of the test substance.

Based on reduced maternal feed consumption values in the 600 mg/kg bw/day dose group, the maternal NOAEL for the test substance was 300 mg/kg bw/day. The developmental NOAEL was 900 mg/kg bw/day, the highest dose tested for which there were no adverse effects on embryo-fetal viability, body weight or morphology.

CA 5.7 Neurotoxicity Studies

Imazamox belongs to the chemical class of imidazolines. This class of compounds is not known to induce neurotoxicity in animals. Accordingly, there was no evidence of neurotoxicity in any of the studies conducted with Imazamox. Therefore, no further studies regarding neurotoxicity were performed.

CA 5.7.1 Neurotoxicity studies in rodents

In the absence of neurotoxicity in any of the studies conducted with Imazamox no further studies were performed or considered to be necessary. Imazamox is not neurotoxic.

CA 5.7.2 Delayed polyneuropathy studies

In the absence of neurotoxicity in any of the studies conducted with Imazamox no further studies were performed or considered to be necessary. Imazamox is not neurotoxic.

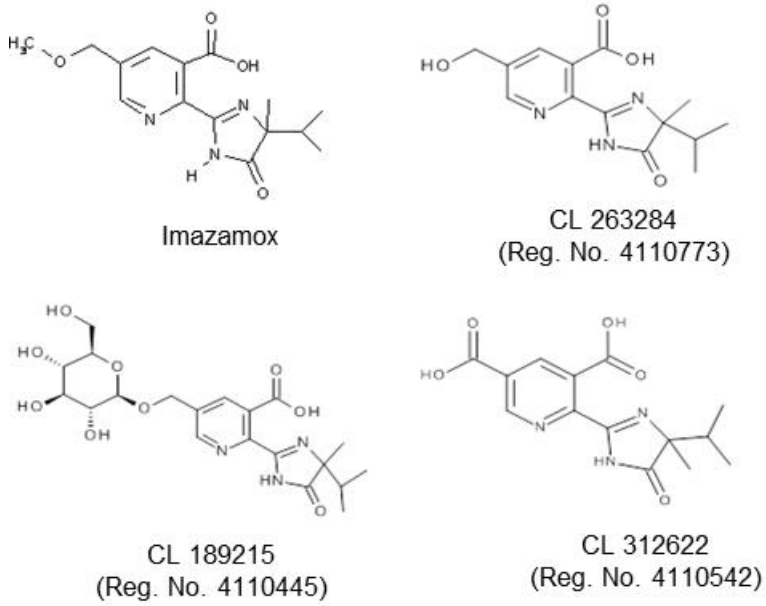
CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Imazamox structurally related plant metabolites are the hydroxymethyl metabolite (CL 263284), its glucoside (CL 189215) and the dicarboxylic acid metabolite (CL 312622) (see chapter CA 6.2.1 , and Doc N1). CL 312622 is also a groundwater metabolite (see respective chapters CP 9.2.4.1 (related to both Formulations), as well as Doc N1). The metabolites CL 312622 and CL 263284 have been demonstrated to be formed in rats after administration of Imazamox but only in in minor amounts (see also chapter CA 5.1) and therefore are not regarded as covered by toxicological testing of the parent compound.

The chemical structures of Imazamox and its metabolites are shown in Figure 5.8.1-1.

Figure 5.8.1-1: Chemical structures of Imazamox and its plant and groundwater metabolites



Studies on metabolites presented in the original Annex II Dossier (1997):
Several studies (acute oral toxicity and Ames test) are available for the three major plant metabolites CL 263284, CL 189215, and CL 312622. Additionally, an acute oral toxicity study in the rat was performed on the soil metabolite CL 354825. These studies have been evaluated by the Rapporteur Member State France and EU authorities during the last submission and are considered to be acceptable.

New studies (not yet peer-reviewed):
With regard to the plant metabolites a relevance assessment has been performed according to the TTC (Threshold of Toxicological Concern) concept, which is the approach as described in the 'Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment' (EFSA Journal 2012; 10(07): 2799). It is laid down in this EFSA Scientific Opinion that 'the TTC approach is the most appropriate tool to evaluate the toxicological relevance of metabolites associated with chronic dietary exposure. The TTC values, established for genotoxic and toxic compounds based on the Cramer et al., (1978; Food Cosmet. Toxicol. 16**,** 255–*276*) scheme, were considered sufficiently conservative'.
In general, the Imazamox plant metabolites show no known chemical structural alerts. According to the presented assessments (see chapter CA 6.9) exposure of the Imazamox metabolites CL 263284, CL 189215 or CL 312622 remains well below the acceptable long-term exposure threshold value of 1.5 µg/kg bw/d for Cramer Class III compounds.
However, all three main plant metabolites exceeded the genotoxicity threshold value of 0.0025 µg/kg bw/d. Thus, appropriate genotoxicity testing *in vitro* was performed. In addition to the previously performed Ames test an *in vitro* micronucleus test was conducted with each of the three plant metabolites according to the recommendations in the EFSA Scientific opinion and in agreement with the RMS. In the case of a positive result in the *in vitro* micronucleus test, a higher tier *in vivo* micronucleus test was conducted in mice to clarify the results seen in the *in vitro* system.

Moreover, for the major plant metabolite CL 263284 also a 28-day toxicity study in rats was performed as a specific request by the RMS, since the metabolite is part of the residue definition (Imazamox + CL 263284 in parent equivalents) and no information of the metabolite's toxicological profile after repeated exposure was available.
Treatment with CL 263284 up to the limit dose for 28-days in rats resulted in decreased body weight and body weight change at the highest dose tested (12000 ppm, 1004 mg/kg bw/d) in males as the only treatment-related effect in this study. Therefore, CL 263284 is considered to show similar toxicity when compared to Imazamox, with effects on body weight as the only observation in the repeated dose studies with the active ingredient (for comparison see also chapter CA 5.4).

Moreover, the metabolite CL 312622 has also been found in the groundwater (for further details see respective chapters CP 9.2.4.1/2) at concentrations exceeding 0.1µg/L for most scenarios. Therefore, a genotoxicity screening had to be performed according to the Sanco Guidance Document on Relevant Metabolites in Groundwater (Sanco/221/2000 –rev.10- final 25 February 2003). In addition to the Ames test and the *in vitro* micronucleus test a gene mutation test with mammalian cells was conducted to fulfill the criteria as outlined in this Guidance Document. Thus, the HPRT test was performed with CL 312622 to complete the package of *in vitro* genotoxicity studies for groundwater metabolites.

For few scenarios the groundwater concentration was found to be greater than 0.75 µg/L but below 10 µg/L (see BAS 831 00 H dossier, chapter CP 9.2.4.1/2). The CL 312622 metabolite shows strong structural similarity with the plant metabolite CL 263284 (oxidation reaction), for which a specific 28-day study in rats has been performed (see chemical structures in Figure 5.8.1-1). Results of this repeated dose study in rats resulted in toxicity comparable to the parent molecule and apart from effects on body weight and body weight change at the highest dose tested (limit dose) no other treatment-related effects have been observed. Therefore, for exposure assessments the ADI of Imazamox is considered to be applicable to both metabolites, CL 263284 and CL 312622.

Overall, the absence of genotoxic properties has been shown for all three metabolites under the experimental condictions of the respective *in vitro* and *in vivo* tests performed.
Table 5.8.1-1 provides an overview of the results from the acute oral LD_{50}, and the genotoxicity studies, and sub-acute oral repeated dose study conducted with metabolites of Imazamox. Newly performed studies (not yet peer-reviewed) are marked in table with an asterisk(*).

Table 5.8.1-1: Summary of toxicity studies with Imazamox metabolites

Study	Metabolite	Species (Strain)/Test system	Findings	References
Acute oral LD_{50}	CL 312622 (Reg. No. 4110542)	Rat (Sprague-Dawley)	> 5000 mg/kg bw (males and females)	[redacted] (1995b) DocID ID-470-001
Acute oral LD_{50}	CL 354825 (Reg. No. 4110603)	Rat (Sprague-Dawley)	2313 mg/kg bw/d (males); 2121 mg/kg bw/d (females)	[redacted] (1995c) Doc ID-470-006
Acute oral LD_{50}	CL 263284 (Reg. No. 4110773)	Mouse (CD-1)	> 5000 mg/kg bw (males and females)	[redacted] (1995) DocID ID-470-007
Microbial Mutagenicity Assay (Ames)	CL 312622 (Reg. No. 4110542)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli* WP2 *uvrA*-	+S9[a]:Negative -S9[b]:Negative (at dose levels of 312.5, 625, 1250, 2500 and 5000 µg/plate)	Mulligan, E. (1995a) DocID ID-470-005
Microbial Mutagenicity Assay (Ames)	CL 263284 (Reg. No. 4110773)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli* WP2 *uvrA*-	+S9[a]:Negative -S9[b]:Negative (at dose levels of 312.5, 625, 1250, 2500 and 5000 µg/plate)	Mulligan, E. (1995b) DocID ID-470-003
Microbial Mutagenicity Assay (Ames)	CL 189215 (Reg. No. 4110445)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli* WP2 *uvrA*-	+S9[a]:Negative -S9[b]:Negative (at dose levels of 312.5, 625, 1250, 2500 and 5000 µg/plate)	Mulligan, E. (1995c) DocID ID-470-004

Study	Metabolite	Species (Strain)/Test system	Findings	References
In vitro /Micronucleus test	CL 312622 (Reg. No. 4110542)	Chinese hamster V79 cells	+S9[a]:Negative -S9[b]:Negative (at dose levels/concentrations of 4.5, 8.9, 17.9, 35.8, 71.6, 143.2, 286.4, 572.8P, 1145.5, and 2291.0 µg/mL)	Bohnenberger, S. (2013a) DocID 2013/1113583 *
In vitro /Micronucleus test	CL 189215 (Reg. No. 4110445)	Chinese hamster V79 cells	+S9[a]:Negative -S9[b]:Negative (at dose levels/concentrations of 11.4, 22.8, 45.5, 91.1, 182.2, 364.4, 728.8, 1457.5, 2915.0, and 5830.0 µg/mL)	Bohnenberger, S. (2013b) DocID 2013/1113582 *
In vitro /Micronucleus test	CL 263284 (Reg. No. 4110773)	Chinese hamster V79 cells	+S9[a]:Positive -S9[b]:Negative (at dose levels/concentrations of 5.0, 10.0, 20.1, 40.2, 80.3, 100.0, 160.6, 200.0, 300.0, 321.3, 400.0, 500.0, 600.0, 642.5, 800.0, 1200.0, 1285.0, 1400.0, 1600.0, 2000.0, and 2570.0 µg/mL)	Bohnenberger, S. (2013c) DocID 2013/1113581; First Amendment: Bohnenberger, S. (2013d) DocID 2013/1165894 *
Gene mutation assay (HPRT)	CL 312622 (Reg. No. 4110542)	Chinese hamster ovary CHO cells	+S9[a]:Negative -S9[b]:Negative (at dose levels/concentrations of 218.8, 437.5, 875.0, 1750.0 and 3500.0 µg/mL and 500.0, 1000.0 2000.0 and 3500.0 µg/mL (-S9 only))	Kapp, M.J. and Landsiedel, R. (2013) DocID 2013/1235040 *
In vivo /Micronucleus test	CL 263284 (Reg. No. 4110773)	NMRI mice (bone marrow)	Negative (at dose levels of 500, 1000 or 2000 mg/kg bw); bioavailability of test substance was confirmed in the plasma	[redacted] (2013) DocID 2013/1235041 *
28-day toxicity study	CL 263284 (Reg. No. 4110773)	Rat (Crl:WI(Han))	males: 4000 ppm based on body weight effects (333 mg/kg bw/d) females: 12000 ppm (1028 mg/kg bw/d) Dose levels: 1200, 4000 and 12000 ppm	[redacted] (2013) DocID 2013/1235042 *

[a] with metabolic activation; [b] without metabolic activation
*new studies

Detailed study summaries of the newly performed toxicological studies (marked with an asterisk (*) above) are presented below. For convenience of the reader a short study summary of the studies already EU-peer reviewed as extracted from the Draft Monograph (FR-RMS, 1999) are also presented.

Toxicological testing of CL 354825 (Reg.No. 4110603)

[redacted] **(1995c):** Oral LD_{50} study in Albino Rats with AC 354,825; BASF Doc ID-470-006

Groups of 5 male and 5 female Sprague-Dawley rats (Crl CD (SD) BR strain) were administered, by gavage, a single dose of 750, 1500, and 3000 mg/kg bw of CL 354825 (90% purity; batch AC 9918-101) prepared as a 7.5%; 15%, and 30% w/v dispersion in distilled water (dose volume of 10 ml/kg bw) and were observed for 14 days. Mortality occurred from postdosing day 2 to day 6 for both sexes (4 /5 males and 3/5 females) at the 3000 mg/kg level and for females (2/5) at the 1500 mg/kg level.
Clinical signs of toxicity were seen in both sexes at 3000 mg/kg level (from hour 1 through day 5 postdosing), in females at the 1500 mg/kg dose level (from day 3 to day 4 postdosing), and at the 750 mg/kg dose level (hour 2 through day 3 postdosing). Clinical findings comprised decreased activity, discolored urine, tremors, chromodacryorrhea and brown material around the nose for males, ptosis, diuresis, ataxia and prostration. All surviving animals gained weight during the study. No gross pathological changes were observed for surviving animals at study termination, gross pathological changes in decedents consisted of test material-filled stomachs and intestinal tracts.
Thus, the oral LD_{50} of CL 354825 was 2313 mg/kg bw for males, 2121 mg/kg for females, and 2274 [1.694 - 3,051] mg/kg for combined sexes in rats.

Toxicological testing of CL 312622 (Reg.No. 4110542)

[redacted] **(1995b):** Oral LD50 study in Albino Rats with AC 312622;
BASF DocID-470-001

The test substance, AC 312622 (CL 312622, 97.2% purity; batch AC 9416-75C), was tested for its acute oral toxicity in 5 male and 5 female Sprague-Dawley rats when administered a single dose of 5000 mg/kg bw via gavage prepared as a 50% w/v dispersion in corn oil. Two deaths (1 male and 1 female) occurred and clinical signs of toxicity were observed during the first hour following dosing (salivation in 9 rats and writhing in 2 rats) which resolved by 2 hours postdosing. All surviving animals gained weight during the study period, and there were no gross pathological changes at necropsy that were attributed to administration of the test material.

Thus, the oral LD50 of CL 312622 was greater than 5000 mg/kg bw for both sexes in SD rats. According to Regulation (EC) 1272/2008 [CLP] the test substance, CL 312622, is not to be classified for acute oral toxicity.

Mulligan A. (1995a): Microbial mutagenicity plate incorporation assay of CL 312,622; BASF DocID ID-470-005

Bacterial reverse mutation tests were performed on CL 312622 (Reg. No. 4110542; 97.2% purity; batch AC 9416-75C) in test strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) and E. coli (WP2 *uvrA*). Experiments were carried out with and without metabolic activation (S9 mix) at dose levels of 312.5, 625, 1250, 2500, and 5000 µg/plate in dimethyl sulfoxide (DMSO), or with DMSO alone as the negative control for three replicates per dose. CL 312622 did not induce toxicity and no insolubility was observed at any dose level. The results obtained from both trials showed no significant or dose-related increases in revertant frequencies in the presence or absence of metabolic activation. The positive controls (2.5 µg/plate of 2-Aminoanthracene with metabolic activation administered to TA98; TA100; TA1535; TA1537 and TA1538 and 10 µg/plate administered to WP2 *uvrA*-; 10 µg/plate of N-methyl-N'-nitro-N-nitrosoguanidine without metabolic activation administered to WP2 *uvrA*-; TA100 and TA1535; 20 µg/plate 2-Nitrofluorene without metabolic activation administered to TA98 and TA1538; and 50 µg/plate 9-Aminoacridine without metabolic activation administered to TA1537) elicited the expected increase in revertant frequencies indicating that the test system was capable of detecting base-pair and frameshift mutations and that the metabolic activation system was functioning properly. The vehicle control revertants colonies were within the expected acceptable ranges.

In conclusion, CL 312622 (Reg. No. 4110542) did not show any genotoxic potential in 6 tester strains of bacteria at concentrations up to and including 5000 µg/plate in the presence and absence of S-9 metabolic activation.

Report:	CA 5.8.1/1 Bohnenberger S., 2013a In vitro micronucleus test in Chinese hamster V79 cells with Reg.No. 4110542 (metabolite of BAS 720 H, Imazamox) 2013/1113583
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The test item, Reg. No. 4110542 (CL312622; metabolite of BAS 720 H, Imazamox) was assessed for its potential to induce micronuclei in V79 cells of the Chinese hamster *in vitro* in 2 independent experiments in the absence and presence of metabolic activation by S9 mix (Experiment I, 4 h-exposure with or without S9; Experiment II, 24 h-exposure without S9 and 4 h-exposure with S9). With and without metabolic activation, no cytotoxicity was observed up to the highest applied concentration (2291.0 µg/mL). With and without metabolic activation, no mutagenicity was observed. Appropriate mutagens were used as positive controls. They induced statistically significant increases in the percentage of micronucleated cells. Under the experimental conditions reported, the test item Reg. No. 4110542 (CL312622; metabolite of BAS 720 H, Imazamox) with or without metabolic activation did not induce micronuclei in V79 cells (Chinese hamster cell line) *in vitro*.

In conclusion, Reg. No. 4110542 (CL312622; metabolite of BAS 720 H, Imazamox) is considered to be non-mutagenic in this in vitro test system.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Imazamox , Reg.No. 4110542 (CL312622; metabolite of BAS 720 H, Imazamox)
Description:	Solid, white
Lot/Batch #:	L82-7
Purity:	88.4% (tolerance ±1%)
Stability of test compound:	The stability of the test item in DMSO was verified (see BASF study number 01Y0732/12Y03). Expiration date: 01-Nov-2013. Homogeneity of the preparations was ensured by mixing.
Solvent used:	Dimethyl sulfoxide; DMSO

2. Control Materials:

Negative and solvent control: DMSO (0.5% (v/v) in culture medium)

Positive control:

Without metabolic activation:

Mytomycin C (MMC) 0.3 µg/mL in deionized water

Griseofulvin 9 µg/mL in DMSO

With metabolic activation (S9):

Cyclophosphamide (CPA) 20 µg/mL in saline

The stability of the positive control substances in solution was proven by the mutagenic response in the expected range.

3. Activation:

S9 was produced from livers of 8–12 weeks old male Wistar rats. The rats received 80 mg/kg bw phenobarbital i.p. and 80 mg/kg bw ß-naphthoflavone p.o. daily on three consecutive days and were sacrificed 24 hours after the last administration. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g and stored at −80 °C Small numbers of the ampoules were kept at −20 °C for up to one week. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

The protein concentration was 27.9 mg/mL for Experiment I and 25.3 mg/mL for Experiment II.

The S9-mix was prepared immediately before use and had the following composition:

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures.

Component	*Concentration*
Sodium ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	Not specified

During the experiment the S9 mix was stored on ice.

4. Test organisms: Chinese hamster V79 cells

For seeding and treatment of the cell cultures the culture medium was MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM) and 10 % (v/v) fetal bovine serum (FBS). Additionally, the medium was supplemented with penicillin/streptomycin (100 U/mL/100 µg/mL). All cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO_2 (98.5% air).

5. Test concentrations:

The highest applied concentration (2291.0 µg/mL; approx. 7.5 mM) was chosen with regard to the solubility properties of the test item and with respect to the OECD Guideline No. 487.

The concentrations of Reg. No. 4110542 (Metabolite of BAS 720 H, Imazamox) used in the Micronucleus test in V79 cells are depicted in the following table.

Exposure period	**Experiment**	**Concentration in µg/mL**
Without S9 mix		
4 h	I	4.5, 8.9, 17.9, 35.8, 71.6, 143.2, **286.4**, **572.8^{P}**, **1145.5^{P}**, **2291.0^{P}**
24 h	II	4.5, 8.9, 17.9, 35.8, **71.6**, **143.2**, **286.4^{P}**, **572.8^{P}**, 1145.5^{P}, 2291.0^{P}
With S9 mix		
4 h	I	4.5, 8.9, 17.9, 35.8, 71.6, 143.2, **286.4**, **572.8^{P}**, **1145.5^{P}**, 2291.0^{P}
4 h	II	71.6, **143.2**, **286.4**, **572.8^{P}**, 1145.5^{P}, 2291.0^{P}

Evaluated experimental points are shown in bold characters
^{P} Precipitation occurred microscopically at the end of treatment

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 16-Jan-2013 to 20-Mar-2013

2. Micronucleus test:

Seeding: The cells were seeded into Quadriperm dishes that contained microscopic slides. Into each chamber 1.0–1.5 × 10^5 cells were seeded with regard to the preparation time.

Treatment: 4 h-exposure:

The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added. Concurrent solvent and positive controls were performed. After 4 h the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then, the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time.

24 h-exposure:

The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparation of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25% (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Micronuclei Analysis: Evaluation of the cultures was performed manually using NIKON microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The criteria for the evaluation of micronuclei are described in Countryman and Heddle (1976), *Mutation Research*, 41, 321–332. The micronuclei were briefly stained in the same way as the main nucleus. The area of the micronucleus did not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2–8 cells were analyzed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity: Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3–4 cells (c4), and 5–8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below [Kirsch-Volders *et al.* (2003) *Mutation Research*, 540, 153–163; Kirsch-Volders *et al.* (2004) *Mutation Research*, 564, 97–100]). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

The proliferation index (*PI*) is

$$PI = \frac{(c1 * 1) + (c2 * 2) + (c4 * 3) + (c8 * 4)}{c1 + c2 + c4 + c8}$$

for the number of clones with *x* cells (*cx* with *x*: 1, 2, 4, or 8).

4. Statistics:

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the Chi square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item can be classified as mutagenic if:

- the number of micronucleated cells is not in the range of the historical control data (see table: historical laboratory control data in RESULTS AND DISCUSSION) and
- either a concentration-related increase in three test groups or a statistically significant increase in the number of micronucleated cells is observed.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data (see table: historical laboratory control data in RESULTS AND DISCUSSION) and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance under storage conditions throughout the study period was proven. The stability in solvent has been verified analytically (see BASF study number 01Y0732/12Y03).

B. SOLUBILITY

In Experiment I, visible precipitation of the test item in the culture medium was observed at 572.8 µg/mL and above in the absence and presence of S9 mix. In addition, precipitation occurred in Experiment II, at 286.4 µg/mL and above in the absence of S9 mix and at 572.8 µg/mL in the presence of S9 mix.

C. CYTOTOXICITY

In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.

D. MICRONUCLEUS ASSAY

In both experiments in the absence and presence of S9 mix no mutagenicity was observed. The rates of micronucleated cells after treatment with the test item (0.20–1.50%) were close to the rates of the solvent control values (0.35−1.65%) and within the range of the laboratory historical solvent control data (see Table **5.8.1-3**).
Mitomycin C (0.3 µg/mL), Griseofulvin (9.0 µg/mL) or CPA (20.0 µg/mL) were used as concurrent positive controls and showed a distinct increase in the percentage of micronucleated cells.

The results are summarized in the following table.

Table 5.8.1-2: Summary of results of the micronucleus test with Reg. No. 4110542

Experiment	Test item concentration in µg/mL	Proliferation index	Micronucleated cells in %
Exposure period 4 h without S9 mix			
I	Solvent control[1]	2.71	0.35
	Positive control[2]	2.22	7.70[S]
	286.4	2.75	0.75
	572.8[P]	2.77	0.70
	1145.5[P]	2.85	0.70
	2291.0 [P]	2.83	n.e.
Exposure period 24 h without S9 mix			
II	Solvent control[1]	3.01	0.90
	Positive control[3]	2.47	12.70[S]
	71.6	2.98	0.90
	143.2	3.03	1.20
	286.4[P]	2.96	0.70
	2291.0[P]	2.57	n.e.
Exposure period 4 h with S9 mix			
I	Solvent control[1]	2.04	0.35
	Positive control[4]	1.51	3.10[S]
	286.4	1.84	0.45
	572.8[P]	1.86	0.50
	1145.5[P]	2.04	0.20
	2291.0 [P]	1.96	n.e.
II	Solvent control[1]	1.73	1.65
	Positive control[4]	1.48	12.35[S]
	143.2	1.75	1.40
	286.4	1.79	1.50
	572.8[P]	1.91	0.95
	2291.0[P]	2.05	n.e.

* The number of micronucleated cells was determined in a sample of 2000 cells
[S] Number of micronucleated cells statistically significantly higher than corresponding control values
[P] Precipitation occurred microscopically at the end of treatment
[1] Deionized water 10.0% (v/v)
[2] Mitomycin C 0.3 µg/mL
[3] Griseofulvin 8.0 µg/mL
[4] Cyclophosphamide 20.0 µg/mL
n.e. not evaluated

A summary of the historical controls is given in the following table.

Table 5.8.1-3: Historical laboratory control data – Percentage of micronucleated cells in Chinese hamster V79 cell cultures (October 2006 to August 2011)

	No. of studies	Range	Mean	Calc. Range[1]
Without S9 mix: preparation interval 24h, treatment 4 h				
Solvent control				
Aqueous sol.[2]	19	0.35–1.50	0.67	0.41–0.94
Organic solv.[3]	28	0.15–1.45	0.63	0.31–0.95
Total	47	0.15–1.50	0.65	0.35–0.94
Positive control				
Mitomycin C 0.03–0.1 µg/mL	44	0.85–18.15	7.65	3.60–11.70
Without S9 mix: preparation interval 24 h, treatment 24 h				
Solvent control				
Aqueous sol.[2]	15	0.15–1.50	0.62	0.23–1.02
Organic solv.[3]	140	0.05–1.50	0.61	0.30–0.91
Total	155	0.05–1.50	0.61	0.29–0.92
Positive control				
Mitomycin C 0.03–0.1 µg/mL	109	2.10–53.40	13.09	5.84–20.33
Griseofulvin 6.3–25.0 µg/mL	113	2.50–31.70	10.63	4.40–16.87
With S9 mix: preparation interval 24 h, treatment 4 h				
Solvent control				
Aqueous sol.[2]	33	0.15–1.70	0.79	0.46–1.12
Organic solv.[3]	176	0.05–1.60	0.7/8	0.44–1.13
Total	209	0.05–1.70	0.78	0.44–1.13
Positive control				
Cyclophosphamide 2.5–25.0 µg/mL	211	0.90–38.30	8.54	3.67–13.42

[1] Mean ± standard deviation
[2] Aqueous solvents: deionised water (10 % v/v), 0.9 % (w/v) saline, and culture medium MEM
[3] Organic solvents: dimethyl sulfoxide, acetone, ethanol, and tetrahydrofurane (0.5 % v/v)

III. CONCLUSION

Under the experimental conditions of this study Reg.No. 4110542 (CL 312622; metabolite of BAS 720 H, Imazamox) was judged to be negative for inducting micronuclei in V79 cells (Chinese hamster cell line) in the absence and presence of metabolic activation *in vitro*. **Thus, Reg.No. 4110542 (CL 312622) is considered to be non-mutagenic in this *in vitro* test system.**

Report:	CA 5.8.1/2 Kapp M.-D.,Landsiedel R., 2013a Reg.No. 4110542 (metabolite of BAS 720 H, Imazamox) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2013/1235040
Guidelines:	OECD 476, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The test item Reg.No. 4110542 (or CL 312622, metabolite of BAS 720 H, Imazamox) was assessed for its potential to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro* in two independent experiments in the absence and presence of metabolic activation by S9 mix (Experiment I, 4 h-exposure with or without S9; Experiment II, 24 h-exposure without S9 and 4 h-exposure with S9). The vehicle controls gave mutant frequencies within the range expected for the CHO cell line. Both positive control substances, EMS and DMBA, led to the expected increase in the frequencies of forward mutations. With and without metabolic activation, no cytotoxicity was observed up to the highest applied concentration (500.0 µg/mL).

Under the test conditions the test item Reg.No. 4110542 (metabolite of BAS 720 H, Imazamox) is not mutagenic in the HPRT locus assay under *in vitro* conditions in CHO cells in the absence and presence of metabolic activation.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg.No. 4110542 (Metabolite of BAS 720 H, Imazamox) or CL 312622
Description:	Solid, white
Lot/Batch #:	L82-7
Purity:	88.4% (tolerance ±1%)
Stability of test compound:	The stability of the test substance under storage conditions was guaranteed until 01 Nov 2013. Homogeneity of the preparations was ensured by mixing.
Solvent used:	dimethyl sulfoxide, DMSO

2. Control Materials:

Negative and solvent control: DMSO (1% (v/v) in cell culture medium)

Positive control: Without metabolic activation:

Ethyl methanesulfonate (EMS) 300 µg/mL in Ham's F12 medium without FCS

With metabolic activation (S9):

7,12-Dimethylbenz[a]anthracene (DMBA) 1.25 µg/mL in DMSO

The stability of EMS and DMBA is well-defined under the selected culture conditions since both positive control substances are well-established reference mutagens.

3. Activation:

S9 was produced from livers of male Wistar rats. The rats received 80 mg/kg bw phenobarbital i.p. and ß-naphthoflavone p.o. daily on three consecutive days and were sacrificed 24 hours after the last administration. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g and stored at −80 °C.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sphosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	Not specified

The phosphate buffer was prepared by mixing a Na_2HPO_4 solution with a NaH_2PO_4 solution in a ratio of about 4:1.

During the experiment the S9 mix was stored on ice.

4. Test organisms:

Chinese hamster ovary (CHO) cells

All media were supplemented with 1% penicillin/streptomycin and 1% amphotericine B. For seeding and treatment (without S9 mix) of the cell cultures the culture medium was Ham's F12 medium containing stable glutamine and hypoxanthine supplemented with 10% fetal calf serum (FCS). Treatment medium with S9 mix did not contain FCS. The pretreatment medium ("HAT" medium) was Ham's F12 medium supplemented with:

Component	Concentration (mg/mL)
Hypoxanthine	13.6×10^{-3}
Aminopterin	0.18×10^{-3}
Thymidine	3.88×10^{-3}
10% FCS	

All cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO_2 (98.5% air).

5. Test concentrations:

The highest applied concentration (3500.0 µg/mL) was based on a pretest and taking into account the current guidelines. At 3500.0 µg/ml no precipitation of the test substance in the vehicle was observed.

The concentrations of Reg. No. 4110542 (Metabolite of BAS 720 H, Imazamox) used in the HPRT test in CHO cells are depicted in the following table.

Exposure period	**Experiment**	**Concentration in µg/mL**
Without S9 mix		
4 h	I	**0,** 218.8**, 437.5, 875.0, 1750.0, 3500.0**
24 h	II	**0,** 218.8**, 437.5, 875.0, 1750.0, 3500.0**
With S9 mix		
4 h	I	**0,** 218.8**, 437.5, 875.0, 1750.0, 3500.0**
4 h	II	**500.0, 1000.0, 2000.0, 3500.0**

Evaluated experimental points are shown in bold characters

B. TEST PERFORMANCE

1. Dates of experimental work: 15-Jul-2013 to 16-Sep-2013

2. HPRT test:

Pretreatment period: During the week prior to treatment, any spontaneous HPRT-deficient mutants were eliminated by pretreatment with "HAT" medium. 3 – 5x10^5 cells were seeded per flask (75 cm²) and incubated with "HAT" medium for 3 - 4 days. A subsequent passage in Ham's F12 medium incl. 10% (v/v) FCS was incubated for a further 3 - 4 days.

Attachment period: For each test group, about 1x10^6 logarithmically growing cells per flask (175 cm²) were seeded into about 20 mL Ham's F12 medium supplemented with 10% (v/v) FCS and incubated for about 20 - 24 hours. Two flasks (one flask referred to as A and one flask referred to as B) were used for each test group.

Exposure: After the attachment period, the medium was removed from the flasks and the treatment medium was added (see table below). The cultures were incubated for the respective exposure period at 37 °C, 5% (v/v) CO_2 and ≥90% humidity.

Expression period: The exposure period was completed by rinsing several times with HBSS. Then the flasks were topped up with at least 20 mL Ham's F12 medium incl. 10% (v/v) FCS and left to stand in the incubator for about 3 days (4-hour treatment) or 2 days (24-hour treatment). This was followed by the 1st passage. After an entire expression period of 7 – 9 days the cells were transferred into selection medium (2nd passage).

Selection period: For selection of the mutants, six 75 cm^2 flasks with 3x10^5 cells each from every treatment group, if possible, were seeded in 10 mL selection medium ("TG" medium) at the end of the expression period. The flasks were returned to the incubator for about 6 - 7 days. At the end of the selection period, the medium was removed and the remaining colonies were fixed with methanol, stained with Giemsa and counted.

Cytotoxicity:

The procedure for the determination of the cloning efficiency in the pre-experiment was similar to that described for the determination of the cloning efficency 1 (CE_1) in the main experiments, excepting that every dose group contained only two cultures. For the determination of the influence of the test substance directly after the exposure period, about 200 cells per dose group were seeded in 25 cm² flasks in duplicate using 5 mL Ham's F12 medium incl. 10% (v/v) FCS. Following cell attachment for 20 – 24 hours, cells were treated with the vehicle, test substance or positive control for 4 hours or 24 hours. Following exposure, cells were rinsed several times with HBSS. Finally, cells were cultured in 5 mL Ham's F12 medium incl. 10% (v/v) FCS. For the determination of the mutation rate after the expression period, two aliquots of about 200 cells each were reserved from the transfer into selection medium (after 7 – 9 days) and seeded in two flasks (25 cm^2) containing 5 mL Ham's F12 medium incl. 10% (v/v) FCS. In all cases, after seeding the flasks were incubated for 5 - 8 days to form colonies. These colonies were fixed, stained and counted. The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_{absolute} = \frac{total\ number\ of\ colonies\ in\ the\ test\ group}{total\ number\ of\ seeded\ cells\ in\ the\ test\ group} * 100$$

$$CE_{relative} = \frac{CE_{absolute}\ of\ the\ test\ group}{CE_{absolute}\ of\ the\ vehicle\ or\ negative\ control} * 100$$

The number of colonies in every flask was counted and recorded. Using the formula above the values of absolute cloning efficiencies ($CE_{absolute}$, $CE1_{absolute}$ and/or $CE2_{absolute}$) were calculated. Based on these values the relative cloning efficiencies ($CE_{relative}$, $CE1_{relative}$ and/or $CE2_{relative}$) of the test groups were calculated and reported as a percentage of the respective $CE_{absolute}$ value of the corresponding vehicle/negative control (vehicle/negative control = 100%).

Mutant frequency: The number of colonies in every flask was counted and recorded. The sum of the mutant colony counts within each test group was subsequently normalized per every 10^6 cells seeded. The uncorrected mutant frequency ($MF_{uncorr.}$) per 10^6 cells was calculated for each test group as follows:

$$MF_{uncorr.} = \frac{total\ number\ of\ mutant\ colonies}{number\ of\ seeded\ cells} * 10^6$$

The uncorrected mutant frequency was corrected with the absolute cloning efficiency 2 for each test group to get the corrected mutant frequency ($MF_{corr.}$):

$$MF_{corr.} = \frac{MF_{uncorr.}}{CE_{2\ absolute}} * 100$$

4. Statistics:

Statistical significance at the one-sided five per cent level ($p < 0.05$) was evaluated by an appropriate trend-test. However, both, biological and statistical significance will be considered together.

5. Evaluation criteria:

A finding is assessed as positive if the following criteria are met:

- Increase in the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent negative control values and our historical negative control data range.
- Evidence of the reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above our historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

The test substance is considered non-mutagenic according to the following criteria:

- The corrected mutation frequency ($MF_{corr.}$) in the dose groups is not statistically significantly increased above the concurrent negative control and is within our historical negative control data range

II. RESULTS AND DISCUSSION

A. CYTOTOXICITY

No cytotoxic effects, as indicated by clearly reduced cloning efficiencies of about or below 20% of the respective negative control values were observed in both experiments in the presence and absence of S9 mix, up to the highest applied concentrations.

B. CELL MORPHOLOGY

In the presence of S9 mix, after 4 hours treatment in the 1st and 2nd experiment the morphology and attachment of the cells was adversely influenced from 875.0 µg/mL and 1000 µg/mL onward, respectively.

C. MUTAGENICITY

No relevant increase in the number of mutant colonies was observed with or without S9 mix. In both experiments after 4 and 24 hours treatment with the test substance the values for the corrected mutation frequencies ($MF_{corr.}$: 0.35 – 5.42 per 10^6 cells) were close to the respective vehicle control values ($MF_{corr.}$: 1.01 – 3.24 per 10_6 cells) and clearly within the range of the historical negative control data (without S9 mix: $MF_{corr.}$: 0.00 – 19.54 per 10^6 cells; with S9 mix: $MF_{corr.}$: 0.00 – 15.83 per 10^6 cells).

The positive control substances EMS (without S9 mix; 300 µg/mL) and DMBA (with S9 mix; 1.25 µg/mL) induced a clear increase in mutation frequencies, as expected. The values of the corrected mutant frequencies (without S9 mix: $MF_{corr.}$: 268.09 – 635.81 per 10^6 cells; with S9 mix: $MF_{corr.}$: 322.10 – 333.39 per 10^6 cells) were clearly within BASF historical positive control data range (without S9 mix: $MF_{corr.}$: 49.94 – 1 366.01 per 10^6 cells; with S9 mix:$MF_{corr.}$: 106.31 – 1 250.00 per 10^6 cells).

The results are summarized in the following table.

Table 5.8.1-4: Summary of results from the HPRT locus assay with Reg. No. 110542

Experiment	Exposure period [h]	Test groups [µg/mL]	S9 mix	Genotoxicity* $MF_{corr.}$ [per 10^6 cells]	Cytotoxicity** CE_1 [%]	Cytotoxicity** CE_2 [%]
1	4	Vehicle control[1]	-	2.48	100.0	100.0
		218.8	-	n.c.	105.1	n.c.
		437.5	-	4.44	114.6	94.6
		875.0	-	1.97	114.1	98.7
		1750.0	-	1.83	108.8	96.3
		3500.0	-	1.95	104.9	91.3
		Positive control[2]	-	268.09	103.9	76.0
2	24	Vehicle control[1]	-	3.08	100.0	100.0
		218.8	-	n.c.	106.5	n.c.
		437.5	-	1.09	108.7	82.1
		875.0	-	0.35	105.0	84.7
		1750.0	-	3.96	101.0	92.5
		3500.0***	-	1.94	102.5	97.0
		Positive control[2]	-	635.81	97.8	49.9
1	4	Vehicle control[1]	+	3.24	100.0	100.0
		218.8	+	n.c.	111.4	n.c.
		437.5	+	2.61	105.9	108.0
		875.0	+	1.53	115.3	106.0
		1750.0	+	0.69	121.2	116.9
		3500.0	+	3.03	124.8	94.2
		Positive control[3]	+	322.10	46.0	90.3
2	4	Vehicle control[1]	+	1.01	100.0	100.0
		500.0	+	3.91	113.8	91.8
		1000.0	+	5.42	123.1	93.7
		2000.0	+	0.35	136.2	96.3
		3500.0	+	2.92	117.8	100.3
		Positive control[3]	+	333.39	119.0	68.5

*: Mutant frequency MFcorr.: mutant colonies per 10^6 cells corrected with the CE_2 value
**: Cloning efficiency related to the respective vehicle control
***: Precipitation in culture medium at the end of exposure period
[1]: DMSO 1%
[2]: EMS 300 µg/mL
[3]: DMBA 1.25 µg/mL

III. CONCLUSION

Thus, in the absence and presence of metabolic activation Reg.No. 4110542 (CL 312622; metabolite of BAS 720 H, Imazamox) is not a mutagenic substance in the HPRT locus assay using CHO cells under the experimental conditions chosen.

Toxicological testing of CL 189215 (Reg.No. 4110445)

Mulligan A. (1995c): Microbial mutagenicity plate incorporation assay of CL 189,215
BASF DocID ID-470-004

CL 189215 (Reg. No. 4110445; 91% purity; batch 9087-43A) was tested in a standard plate incorporation assay using test strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) and E. coli (WP2 *uvrA*-). Experiments were carried out with and without metabolic activation (S9 mix) at dose levels of 312.5, 625, 1250, 2500, and 5000 µg/plate in dimethyl sulfoxide (DMSO), or with DMSO alone as the negative control for three replicates per dose. CL 189215 did not induce toxicity and no insolubility was observed at any dose level. Administered doses of 343.44; 686.88; 1373.75; 2747.50 and 5495.00 µg/plate reflected use of a correction factor for the stated purity of 91% test material. The results obtained from both trials showed no significant or dose-related increases in revertant frequencies in the presence or absence of metabolic activation. The positive controls (2.5 µg/plate of 2-Aminoanthracene (2-AA; 85% purity with metabolic activation administered to TA98; TA100; TA1535; TA1537 and TA1538 and 10 µg/plate administered to WP2 *uvrA*-; 10 µg/plate of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; 97% purity) without metabolic activation administered to WP2 *uvrA*-, TA100 and TA1535; 20 µg/plate 2-Nitrofluorene (2-NF; 98% purity) without metabolic activation administered to TA98 and TA1538; and 50 µg/plate 9-Aminoacridine (9-AA ; 98% purity) without metabolic activation administered to TA1537) elicited the expected increase in revertant frequencies indicating that the test system was capable of detecting base-pair and frameshift mutations and that the metabolic activation system was functioning properly. The vehicle control revertants colonies were within the expected acceptable ranges.

In conclusion, CL 189215 (Reg. No. 4110445) did not show any genotoxic potential in 6 tester strains of bacteria at concentrations up to and including 5000 µg/plate in the presence and absence of S-9 metabolic activation.

Report:	CA 5.8.1/3 Bohnenberger S., 2013b Reg. No. 4110445 (metabolite of BAS 720 H, Imazamox) - In vitro micronucleus test in Chinese hamster V79 cells 2013/1113582
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The test item, Reg. No. 4110445 (CL189215; metabolite of BAS 720 H, Imazamox) was assessed for its potential to induce micronuclei in V79 cells of the Chinese hamster *in vitro* in 2 independent experiments in the absence and presence of metabolic activation by S9 mix (Experiment I, 4 h-exposure with or without S9; Experiment II, 24 h-exposure without S9 and 4 h-exposure with S9). With and without metabolic activation no cytotoxicity was observed up to the highest applied concentration (5830.0 µg/mL). No mutagenicity was observed with and without metabolic activation. In Experiment I in the absence of metabolic activation one statistically significant increase (1.10% micronucleated cells) was observed after treatment with 5830.0 µg/mL of the test item. Since the value is clearly within the range of the laboratory historical control data (0.15–1.50 % micronucleated cells), the finding is regarded as biologically irrelevant. Appropriate mutagens were used as positive controls. They induced statistically significant increases in the percentage of micronucleated cells. Under the experimental conditions reported, the test item Reg. No. 4110445 (CL189215: metabolite of BAS 720 H, Imazamox) did not induce micronuclei in V79 cells (Chinese hamster cell line) *in vitro* in the absence and presence of metabolic activation.
In conclusion, Reg. No. 4110445 (CL189215; metabolite of BAS 720 H, Imazamox) is considered to be non-mutagenic in this *in vitro* test system.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg.No. 4110445 (synonym: CL189215; metabolite of BAS 720 H, Imazamox)
Description:	Solid, beige to brown
Lot/Batch #:	AC11951-85
Purity:	77.8% (tolerance ±1%)
Stability of test compound:	The stability of the test item in deionized water was verified. Homogeneity of the preparations was ensured by mixing.
Solvent used:	Deionized water

2. Control Materials:

Negative and solvent control: Deionized water (10% (v/v) in cell culture medium)

Positive control:

Without metabolic activation:

Mytomycin C (MMC) 0.3 µg/mL in deionized water

Griseofulvin 8 µg/mL in DMSO

With metabolic activation (S9):

Cyclophosphamide (CPA) 20 µg/mL in saline

The stability of the positive control substances in solution was proven by the mutagenic response in the expected range.

3. Activation:

S9 was produced from livers of 8–12 weeks old male Wistar rats. The rats received 80 mg/kg bw phenobarbital i.p. and 80 mg/kg bw ß-naphthoflavone p.o. daily on three consecutive days and were sacrificed 24 hours after the last administration. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g and stored at −80 °C Small numbers of the ampoules were kept at −20 °C for up to one week. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

The protein concentration was 27.9 mg/mL.

The S9-mix was prepared immediately before use and had the following composition:

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures.

Component	*Concentration*
Sodium ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	Not specified

During the experiment the S9 mix was stored on ice.

4. Test organisms: Chinese hamster V79 cells

For seeding and treatment of the cell cultures the culture medium was MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM) and 10% (v/v) fetal bovine serum (FBS). Additionally, the medium was supplemented with penicillin/streptomycin (100 U/mL/100 µg/mL). All cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO_2 (98.5% air).

5. Test concentrations:

The highest applied concentration (5830.0 µg/mL; approx. 10 mM) was chosen with regard to the molecular weight and the purity (77.8 %) of the test item and with respect to the OECD Guideline No. 487.

The concentrations of Reg. No. 4110445 (Metabolite of BAS 720 H, Imazamox) used in the Micronucleus test in V79 cells are depicted in the following table.

Exposure period	Experiment	Concentration in µg/mL
Without S9 mix		
4 h	I	11.4, 22.8, 45.5, 91.1, 182.2, 364.4, 728.8, **1457.5**, **2915.0**, **5830.0**
24 h	II	11.4, 22.8, 45.5, 91.1, 182.2, 364.4, 728.8, **1457.5**, **2915.0**, **5830.0**
With S9 mix		
4 h	I	11.4, 22.8, 45.5, 91.1, 182.2, 364.4, 728.8, **1457.5**, **2915.0**, **5830.0**[P]
4 h	II	182.2, 364.4, 728.8, **1457.5**, **2915.0**, **5830.0**

Evaluated experimental points are shown in bold characters
[P] Precipitation occurred microscopically at the end of treatment

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 9-Jan-2013 to 21-Feb-2013

2. Micronucleus test:

Seeding: The cells were seeded into Quadriperm dishes that contained microscopic slides. Into each chamber 1.0–1.5 × 10^5 cells were seeded with regard to the preparation time.

Treatment: 4 h-exposure:

The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added. Concurrent solvent and positive controls were performed. After 4 h the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then, the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time.

24 h-exposure:

The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparation of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionized water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25% (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labeled with a computer-generated random code to prevent scorer bias.

Micronuclei Analysis: Evaluation of the cultures was performed manually using NIKON microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The criteria for the evaluation of micronuclei are described in Countryman and Heddle (1976), *Mutation Research*, 41, 321–332. The micronuclei were briefly stained in the same way as the main nucleus. The area of the micronucleus did not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2–8 cells were analyzed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity: Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3–4 cells (c4), and 5–8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below [Kirsch-Volders *et al.* (2003) *Mutation Research*, 540, 153–163; Kirsch-Volders *et al.* (2004) *Mutation Research*, 564, 97–100]). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

The proliferation index (*PI*) is

$$PI = \frac{(c1 * 1) + (c2 * 2) + (c4 * 3) + (c8 * 4)}{c1 + c2 + c4 + c8}$$

for the number of clones with *x* cells (*cx* with *x*: 1, 2, 4, or 8).

4. Statistics:

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the Chi square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item can be classified as mutagenic if:

- the number of micronucleated cells is not in the range of the historical control data (see table: historical laboratory control data in RESULTS AND DISCUSSION) and
- either a concentration-related increase in three test groups or a statistically significant increase in the number of micronucleated cells is observed.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data (see table: historical laboratory control data in RESULTS AND DISCUSSION) and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATION

The stability of the test substance under storage conditions throughout the study period was proven. The stability in solvent has been verified analytically.

B. CYTOTOXICITY

In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.

C. MICRONUCLEUS ASSAY

No mutagenicity was observed with and without S9 mix. The rates of micronucleated cells after treatment with the test item (0.45–1.50%) were close to the rates of the solvent control values (0.40–1.10%) and within the range of the laboratory historical solvent control data (4 hours treatment without S9 mix: 0.15–1.50% micronucleated cells, 24 hour treatment without S9 mix: 0.05–1.50% micronucleated cells, 4 hour treatment with S9 mix: 0.05–1.70% micronucleated cells). In Experiment I in the absence of S9 mix one statistically significant increase (1.10% micronucleated cells) was observed after treatment with a dose of 5830.0 µg/mL. Since the value is clearly within the range of the laboratory historical control data (0.15–1.50 % micronucleated cells) the finding is regarded as biologically irrelevant. Mitomycin C (0.3 µg/mL), Griseofulvin (8.0 µg/mL) or CPA (20.0 µg/mL) were used as positive controls and showed a distinct increase in the percentage of micronucleated cells.

The results are summarized in the following table.

Table 5.8.1-5: Summary of results of the micronucleus test *in vitro* with Reg.No. 4110445

Experiment	Test item concentration in µg/mL	Proliferation index	Micronucleated cells in %
Exposure period 4 h without S9 mix			
I	Solvent control[1]	2.97	0.40
	Positive control[2]	2.16	12.75[S]
	1457.5	2.96	0.45
	2915.0	2.91	0.50
	5830.0	2.83	1.10[S]
Exposure period 24 h without S9 mix			
II	Solvent control[1]	3.15	0.55
	Positive control[3]	2.71	13.25[S]
	1457.5	3.14	0.50
	2915.0	3.05	0.50
	5830.0	2.91	0.70
Exposure period 4 h with S9 mix			
I	Solvent control[1]	1.93	1.10
	Positive control[4]	1.54	6.90[S]
	1457.5	2.16	0.90
	2915.0	2.21	0.45
	5830.0[P]	2.18	0.55
II	Solvent control[1]	2.12	1.05
	Positive control[4]	1.69	11.70[S]
	1457.5	2.08	1.50
	2915.0	2.02	1.45
	5830.0[P]	2.05	1.35

* The number of micronucleated cells was determined in a sample of 2000 cells
[S] Number of micronucleated cells statistically significantly higher than corresponding control values
[P] Precipitation occurred microscopically at the end of treatment
[1] Deionized water 10.0% (v/v)
[2] Mitomycin C 0.3 µg/mL
[3] Griseofulvin 8.0 µg/mL
[4] Cyclophosphamide 20.0 µg/mL

A summary of the historical controls is summarized in the following table.

Table 5.8.1-6: Historical laboratory control data – Percentage of micronucleated cells in Chinese hamster V79 cell cultures (October 2006 to August 2011)

	No. of studies	Range	Mean	Calc. Range[1]
Without S9 mix: preparation interval 24h, treatment 4 h				
Solvent control				
Aqueous sol.[2]	19	0.35–1.50	0.67	0.41–0.94
Organic solv.[3]	28	0.15–1.45	0.63	0.31–0.95
Total	47	0.15–1.50	0.65	0.35–0.94
Positive control				
Mitomycin C 0.03–0.1 µg/mL	44	0.85–18.15	7.65	3.60–11.70
Without S9 mix: preparation interval 24 h, treatment 24 h				
Solvent control				
Aqueous sol.[2]	15	0.15–1.50	0.62	0.23–1.02
Organic solv.[3]	140	0.05–1.50	0.61	0.30–0.91
Total	155	0.05–1.50	0.61	0.29–0.92
Positive control				
Mitomycin C 0.03–0.1 µg/mL	109	2.10–53.40	13.09	5.84–20.33
Griseofulvin 6.3–25.0 µg/mL	113	2.50–31.70	10.63	4.40–16.87
With S9 mix: preparation interval 24 h, treatment 4 h				
Solvent control				
Aqueous sol.[2]	33	0.15–1.70	0.79	0.46–1.12
Organic solv.[3]	176	0.05–1.60	0.7/8	0.44–1.13
Total	209	0.05–1.70	0.78	0.44–1.13
Positive control				
Cyclophosphamide 2.5–25.0 µg/mL	211	0.90–38.30	8.54	3.67–13.42

[1] Mean ± standard deviation
[2] Aqueous solvents: deionized water (10 % v/v), 0.9 % (w/v) saline, and culture medium MEM
[3] Organic solvents: dimethyl sulfoxide, acetone, ethanol, and tetrahydrofurane (0.5 % v/v)

III. CONCLUSION

Under the experimental conditions of this study, Reg. No. 4110445 (or CL189215; metabolite of BAS 720 H, Imazamox) was judged to be negative for inducting micronuclei in V79 cells (Chinese hamster cell line) in the absence and presence of metabolic activation *in vitro*.

Therefore, Reg. No. 4110445 (CL189215) is considered to be non-mutagenic in this *in vitro* test system.

Toxicological testing of CL 263284 (Reg.No. 4110773)

[redacted]**. (1995c):** Oral LD50 study in Albino Mice with CL 263284;
BASF DocID ID-470-007

The test substance, CL 263284 (95% purity; batch AC 9745-61A), was tested for its acute oral toxicity in 5 male and 5 female CD-1 mice when administered a single dose of 5000 mg/kg bw prepared as a 20% w/v dispersion in corn oil via gavage. 3 of the 5 females died 1 to 2 days after dosing. Gross necropsy confirmed that for 1 of these 3 females, death was attributed to a dosing accident. Clinical signs of toxicity were limited to decreased activity, ataxia and dyspnoea in 2 of the 3 female decedents only. None of the male mice died or exhibited clinical signs of toxicity. Because of these deaths in females and the lack of mortality in males at 5000 mg/kg bw, additional 5 females were dosed at 5000 mg/kg bw. 5 additional females also received CL 263284 at 3500 mg/kg. There were no mortalities or clinical signs of toxicity noted for any of the females dosed at 5000 mg/kg bw (second trial) or 3500 mg/kg bw. All animals which survived to study termination gained weight. Moreover, there were no gross pathological findings for animals sacrificed at study termination. Excluding the female mouse whose death was definitely attributed to a dosing accident, the combined mortality for females from the first and second trials at 5000 mg/kg bw was 2 of 9.

Thus, the oral LD50 of CL 263284 was greater than 5000 mg/kg bw for male and female mice. According to Regulation (EC) 1272/2008 [CLP] the test substance, CL 263284, is not to be classified for acute oral toxicity.

Mulligan A. (1995b): Microbial mutagenicity plate incorporation assay of CL 263,284;
BASF DocID ID-470-003

Reverse mutation tests were performed on CL 263284 in test strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) and E. coli (WP2 *uvrA*). Experiments were carried out with and without metabolic activation (S9 mix) at dose levels of 312.5, 625, 1250, 2500, and 5000 µg/plate. CL 263284 did not induce toxicity, and the results obtained from both trials showed no positive responses or dose-related increases in revertant frequencies in the presence or absence of metabolic activation.

In conclusion CL 263284 was negative for inducing base-pair or frame shift mutations in the 6 tested strains of bacteria up to and including 5000 µg/plate in the presence and absence of S-9metabolic activation.

Report: CA 5.8.1/4
Bohnenberger S., 2013c
Reg.No. 4110773 (metabolite of BAS 720 H, Imazanox) - In vitro micronucleus test in Chinese hamster V79 cells
2013/1113581

Report: CA 5.8.1/5
Bohnenberger S., 2013e
First amendment - Reg. No. 4110773 (metabolite of BAS 720 H, Imazamox) - In vitro micronucleus test in Chinese hamster V79 cells
2013/1165894

The amendment corrected information regarding the stability of the test item in solvent, osmolarity and pH values. These changes have no impact on the outcome of the study.

Guidelines: OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012
GLP: Yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The test item, Reg. No. 4110773 (CL263284; metabolite of BAS 720 H, Imazamox) was assessed for its potential to induce micronuclei in V79 cells of the Chinese hamster *in vitro* in 4 independent experiments in the absence and presence of metabolic activation by S9 mix (Experiment IB, 4 h-exposure w/o S9; Experiment IIA, 24 h-exposure w/o S9; Experiment IA and Experiment IIB, 4 h-exposure with S9). Without metabolic activation and in Experiment IIB with metabolic activation, no cytotoxicity was observed up to the highest applied concentration (3220.0 µg/mL). In Experiment IA with metabolic activation, treatment that resulted in clear cytotoxic effects was not evaluable for cytogenetic damage. In the absence of S9 mix no mutagenicity was observed. In Experiment IA in the presence of metabolic activation treatment with 321.3 µg/mL of the test item resulted in a single statistically significant increase (4.55% micronucleated cells) that clearly exceed the historical solvent control data range (0.05–1.70% micronucleated cells). In Experiment IIB in the presence of metabolic activation treatment with 1200.0, 1400.0, 1600.0, 2000.0, and 2570.0 µg/mL resulted in statistically significant increases (2.00, 2.50, 2.40, 2.30, and 2.50% micronucleated cells, respectively) that clearly exceed the historical solvent control data range. Appropriate mutagens were used as positive controls. They induced statistically significant increases in the percentage of micronucleated cells.
In conclusion, the test item Reg. No. 4110773 (CL263284; metabolite of BAS 720 H, Imazamox) induced micronuclei in V79 cells (Chinese hamster cell line) *in vitro* only in the presence of metabolic activation.
Therefore, Reg. No. 4110773 (CL263284; metabolite of BAS 720 H, Imazamox) is considered to be mutagenic in this *in vitro* test system.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 4110773 (synonym: CL263284; metabolite of BAS 720 H, Imazamox)

Description: Solid, beige

Lot/Batch #: L76-148

Purity: 90.5% (tolerance ±1%)

Stability of test compound: The stability of the test substance under storage conditions is guaranteed for the duration of the study.

The stability of the test item in DMSO was verified.

Homogeneity of the preparations was ensured by mixing.

Solvent used: DMSO

2. Control Materials:

Negative and solvent control: Dimethyl sulfoxide; DMSO (0.5% (v/v) in cell culture medium)

Positive control: Without metabolic activation:

Mytomycin C (MMC) 0.3 µg/mL in deionized water

Griseofulvin 8 µg/mL in DMSO

With metabolic activation (S9):

Cyclophosphamide (CPA) 15 µg/mL (Experiment IA) and 20 µg/mL (Experiment IIB) in saline

The stability of the positive control substances in solution was proven by the mutagenic response in the expected range.

3. Activation: S9 was prepared from livers of 8–12 weeks old male Wistar rats. The rats received 80 mg/kg bw phenobarbital i.p. and 80 mg/kg bw ß-naphthoflavone p.o. daily on three consecutive days and were sacrificed 24 hours after the last administration. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g and stored at −80 °C Small numbers of the ampoules were kept at −20 °C for up to one week. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

The protein concentration was 29.8 mg/mL for Experiment IA and 25.3 mg/mL for Experiment IIB.

The S9-mix was prepared immediately before use and had the following composition:

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures.

Component	*Concentration*
Sodium ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	Not specified

During the experiment the S9 mix was stored on ice.

4. Test organisms:

Chinese hamster V79 cells

For seeding and treatment of the cell cultures the culture medium was MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM) and 10% (v/v) fetal bovine serum (FBS). Additionally, the medium was supplemented with penicillin/streptomycin (100 U/mL/100 µg/mL). All cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO_2 (98.5% air).

5. Test concentrations:

The highest treatment concentration in this study, 3220.0 µg/mL (approx. 10 mM), was chosen with regard to the molecular weight and the purity (90.5 %) of the test item and with respect to the OECD Guideline for *in vitro* mammalian cytogenetic tests. No precipitation of the test item in the culture medium was observed. No relevant influence on osmolarity was observed. The pH value was adjusted to physiological values using small amounts of 2 N NaOH.

The concentrations of Reg. No. 4110773 (Metabolite of BAS 720 H, Imazamox) used in the Micronucleus test in V79 cells are depicted in the following table.

Exposure period	Experiment	Concentration in µg/mL
Without S9 mix		
4 h	IA*	5.0, 10.0, 20.1, 40.2, 80.3, 160.6, 321.3, 642.5, 1285.0, 2570.0
4 h	IB	160.6, 321.3, 642.5, **1285.0**, **2570.0**, **3220.0**#
24 h	IIA	10.0, 20.1, 40.2, 80.3, 160.6, 321.3, 642.5, **1285.0**, **2570.0**, **3220.0**#
With S9 mix		
4 h	IA	5.0, 10.0, 20.1, 40.2, **80.3**, **160.6**, **321.3**, 642.5, 1285.0, 2570.0
4 h	IIA**	50.0, 100.0, 200.0, 300.0, 400.0, 500.0, 600.0, 800.0, 1000.0, 1200.0
4 h	IIB	100.0, 300.0, **600.0**, **800.0**, **1000.0**, **1200.0**, **1400.0**, **1600.0**, **2000.0**, **2570.0**

Evaluated experimental points are shown in bold characters
* Was repeated since no cytotoxicity or precipitation was observed
** Was repeated due to lack of cytotoxicity
# Applied as inhomogeneous suspension

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 12-Dec-2012 to 26-Mar-2013

2. Micronucleus test:

Seeding: The cells were seeded into Quadriperm dishes that contained microscopic slides. Into each chamber 1.0 × 10^5 – 1.5 × 10^5 cells were seeded with regard to the preparation time.

Treatment:

4 h-exposure:

The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added. Concurrent solvent and positive controls were performed. After 4 h the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then, the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time.

24 h-exposure:

The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparation of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25% (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Micronuclei Analysis: Evaluation of the cultures was performed manually using NIKON microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The criteria for the evaluation of micronuclei are described in Countryman and Heddle (1976), *Mutation Research*, 41, 321–332. The micronuclei were briefly stained in the same way as the main nucleus. The area of the micronucleus did not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2–8 cells were analyzed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity: Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3–4 cells (c4), and 5–8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below [Kirsch-Volders *et al.* (2003) *Mutation Research*, 540, 153–163; Kirsch-Volders *et al.* (2004) *Mutation Research*, 564, 97–100]). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

The proliferation index (*PI*) is

$$PI = \frac{(c1 * 1) + (c2 * 2) + (c4 * 3) + (c8 * 4)}{c1 + c2 + c4 + c8}$$

for the number of clones with *x* cells (*cx* with *x*: 1, 2, 4, or 8).

4. Statistics:

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the Chi square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item can be classified as mutagenic if:

- the number of micronucleated cells is not in the range of the historical control data (see table: historical laboratory control data in RESULTS AND DISCUSSION) and
- either a concentration-related increase in three test groups or a statistically significant increase in the number of micronucleated cells is observed.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data (see table: historical laboratory control data in RESULTS AND DISCUSSION) and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATION

The stability of the test substance under storage conditions throughout the study period was proven. The stability in solvent has been verified analytically.

B. CYTOTOXICITY

In Experiment IB and IIA in the absence of S9 mix and in Experiment IIB in the presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration. In Experiment IA in the presence of S9 mix concentrations showing clear cytotoxic effects were not evaluable for cytogenetic damage.

C. MICRONUCLEUS ASSAY

In the absence of S9 mix no mutagenicity was observed. The rates of micronucleated cells after treatment with the test item (0.40–1.10%) were close to the rates of the solvent control values (0.45–1.15%) and within the range of the laboratory historical solvent control data (4 h treatment without S9 mix: 0.15–1.50% micronucleated cells, 24 h treatment without S9 mix: 0.05–1.50% micronucleated cells). In Experiment IA, treatment with 321.3 µg/mL of the test item in the presence of metabolic activation resulted in a single statistically significant increase (4.55% micronucleated cells) that clearly exceed the historical solvent control data range (0.05–1.70% micronucleated cells). In Experiment IIB treatment with 1200.0, 1400.0, 1600.0, 2000.0, and 2570.0 µg/mL of the test item in the presence of metabolic activation resulted in statistically significant increases that clearly exceed the historical solvent control data range (2.00, 2.50, 2.40, 2.30, and 2.50% micronucleated cells, respectively). Mitomycin C (0.3 µg/mL), Griseofulvin (8.0 µg/mL) or CPA (15.0 or 20.0 µg/mL) were used as positive controls and showed a distinct increase in the percentage of micronucleated cells.

The results are summarized in the following table.

Table 5.8.1-7: Summary of results of the *in vitro* micronucleus test with Reg. No. 4110773

Experiment	Test item concentration in µg/mL	Proliferation index	Micronucleated cells* in %
Exposure period 4 h without S9 mix			
IB	Solvent control[1]	2.85	0.45
	Positive control[2]	2.24	11.95[S]
	1285.0	2.69	0.70
	2570.0	2.68	0.65
	3220.2[#]	2.70	0.40
Exposure period 24 h without S9 mix			
IIA	Solvent control[1]	2.55	1.15
	Positive control[3]	2.59	11.55[S]
	1285.0	2.77	0.85
	2570.0	2.69	0.55
	3220.0[#]	2.56	1.10
Exposure period 4 h with S9 mix			
IA	Solvent control[1]	2.06	0.60
	Positive control[4]	1.28	5.20[S]
	80.3	1.92	0.50
	160.6	1.87	0.50
	321.3	1.83	**4.55[S]**
IIB	Solvent control[1]	1.76	0.55
	Positive control[5]	1.31	5.15[S]
	600.0	1.85	0.70
	800.0	1.98	0.90
	1000.0	1.96	1.00
	1200.0	2.02	**2.00[S]**
	1400.0	2.06	**2.50[S]**
	1600.0	2.01	**2.40[S**]**
	2000.0	2.05	**2.30[S**]**
	2570.0	2.06	**2.50[S**]**

* The number of micronucleated cells was determined in a sample of 2000 cells
** The number of micronucleated cells was determined in a sample of 4000 cells
# Applied as inhomogeneous suspension
S Number of micronucleated cells statistically significantly higher than corresponding control values
1 DMSO 0.5 % (v/v)
2 Mitomycin C 0.3 Lg/mL
3 Griseofulvin 8.0 Lg/mL
4 CPA 15.0 µg/mL
5 CPA 20.0 µg/mL

A summary of the historical controls is presented in the following table.

Table 5.8.1-8: Historical laboratory control data – Percentage of micronucleated cells in Chinese hamster V79 cell cultures (October 2006 to August 2011)

	No. of studies	Range	Mean	Calc. Range[1]
Without S9 mix: preparation interval 24h, treatment 4 h				
Solvent control				
Aqueous sol.[2]	19	0.35–1.50	0.67	0.41–0.94
Organic solv.[3]	28	0.15–1.45	0.63	0.31–0.95
Total	47	0.15–1.50	0.65	0.35–0.94
Positive control				
Mitomycin C 0.03–0.1 µg/mL	44	0.85–18.15	7.65	3.60–11.70
Without S9 mix: preparation interval 24 h, treatment 24 h				
Solvent control				
Aqueous sol.[2]	15	0.15–1.50	0.62	0.23–1.02
Organic solv.[3]	140	0.05–1.50	0.61	0.30–0.91
Total	155	0.05–1.50	0.61	0.29–0.92
Positive control				
Mitomycin C 0.03–0.1 µg/mL	109	2.10–53.40	13.09	5.84–20.33
Griseofulvin 6.3–25.0 µg/mL	113	2.50–31.70	10.63	4.40–16.87
With S9 mix: preparation interval 24 h, treatment 4 h				
Solvent control				
Aqueous sol.[2]	33	0.15–1.70	0.79	0.46–1.12
Organic solv.[3]	176	0.05–1.60	0.7/8	0.44–1.13
Total	209	0.05–1.70	0.78	0.44–1.13
Positive control				
Cyclophosphamide 2.5–25.0 µg/mL	211	0.90–38.30	8.54	3.67–13.42

[1] Mean ± standard deviation
[2] Aqueous solvents: deionised water (10 % v/v), 0.9 % (w/v) saline, and culture medium MEM
[3] Organic solvents: dimethyl sulfoxide, acetone, ethanol, and tetrahydrofurane (0.5 % v/v)

III. CONCLUSION

Under the experimental conditions reported, the test item Reg. No. 4110773 (CL263284; metabolite of BAS 720 H, Imazamox) was judged to be positive for inducing micronuclei in V79 cells (Chinese hamster cell line) *in vitro* in the presence of metabolic activation.
Therefore, Reg. No. 4110773 (CL263284; metabolite of BAS 720 H, Imazamox) has to be considered as mutagenic in this *in vitro* test system in the presence of metabolic activation, when tested up to the highest evaluable concentrations.

Report:	CA 5.8.1/6 [redacted] 2013a Reg.No. 4110773 (Metabolite of BAS 720 H, Imazamox) - Micronucleus test in bone marrow cells of the mouse 2013/1235041
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395, OECD 474 (1997)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The test item, Reg. No. 4110773 (synonym: CL263284; metabolite of BAS 720 H, Imazamox) was assessed for its potential to induce chromosomal damage (clastogenicity) or spindle poison effects (aneugenic activity) in NMRI mice using the micronucleus test method. Male mice received a single oral dose of 500, 1000 or 2000 mg/kg bw of the test item or positive (cyclophosphamide for clastogenicity and vincristine sulfate for spindle poison effects) and negative (DMSO/corn oil (2:3)) controls.

Bone marrow of the two femora was prepared 24 and 48 hours after administration in the highest dose group of 2000 mg/kg bw and in the vehicle controls. In the test groups of 1000 mg/kg bw and 500 mg/kg bw and in the positive control groups, the 24-hour sacrifice interval was investigated only. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded. In addition, blood samples taken immediately after sacrifice were analyzed to verify the bioavailability of the test substance in the target organ.

Both positive control substances led to the expected increase in the rate of polychromatic erythrocytes containing small or large micronuclei. No distinct inhibition of erythropoiesis determined from the ratio of polychromatic to normochromatic erythrocytes was detected. However, the bioavailability of the test substance in blood after oral administration was clearly confirmed. The rate of micronuclei in animals that received the test item was close to the range of the concurrent vehicle control in all dose groups and at all sacrifice intervals and within the range of the historical vehicle control data.

Thus, under the experimental conditions of this study, the test substance Reg. No. 4110773 (CL263284; metabolite of BAS 720 H, Imazamox) does not induce cytogenetic damage in bone marrow cells of NMRI mice *in vivo*.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 4110773 (or CL 263284, metabolite of BAS 720 H, Imazamox)

Description:	Solid, beige
Lot/Batch #:	L82-116
Purity:	97.9% (tolerance ±1%)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 May 2015.
Solvent used:	DMSO

2. Vehicle and/or positive control:

Vehicle:	DMSO in corn oil (ratio 2:3)
Positive controls:	20 mg/kg bw cyclophosphamide 0.15 mg/kg bw vincristine sulfate

3. Animals:

Species:	Mouse
Strain:	Crl:NMRI
Age:	5 – 8 weeks
Sex:	male
Total number of animals:	40 animals
Weight at dosing:	28.58 g
Acclimatization time:	at least 5 days
Supplier:	Charles River Laboratories, 97633, Sulzfeld, Germany
Housing:	Individually in Makrolon cages, type M II
Diet:	Standardized pelleted feed (Maus/Ratte Haltung "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Drinking water:	Tap water, *ad libitum*
Bedding:	Dust-free wooden bedding, specified and documented in the raw data

4. Dosing:

Route:	oral
Doses:	500, 1000, 2000 mg/kg bw (single application)
Application volume:	10 mL/kg bw

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 05 Aug 2013 to 18 Oct 2013

2. Micronucleus test:

Preparation of bone marrow: Two femora were prepared by dissection and removing all soft tissues. After cutting off the epiphyses, the bone marrow was flushed out of the diaphysis into a centrifuge tube using a cannula filled with fetal calf serum (FCS) which was preheated up to 37 °C (about 2 mL/femur). The suspension was mixed thoroughly with a pipette and centrifuged at 300 x g for 5 minutes. The supernatant was removed and the precipitate was resuspended in about 50 µL fresh FCS. One drop of this suspension was dropped onto clean microscopic slides, using a Pasteur pipette. Smears were prepared using slides with ground edges. The preparations were dried in the air and subsequently stained.

Slide staining: The slides were stained with eosin and methylene blue for about 5 minutes. After briefly rinsing in deionized water, the preparations were soaked in deionized water for about 2 - 3 minutes. Subsequently, the slides were stained with Giemsa solution (15 mL Giemsa plus 185 mL deionized water) for about 15 minutes. After rinsing twice in deionized water and clarifying in xylene, the preparations were mounted in Corbit-Balsam.

Microscopic evaluation: In general, 2000 polychromatic erythrocytes (PCE) were evaluated for the occurrence of micronuclei from each animal of every test group, so in total 10000 PCEs were scored per test group. The normochromatic erythrocytes (= normocytes / NCE) were also scored. Recorded were the number of polychromatic and normochromatic erythrocytes and the respective number containing micronuclei, the ratio of polychromatic to normochromatic erythrocytes and the number of small micronuclei ($d < D/4$) and of large micronuclei ($d \geq D/4$) [d = diameter of micronucleus, D = cell diameter]

4. Statistics:

The asymptotic U test according to Mann-Whitney (modified rank test according to Wilcoxon) was carried out to clarify the question whether there are statistically significant differences between the untreated control group and the treated dose groups with regard to the micronucleus rate in polychromatic erythrocytes. The relative frequencies of cells containing micronuclei of each animal were used as a criterion for the rank determination for the U test. Statistical significances were established at $p \leq 0.05$ (*) and $p \leq 0.01$(**).

5. Evaluation criteria:

The mouse micronucleus test is considered valid if the following criteria are met:

- The quality of the slides must allow the evaluation of a sufficient number of analyzable Cells; i.e. ≥ 2000 PCEs per animal and a clear differentiation between PCEs and NCEs.
- The ratio of PCEs/NCEs in the concurrent vehicle control animals has to be within the normal range for the animal strain selected.
- The number of cells containing micronuclei in vehicle control animals has to be within the range of the historical vehicle control data for PCEs.
- The two positive control substances have to induce a distinct increase in the number of PCEs containing small and/or large micronuclei within the range of the historical positive control data or above.

A finding is considered positive if the following criteria are met:

- Statistically significant and dose-related increase in the number of PCEs containing micronuclei.
- The number of PCEs containing micronuclei has to exceed both the concurrent vehicle control value and the range of the historical vehicle control data.

A test substance is considered negative if the following criteria are met:

- The number of cells containing micronuclei in the dose groups is not statistically significant increased above the concurrent vehicle control value and is within the range of the historical vehicle control data

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATION

The stability of a comparable batch (L76-148, PSN No. 12/0728-1) at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically. The homogeneity of the test substance in the vehicle was quantified indirectly by analytical determination of three individual samples of each concentration. The test substance formulations were constantly stirred during sampling. The concentration control analyses of all concentrations revealed that the mean values were in the expected range of the target concentrations, i.e. were always in a range of 90% - 110% of the nominal concentration. Based on the recovery rates it has to be considered that the test substance was stable at room temperature in the vehicle DMSO/corn oil at least over a period of 35 minutes.

B. PLASMA ANALYSIS

The bioavailability of the test substance in blood after oral administration was clearly demonstrated by analysis of plasma samples taken at the respective sampling intervals.

C. CLINICAL SIGNS

The single oral administration of the vehicle in a volume of 10 mL/kg bw was tolerated by all animals without any clinical observations. The administration of the test substance did not lead to any clinical signs of toxicity. Neither the single administration of the positive control substance cyclophosphamide in a dose of 20 mg/kg bw nor that of vincristine sulfate in a dose of 0.15 mg/kg bw caused any evident signs of toxicity.

D. MICRONUCLEUS ASSAY

The single oral administration of the vehicle DMSO/corn oil in a volume of 10 mL/kg bw led to 0.9‰ polychromatic erythrocytes containing micronuclei after the 24-hour sacrifice interval or to 1.6‰ after the 48-hour sacrifice interval, respectively.
After the single administration of the highest dose of 2000 mg/kg bw, 1.3‰ polychromatic erythrocytes containing micronuclei were found after 24 hours and 2.0‰ after 48 hours.
In the two lower dose groups, rates of micronuclei of 1.9‰ (1000 mg/kg bw group) and 1.6‰ (500 mg/kg bw group) were detected at a sacrifice interval of 24 hours in each case.
The positive control substance for clastogenicity, cyclophosphamide, led to a statistically significant increase (19.5‰) in the number of polychromatic erythrocytes containing exclusively small micronuclei, as expected.
Vincristine sulfate, a spindle poison, produced a statistically significant increase (26.3‰) in the number of polychromatic erythrocytes containing micronuclei. A significant portion increase, 6.2‰ was attributable to large micronuclei.
The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the vehicle control group or in the various dose groups at any of the sacrifice intervals.
No distinct inhibition of erythropoiesis induced by the treatment of mice with Reg.No. 4110773 (metabolite of BAS 720 H, Imazamox) was detected. The results are summarized in the following table.

Table 5.8.1-9: Induction of micronuclei by Reg. No. 4110773 in bone marrow cells of mice

Test group	Sacrifice interval [h]	Animal No.	PCE with micronuclei		PCEs per 2000 erythrocytes[c]
			total[a] [‰]	total[b] [‰]	
DMSO/corn oil	24	5	0.9	0.1	1355
Test substance 500 mg/kg bw.	24	5	1.6	0.1	1334
Test substance 1000 mg/kg bw.	24	5	1.9	0.1	1296
Test substance 2000 mg/kg bw.	24	5	1.3	0.0	1310
Cyclophosphamide 20 mg/kg bw.	24	5	19.5**	0.0	1286
Vincristine sulfate 0.15 mg/kg bw.	24	5	26.3**	6.2**	1212
DMSO/corn oil	48	5	1.6	0.4	1388
Test substance 2000 mg/kg bw.	48	5	2.0	0.5	1293

PCE: polychromatic erythrocytes
NCE: normochromatic erythrocytes
[a]: sum of small and large micronuclei
[b]: large micronuclei (indication for spindle poison effect)
[c]: calculated number of PCEs per 2000 erythrocytes (PCE + NCE) when scoring a sample of up to 10000 PCE per test group
* = $p \leq 0.05$
** = $p \leq 0.01$

III. CONCLUSION

Under the experimental conditions of this study the test substance Reg. No. 4110773 (CL263284; metabolite of BAS 720 H, Imazamox) does not induce cytogenetic damage in bone marrow cells of NMRI mice *in vivo*.

Report:	CA 5.8.1/7 [redacted] 2013a Reg.No. 4110773 (metabolite of BAS 720 H, Imazamox) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2013/1235042
Guidelines:	OECD 407, EPA 870.3050, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of Reg. No. 4110773 (CL 263284, metabolite of BAS 720 H, Imazamox) (L82-116; 97.9%) to Crl:WI(Han) rats at dietary dose levels of 0, 1200, 4000 and 12000 ppm for at least 28 days decreased body weight (- 7.7%; not statistically significantly) and body weight change values (- 17.0%; statistically significantly) in top dose males (12000 ppm; equivalent to 1004 mg/kg bw/d). Clinical examinations, pathology and blood and urine analyses revealed no further treatment-related adverse findings. No treatment-related adverse effects were noted in females at any dose level or in males at lower dose levels. Based on the body weight changes observed in males at 12000 ppm, the NOAEL under the conditions of the present study was 4000 ppm (333 mg/kg bw/d) in male Wistar rats. The NOAEL was 12000 ppm (1028 mg/kg bw/d) for females Wistar rats.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg.No. 4110773 (CL 263284Metabolite of BAS 720 H, Imazamox)
Description:	solid/ beige
Lot/Batch #:	L82-116
Purity:	97.9% (tolerance +/- 1.0%)
Stability of test compound:	The test substance was stable over the study period (Expiry date 01 May 2015).
2. Vehicle control:	Rodent diet

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Sex:	Male and female
Age:	42 ± 1 days at start of administration
Weight at dosing:	♂: 155 g, ♀: 127 g (means)
Source:	Charles River, Germany
Acclimation period:	6 days
Diet:	Kliba maintenance diet for mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, ad libitum
Housing:	In groups (5 animals/cage) in polysulfonate cages (TECNIPLAST, Hohenpeißenberg, Germany), floor area about 2065 cm^2. Motor activity measurements were conducted in Polycarbonate cages and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	15 / h
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 16 Jul 2013 to 14 Nov 2013
(In life dates: 22 Jul 2013 to 20 Aug 2013)

2. Animal assignment and treatment:

Reg.No. 4110773 (Metabolite of BAS 720 H, Imazamox) was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200, 4000 and 12000 ppm for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:

The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. One diet preparation per dose was performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 4 days at room temperature.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration.

Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Name	Concentration [ppm] Mean ± SD#	Nominal concentration [%]	Mean[%]	Relative standard deviation [%]
1200	sample 3	1078.400	89.9		
1200	sample 4	1126.027	93.8		
1200	sample 5	1083.367	90.3	91.3	2.4
4000	sample 6	4549.322	113.7		
12000	sample 7	12646.724	105.4		
12000	sample 8	12251.593	102.1		
12000	sample 9	12166.877	101.4	103.0	2.1

Relative standard deviations of the homogeneity samples in the range of 2.1 to 2.4% indicate the homogenous distribution of Reg.No. 4110773 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 91.3 to 103.0% of the nominal concentrations.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
Body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
Feces, rearing, grip strength forelimbs, grip strength hindlimbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON test (two-sided) for the equal medians

Statistics of clinical pathology

Parameter	Statistical test
Blood parameters	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urinalysis parameters (apart from pH, urine volume, specific gravity, color and turbidity	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urine pH, volume, specific gravity, color and turbidity	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians. Urine color and turbidity are not evaluated statistically.

Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmus
15. feces (appearance/consistency)
16. urine
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption and compound intake:

Individual food consumption was determined weekly over a period of 3 days and calculated as mean food consumption in grams per animal and day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and mean food consumption per cage.

$$\text{Substance intake for day } x = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g) on day x; C as the concentration in the diet on study day x (mg/kg); BW_x as body weight on day x of the study (in g).

4. Water consumption:

Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume.

5. Functional observation battery (FOB):

A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 h. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm height) and observed for at least 2 minutes. The following parameters were examined:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements / stereotypes
5. nasal discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces excreted within two minutes (number/appearance/consistency)
8. posture	17. urine excreted within two minutes (amount/color)
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response	8. behavior during handling
2. touch response	9. vocalization
3. vision (visual placing response)	10. pain perception (tail pinch)
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hind limbs
6. audition (auditory startle response)	13. landing foot-splay test
7. coordination of movements (righting response)	14. other findings

6. Motor activity measurement:

Motor activity (MA) was also measured from 14:00 h onwards on the same day as the FOB was performed. The examinations were performed using the TSE Labmaster System supplied by TSE Systems GmbH, Bad Homburg, Germany. For this purpose, the rats were placed in new clean polycarbonate cages with a small amount of bedding for the duration of the measurement. Eighteen beams were allocated per cage. The number of beam interrupts was counted over 12 intervals for 5 minutes per interval. The sequence in which the rats were placed in the cages was selected at random. On account of the time needed to place the rats in the cages, the starting time was "staggered" for each animal. The measurement period began when the 1st beam was interrupted and finished exactly one hour later. No food or water was offered to the rats during these measurements and the measurement room was darkened after the transfer of the last rat.

7. Hematology and clinical chemistry:

In the morning blood was taken from the retro-bulbar venous plexus from fasted animals. The animals were anaesthetized using isoflurane (Isoba®, Essex GmbH Munich, Germany). The blood sampling procedure and subsequent analysis of blood and serum samples were carried out in a randomized sequence. For urinalysis the individual animals were transferred to metabolism cages (withdrawal of food and water) and urine was collected overnight. Urine samples were evaluated in a randomized sequence

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test)
✓	Hemoglobin (HGB)	✓	Neutrophils (differential)	✓	Platelet count (PLT)
✓	Hematocrit (HCT)	✓	Eosinophils (differential)		
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Differential blood count	✓	Large unstained cells (LUCA)		
✓	Reticulocytes (RET)				

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium (CA)	✓	Albumin (ALB)	✓	Alanine aminotransferase (ALT)
✓	Chloride (CL)	✓	Total bilirubin (TBIL)	✓	Aspartate aminotransferase (AST)
✓	Inorganic phosphate (INP)	✓	Cholesterol (CHOL)	✓	Alkaline phosphatase (ALP)
✓	Potassium (K)	✓	Creatinine (CREA)	✓	γ-glutamyltransferase (GTT)
✓	Sodium (NA)	✓	Globulin (by calculation, GLOB)		
		✓	Glucose (GLUC)		
		✓	Total protein (TPROT)		
		✓	Triglycerides (TRIG)		
		✓	Urea (UREA)		
		✓	Bile acids (TBA)		

8. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin (BIL)	✓	pH
✓	Specific gravity	✓	Blood	✓	Urobilinogen (UBG)
		✓	Glucose (GLU)	✓	Sediment (microscopical exam.)
		✓	Ketones (KET)	✓	Color and turbidity (visual exam.)
		✓	Protein (PRO)		

9. Sacrifice and pathology:

The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓			lachrymal glands[%]	✓		#	Sciatic nerve
✓			aorta	✓			larynx	✓			salivary glands*
✓		#	bone marrow[§]	✓	✓	✓	liver	✓	✓	#	seminal vesicles with coagulating glands
✓	✓	#	brain	✓		#	lung	✓		#	skeletal muscle
✓		#	caecum	✓		#	lymph nodes[#]	✓			skin
✓		#	colon	✓			mammary gland	✓		#	spinal cord (3 levels)[@]
✓		#	duodenum	✓			muscle, skeletal	✓	✓	#	spleen
✓	✓	#	epididymides					✓		#	sternum with marrow
✓			esophagus	✓			nose/nasal cavity	✓		#	stomach (fore- & glandular)
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and ovidcuts	✓	✓	#	testes
✓			femur (with knee joint)	✓			pancreas	✓	✓	#	thymus
			gall bladder	✓			parathyroid	✓	✓	#	thyroid glands
✓		✓	gross lesions	✓			pharynx	✓		#	trachea
✓	✓	#	heart					✓		#	urinary bladder
✓		#	ileum	✓		#	pituitary	✓	✓	#	uterus with cervix
✓		#	jejunum (w. Peyer's patches)	✓	✓	#	prostate	✓		#	vagina
✓	✓	#	kidneys	✓		#	rectum		✓		body (anesthetized animals)

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed and no histopathologically examined; [%] extraorbital,

The organs or tissues were fixed in 4% formaldehyde or in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Whenever the histopathological evaluation of the immunorelevant organs and tissues did not reveal a morphologic alteration of these items and/or whenever no other pathologic finding was noted, these organs were diagnosed as "no abnormalities detected".
Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina. The organs were trimmed according to the "Revised guides for organ sampling and trimming in rats and mice" (Ruel-Fehlert *et al.*, 2003; Kittel *et al.*, 2004; Morawietz *et al.*, 2004). A correlation between gross lesions and histopathological findings was attempted.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section Study Design and Methods B.3 above.

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensorimotor tests and reflexes. Most deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus, were considered to be incidental. No statistically significant differences of overall motor activity between control and treated animals were observed.

4. Body Weight and Body Weight Gain

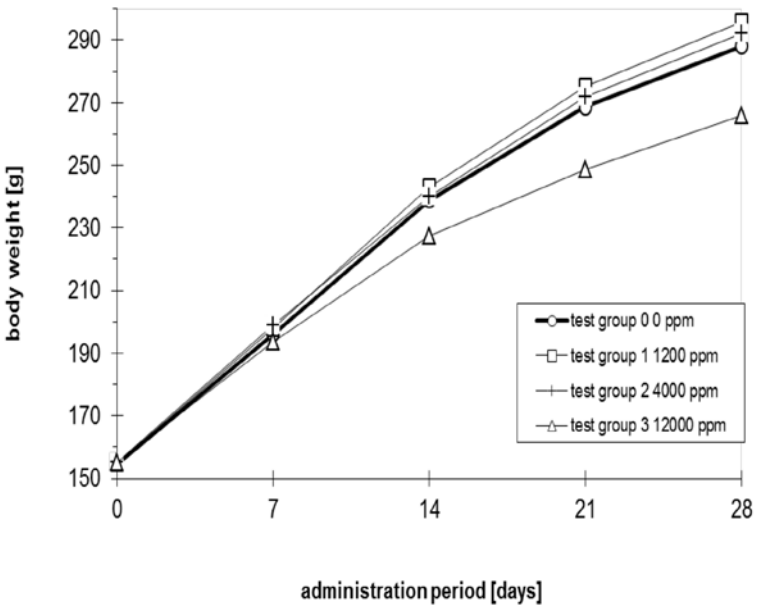
At 12000 pm in male animals, mean body weight were lower (not significantly) on study days 21 and 28 compared to control. However, the respective mean body weight changes were significantly lower in males on study days 21 and 28 ($p \leq 0.05$).
These findings were assessed as being related to treatment. No changes in body weight parameters were observed for male animals treated with 1200 or 4000 ppm. The same was true for female animals treated with 1200, 4000, and 12000 ppm. The significantly lower mean body weight of female animals treated with 1200 ppm on study day 7 was assessed to be incidental and not related to treatment as neither a dose response-relationship nor a clear trend in body weight development occurred. (seeTable 5.8.1-10 and Figure **5.8.1-2**)

Table 5.8.1-10: Mean body weight and body weight change of rats administered Reg.No. 4110773 for 28 days

	Males				Females			
Dose level [ppm]	0	1200	4000	12000	0	1200	4000	12000
Body weight [g]								
- Day 0	154.7	154.7	155.2	155.2	128.1	125.9	128.4	125.9
- Day 7 (Δ% of control)	196.2	198.1 (1.0)	198.9 (1.4)	193.6 (-1.3)	147.2	**142.0* (-3.5)**	147.0 (-0.1)	146.8 (-0.2)
- Day 14 (Δ% of control)	239.0	243.0 (1.7)	240.1 (0.5)	227.4 (-4.9)	160.9	159.2 (-1.1)	157.4 (-2.2)	160.6 (-0.2)
- Day 21(Δ% of control)	268.6	275.2 (2.4)	271.8 (1.2)	248.8 (-7.4)	174.3	172.0 (-1.3)	176.0 (1.0)	174.6 (0.2)
- Day 28 (Δ% of control)	288.1	295.6 (2.6)	292.1 (1.4)	266.0 (-7.7)	185.5	181.1 (-2.4)	188.5 (1.6)	184.5 (-0.5)
Body weight change d0-d21 [g] (Δ% of control)	113.9	120.5 (5.8)	116.6 (2.3)	**93.6* (-17.9)**	46.1	46.1 (-0.1)	47.6 (3.3)	48.7 (5.5)
Overall body weight gain d0-d28 [g] (Δ% of control)	133.4	141.0 (5.7)	136.9 (2.6)	**110.8* (-17.0)**	57.4	55.2 (-3.8)	60.1 (4.7)	58.6 (2.1)

* p<= 0.05 (Dunnett test, two-sided)

Figure 5.8.1-2: Body weight development of rats administered Reg.No. 4110773 for at least 28 days



5. Food Consumption and Compound Intake

No test substance-related effects on food consumption were observed in male and female animals of all test groups.
The mean daily test substance intake was calculated to have been 102, 333, and 1004 mg/kg bw/d in males and 104, 339, and 1028 mg/kg bw/d in females at dietary dose levels of 1200, 4000, and 12000 ppm, respectively.

6. Water Consumption

No test substance-related effects on food consumption were observed in male and female animals of all test groups.

7. Blood Analysis

a. Hematological findings

At the end of the study red blood cell (RBC) counts were significantly lower in male animals treated with 1200 ppm. Treatment with 1200 and 4000 ppm resulted in significantly increased relative large unstained cell (LUC) counts in male animals compared to controls. Both parameters were not dose-dependently changed and therefore, these alterations were regarded as incidental and not treatment-related.

b. Clinical chemistry findings

At the end of the study, inorganic phosphate levels were significantly increased in male animals treated with 1200 ppm but the alteration was not dose-dependent and, therefore, this change was regarded as incidental and not treatment-related. In female animals urea levels were significantly higher after treatment with 12000 ppm compared to controls. This was the only changed parameter in these individuals and therefore, this alteration was regarded as treatment-related, but not adverse.

8. Urinalysis

No treatment-related changes of urinalysis parameters were observed.

9. Plasmakinetics

Reg.No. 4110773 was detectable in all plasma samples of treated animals.

C. NECROPSY

1. Organ weight

Terminal body weights of treated rats displayed no statistically significant differences to the controls (Table **5.8.1-11**). The mean absolute weights of the liver and testes were significantly increased in males at the 1200 ppm dose group compared to the control group. Relative (to body weight) testes weights were significantly increased in males at 1200 ppm. All other weight parameters in male animals and all weight parameters in female animals did not show a significant difference compared to control. In the absence of any dose-response relationship, histomorphological changes as well as clinico-chemical parameters, the increased mean absolute liver weight and the increased mean absolute and relative testes weight observed in male animals at the low dose (1200 ppm) were regarded as incidental and not treatment-related.

Table 5.8.1-11: Selected mean absolute and relative organ weights of rats administered Reg.No. 4110773 for at least 28 days

Sex		Males			
Organ weight	Dose [ppm]	Absolute weight [mg]	% of control	Relative weight [% of bw]	Δ%
Terminal Weight [g]	0	262.86		100	100
	1200	270.24	103	100	100
	4000	267.64	102	100	100
	12000	240.26	91	100	100
Liver [mg]	0	6916		2.636	
	1200	7610	**110***	2.819	107
	4000	7158	103	2.675	102
	12000	6242	90	2.59	98
Testes[mg]	0	2914		1.112	
	1200	3458	**119****	**1.28****	**115****
	4000	3222	111	1.205	108
	12000	3120	107	1.305	117

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis H and Wilcoxon-test, two sided)

2. Gross and histopathology

All findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.

III. CONCLUSION

The administration of Reg.No. 4110773 (CL 263 284, metabolite of BAS 720 H, Imazamox) via the diet to male and female Wistar rats for 4 weeks revealed a treatment-related decrease in body weight in males animals at a concentration of 12000 ppm (1004 mg/kg bw/d) as the only effect observed with regard to clinical examinations. Body weights were decreased, but not significantly, in males at the highest concentration at study weeks 2, 3, and 4 (-4.9%, -7.4% and -7.7%, respectively). Body weight gain decreases were only significant in males at the highest concentration at weeks 3 and 4 (-17.9% and -17.0%, respectively). No other adverse effects were observed on clinical pathology or pathology. Moreover, there were no effects observed in females at 12000 ppm, or at lower dose levels in both sexes.
Therefore, under the conditions of the present study, the No Observed Adverse Effect Level (NOAEL) was 4000 ppm (333 mg/kg bw/d) in male and 12000 ppm (1028 mg/kg bw/d) in female Wistar rats.

CA 5.8.2 Supplementary studies on the active substance

Due to the overall low toxicity of Imazamox and in the absence of target organ toxicity, it was not necessary to conduct any supplementary studies to clarify observed effects.

CA 5.8.3 Endocrine disrupting properties

Considering the complete toxicological database of Imazamox, the active ingredient is of low toxicity and no target organs were identified up to and exceeding the limit dose. There were no effects on endocrine organs or endocrine systems observed in the context of the regulatory toxicological studies. Moreover, in the external literature search no publications of any toxicological relevance were identified. Therefore, Imazamox is not considered to have an endocrine potential and no further studies were performed.

CA 5.9 Medical Data

A general pharmacology study has already been submitted in the first EU dossier and has been evaluated by France (as RMS) and by European authorities. This study was performed to evaluate the potential for and characteristics of acute poisoning of Imazamox and to provide useful information for the treatment of acute poisoning. A short summary of the results is presented in Table 5.9-1.

For convenience of the reviewer a study summary as extracted from the Monograph (1999) is presented below in chapter CA 5.9.7.

Table 5.9-1: Summary of already peer-reviewed general Pharmacology study with Imazamox

Study	Species/ Test System	Findings	References
General pharmacology	ICR mice; Japanese white rabbits	Low potential to produce serious acute poisoning in humans (at dose levels of 0, 78.1, 313, 1250, and 5000 mg/kg bw)	[redacted] (1995) ID-452-001

Moreover, a search in the databases listed below has been performed on September, 9th 2013 for the following terms and no relevant information have been retrieved:

1. Databases searched via DIMDI-Host (Cologne)

Used search terms:
cr=114311-32-9 or ft=imazamox
restricted with pps=human or ct d human or te=human or ut=human

ME66	MEDLINE	NLM
EM74	EMBASE	2005 Elsevier B.V.
EA08	EMBASE Alert	2005 Elsevier B.V.
GA03	gms	gms
BA26	BIOSYS	Thomson Reuters
KA00	Chemikalien und Kontaktallergie	BfR
IA70	IPA	Thomson Reuters
TB69	Toxbio	Thomson Reuters
T165	XTOXLINE	DIMDI
CCTR93	Cochrane Library - Central	Cochrane
CR00	CCRIS (Chemical Carcinogenesis Research Information System)	NCI
AR96	Deutsches Ärzteblatt	

2. Crosscheck via ChemIDplus (chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)
3. Crosscheck via PubMed (www.ncbi.nlm.nih.gov/sites/entrez)
4. GUA-internal literature database “Faust”
5. Regarding the databases HSDB (NLM) and GESTIS (BGIA)
6. Register of the internal medical ward

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of Imazamox. Thus, the medical monitoring programme is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.
The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to Imazamox exposure have not been observed.

Operating personnel would have potential exposure to Imazamox in the site manufacturer unit by:

- Imazamox slurry - tasks: collect slurry samples, prepare equipment for maintenance work
- Imazamox wet cake - tasks: collect wet cake samples, centrifuge cloth cleaning, wet cake conveyor cleaning, table feeder cleaning, dryer loop clean-outs, prepare equipment for maintenance work & campaign changes
- Imazamox dried product - tasks: collect technical product samples, packaging (filling drums or super sacks), product hopper clean-outs, baghouse bags changes & clean-outs, prepare equipment for maintenance work & campaign changes.

Operating personnel expected qualitative exposure rating level for the above tasks is from Low Exposure (Infrequent contact with material at low concentrations and/or Operator works in areas where material is contained in closed system) to Moderate Exposure (Infrequent contact with material at high concentrations and/or Handles low dust potential material regularly without ventilation).

Quality Control Laboratory personnel would have potential exposure to Imazamox in the site laboratory by:

- Sample analysis of Imazamox slurry, Imazamox wet cake, & Imazamox dried product

Laboratory personnel expected Qualitative Exposure level for the above task is Low Exposure (Infrequent contact with material at low concentrations - handling small quantities in laboratory hood).

Over years of Imazamox manufacturing, the site has not had any reports of health effects due to Imazamox.

CA 5.9.2 Data collected on humans

No human cases of intoxication or poisoning deriving from Imazamox are known to BASF.

CA 5.9.3 Direct observations

No data on exposure of the general public are known to BASF.

CA 5.9.4 Epidemiological studies

No epidemiologic studies are available for BASF, nor is BASF aware on any epidemiologic studies performed by third parties.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known.

CA 5.9.7 Expected effects of poisoning

Expected effects were derived for acute and subacute studies in animals. A general pharmacology study has been conducted to evaluate the potential for and characteristics of acute poisoning of Imazamox and to provide useful information for the treatment of acute poisoning.

[redacted] **(1995):** AC 299,263 Technical: General Pharmacology Study; BASF DocID ID-452-001.

Imazamox (97.1% purity; batch AC 6935-63) was tested for its acute toxic potential in groups of 3 male and 3 female ICR mice when administered a single intraperitoneal dose at levels of 0, 78.1, 313, 1250, and 5000 mg/kg bw test substance prepared as a suspension in 0.5% CMC aqueous solution. Additionally, groups of 4 male Japanese white strain of rabbits were dosed once orally with 0 and 5000 mg/kg bw Imazamox.
Findings in mice: In both sexes, intraperitoneal doses of 1,250 and 5,000 mg/kg bw induced decreases in awareness and motor activity, abnormal posture, lack of motor coordination, decreases in muscle tone and reflexes and inhibitory abnormal autonomic signs (decreases in alertness and visual placing; passivity; decreases in spontaneous activity, reactivity, touch response and pain response; staggering gait; decreases in righting reflex, limb tone, grip strenght, body tone, abdominal tone, corneal reflex and ipsilateral flexor reflex; hypothermia; abnormal skin color (cianosis) and decrease in respiratory rate). These signs were noted 30 minutes following administration of Imazamox technical. All mice in the 5000 mg/kg bw dose group died within 6 hours following administration (decreases in respiratory rate, ptosis, and, hypothermia).
In the 1,250 mg/kg bw dose group, mice recovered within 2 days after administration. No abnormal clinical signs were noted at a dose level of 313 mg/kg bw or lower.
Findings in rabbits: Rabbits dosed orally with Imazamox technical at 5,000 mg/kg bw exhibited no abnormal signs with regard to behavior and somatic and autonomic profiles. No changes in bw, respiration, blood pressure, electrocardiogram or heart rate were observed.
Intraperitoneal injection of test material to mice at a dose greater than 1250 mg/kg bw produced non-specific inhibitory abnormal signs (most of which manifested in the lethal dose of 5000 mg/kg bw) which disappeared within 2 days after treatment at 1250 mg/kg bw. Oral administration of 5000 mg/kgbw in rabbits did not produce changes in clinical signs and cardiorespiratory parameters. Therefore, Imazamox induced clinical signs at dose levels close to the lethal dose, which can be reversible. In conclusion, it is suggested that the potential for producing serious acute poisoning in humans is low.

BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
22/July/2014	MCA 6.2.1/5, Interim Report MCA 6.2.1/5 BASF DocID 2013/1251176 (paddy rice metabolism) was replaced by Final Report BASF DocID 2013/1307620	MCP Section 6 BASF DocID2013/1348642 Version 1
24/Feb/2015	MCA 6.2.1/6, BASF DocID ID-640-008 (alfalfa metabolism report) added.	
24/Feb/2015	MCA 6.2.2 and 6.2.3 – added dose levels in mg/kg bw-day units to livestock metabolism studies. BASF DocIDs IA-440-002, ID-440-005, IA-440-001, ID-440-006	
24/Feb/2015	MCA 6.3.2/4 amendment to MCA 6.3.2/3 (2012/1084183) was added	
31/Mar/15	MCA 6.3.2/6 amendment to MCA 6.3.2/5 (2014/1158174) being previously omitted was added	
24/Feb/2015	MCA 6.5.3/4, BASF 2012/1044747 (soybean processing report for CL 189215) added. (erroneously referenced in the dRAR as 2010/1127504 due to a wrong numbering)	
12/Mar/2015	CA 6.3.2/7 BASF Doc IDs 2013/1405201 and CA 6.3.2/8 BASF DocID 2014/1162727added	
24/Feb/2015	MCA 6.6.1/2 amendment to MCA 6.6.1/1 (2013/1085609) was added	
24/Feb/2015	Appendix 1 – Tier 1 Summaries for alfalfa: growth stages at harvest added	
14/Mar/2015	CA 6.7.3 MRL for sunflower amended	

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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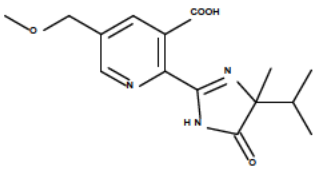
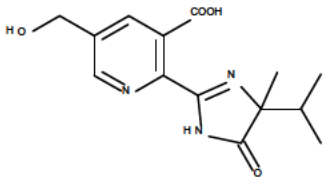
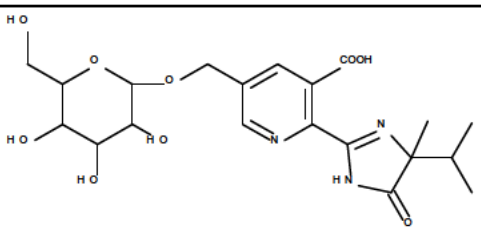
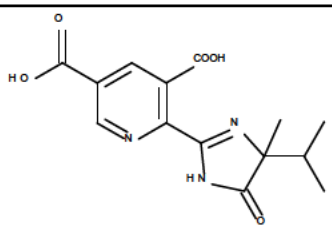
CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

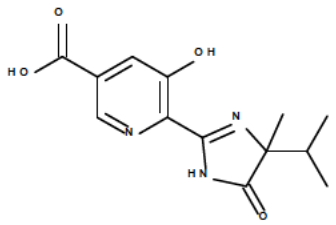
According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. For those studies which are considered still relevant for this Section to give a complete picture of the metabolism and residue behaviour of Imazamox in plants and livestock animals, short summaries have been included for the reviewer's convenience.

Since over time different systems for metabolite designation evolved, the following concordance list has been included summarizing all metabolites observed in plants and livestock studies together with their various designations.

Notations of parent and metabolites of imazamox

Metabolite Designation				Structure/Name
BASF Code/ Synonym (Mol. Weight)	**Reg. No.**	**Metabolite Code**	**CAS-Nr.**	
BAS 720 H CL 299263 (305)	**4096483**	-	**114311-32-9**	
CL 263284 (291)	**4110773**	**M715H001**	**81335-78-6**	
CL 189215 (453.5)	**4110445**	**M715H002**	**200111-50-8**	
CL 312622 (305)	**4110542**	**M720H002**	**146953-32-4**	

Notations of parent and metabolites of imazamox

Metabolite Designation				Structure/Name
BASF Code/ Synonym (Mol. Weight)	Reg. No.	Metabolite Code	CAS-Nr.	
CL 354825 (277)	4110603	-	-	

CA 6.1 Storage stability of residues

Storage stability in frozen crop matrices was evaluated during the initial EU Review of the active substance imazamox by the RMS France. Imazamox has been shown to be stable in soybean seed for at least 24 months when stored frozen at approximately -10ºC. Interim data showed that imazamox and CL 263284 were stable in oil seed rape (canola) seed for at least 12 months when stored deep frozen. Interim data was also given for maize commodities, but only for the 0-day interval since the study had only started and no further data was available at that time.
In the present Annex I renewal dossier, storage stability data are provided for the matrices rape seed, soya bean, and peanut, representing oilseeds as well as in wheat and maize, representing cereal crops; and in alfalfa, representing forage crops. Storage stability could be demonstrated for parent imazamox as well as hydroxy metabolite CL 263284 for all matrices. None of the studies summarized below were included in the original Annex II dossier (1997) and thus were not part of the last peer review for Imazamox.

Wheat

Report:	CA 6.1/1 Bibo X., 2002a Freezer storage stability of CL 299236 and CL 263284 in wheat forage, Hay, straw, and grain 2002/5004279
Guidelines:	EPA 860.1380
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

A freezer storage stability study was performed investigating wheat samples spiked with imazamox (BAS 720 H) and its metabolite CL 263284 (M715H001) at 0.5 mg/kg. The study was performed over a period of 48 months. The samples were analysed for imazamox and CL 263284 with Method M3098, which determines the analytes by means of CE-UV.
The data indicate that residues of imazamox and CL 263284 are stable for at least 48 months in wheat matrices.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Imazamox (CL 299263, BAS 720 H), CL 263284 (M715H001)

Description:
Lot/Batch #: AC9745-124A (imazamox), AC12820-7, AC11026-78 (CL 263284)
Purity: 98.3%, 99.5%, 82%
CAS#: 114311-32-9 (imazamox), 81335-78-6 (CL 263284)
Spiking levels: 0.5 mg/kg

2. Test Commodity:
Crop: Wheat (forage, hay, straw and grain)
Sample size: Not reported

B. STUDY DESIGN

1. Test procedure

The stability of imazamox and its metabolite CL 263284 was investigated under deep frozen conditions over a time period of 48 months for wheat (forage, hay, straw and grain).
Wheat samples were spiked with the test items at a concentration level of 0.5 mg/kg. The spiked samples were stored frozen in glass bottles under the usual storage conditions for residue samples from field studies and analyzed after different intervals (0, 3, 6, 12, 18, 24, 37 and 48 months).

2. Description of analytical procedures

The samples were analysed by Method M 3098 which determines the analytes by means of CE-UV. The validated LOQ of the method is 0.05 mg/kg per compound.
Residues of the test compounds were extracted with acidic methanol followed by clean-up steps which included solid-phase extraction techniques and liquid-liquid partitioning. Measurement of imazamox and CL 263284 was accomplished by capillary electrophoresis (CE) equipped with a high sensitivity flow cell and a UV detector set at 240 nm.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition.
After 18 and 48 months, the mean recovery rate of imazamox residues from wheat hay was below 70%. When correcting the recovery in the stored samples for the respective procedural recovery, mean recoveries of 65% and 80% result. After 24 and 37 months 93% and 74% of imazamox have been recovered. Therefore the results indicate that imazamox is stable frozen in wheat (grain, straw, forage and hay) for at least 48 months.

The residue recovery rate for metabolite CL 263284 was below 70% in grain after 48 months, in straw after 18 and 48 months, in forage after 18 and 48 months and in hay after 6, 12 18 and 48 months. When correcting the recovery in the stored samples for the respective procedural recovery, mean recoveries of 70-94% result. These results indicate that the metabolite CL 263284 is stable frozen in wheat (grain, straw, and forage) for at least 48 months. The tables below present a summary of the recoveries from the stored fortified samples:

Table 6.1-1 Storage stability of imazamox (BAS 720 H) in wheat matrices

Mean Recovery (%)								
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample			
Months	**A**	B	**A**	B	**A**	B	**A**	B
Method M3098								
	Grain		**Straw**		**Hay**		**Forage**	
0	**82**	95	**83**	87	**88**)	93	**80**	86
3	**101**	102	**92**	94	**95**	90	**90**	94
6	**108**	103	**95**	93	**75**	91	**90**	108
12	**109**	100	**99**	92	**76**	98	**88**	93
18	**98**	92	**76**	88	**64** (65)	98	**79**	90
24	**110**	108	**91**	99	**93**	95	**88**	106
37	**87**	92	**80**	98	**74**	88	**91**	99
48	**84**	84	**81**	82	**65** (80)	81	**72**	83

() values in parenthesis were corrected for procedural recovery

Table 6.1-2 Storage stability of metabolite CL 263284 (M715H001)

Mean Recovery (%)								
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample			
Month	**A**	B	**A**	B	**A**	B	**A**	B
Method M 3098								
	Grain		**Straw**		**Hay**		**Forage**	
0	**71**	84	**70**	75	**78**	80	**70**	77
3	**83**	82	**78**	74	**77**	78	**76**	71
6	**97**	85	**81**	82	**62** (75)	82	**75**	85
12	**89**	88	**79**	75	**61** (71)	85	**75**	83
18	**81**	75	**62** (85)	73	**57** (70)	82	**69** (84)	82
24	**98**	94	**81**	89	**76**	92	**82**	91
37	**88**	84	**84**	85	**70**	79	**82**	95
48	**68** (94)	72	**61** (92)	66	**57** (76)	75	**62** (91)	68

() values in parenthesis were corrected for procedural recovery

III. CONCLUSION

Frozen storage stability for imazamox and the metabolite CL 263284 could be proven for at least 48 months in all tested wheat matrices.

The following study has been included to show storage stability data for metabolite CL 189215.

Report:	CA 6.1/2 Nejad H., 1999a CL 263,222 (Imazapic): Freezer stability of residues of CL 263,222, CL 263,284 and CL 189,215 in wheat green forage, wheat hay, wheat straw, and wheat grain IA-730-011
Guidelines:	EPA 40 CFR 158.240, EPA 860.1380
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

The metabolites CL 263284 (M715H001) and CL 189215 (M715H002) are formed by both imazamox (BAS 720 H) and imazapic (BAS 715 H). A freezer storage stability study for imazapic and its metabolites included wheat samples spiked with metabolite CL 263284 and CL 189215 at 0.5 mg/kg. The study was performed over a period of 24 months. The samples were analysed for CL 263284 and CL 189215 with Method M2463, which determines the analytes by means of CE-UV. The data indicate that residues of CL 263284 and CL 189215 are stable for at least 24 months in wheat matrices. Information on imazapic is not reported any further since it is not relevant for this dossier.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** CL 263284 (M715H001), CL 189215 (M715H002)
   Description:
   Lot/Batch #: AC 9745-61A, AC 9087-43A
   Purity: 95.0%, 91.0%
   CAS#: 81335-78-6
   Spiking levels: 0.5 mg/kg

2. **Test Commodity:**
   Crop: Wheat (green forage, hay, straw and grain)
   Sample size: Not reported

B. STUDY DESIGN

1. Test procedure

The stability of the metabolites CL 263284 and CL 189215 was investigated under deep frozen conditions at -10 C or lower over a time period of 24 months for wheat (green forage, hay, straw and grain).
Wheat samples were spiked with the test items at a concentration level of 0.5 mg/kg. The spiked samples were stored frozen in glass bottles under the usual storage conditions for residue samples from field studies (≤-10°C) and analyzed after different intervals (14, 18 and 24 months).

2. Description of analytical procedures

The samples were analysed by Method M2463 which determines the analytes by means of CE-UV. The validated LOQ of the method is 0.1 mg/kg per compound.
Residues of the test compounds were extracted with acidic water-methanol followed by clean-up steps which included precipitation, centrifugation and solid-phase extraction techniques. Measurement of CL 263284 and CL 189215 was accomplished by capillary electrophoresis (CE) equipped with a high sensitivity flow cell and a UV detector set at 240 nm.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition.
The results obtained from the storage of fortified samples indicate that CL 263284 and CL 189215 are stable frozen in wheat (grain, straw, hay and forage) for at least 24 months. The table below presents a summary of the recoveries from the stored fortified samples:

Table 6.1-3 Storage stability of CL 263284 (M715H001) in wheat matrices

Mean Recovery (%)								
A: in stored samples, % of nominal B: procedural, in freshly spiked sample								
Months	**A**	B	**A**	B	**A**	B	**A**	B
Method M2463								
	Grain		**Straw**		**Hay**		**Forage**	
14	**91**	91	**92**	91	**82**	92	**79**	89
18	**102**	104	**93**	98	**75**	89	**97**	87
24	**112**	106	**92**	92	**84**	108	**70**	87

Table 6.1-4 Storage stability of CL 189215 (M715H002) in wheat matrices

Mean Recovery (%)								
A: in stored samples, % of nominal B: procedural, in freshly spiked sample								
Months	**A**	B	**A**	B	**A**	B	**A**	B
Method M2463								
	Grain		**Straw**		**Hay**		**Forage**	
14	**83**	90	**79**	78	**83**	92	**79**	85
18	**82**	98	**80**	89	**77**	95	**84**	95
24	**85**	103	**87**	77	**88**	108	**71**	90

III. CONCLUSION

After 24 months CL 263284 and CL 189215 proved to be stable under frozen storage conditions in all wheat matrices tested.

Maize

Report:	CA 6.1/3 Rawle N.W., 2003a Freezer stability of AC 299263 and CL 263284 in maize grain, ear and immature whole plant samples 2003/1030079
Guidelines:	EEC 7032/VI/95 rev. 5
GLP:	Yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

A freezer storage stability study was performed investigating maize samples spiked with imazamox (BAS 720 H) and its metabolite CL 263284 (M715H001) at 0.5 mg/kg. The study was performed over a period of up to 24 months. The samples were analysed for imazamox and CL 263284 with Method SOP CEM-236/002, which determines the analytes by means of HPLC-UV.
The data indicate that residues of imazamox and CL 263284 are stable for at least 24 months in maize commodities.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Imazamox (BAS 720 H, AC 299263), M715H001 (CL 263284)
Description:
Lot/Batch #: AC9745-124A (imazamox), AC 9745-61A (CL 263284)
Purity: 98.3%, 95.0%
CAS#: 114311-32-9 (imazamox), 81335-78-6 (CL 263284)
Spiking levels: 0.5 mg/kg

2. Test Commodity:
Crop: Maize (grain, ear, immature plant)
Sample size: 20 g (stored sample)

B. STUDY DESIGN

1. Test procedure

The stability of imazamox and the metabolite CL 263284 was investigated under deep frozen conditions over a time period of 24 months. Grain, ears and whole immature plants of maize were spiked with the test items at a concentration level of 0.5 mg/kg. The spiked samples were stored in a cold chamber under the usual storage conditions for laboratory samples (≤-18 °C in the dark) and analyzed after different intervals (0 day, 3, 6, 12, 18 and 24 months).

2. Description of analytical procedures

The samples were analysed by CEMAS SOP CEM-236/002 which determines the analytes by means of HPLC-UV.
Residues of imazamox and CL 263284 were extracted by maceration with extraction solution (methanol/water/1M hydrochloric acid). After filtration, extracts were re-suspended in methanol and hydrochloric acid before they were partitioned into dichloromethane. After evaporation to dryness, the residuum was reconstituted in methanol and acetonitrile before being extracted into hexane. The hexane layer was evaporated to dryness and the residue was redissolved into 0.2% hydrochloric acid. The extracts were cleaned up by solid phase extraction. The quantitation of residues was performed by HPLC-UV at 254 nm.
The method has a quantitation limit of 0.05 mg/kg for each compound in maize.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition.
In the analysis after 12 month less than 70% of imazamox and CL 263284 were recovered in maize ears. However, when correcting the recovery in the stored samples for the respective procedural recovery, mean recoveries of 92% for imazamox and 85% for CL 263284 result. The uncorrected values obtained after 18 and 24 months remained unaffected. In total, the results obtained from the stored fortified samples indicate that imazamox (BAS 720 H) and CL 263284 are stable in maize commodities for at least 24 months when stored under frozen conditions. The tables below present a summary of the recoveries from the stored fortified samples and the procedural recoveries:

Table 6.1-5 Storage stability of imazamox (BAS 720 H) in maize

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
SOP CEM-236/002						
	Grain		**Ear**		**Immature plant**	
0	**86**	82	**86**	89	**80**	100
3	**85**	82	**84**	90	**83**	72
6	**80**	78	**95**	93	**86**	78
12	**71**	75	**69** (92)	75	**85**	81
18	**87**	89	**79**	89	**80**	76
24	**83**	88	**86**	94	**87**	87

()values in parenthesis have been corrected for procedural recovery

Table 6.1-6 Storage stability of metabolite CL 263284 (M715H001) in maize

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
SOP CEM-236/002						
	Grain		**Ear**		**Immature plant**	
0	**75**	72	**77**	75	**75**	87
3	**75**	71	**75**	88	**77**	76
6	**74**	76	**92**	93	**81**	76
12	**71**	71	**64** (85)	74	**80**	74
18	**83**	90	**80**	88	**77**	78
24	**79**	92	**80**	90	**80**	87

() values in parenthesis have been corrected for procedural recovery

III. CONCLUSION

After 24 months, imazamox and CL 263284 proved to be stable under frozen storage conditions in maize matrices.

Soya bean

Report:	CA 6.1/4 Bixler T.A.,Safarpour H., 2000a Freezer stability of residues of CL 299, 263 and CL 263, 284 in soybean commodities (seed, forage and hay) ID-720-070
Guidelines:	EPA 40 CFR 158, EPA 860.1380
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

A freezer storage stability study was performed investigating soya bean seed, forage and hay spiked with imazamox (BAS 720 H) and metabolite CL 263284 at 0.5 mg/kg. The study was performed over a period of up to 44 months. The samples were analysed for imazamox and CL 263284 with method M 2503 which determines the analytes by means of capillary electrophoresis (UV detector).
The data indicate that residues of imazamox and CL 263284 are stable for at least 44 months in all frozen soya bean matrices.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Imazamox (CL 299263, BAS 720 H), CL 263284 (M715H001)
   Description:
   Lot/Batch #: AC9745-124A (imazamox), AC9745-61A (CL 263284),
   Purity: 98.3% (imazamox), 95% (CL 263284)
   CAS#: 114311-32-9 (imazamox), 81335-78-6 (CL 263284)
   Spiking levels: 0.5 mg/kg

2. **Test Commodity:**
   Crop: Soya bean (seed, forage, hay)
   Sample size: Not stated

B. STUDY DESIGN

1. Test procedure

The stability of imazamox and metabolite CL 263284 was investigated under deep frozen conditions over a time period of about 44 months for soya bean (seeds, forage and hay).
Soya bean samples were spiked with the test items at a concentration level of 0.5 mg/kg. The spiked samples were stored in a freezer chamber under the usual storage conditions for laboratory samples (≤-15°C in the dark) and analyzed after different intervals (32, 35, 38, and 44 months.

2. Description of analytical procedures

The samples were analysed by method M2503 which determines the analytes by means of CE-UV.
Residues of a soya bean samples were extracted with acidic water methanol. After filtration, solid phase extraction and liquid-liquid portioning techniques the final quantitation was performed by CE-UV (240 nm). The method has a quantitation limit of 0.5 mg/kg for each compound in soya bean.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition. Some values below 70% recovery rate have been obtained for CL 263284 in forage and hay. When correcting the recovery in the stored samples for the respective procedural recovery, mean recoveries of 76-86% result. After 33 and 38 months the uncorrected mean recoveries from the same samples in forage were >70%. In hay the uncorrected mean recoveries were 73% after 44 months. Therefore the results obtained from the stored fortified samples indicate that imazamox and CL 263284 are stable in frozen soya bean (seeds, forage and hay) for at least 44 months. The tables below present a summary of the recoveries from the stored fortified samples and the procedural recoveries:

Table 6.1-7 Storage stability of imazamox (BAS 720 H) in soya bean matrices

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
Method M2503						
	Seed		**Forage**		**Hay**	
32	**119**	78	**91**	79	**92**	87
33	**134**	87	**109**	89	**112**	93
38	**124**	90	**105**	90	**106**	90
44	**124**	88	**100**	85	**107**	86

Table 6.1-8 Storage stability of metabolite CL 263284 in soya bean matrices

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
Method M2503						
	Seed		**Forage**		**Hay**	
32	**80**	74	**61** (83)	74	**57** (76)	75
33	**91**	79	**73**	74	**64** (81)	79
38	**86**	85	**70**	81	**69** (83)	83
44	**80**	79	**66** (86)	77	**73**	84

() values in parenthesis were corrected for procedural recovery

III. CONCLUSION

After 44 months, imazamox and CL 263284 proved to be stable under storage conditions in all soya bean matrices tested.

The following study has been included to show storage stability data for metabolite CL 189215.

Report:	CA 6.1/5 Leite R.,Alves M., 2011a Investigation study of the storage stability of Imazapyr (BAS 693 H), Imazapic (BAS 715 H) and its metabolites CL 263,284 and CL 189,215 in soybean and processed fractions 2011/1207286
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), FAO manual on submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed - Roma 2002 1st edition
GLP:	Yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The metabolites CL 263284 (M715H001) and CL 189215 (M715H002) are formed by both imazamox (BAS 720 H) and imazapic (BAS 715 H). A freezer storage stability study was performed investigating soya bean samples spiked with imazapic (BAS 715 H), imazapyr (BAS 693 H) and metabolites CL 263284 and CL 189215 at 0.1 mg/kg. The study was performed over a period of up to ten months. The samples were analysed for imazapic, imazapyr and metabolites CL 263284 and CL 189215 with Method SOP-PA.0288 which determines the analytes by means of LC-MS/MS.
The data indicate that residues of CL 263284 and CL 189215 are stable for at least ten months in soya bean grain and for at least three months in several processed soya bean matrices. Information on imazapyr and imazapic is not reported any further since it is not relevant for this dossier.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: CL 263284 (M715H001), CL 189215 (M715H002)
Description:
Lot/Batch #: AC11026-78 (CL 263284), AC11951-85 (CL 189215)
Purity: 81.7% (CL 263284), 77.8% (CL 189215)
CAS#: 81335-78-6 (CL 263284), no CAS# (CL 189215)
Spiking levels: 0.1 mg/kg

2. Test Commodity:
Crop: Soya bean (grain, oil, laminated soybean, defatted meal and toasted defatted meal)
Sample size: 2.5-5 g (stored sample)

B. STUDY DESIGN

1. Test procedure

The stability of metabolites CL 263284 and CL 189215 was investigated under deep frozen conditions over a time period of about 10 months for soybean (grains) and over a time period of about 3 months for soybean processed fractions (oil, laminated soybean, defatted meal and toasted defatted meal), all at -20°C or lower.
Soybean samples were spiked with the test items at a concentration level of 0.1 mg/kg. The spiked samples were stored in a cold chamber under the usual storage conditions for laboratory samples (≤-20 °C in the dark) and analyzed after different intervals.

2. Description of analytical procedures

The samples were analysed by internal procedure SOP-PA.0288 which determines the analytes by means of LC-MS/MS.
Residues of a soya bean sample aliquot were extracted with extraction solution (methanol/water/HCl, 60:39:1, v/v/v). Extraction was conducted on a mechanical shaker. After centrifugation of the samples, one aliquot was taken. The aliquot was transferred to a volumetric flask and filled up with solution 1 (MPA (mobile phase A, 0.1% formic acid in water) / MPB (mobile phase B, 0.1% formic acid in methanol), 50:50, v/v). The final determination was performed by HPLC-MS/MS.
The method has a quantitation limit of 0.01 mg/kg for each compound in soya bean.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition.
The results obtained from the stored fortified samples indicate that CL 263284 and CL 189215 are stable frozen in soybean (grains) for at least 10 months, and in its processed fractions (laminated soybean, defatted meal, toasted defatted meal and oil) for at least 3 months. The tables below present a summary of the recoveries from the stored fortified samples and the procedural recoveries:

Table 6.1-9 Storage stability of CL 263284 in soya bean matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
Method SOP-PA.0288										
	Grain		**Laminated soya bean**		**Defatted meal**		**Toasted defatted meal**		**Oil**	
0	**107**	-	**95**	-	**101**	-	**114**	-	**111**	-
1	**101**	103	**98**	108	**105**	107	**112**	104	**86**	102
2	**92**	99	**-**	-	**-**	-	**-**	-	**-**	-
3	**104**	103	**93**	104	**107**	97	**96**	103	**100**	110
7	**75**	89	**-**	-	**-**	-	**-**	-	**-**	-
10	**111**	123	**-**	-	**-**	-	**-**	-	**-**	-

Table 6.1-10 Storage stability of CL 189215 in soya bean matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
Method SOP-PA.0288										
	Grain		**Laminated soya bean**		**Defatted meal**		**Toasted defatted meal**		**Oil**	
0	**107**	-	**90**		**92**	-	**98**	-	**106**	-
1	**94**	108	**86**	111	**85**	126	**88**	126	**83**	100
2	**88**	106	**-**	-	**-**	-	**-**	-	**-**	-
3	**99**	107	**99**	89	**97**	100	**111**	90	**93**	116
7	**94**	103	**-**	-	**-**	-	**-**	-	**-**	-
10	**100**	124	**-**	-	**-**	-	**-**	-	**-**	-

III. CONCLUSION

CL 263284 and CL 189215 proved to be stable under frozen storage conditions in soya bean processed fractions for at least three months and in soya bean grain for a period of at least ten months.

Peanut

The following study has been included to show storage stability data for metabolite CL 189215 in oilseeds.

Report:	CA 6.1/6 Nejad H.,Xu B., 2000c CL 263,222 (Imazapic): Freezer stability of residues of CL 263,222, CL 263,284 and CL 189,215 in peanut hull and nutmeat IA-740-023
Guidelines:	EPA 40 CFR 158.240, EPA 860.1380
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

A freezer storage stability study was performed investigating peanut samples spiked with imazapic (BAS 715 H) and its metabolites CL 263284 (M715H001) and CL 189215 (M715H002) at 1 mg/kg. The samples were analysed for CL 263284 and CL 189215 with Method M2379, which determines the analytes by means of CE-UV. The study was performed over a period of 24 months.
The data indicate that residues of CL 263284 and CL 189215 are stable for at least 24 months in peanut hulls and nutmeat. Information on imazapic is not reported any further since it is not relevant for this dossier.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** CL 263284 (M715H001), CL 189215 (M715H002)
   Description:
   Lot/Batch #: AC 9745-61A (CL 263284), AC 9087-43A (CL 189215)
   Purity: 95% (CL 263284), 91% (CL 189215)
   CAS#: 81335-78-6 (CL 263284), - (CL 189215)
   Spiking levels: 1 mg/kg

2. **Test Commodity:**
   Crop: Peanut (hull, nutmeat)
   Sample size: 20 g (stored samples)

B. STUDY DESIGN

1. Test procedure

The stability of CL 263284 and CL 189215 was investigated under deep frozen conditions over a time period of 24 months. Peanut (hull and nutmeat) samples were spiked with the test items at a concentration level of 1 mg/kg. The spiked samples were stored in glass bottles at -5 to -25°C and analyzed after different intervals (1, 5, 6, 12, 18 and 24 months).

2. Description of analytical procedures

The samples were analysed by Method M2379 which determines the analytes by means of CE-UV.
Residues of the test compounds were extracted with 1 N HCl/water/methanol solution (1:39:60, v/v/v).

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition. The samples from the 1 month interval were analysed by a different laboratory than the other samples.
The results obtained from the storage fortified samples indicate that CL 263284 and CL 189215 are stable frozen in peanut hulls and nutmeat for at least 24 months. Only after 18 months of storage, recoveries of below 70% were found for CL 263284 in hull and nutmeat and for CL 189215 in nutmeat. However, when correcting the recovery in the stored samples for the respective procedural recovery, mean recoveries of 74-80% result. After 24 months of storage, the uncorrected mean recoveries from those samples ranged from 82 to 85%. The tables below present a summary of the recoveries from the stored fortified samples and the procedural recoveries:

Table 6.1-11 Storage stability of CL 263284 (M715H001) in peanut matrices

Mean Recovery (%)				
	A: in stored samples, % of nominal	B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B
Method M2379				
	Hull		**Nutmeat**	
1	**87**	78	**87**	98
5	**122**	113	**83**	92
6	**86**	88	**76**	85
12	**81**	84	**75**	93
18	**68 (80)**	85	**62 (74)**	84
24	**83**	93	**85**	81

() values in parenthesis were corrected for procedural recovery

Table 6.1-12 Storage stability of CL 189215 (M715H002) in peanut matrices

Mean Recovery (%)				
	A: in stored samples, % of nominal	B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B
Method M2379				
	Hull		**Nutmeat**	
1	**81**	78	**80**	87
5	**84**	79	**84**	91
6	**97**	96	**76**	86
12	**84**	86	**78**	96
18	**74**	85	**66 (80)**	83
24	**83**	96	**82**	77

() values in parentheses were corrected for procedural recoveries

III. CONCLUSION

CL 263284 and CL 189215 proved to be stable under frozen storage conditions in peanut hulls and nutmeat for at least 24 months.

Alfalfa

Report: CA 6.1/7
Fletcher J.S., 2001a
CL 299, 263 (Imazamox): Freezer storage stability of CL 299, 263, CL 263, 284 , CL 189, 215 and CL 312, 622 residues in alfalfa seed, forage and hay
ID-326-024
Guidelines: EPA 860.1380
GLP: <none>

Report: CA 6.1/8
Fletcher J.S., 2001b
CL 299, 263 (Imazamox): Freezer storage stability of CL 299, 263, (and its metabolites) CL 263, 284, CL 189, 215 and CL 312, 622 residues in alfalfa seed, forage and hay - Report amendment No. 01
ID-790-014
Guidelines:
GLP: <none>

Executive Summary

A freezer storage stability study was performed investigating alfalfa seed, forage and hay samples spiked with imazamox (BAS 720 H) and its metabolites CL 263284 (M715H001), CL 189215 (M715H002) and CL 312622 (M720H002) at 1.0 mg/kg, while procedural freshly spiked samples were fortified with 0.5 mg/kg. The study was performed over a period of 18 months. The samples were analysed for imazamox, CL 263284, CL 189215 and CL 312622 with Method M 3178, which determines the analytes by means of LC-MS.
The data indicate that residues of imazamox, CL 263284, CL 189215 and CL 312622 are stable for at least 18 months in all investigated alfalfa commodities.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Imazamox (BAS 720 H), CL 263284 (M715H001), CL 189215 (M715H002), CL 312622 (M720H002)

Description:

Lot/Batch #: AC9745-124A (imazamox); AC11026-78 (CL 263284); AC 9087-43A (Cl 189215), AC11951-85 (CL 189215); AC10194-40A (CL 312622)

Purity: 98% (imazamox); 82% (CL 263284); 77% (CL 189215), 91% (CL 189215); 86% (CL 312622)

CAS#: 114311-32-9 (imazamox); 81335-78-6 (CL 263284); - (CL 189215); - (CL 312622)

Spiking levels: 1 mg/kg

2. Test Commodity:

Crop: Alfalfa (seed, forage and hay)

Sample size: 20 g (stored samples)

B. STUDY DESIGN

1. Test procedure

The stability of imazamox (BAS 720 H) and it metabolites CL 263284, CL 189215 and CL 312622 was investigated under deep frozen conditions over a time period of 18 months. Alfalfa seed, forage and hay samples were spiked with the test items at a concentration level of 1.0 mg/kg, while freshly spiked procedural recoveries were spiked at LOQ of 0.5 mg/kg. The spiked samples were stored frozen at -10 to -35° C and analyzed after different intervals (0, 1, 3, 6, 12and 18 months).

2. Description of analytical procedures

The samples were analysed by Method M 3178.01 which determines the analytes by means of HPLC/LSC. The validated LOQ of the method is 0.5 mg/kg per compound.

Residues of the test compounds were extracted with acetone/water/methanol solution (1:1:1, v/v/v) and purified by solid phase extraction (SPE) techniques. Measurement of imazamox, CL 263284, CL 189215 and CL 312622 was accomplished by LC-MS.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the procedural recovery results are given in addition.
The recovery rate for imazamox in forage (after 0 months) and seeds (after 0, 1 and 3 months) was below 70%. When correcting the recovery of stored samples for the respective procedural recovery, mean recoveries of 82-99% result. After 18 months of storage, the uncorrected mean recoveries from those samples ranged from 75 to 84%.
The recovery rate for CL 263284 was below 70% for extractions from hay (after 3 and 6 months), forage (after 0 months) and seeds (after 12 months). When correcting the recovery of stored samples for the respective procedural recovery, mean recoveries of 74-102% result. After 18 months of storage, the uncorrected mean recoveries from those samples ranged from 74 to 84%.
The recovery rate for CL 189215 in hay (after 0, 3, 6 and 12 months), forage (after 0, 6 and 12 months) and seeds (all trials) was below 70%. When correcting the recovery of stored samples for the respective procedural recovery, mean recoveries of 71-97% result, except after 0 and 12 months in seed and after 12 months in hay. Subsequent extractions after 18 months resulted in recovery rates of 80% for hay, 75% for forage and 97% (corrected) for seed.
The recovery rate for CL 312622 was below 70% for extractions from hay (after 1, 3 and 6 months) and seeds (after 3 and 6 months). When correcting the recovery of stored samples for the respective procedural recovery, mean recoveries of 70-87% result. After 18 months of storage, the uncorrected mean recoveries from those samples ranged from 76 to 101%.
The tables below present a summary of the recoveries from the stored fortified samples and the procedural recoveries:

Table 6.1-13 Storage stability of imazamox (BAS 720 H) in alfalfa

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
Method M3178						
	Hay		**Forage**		**Seed**	
0	**79**	81	**59 (89)**	67	**58 (99)**	59
1	**76**	89	**78**	85	**65 (91)**	72
3	**71**	94	**74**	82	**68 (82)**	83
6	**70**	81	**78**	80	**74**	79
12	**75**	85	**75**	81	**76**	90
18	**75**	92	**76**	87	**84**	89

() values in parenthesis were corrected for procedural recovery

Table 6.1-14 Storage stability of CL 263284 (M715H001) in alfalfa

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
Method M3178						
	Hay		**Forage**		**Seed**	
0	**76**	87	**67 (81)**	82	**76**	78
1	**76**	79	**82**	88	**76**	82
3	**68 (78)**	88	**75**	86	**79**	87
6	**69 (102)**	67	**75**	74	**79**	87
12	**82**	87	**73**	78	**63 (74)**	85
18	**84**	98	**78**	91	**74**	72

() values in parenthesis were corrected for procedural recovery

Table 6.1-15 Storage stability of CL 189215 (M715H002) in alfalfa

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
Method M3178						
	Hay		**Forage**		**Seed**	
0	**69 (79)**	87	**66 (86)**	77	**55 (69)**	80
1	**70**	72	**80**	81	**61 (76)**	80
3	**65 (83)**	78	**78**	82	**57 (71)**	81
6	**60 (94)**	64	**66 (83)**	80	**61 (89)**	68
12	**63 (64)**	98	**68 (96)**	71	**59 (67)**	88
18	**80**	91	**75**	75	**61 (97)**	63

() values in parenthesis were corrected for procedural recovery

Table 6.1-16 Storage stability of CL 312622 (M720H002) in alfalfa

Mean Recovery (%)						
	A: in stored samples, % of nominal			B: procedural, in freshly spiked sample		
Months	**A**	B	**A**	B	**A**	B
Method M3178						
	Hay		**Forage**		**Seed**	
0	**70**	68	**81**	85	**71**	82
1	**65 (70)**	93	**83**	94	**68 (87)**	79
3	**67 (78)**	86	**81**	87	**66 (75)**	88
6	**69 (83)**	83	**77**	79	**75**	79
12	**79**	93	**72**	84	**77**	91
18	**101**	93	**76**	84	**77**	82

() values in parenthesis were corrected for procedural recovery

III. CONCLUSION

Imazamox (BAS 720 H) and it metabolites CL 263284, CL 189215 and CL 312622 proved to be stable under frozen storage conditions in alfalfa hay, forage and seeds for at least 18 months.

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Metabolism, distribution and expression of residues in plants

During the initial EU Review of the active substance imazamox the metabolism of imazamox has been studied in soybean, peas, oil seed rape (canola) and maize (corn) with 6-pyridine-^{14}C labelled imazamox. Based on these studies, in all crops a similar metabolic pathway was observed, starting with the demethylation of the 5-methoxymethyl position to form the hydroxymethyl metabolite CL 263,284 (M715H001). This metabolite is subsequently either oxydized to form the dicarboxylic acid metabolite CL 312622 (M720H002) or conjugated to form the glycoside CL 189215 (M715H002). Minor amounts of the hydroxy-acid, CL 354,825 may also be produced. In all cases parent imazamox and hydroxymethyl metabolite CL 263284 were the predominant residues found in edible commodities, while in immature matrices used as forage imazamox parent dominated.

Although these studies with the pyridine label support the assumption that no cleavage of the molecule between the two rings should occur, nevertheless two new metabolism studies have been performed, one in IMI-tolerant oilseed rape and one in IMI-tolerant wheat, with the molecule radiolabelled in the imidazolinone moiety, in order to capture the fate of this part of the molecule as well. Furthermore, these studies additionally provide information on the enantiomer ratio of imazamox, capturing any possible preferential metabolism of individual enantiomers.

In order to provide a complete picture on the metabolic fate of imazamox in oilseed/pulses, cereals and nongrass feed items, not only the two new studies with the imidazolinone label are shown, but also the respective studies with the pyridine ring label (ID-640-009, ID-640-005 and ID-640-008) in these crops are included. These studies were not part of the previous evaluation either.

Additionally, in order to cover the special conditions of imazamox use in paddy rice, a third new metabolism study was conducted in paddy rice (BASF DocID 2013/1307620).

Report: CA 6.2.1/1
Roman Y., 1999a
AC 299, 263: Metabolism of carbon-14 labelled AC 299, 263 in field grown oil seed rape / canola
ID-640-009
Guidelines: EPA 40 CFR 158.240, EPA 860.1300, EEC 91/414
GLP: <none>

Executive Summary

A small plot field study on the metabolic fate of imazamox in oilseed rape was conducted during the oilseed rape-growing season in 1998. The oilseed rape (imidazolinone herbicide tolerant variety 45A71) was treated with either 51.43 g a.s./ha (0.0459 lb a.s./A) or 89.03 g a.s./ha (0.0795 lb a.s./A). Analysis of the total radioactive residues (TRR) in oilseed rape and soil, and the determination of the nature of residues in oilseed rape samples were conducted at the American Cyanamid Company (now BASF). The solution was sprayed evenly over the entire plot of the oilseed rape at the 3 to 4-leaf stage. The spray volume of 280.5 L/ha (or 30 gal/A) and the application timing simulated the actual field use conditions. Oilseed rape plant specimens were sampled directly after the application (0 DAT), foliage was sampled at 22 DAT and the last sampling of seeds and straw took place at 78 DAT. Soil samples were taken 1 day before application, 2 h after application and 90 DAT. Samples were frozen (-10 down to -35°C) and analysed for imazamox and its metabolites.
The total radioactive residues (TRR) in oilseed rape samples declined significantly from 2.208 and 3.943 mg/kg at 0 DAT to 0.040 and 0.130 mg/kg in foliage at 22 DAT, and 0.088 mg/kg and 0.178 mg/kg in straw at 78 DAT, for plots A and B, respectively. The TRR value in straw was higher than those observed in foliage due to the dehydration of the samples. At harvest (78 DAT), the residue level in oilseed rape seed was 0.004 mg/kg and 0.006 mg/kg for plots A and B, respectively. The incurred radioactive residues in the foliage, and straw were largely (80% to 98%) extractable with aqueous methanol followed by acetone-methanol mixture and by acidified methanol. All plant, foliage and straw analysed gave qualitatively similar metabolic profiles. At 0 (plant) and 22 DAT (forage), the unchanged parent compound accounted for 77.8% to 78.2% and 15.5% to 21.3% of the TRR, for the high and low dose, respectively. At a later sampling period (78 DAT), imazamox was extensively metabolised by the oilseed rape plant to the alcohol metabolite CL 263284. The only significant components of the residue in oilseed rape were imazamox and CL 263284. Therefore the proposed metabolic pathway of imazamox in oilseed rape, involves the O-demethylation of the methoxymethyl group to form the hydroxymethyl metabolite (CL 263284) which undergoes further metabolism via oxidation and/or glucose conjugation to yield very minor amounts of the diacid metabolite (CL 312622), and the glucose conjugate (CL 189215). Several polar metabolites were also present. Only a trace of imazamox-derived residue (<0.001 mg/kg) was detected in oilseed rape oil as determined by hexane extraction.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: ^{14}C-pyridine-labelled (6 position) imazamox (imazamox)
^{13}C-pyridine-labelled (6 position) imazamox
Unlabelled imazamox

Lot/Batch #: isotopic mixture: ^{14}C, ^{13}C and ^{12}C: AC 11396-91
Unlabelled AC 9745-124A

Purity: Radiochemical purity: 96.5% of mixture
Specific activity of mixture: 28.00 μCi/mg

CAS#: 114311-32-9

Stability of test compound: The test item was stable over the test period.

2. Test Commodity:

Crop: Oilseed rape
Type: Oilseed
Variety: 45A71
Botanical name: *Brassica napus L.*
Crop parts(s) or processed commodity: Plant, foliage, straw, seed
Sample size: Not relevant

3. Soil: A sandy clay loam was used. The soil physicochemical properties are described below (see Table 6.2.1-1).

Table 6.2.1-1: Soil physicochemical properties

Soil Series	Soil Type	pH	OM %	Sand %	Silt %	Clay %	Moisture at 1/3 bar %	CEC cmol/kg
* NA	**Loam	*** 8.5	**** 3.8	** 32	** 48	** 20	31.3	37.8

* NA not applied ** USDA scheme *** ($CaCl_2$) ****Organic matter calculated as 1.72 x percent organic carbon

B. STUDY DESIGN

The metabolism study was conducted with [^{14}C]imazamox during 1998. The field phase of the study was performed by the Enviro-Quest facilities in Minto, Manitoba, Canada.

1. Test procedure

The in-life phase of the study was conducted by the Enviro-Quest facilities in Minto, Manitoba, Canada in imidazolinone-resistant oilseed rape variety 45A71.
The study consisted of one control and two treated plots. Control plot C measuring 1.2 m by 4.6 m was established for the treatment of the blank formulation without test substance. Treatment plot A (same size as plot C) was established for a single postemergence (POST) treatment with the formulated carbon-14 labelled imazamox at a nominal rate of 51.43 g a.s./ha (or 0.05 lb a.s./A). Treatment plot B (same size as plot C) was established for a single postemergence (POST) treatment with the formulated carbon-14 labelled imazamox at a nominal rate of 89 g a.s./ha (or 0.08 lb a.s./A). The control plot was separated from the treated plot by at least 15.2 m.
Oilseed rape plant specimens were sampled directly after the application (0 DAT), foliage was sampled at 22 DAT and the last sampling of seeds and straw took place at 78 DAT. Soil samples were taken 1 day before application, 2 h after application and 90 DAT. Samples were frozen (-10 down to -35°C) and analysed for imazamox and its metabolites.

2. Description of analytical procedures

Plant: Green plants (0 and 22 DAT) were cut into small pieces and then ground into a fine powder with dry ice. Straw collected at later sampling intervals was first homogenised in dry ice. The seed was manually cleaned of any remaining chaff. The resulting chaff was combined with straw and processed as part of the straw sample. The seed samples were ground into a fine powder. All processed samples were placed in a freezer to allow dissipation of the dry ice prior to analysis.
Soil: Six soil sample cores taken at each sampling interval were analysed as two pairs of triplicates. Soil cores were thawed and cut into sections of 0-8, 8-15, and 15-30, and 30-46 cm for all samples taken, pretreatment, after application (0 DAT), and harvest (78 DAT). The soils were dislodged from each section and common depths from each triplicate were composited. Fresh weights were recorded and the soil was allowed to air dry overnight. The dry weight of each soil sample was taken and the soil was ground with dry ice. The ground soil samples were stored frozen in plastic bags until analysis.
Radioanalysis: Upon complete dissipation of the dry ice, aliquots of the ground plant tissue or soil samples were weighed and were then combusted in triplicate using an oxidizer and an absorber/scintillant mixture. The liberated $^{14}CO_2$ was trapped by an amine and quantitated by liquid scintillation counting (LSC).

Extraction: Finely ground oilseed rape samples were weighed and extracted with methanol/water (80:20, v/v) overnight. The samples were homogenised and then stirred. The ratio of sample to solvent was 1:10. The homogenate was centrifuged and the supernatant was then decanted (Extract 1). The postextraction solid (RRR) was homogenised in additional aqueous methanol. After separation by centrifugation, the supernatant was labelled as Extract 2. The RRR was re-extracted using the same conditions. The supernatant was labelled as Extract 3. The postextraction solid was then extracted once with a solvent mix of methanol/acetone/water (1:1:1, v/v/v) by homogenizing. The methanol/ acetone/water extract was designated as Extract 4. The RRR were then extracted once with 2% HCl in methanol/water (4:1). The extract was designated as Extract 5. The RRR obtained was referred to as RRR I. After drying in the hood, aliquots of RRR I were combusted in triplicate to determine the non-extractable residue by the organic solvents. The combined radioactivity obtained from the five extracts was used to determine the extractability of the C^{14} residues by the organic solvents. The combined extracts of 1, 2, 3 and 4 (neutral organoextractable) were concentrated. Aliquots of the concentrated extract were assayed in triplicate by liquid scintillation to determine the level of radioactivity. After filtering, the concentrated solution was analysed for the radioactive components by HPLC. Oilseed rape seed from Plot B was extracted with hexane. The resulting extract was concentrated. Aliquots of the concentrated extract were assayed in triplicate by liquid scintillation counting.
Enzymatic Treatment: The dry RRR derived from the acidic methanol extraction of oilseed rape straw samples (except the seed sample) containing radioactivity ranging 17% to 19% of the TRR was treated with cellulase. An aliquot of extracted straw was mixed with appropriate amounts of the 0.05 M sodium acetate buffer, pH 5.0 and cellulase was added. The mixture was incubated at 37°C. At the end of the incubation period, methanol was added to the mixture. The mixture was centrifuged to separate the extract from the solid precipitate. The RRR obtained from the enzyme treatment were subjected to 6 N HCl hydrolysis via reflux. After separation of the residual solids from the reaction mixture by centrifugation, the hydrolysates were sampled for radioanalysis by combustion analysis.
HPLC: Extracts were concentrated, assayed by LSC and characterised by reversed phase HPLC.

3. Identification of metabolites

Foliage collected at 22 DAT (plot B) was used as a source for the isolation of metabolites CL 263284 (M3) and CL 312622 (M5). Several HPLC injections were made from the high dose foliage neutral organoextractable fraction. Fractions from these runs were collected. From the pooled fractions an aliquot was assayed by LSC to locate peak radioactivity. Fractions 46 to 48 were combined and evaporated to dryness. Fractions 60 and 61 were combined and evaporated to dryness. Just prior to LC-MS analysis each sample was reconstituted in a small amount of methanol.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

All control samples were subjected to combustion analysis and no ^{14}C above background was detected in these samples. The treated samples were subjected to combustion analysis and the results are presented in Table 6.2.1-2.

Plant: The total radioactive residue (TRR) in the foliage taken at 0 DAT after a single POST treatment of the oilseed rape plants with carbon-14 labelled imazamox was 2.208 and 3.943 mg/kg, for plots A and B, respectively. The level of the radioactive residues declined to 0.040 mg/kg and 0.130 mg/kg at 22 DAT but increased to 0.088 mg/kg and 0.178 mg/kg in the straw presumably due to dehydration. The concentration of the total radioactive residues in the mature seed was relatively low, 0.004 mg/kg and 0.006 mg/kg, for plots A and B, respectively.

Soil: The concentrations of the carbon-14 residues in the top 8-cm layer of the soil taken from the treatment Plot A ranged from 0.075 mg/kg to 0.097 mg/kg, and from treatment Plot B ranged from 0.030 mg/kg to 0.078 mg/kg approximately 5 h after application. The TRR values in the rest of the soil fractions (8-15 and 15-30 cm layers) were found to be <0.001 mg/kg to 0.001 mg/kg. At harvest, the level of the carbon-14 residues in the top 8-cm layer of the soil from the treatment plots declined to range from 0.032 mg/kg to 0.043 mg/kg. The TRR values in the 8-15 cm layer of the soil were approximately 0.01 mg/kg. Total radioactive residues in the rest of the soil fractions (15-30 and 30-46 cm layers) were 0.001 mg/kg to 0.01 mg/kg. The purpose of analyzing soil samples was to demonstrate deposition of [pyridine-6] imazamox related materials on soil. No attempts were made to further characterise the nature of radioactive residues in the soil following application, but rather to use as a guide for soil remediation.

Table 6.2.1-2: Total radioactive residues (TRRs) in oilseed rape plant, foliage, straw, seed and soil samples after application of [^{14}C] imazamox

TRRs in treated oilseed rape plant, foliage, straw, seed and soil				
Matrix	**DAT/ Soil depth [cm]**		**TRR determined by direct combustion [mg/kg]**	
			Plot A (51.43 g a.s./ha)	**Plot B (89.03 g a.s./ha)**
Plant	0		2.208	3.943
Foliage	22		0.040	0.130
Straw	78		0.088	0.178
Seed	78		0.004	0.006
Soil*	-1 DAT	0-8	<0.001	<0.001
		8-15	<0.001	<0.001
		15-30	<0.001	<0.001
		30-46	<0.001	<0.001
	0 DAT	0-8	0.086	0.054
		8-15	0.001	<0.001
		15-30	<0.001	<0.001
		30-46	<0.001	<0.001
	78 DAT	0-8	0.038	0.034
		8-15	0.011	0.011
		15-30	0.007	0.004
		30-46	0.004	0.002

DAT = Days after treatment
* Mean of two values

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The extractabilities of radioactive residues from oilseed rape plant, foliage, straw and seed are summarized in Table 6.2.1-3.

Table 6.2.1-3: Extraction efficiency of residues of [^{14}C]imazamox in oilseed rape samples

Matrix	DAT	TRR calc. *	Distribution of Radioactive Residues				ERR		RRR	
			Neutral Organo-extractable[1]		2% HCL in Methanol					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Plot A: 51.43 g a.s./ha										
Plant	0	2.208	2.142	97.00	0.026	1.17	2.168	98.17	0.040	1.83
Foliage	22	0.040	0.037	92.11	0.001	2.56	0.038	94.67	0.002	5.34
Straw	78	0.088	0.067	75.89	0.004	4.60	0.071	80.49	0.017	19.51
Seed	78	0.004	n.d.		n.d.		n.d.			
Plot B: 89.03 g a.s./ha										
Plant	0	3.943	3.840	97.38	0.049	1.24	3.889	98.62	0.055	1.39
Foliage	22	0.130	0.120	92.04	0.006	4.51	0.126	96.55	0.004	3.45
Straw	78	0.178	0.140	78.42	0.007	4.13	0.147	82.55	0.031	17.44
Seed	78	0.006	0.003	56.96	0.000	5.17	0.003	62.13	0.002	37.86

DAT = Days after treatment
* TRR was calculated as the sum of ERR + RRR
1) The extraction was done with 80% aqueous methanol three times, methanol/acetone/water once.
n.d. = not detected

1. Extraction and characterisation of residues in oilseed rape

Extraction:
The radioactive residues in oilseed rape samples (foliage, straw and seed) were extracted with aqueous organic solvent mixtures that contained methanol, acetone, and 2% HCl in various proportions. The results show that 62% to 99% of the total radioactive residues (TRR) in foliage, straw and seed were extracted. The unextracted radioactivity in the RRR I (residual radioactive residues) from oilseed rape foliage and straw accounted for 1.39% to 19.5% of the TRR. The extractability of the carbon-14 residues from the harvest oilseed rape seed was 62% of the TRR (or 0.004 mg/kg). The radioactivity remaining in the RRR I from seed accounted for 38% of the TRR (or 0.002 mg/kg). The dry straw RRR I samples were each treated with Penicillium cellulase. The enzymes released a small amount (2.01% to 2.46% of TRR) of the bound carbon-14 residues from the RRR. The results suggest that the presence of glycosidic linkages of the residue with endocons in the oilseed rape samples is insignificant. Due to the very low residue levels in the enzyme hydrolysates (0.002 mg/kg to 0.004 mg/kg) no attempts were made to further characterise the nature of the residues. The determination of the residue levels of imazamox and the various radiocomponents in the extracts of foliage, straw and seed was based on total radioactive residues (TRR) obtained by combustion of the sample, the extractability of carbon-14 residues in the oilseed rape samples and the distribution of radioactivity recovered from HPLC analysis of the extract. At 0 DAT, the unchanged imazamox accounted for approximately 78% of the TRR. All other components detected were found to be minor with none exceeding 2.4% of the total residues. The radioprofiles are qualitatively similar for the extracts from foliage, and straw collected at various growth stages. Imazamox was extensively metabolised in the late growing and matured oilseed rape plants. In addition to the unchanged parent compound, three main radioactive components, two polar yeaks/regions as well as many other minor components were separated by the C-18 reversed-phase HPLC.

Characterisation:
To elucidate the nature of the carbon-14 residues, HPLC of the oilseed rape foliage, straw and seed extracts was performed on a reversed-phase HPLC column using an acidic water-acetonitrile gradient system.
Soil: The concentrations of the carbon-14 residues in the top 8-cm layer of the soil taken from the treatment Plot A ranged from 0.075 mg/kg to 0.097 mg/kg, and from treatment Plot B ranged from 0.030 mg/kg to 0.078 mg/kg approximately 5 h after application. The TRR values in the rest of the soil fractions (8-15 and 15-30 cm layers) were found to be <0.001 mg/kg to 0.001 mg/kg. At harvest, the level of the carbon-14 residues in the top 8-cm layer of the soil from the treatment plots declined to range from 0.032 mg/kg to 0.043 mg/kg. The TRR values in the 8-15 cm layer of the soil were approximately 0.01 mg/kg. Total radioactive residues in the rest of the soil fractions (15-30 and 30-46 cm layers) were 0.001 mg/kg to 0.01 mg/kg. The purpose of analyzing soil samples was to demonstrate deposition of [pyridine-6] imazamox related materials on soil. No attempts were made to further characterise the nature of radioactive residues in the soil following application, but rather to use as a guide for soil remediation.

Table 6.2.1-4: Metabolites detected in oilseed rape matrices following foliar application of 51.43 g a.s./ha [^{14}C]-imazamox

Components	Plant (0 DAT)		Foliage (22 DAT)		Straw (78 DAT)		Seed (78 DAT)	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Imazamox	1.726	78.2	0.008	21.3	0.002	2.1	n.d.	
CL 189215	0.028	1.3	0.001	3.1	0.002	1.7	n.d.	
CL 263284	0.053	2.4	0.014	37.4	0.044	49.6	n.d.	
CL 312622	0.045	2.0	0.003	8.6	0.001	1.4	n.d.	
Total identified from ERR	1.852	83.9	0.026	70.4	0.049	54.8	n.d.	
Total characterised from ERR [1)]	0.290	13.1	0.009	21.7	0.019	21.2	n.d.	
Total identified and/or characterised from ERR [1)]	2.142	97.0	0.035	92.1	0.068	76.0	n.d.	
2% HCl in aqueous MeOH (unidentified compounds)	0.026	1.17	0.001	2.56	0.004	4.60	n.d.	
Enzyme-released (cellulase)	n.a.	n.a.	n.a.	n.a.	0.002	2.46	n.d.	
6 N HCL Reflux	n.a.	n.a.	n.a.	n.a.	0.008	9.37	n.d.	
Unextractable (RRR) [2)]	0.040	1.83	0.002	5.34	0.006	6.43	n.d.	
Grand total	2.208	100.0	0.038	100.0	0.0088	98.86	0.004	100

1 ERR = extractable radioactive residue
2 RRR = residual radioactive residue
n.d. = not detected
n.a. = not analysed

Table 6.2.1-5: Metabolites detected in oilseed rape matrices following foliar application of 89.03 g a.s./ha [^{14}C]-imazamox

Components	Plant (0 DAT)		Foliage (22 DAT)		Straw (78 DAT)		Seed (78 DAT)	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Imazamox	3.068	77.8	0.020	15.5	0.003	1.7	0.001	12.6
CL 189215	0.045	1.2	0.002	1.7	0.002	1.1	0.001	20.5
CL 263284	0.104	2.6	0.047	36.0	0.083	46.4	0.000	4.6
CL 312622	0.061	1.6	0.004	3.2	0.001	0.8	0.000	2.1
Total identified from ERR	3.278	83.2	0.073	56.4	0.089	50.0	0.002	39.8
Total characterised from ERR [1)]	0.561	14.2	0.046	35.7	0.050	28.4	0.002	17.4
Total identified and/or characterised from ERR [1)]	3.839	97.4	0.119	92.1	0.139	78.4	0.004	57.2
2% HCl in aqueous MeOH (unidentified compounds)	0.049	1.24	0.006	4.51	0.007	4.13	0.000	5.17
Enzyme-released (cellulase)	n.a.	n.a.	n.a.	n.a.	0.004	2.01	n.a.	n.a.
6N HCL Reflux	n.a.	n.a.	n.a.	n.a.	0.013	7.40	n.a.	n.a.
Unextractable (RRR) [2)]	0.055	1.39	0.004	3.45	0.010	5.67	0.002	37.86
Grand total	3.943	100.03	0.130	100.06	0.173	97.61	0.006	100.23

[1] ERR = extractable radioactive residue
[2] RRR = residual radioactive residue
n.d. = not detected
n.a. = not analysed

2. Identification and quantification of extractable residues in oilseed rape

The nature of the main radioactive components in the extracts of oilseed rape samples was first characterised by matching the HPLC profile with the available synthetic reference compounds. Identification of metabolites was then accomplished by isolating the radiocomponents from the oilseed rape foliage extract. Structural characterisation of the isolated metabolites was confirmed by mass spectrometric analysis. For LC-MS analysis of the HPLC isolate corresponding to imazamox, a peak was observed at approximately 7.4 min. This peak generated an ion doublet at m/z $306^+/307^+$. These ions correspond to the $(M+H)^+$ ions resulting from approximately equimolar mixture of unlabelled and C^{13} labelled imazamox. For LC-MS analysis of the HPLC isolate corresponding to CL 263284 a peak was observed at approximately 6.0 min. This peak generated an ion doublet at m/z $292^+/293^+$ corresponding to the $(M+H)^+$ ions of CL 263284.

3. Proposed metabolic pathway

The proposed metabolic pathway of imazamox in oilseed rape, involves the O-demethylation of the methoxymethyl group to form the hydroxymethyl metabolite (CL 263284) which undergoes further metabolism via oxidation and/or glucose conjugation to yield very minor amounts of the di-acid metabolite (CL 312622), and the glucose conjugate (CL 189215). Several polar metabolites were also present. Only a trace of imazamox-derived residue (<0.001 mg/kg) was detected in the oilseed rape oil as determined by hexane extraction. The proposed metabolic pathway is shown in Figure 6.2.1-1.

4. Storage stability

All samples were stored in a freezer at approximately -20°C until used. The crop samples were processed, extracted, and analysed within 9 to 401 days of harvest.

III. CONCLUSION

A single post application of [^{14}C]imazamox at the application rate of 51.43 g a.s./ha (or 0.0459 lb a.s./A) or 89.03 g a.s./ha (or 0.082 lb a.s/A) was applied to IMI-tolerant oilseed rape at the 3-4 leaf stage. The total radioactive residues (TRR) in the oilseed rape samples declined significantly from 2.208 and 3.943 mg/kg at 0 DAT to 0.040 and 0.130 mg/kg in foliage at 22 DAT, and 0.088 mg/kg and 0.178 mg/kg in straw at 78 DAT, for plots A and B, respectively. The TRR value in straw was higher than those observed in foliage due to the dehydration of the samples. At harvest (78 DAT), the residue level in oilseed rape seed was 0.004 mg/kg and 0.006 mg/kg for plots A and B, respectively. The incurred radioactive residues in foliage, and straw were largely (80% to 98%) extractable with aqueous methanol followed by acetone-methanol mixture and by acidified methanol. All plant, foliage and straw analysed gave qualitatively similar metabolic profiles. At 0 and 22 DAT, the unchanged parent compound accounted for 77.8% to 78.2% and 15.5% to 21.3% of the TRR, for the high and low dose, respectively. At a later sampling period (78 DAT), imazamox was extensively metabolised by the oilseed rape plant to the alcohol metabolite CL 263284. The only significant components of the residue in oilseed rape were imazamox and CL 263284. Therefore the proposed metabolic pathway of imazamox in oilseed rape (shown in Figure 6.2.1-1) involves the O-demethylation of the methoxymethyl group to form the hydroxymethyl metabolite (CL 263284) which undergoes further metabolism via oxidation and/or glucose conjugation to yield very minor amounts of the di-acid metabolite (CL 312622) and the glucose conjugate (CL 189215). Several polar metabolites were also present. Only a trace of imazamox-derived residue (<0.001 mg/kg) was detected in oilseed rape oil as determined by hexane extraction.

Report:	CA 6.2.1/2 Radzom M., 2013a Metabolism of 14C-Imazamox in rapeseed 2011/1281377
Guidelines:	EPA 860.1300: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1000: Background - PMRA Section 97.2 (Canada): Residue Chemistry Guidelines: Plants and Lifestock (June 1997), JMAFF No 59 NohSan No 4200, BBA IV 3-2, Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft), OECD 501
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A metabolism study was conducted to investigate the amount of imazamox residues and the nature of its degradation products in summer oilseed rape after foliar application. Oilseed rape (imidazolinone herbicide tolerant variety Salsa CL) was treated once with ^{14}C, ^{15}N-labelled imazamox (labelled in the imidazolinone ring) at growth stage BBCH 10-18 with a nominal application rate of 75 g a.s./ha in combination with the adjuvant DASH. Rape forage was sampled 12 days after treatment at BBCH growth stage 39. Samples of seed, straw and hull were collected 90 days after treatment (BBCH 89).

The highest levels of total radioactive residues (TRR) were found in oilseed rape hull with 2.527 mg/kg followed by straw with 1.134 mg/kg. Lower levels of 0.889 mg/kg and 0.152 mg/kg were detected in forage and seed, respectively. For the investigations of metabolic patterns, subsamples of the four oilseed rape matrices forage, straw, hull and seed were extracted with methanol and water. The extractability of the radioactive residues was very high for forage (97.7% TRR), high for straw and hull (77.6% and 78.8% TRR) and moderate for seed (58.3% TRR). The residual radioactive residues after solvent extraction (RRR) were 2.3% of the TRR for forage, 22.4% and 21.2% TRR for straw and hull, and 41.7% TRR for seed. The residues of straw, hull and seed after solvent extraction were further solubilised by a sequential solubilisation procedure with aqueous ammonia, protease (only seed) and a mixture of macerozyme and cellulase, whereby 3.2% to 11.9% TRR were additionally released within the single solubilisation steps. Altogether 7.7% to 26.5% TRR were released by the sequential solubilisation procedures from straw, hull and seed. Parent compound imazamox was detected in oilseed rape forage only at levels of 0.373 mg/kg or 41.9% TRR. In all oilseed rape matrices metabolite CL 263284, which is generated by demethylation of parent imazamox, was the main component identified. CL 263284 was detected in concentrations of 0.476 mg/kg (53.5% TRR) in forage, 0.496 mg/kg (43.7% TRR) in straw, 1.776 mg/kg (70.3% TRR) in hull and 0.047 mg/kg (31.0% TRR) in seed, respectively. Additionally, dicarboxylic acid CL 312622, which resulted from oxidation of CL 263284, was found in forage at 0.076 mg/kg (8.6% TRR), in straw at 0.296 mg/kg (26.2% TRR), in hull at 0.137 mg/kg (5.4% TRR) and in seed at 0.004 mg/kg (2.8% TRR). In order to investigate whether a cleavage of the imidazoline moiety from the parent molecule occurs, the reference item dimethylhydantoin was analysed by HPLC to identify chromatographic properties of such degradation products.

However, according to retention time comparison, no such cleavage products were observed in the oilseed rape extracts
Enantiomer-specific HPLC analyses were performed to determine whether one enantiomer of the parent compound imazamox was preferentially metabolised in oilseed rape. Analysis of the diluted application solution resulted in an uniformly distributed enantiomer ratio of the test item imazamox (ratio about 50:50), while the enantiomer ratio of imazamox in forage extract was found to be about 30:70 and the enantiomer ratio of its metabolite CL 263284 was about 40:60. The same enantiomer ratio of CL 263284 was found in straw extract (ratio about 40:60). For hull and seed the enantiomer ratio of CL 263284 was approximately 60:40. These results show no significant trend for a stereospecific metabolism, but only slight differences of the plant matrices compared to the application solution.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Imidazolinone-5-^{14}C, 3-^{15}N-labelled imazamox
Lot/Batch #:	1003-1001
Purity:	Radiochemical purity: 98.7% Chemical purity: 95.3% Specific activity: 7.0 MBq/mg
CAS#:	114311-32-9
Stability of test compound:	The test item was stable over the test period.

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	Salsa CL
Botanical name:	*Brassica napus L.*
Crop parts(s) or processed commodity:	Seed, forage, straw, hull
Sample size:	Not relevant

3. Soil: A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.2.1-6).

Table 6.2.1-6: Soil physicochemical properties

Soil Series	Soil Type	pH	OM %	Sand %	Silt %	Clay %	Maximal water holding capacity	CEC[1] cmol/kg
* NA	**sandy loam	*** 7.1	**** 2.5	** 67.1	** 22.1	** 10.8	28.8	12.6

* NA not applied ** USDA scheme *** ($CaCl_2$) ****Organic matter calculated as 1.72 x percent organic carbon
1) Cation exchange capacity

B. STUDY DESIGN

The oilseed rape metabolism study was carried out at the Agricultural Research Center of BASF in Limburgerhof, Germany. The cultivation of the crop and the plant uptake part of the study took place in plastic containers located in climatic chambers (phytotrons). The phytotrons simulated the natural climatic conditions of a typical rapeseed-growing area (virtuel starting date: April 01 at sowing on October 08, 2010 and May 04 at application on November 18, 2010).

1. Test procedure

Oilseed rape (imidazolinone herbicide-tolerant variety: Salsa CL) was sowed into ten containers (total test area, external size = 2.4 m^2) filled with sandy loam soil. The maintenance of the crop was performed in accordance with normal agricultural practice; fertilizers and additional pesticides were applied to achieve an adequate plant growth. Immediately after sowing, the soil was watered. According to the study protocol, the crop was treated once with imazamox at a nominal rate of 75 g a.s./ha (approximately 0.067 Ib/A) at growth stage BBCH 10-18. Twelve days after application forage was sampled at BBCH growth stage 39. Harvest of mature oilseed rape (straw, hull and seed) was accomplished 90 days after treatment.
In order to achieve a nominal target rate of 75 g a.s./ha, calculated amounts of ^{14}C,^{15}N-imazamox and unlabelled ^{12}C-imazamox were weighed and mixed to obtain a ratio of 2:1 (^{14}C,^{15}N-imazamox : ^{12}C-imazamox). Afterwards a mixture of calculated amounts of BAS 720 AC H blank formulation (40 g a.s./kg blank formulation), the adjuvant DASH or BAS 160 00 S (1 L/ha) and water was added and ultrasonic treated to achieve a homogeneous and stable emulsion. The application solution was monitored for purity by HPLC the isotope pattern was determined by mass spectrometry. The application formulation was applied with an automatic spray track at an actual application rate of 75 g a.s./ha (corresponding to a spray volume of 216 L/ha). The application was carried out at BBCH 10-18. Forage samples were taken 12 days after treatment (BBCH 39). At harvest (90 DAT, BBCH 89), mature oilseed rape pods and straw were cut off. Hull and seed were separated using a thresher. All samples were stored in a freezer at approximately -18°C or below. The storage conditions stayed the same until analysis started and during the whole period of the metabolism study. Extracts were stored in a refrigerator or, for longer periods, in a freezer.

2. Description of analytical procedures

Homogenisation
All samples (forage, straw, hull and seed) were homogenised with a mill upon addition of dry ice. After sublimation of the dry ice, the samples were weighed, mixed and divided into aliquots. Appropriate portions of the homogenised plant material were stored frozen. In order to determine the TRR values by combustion analysis, five small aliquots of the homogenised material were combusted to $^{14}CO_2$ and H_2O.

Solvent extraction with methanol and water
Subsamples of homogenised plant material (forage, straw, hull and seed) were extracted with methanol and water. The supernatants were combined yielding the methanol extract and the water extract, respectively. Aliquots of the combined extracts were measured by LSC. The combined results of methanol extractions and water extractions are referred to as extractable radioactive residues (ERR). The residual radioactive residues (RRR) after solvent extraction were dried in a fume hood, homogenised and aliquots were combusted for the determination of radioactive residues.

Solubilisation of the RRR
In order to release the residual radioactive residues after solvent extraction, a sequential solubilisation procedure was applied for the RRR of oilseed rape straw, hull and seed. In general, weighed aliquots and in some cases the entire sample of the RRR were treated under continuous shaking with aqueous ammonia solutions or suspended aqueous buffers and incubated with hydrolyzing enzymes as described below:
Treatment with aqueous ammonia:
The residual radioactive residues after solvent extraction were extracted with aqueous ammonia solution (1%). After centrifugation and decantation the remaining residues were washed with water, the volumes of the supernatants were determined and aliquots were measured by LSC. The remaining insoluble residue was air-dried, homogenised and subjected to enzyme treatments as described below.
Treatment with protease (only seed):
The residue after aqueous ammonia treatment was suspended in TRIS buffer (pH 7.5) and incubated with protease under continuous shaking at 37°C for 48 h. Extract and the residue were separated by centrifugation. The residues were washed with water and again centrifuged. The supernatants were combined, adjusted to a defined volume and analysed by LSC. The remaining residue was homogenised and treated with cellulase and macerozyme as described below.
Treatment with cellulase and macerozyme:
The residues after aqueous ammonia treatment or in case of seed after protease treatment were suspended in sodium-acetate buffer (pH 6) and incubated with a mixture of cellulose and macerozyme R-10. Afterwards the mixture was cooled down to 4°C or room temperature followed by a separation of the extract and the residue by centrifugation. The residues were washed with water and again centrifuged. The supernatants were combined, adjusted to a defined volume and analysed by LSC. The residue was dried, homogenised and radioassayed.

3. Identification of metabolites

Aliquots of concentrated methanol extracts of oilseed rape forage, straw and hull were directly investigated by LC-MS. Analyses resulted in the identification of parent compound imazamox and the two metabolites CL 263284 and CL 312622. Metabolite CL 263284 results from cleavage of the ether group of imazamox. Oxidation of the hydroxyl group of CL 263284 to a carboxyl group results in CL 312622. Peak assignment in the other samples was done by comparing the metabolite patterns with those of the extracts investigated by MS-analysis and by comparison of the retention times of the identified components with the ^{14}C-signals of the quantitative and confirmatory HPLC analyses. Additionally, a co-chromatography experiment with the reference items CL 312622 and CL 263284 was performed with the water extract of oilseed rape straw. In order to investigate, if cleavage of the imazamox ring systems occurs, reference item dimethylhydantoin was studied with respect to its chromatographic properties to check for similar degradation products. According to retention time comparison, in the HPLC chromatograms of oilseed rape matrices no corresponding signals were detected and therefore no formation of cleavage products was observed.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

All control samples were subjected to combustion analysis and no ^{14}C residues above background were detected in these samples (except for foliage: 0.003-0.005 mg/kg). The treated samples were subjected to combustion analysis and the results are presented in Table 6.2.1-7.

Table 6.2.1-7: Total radioactive residues (TRRs) in oilseed rape samples following foliar application of [^{14}C] imazamox

TRRs in treated oilseed rape forage, straw, hull and seed			
Matrix	**DAT**	**TRR determined by direct combustion [mg/kg]**	**TRR calculated [mg/kg] [1]**
Forage	12	1.004	0.889
Straw	90	1.100	1.134
Hull	90	2.488	2.527
Seed	90	0.149	0.152

DAT = days after treatment

[1] Sum of ERR (methanol extract and water extract) and RRR (extraction residue)

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The extractabilities of radioactive residues from oilseed forage, straw, hull and seed are summarized in Table 6.2.1-8.

Table 6.2.1-8: Extraction efficiency of residues of [^{14}C]imazamox in oilseed rape samples

Matrix	DAT	TRR calc.*	Distribution of Radioactive Residues				ERR [1]		RRR [2]	
			Combined Methanol Extract		Combined Aqueous Extract					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Forage	12	0.889	0.823	92.6	0.045	5.0	0.868	97.7	0.021	2.3
Straw	90	1.134	0.573	50.5	0.307	27.1	0.879	77.6	0.254	22.4
Hull	90	2.527	1.458	57.7	0.533	21.1	1.991	78.8	0.536	21.2
Seed	90	0.152	0.036	24.0	0.052	34.3	0.089	58.3	0.063	41.7

DAT = Days after treatment
* TRR was calculated as the sum of ERR + RRR
1 ERR = extractable radioactive residue
2 RRR = residual radioactive residue (after solvent extraction)

1. Extraction and characterisation of residues in oilseed rape

The extractability of oilseed rape forage with methanol and water was very high and accounted for 97.7% TRR with the major part of the residues extracted with methanol (92.6% TRR). The extractability of oilseed rape straw and hull with methanol and water was high with 77.6% and 78.8% TRR, respectively. Again, the major part of the residues was extracted with methanol (50.5% TRR and 57.7% TRR). From rape seed 58.3% TRR were extracted by solvent extraction, whereby more radioactivity was extracted with water (34.3% TRR) than with methanol (24.0% TRR).

Table 6.2.1-9: Metabolites detected in oilseed rape matrices following foliar application of [^{14}C]imazamox

Components	Forage		Straw		Hull		Seed	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Imazamox	0.373	41.9	n.d.		n.d.		n.d.	
CL 263284	0.476	53.5	0.496	43.7	1.776	70.3	0.047	31.0
CL 312622	0.076	8.6	0.296	26.2	0.137	5.4	0.004	2.8
Total identified from ERR [1)]	0.925	104.0	0.792	69.9	1.913	75.7	0.051	33.8
Total characterised from ERR [1)]	0.063	7.1	0.230	20.3	0.261	10.3	0.037	24.2
Total identified and/or characterised from ERR [1)]	0.988	111.2	1.022	90.1	2.174	86.0	0.088	58.0
Unextractable (RRR) [2)]	0.021	2.3	0.131	11.6	0.323	12.8	0.040	26.5
Grand total	1.009	113.5	1.153	101.7	2.496	98.8	0.146	96.0

1 ERR = extractable radioactive residue
2 RRR = residual radioactive residue

2. Identification and quantification of extractable residues in oilseed rape

Forage: Analysis of the methanol extract of oilseed rape forage resulted in a pattern of two major and four minor peaks, of which three were identified by direct LC-MS analysis of the methanol extract. One of the two prominent peaks was identified as metabolite CL 263284 and accounted for 0.476 mg/kg or 53.5% TRR. Parent compound imazamox was the second most abundant component and accounted for 0.373 mg/kg or 41.9%. Metabolite CL 312622 was identified at 8.6% TRR. The remaining components were detected at levels below 1.1% TRR and characterised by their chromatographic properties. The same methanol extract was additionally analysed using HPLC that confirmed the metabolite pattern of the quantitative analysis. The respective water extract and the residue obtained after solvent extraction were not further investigated due to their low amounts of radioactive residues. In the ERR, 104.0% of the total radioactive residues were identified and 7.1% were characterised by HPLC analysis or their extractability with water.

Straw: Analysis of the methanol extract of rape straw resulted in a pattern of one main peak and seven minor peaks. The main peak was identified as metabolite CL 263284 and accounted for 0.398 mg/kg or 35.1% TRR. The metabolite CL 312622 was identified at a level of 0.071 mg/kg or 6.3% TRR. Parent compound imazamox was not detected. Metabolites were identified by direct LC-MS analysis of the methanol extract. The remaining components were detected at levels below 3.7% TRR. The metabolite pattern was additionally confirmed with the same methanol extract. HPLC analysis of the water extract of rape straw yielded a pattern of three peaks. The most abundant peak corresponded to the metabolite CL 312622 (0.212 mg/kg or 8.7% TRR). The second most abundant component was metabolite CL 263284, which accounted for 0.066 mg/kg or 5.8% TRR. Additionally a co-chromatography experiment with the reference items CL 312622 and CL 263284 was performed that confirmed the peak identities. The residue after solvent extraction was further solubilised with ammonia, and a mixture of macerozyme and cellulase. The first solubilisation step with aqueous ammonia solubilised a portion of 0.069 mg/kg (6.1% TRR) of the radioactive residues, which may have been incompletely extracted with methanol and water or weakly associated with insoluble plant material. In the subsequent solubilisation step, the NH_4OH residue was incubated with a mixture of macerozyme and cellulase which released 0.045 mg/kg (4.0% TRR). Analysis of the ammonia extract resulted in the identification of CL 263284 and metabolite CL 312622, which accounted for up to 1.5% of the TRR. In the supernatant obtained after treatment with macerozyme and cellulase, CL 263284 was identified with 1.3% TRR. The metabolite patterns were additionally confirmed with the same extracts. In the ERR of rape straw, 66.0% of the total radioactive residues were identified and 14.4% TRR were characterised by HPLC. In the RRR, additional 3.9% TRR were identified and 5.9% TRR were characterised by their HPLC elution behavior.

Hull: Analysis of the methanol extract of rape hull resulted in a pattern of one main peak and five minor peaks. The main peak was identified as metabolite CL 263284 and accounted for 1.321 mg/kg or 52.3% TRR. The metabolite CL 312622 was identified at a level of 0.084 mg/kg or 3.3% TRR. The remaining components were detected at levels below 1.4% TRR and characterised by their chromatographic properties. The metabolite pattern was confirmed with the same methanol extract. Likewise, in the water extract again the main constituent was CL 263284 at 0.348 mg/kg or 13.8% TRR detected. CL 312622 was identified at 0.049 mg/kg or 1.6% TRR. The remaining five minor peaks were detected at levels at or below 2.0% TRR and characterised by their chromatographic properties.

Analysis of the same water extract confirmed the metabolite pattern of the quantitative analysis. The residue after solvent extraction was further solubilised with ammonia, and a mixture of macerozyme and cellulase. The first solubilisation step with aqueous ammonia solubilised a portion of 0.114 mg/kg (4.5% TRR) and subsequent solubilisation step with macerozyme and cellulase released 0.082 mg/kg (3.2% TRR). Analysis of the ammonia extract resulted in the identification of CL 263284 and metabolite CL 312622, which accounted for up to 2.4% of the TRR. In the supernatant obtained after treatment with macerozyme and cellulase, CL 263284 was identified with 1.8% TRR. The metabolite patterns were additionally confirmed with the same extracts. In the ERR of rape hull, 71.0% of the total radioactive residues were identified and 8.3% TRR were characterised by HPLC. In the RRR, additional 4.7% TRR were identified and 2.0% TRR were characterised by their HPLC elution behavior.

Seed: Analysis of the methanol extract of rape seed resulted in a pattern of two peaks. The peak at 24.8 min was identified as metabolite CL 263284 and accounted for 0.020 mg/kg or 13.3% TRR. One polar component eluted at 3.4 min and was detected at 0.017 mg/kg or 11.1% TRR. The metabolite pattern was confirmed with the same methanol extract. In the confirmatory HPLC chromatogram the peak eluting in the polar region was split up into three components each below or equal to 0.009 mg/kg or 5.9% TRR. For further investigation, the polar fraction was isolated by SPE fractionation and analysed by HPLC. Both chromatograms confirmed that the polar fraction consist of at least two components. In the water extract the main constituent was identified as CL 263284 at 0.027 mg/kg or 17.8% TRR. CL 312622 was identified at 0.004 mg/kg or 2.8% TRR. The remaining non-identified components were detected at levels of up to 0.007 mg/kg or 4.3% TRR. Analysis of the same water extract confirmed CL 263284 as the main component. The residue after solvent extraction was further solubilised with ammonia, protease and a mixture of macerozyme and cellulase. The first solubilisation step with aqueous ammonia solubilised a portion of 0.015 mg/kg (9.8% TRR) and solubilisation with protease and macerozyme / cellulase released 11.9% TRR and 4.8% TRR, respectively. An aliquot of the concentrated protease solubilisate was analysed and some minor components below or equal to 0.003 mg/kg or 1.9% TRR eluting in the polar region were detected. In the ERR, 33.8% of the total radioactive residues were identified and 24.2% were characterised by HPLC. In the RRR, additional 9.4% TRR were characterised by HPLC analysis of the protease supernatant. In addition, 14.6% TRR were characterised by solubilisation with aqueous ammonia and a mixture of macerozyme and cellulase.

Enantiomer Ratio of imazamox and CL 263284

In order to analyse whether one enantiomer of imazamox and CL 263284 was preferably metabolised in oilseed rape, enantiomer-specific analyses were performed in all four matrices (forage, straw, hull and seed). For the test item and the unlabelled reference item CL 263284 the ratio of enantiomer 1 to enantiomer 2 was found to be approximately 50:50. For the determination of the enantiomer ratio in the different matrices, the parent compound imazamox and metabolite CL 263284 were isolated from the methanol extracts and analysed using HPLC. The enantiomer ratio of imazamox in forage was found to be about 30:70 and the enantiomer ratio of its metabolite CL 263284 was found to be between approximately 40:60 in forage and straw and about 60:40 in hull and seed.

3. Proposed metabolic pathway

The proposed metabolic pathway of imazamox in oilseed rape is shown in Figure 6.2.1-1. The key step of the metabolism of imazamox in oilseed rape after foliar application was the cleavage of the methyl ether group (demethylation) resulting in metabolite CL 263284. Subsequently, oxidation of the hydroxyl group of CL 263284 generated the dicarboxylic acid metabolite CL 312622.

4. Extractability of residues according to residue analytical methods

Homogenised subsamples of straw and seed were additionally extracted according to BASF Method No. M 3519, QuEChERS method and DFG S19 method. For straw samples the extractability with BASF Method No. M 3519 (80.2% TRR) and QuEChERS method (64.8%TRR) was comparable to the extractability with methanol and water (77.6% TRR). For seed BASF Method No. M 3519 extracted about one half (31.0% TRR) and QuEChERS method one third (17.2% TRR) of the radioactive residues extracted with methanol and water (58.3% TRR). For both matrices the DFG S19 method extracted the lowest amounts of radioactive residues with 16.9% TRR for straw and 8.0% TRR for seed. HPLC analyses of the straw and seed extracts obtained from extraction with BASF Method No. M 3519, QuEChERS and DFG S19 method (for straw only) showed a similar metabolite pattern compared to methanol extracts used for metabolism investigation, with CL 263284 as the main component. This data demonstrates that among the tested residues methods the data generation method M 3519 has the capability to extract the relevant residues of imazamox in the most appropriate way.

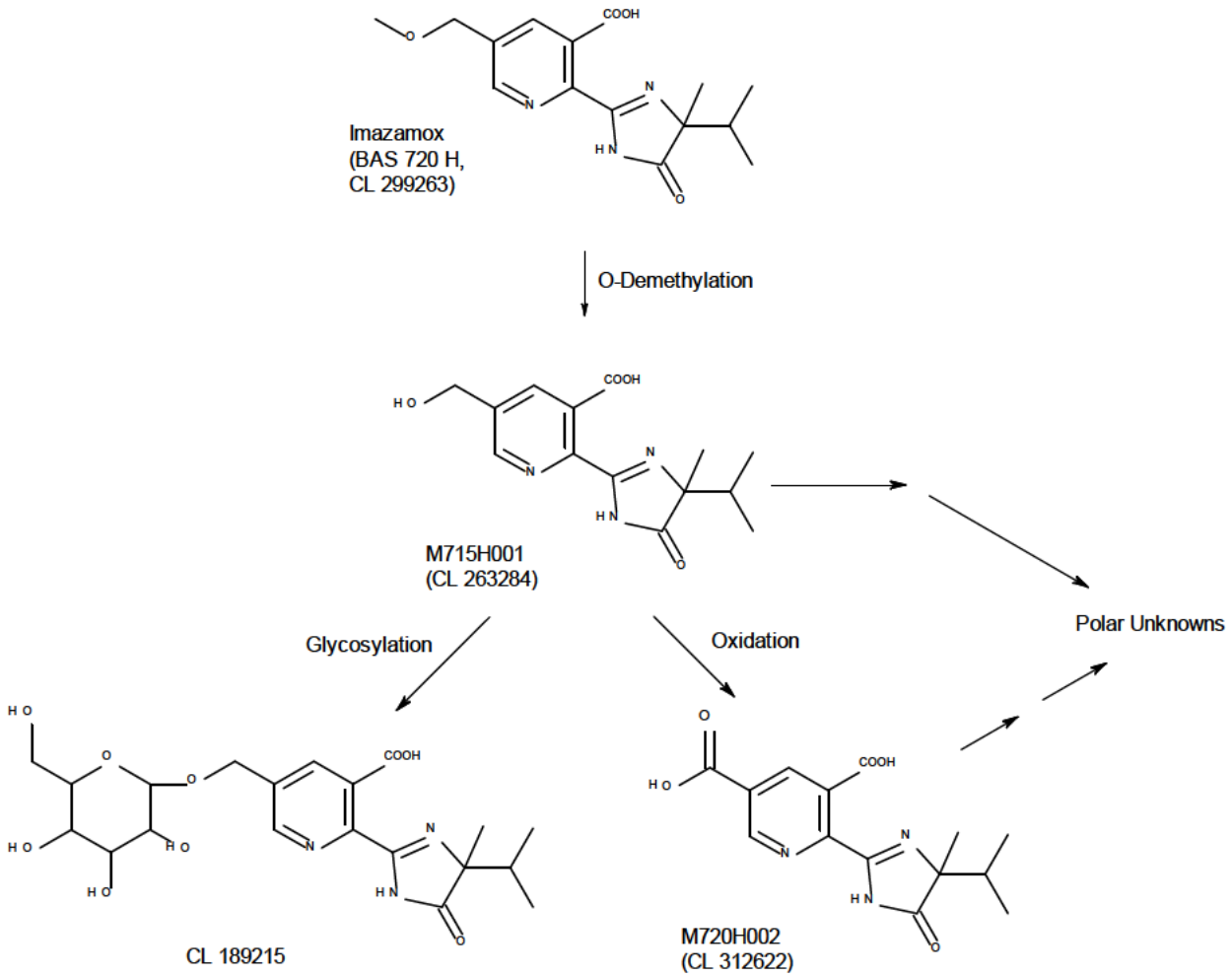
5. Storage stability

Initial analyses of the methanol extracts of oilseed rape straw, hull and seed were carried out within 36 days after sampling and for rape forage within 114 days after sampling. The stored methanol extracts were re-analysed 337 days (about 11 months) after the first analysis. In all cases the metabolite patterns were in very good accordance with those obtained by the initial analyses. The water extract of oilseed rape straw was analysed 338 days after sampling and 470 days after sampling. The water extract was additionally used for co-chromatography experiments, resulting in a storage time of 551 days (about 18 months) after extraction. Based on the latter analyses it can be concluded, that metabolites in the water extract are stable for at least 18 months. The results demonstrated that the metabolite pattern was stable in the solvent extracts over a period of at least 11 months under the chosen conditions. In the water extract of rape straw, metabolites were actually found to be stable for at least 18 months.

III. CONCLUSION

^{14}C,^{15}N-labelled imazamox (labelled in the imidazolinone ring) was applied to oilseed rape (imidazolinone herbicide tolerant variety Salsa CL) by one foliar application at a rate of 75 g a.s./ha at growth stage BBCH 10-18. Forage was sampled 12 days after treatment, while straw, hull and seed were harvested 90 days after treatment. The highest levels of total radioactive residues (TRR) were found in oilseed rape hull accounting for 2.527 mg/kg followed by straw with 1.134 mg/kg. Lower levels of 0.889 mg/kg and 0.152 mg/kg were detected in forage and seed, respectively. The extractability of the radioactive residues with methanol and water was very high for rape forage (>97% TRR) and high for straw and hull (>77% TRR), while moderate extractability was observed in rape seed (about 58% TRR). From rape forage samples the predominant part of the radioactive residues was extracted with methanol (>92% TRR), and only small portions were extracted with water. Methanol extracted about 51% and 58% TRR from straw and hull, while 27% and 21% TRR were extracted with water. For rape seed, the water-soluble fraction was higher (34% TRR) than the portion of radioactive residues extracted with methanol (24% TRR). For identification of metabolites, aliquots of concentrated methanol extracts of rape forage, straw and hull were directly investigated by HPLC-MS. Analyses resulted in the identification of parent compound imazamox and the two metabolites CL 263284 and CL 312622. In all oilseed rape matrices metabolite CL 263284 was the main component identified, representing 31.0 to 70.3% TRR. It was generated by cleavage of the ether bond of parent imazamox. The dicarboxylic acid CL 312622, resulting from oxidation of CL 263284, was found in forage (8.6% TRR), straw (26.2% TRR), hull (5.4% TRR) and seed (2.8% TRR). The parent compound imazamox was detected only in rape forage with 41.9% TRR.
No cleavage of the imidazoline moiety from the parent molecule was observed. Enantiomer-specific HPLC analyses of the enantiomer ratio of the parent compound imazamox and metabolite CL 263284, which were isolated from the methanol extracts of oilseed rape matrices, showed only slight differences of the plant matrices compared to the application solution. Straw and seed samples were additionally extracted according to three methods (M 3519, QuEChERS and DFG S19). For straw samples the extractability with M 3519 and QuEChERS was comparable to the extractability with methanol and water performed in the current study, while for seed about one half and one third of the radioactive residues were extracted with the latter methods. For both matrices the DFG S19 extracted the lowest amounts. HPLC analyses of the straw and seed extracts obtained from extraction with M 3519 and QuEChERS showed, at lower absolute radioactivity amounts, CL 263284 as the main component. This data demonstrates that among the tested residues methods the data generation method M 3519 (using acidic methanol/water solution, identical to the extraction solvent used by the new data generation and proposed enforcement method L0188/01) has the capability to extract the relevant residues of imazamox in the most appropriate way. Storage stability investigations were performed in extracts of all four oilseed rape matrices showing that there was no relevant change in the peak patterns during the storage of sample extracts over a period of at least 11 months.

Figure 6.2.1-1: Proposed Metabolic Pathway of Imazamox in (IMI-tolerant) Oilseed rape



Report: CA 6.2.1/3
Johnson D.H., 1996a
CL 299, 263: Metabolism of carbon-14 labelled CL 299, 263 in wheat under field conditions - Report amendment number 1 (Amendment number 1 included - in the first place)
ID-640-005
Guidelines: EPA 171-4(a)
GLP: <none>

Executive Summary

A small plot field study was conducted on sandy loam soil in Princeton, New Jersey, USA during the spring and summer of 1993. The study was to determine the residues and metabolism of imazamox in imidazolinone-tolerant wheat after a single spray application of [6-pyridine-^{14}C]-imazamox as a post-emergence (POST) treatment. The [6-pyridine-^{14}C]-imazamox was applied at the exaggerated treatment rate of 0.14 kg a.s./ha. Wheat seeds were planted and allowed to grow with supplemental irrigation as needed. The [6-pyridine-^{14}C]-imazamox was sprayed as a postemergence treatment. Formulation blank was applied to the control plot. The spray application simulated actual use conditions. Wheat forage, hay, straw, and grain were harvested 28, 45 and 70 days after treatment (DAT) with [6-pyridine-^{14}C]-imazamox, respectively. Additionally, plant samples were taken the same day of treatment (0 DAT) to demonstrate the application of [6-pyridine-^{14}C]-imazamox to the plants and the stability of the [6-pyridine-^{14}C]-imazamox in the spray solution. No symptoms of imazamox injury where observed on the wheat plants. The total radioactive residues (TRR) in the plant samples were determined by combustion of aliquots followed by liquid scintillation counting (LSC). Initial extractions were done with aqueous methanol. The TRR in the extracts and the residual radioactive residues (RRR) were determined by combustion followed by LSC. LSC was used to analyze fractions from HPLC. The extractability of radioactive residues was 82.3, 75.6, 75.3, and 46.2% for forage, hay, grain and straw samples, respectively. The RRR from the straw sample was further extracted with dilute acid HCl, X-100 surfactant, and 6 N HCl reflux. The identity of the extractable residues components was determined by co-chromatography with known reference compounds on HPLC and mass spectrometry analysis of some of the isolates. These results show that [6-pyridine-^{14}C]-imazamox-derived residues translocated in the wheat plant to all sampled plant parts, except for CL 312622, which was not detected in grain. HPLC results showed the radioactive components consisted of unaltered parent imazamox, the hydroxymethyl metabolite CL 263284, the dicarboxylic acid metabolite CL 312622, and CL 189215, which is the glucose conjugate of CL 263284. CL 312622 or CL 189215 never accounted for 7% of the TRR in any crop sample. Imazamox was the highest-concentration component in hay, forage and grain, while in straw the highest concentration identified component was CL 263284. The unknowns in the samples were mostly highly polar compounds. For the straw sample, the dilute acid, and 6 N acid extracts produced many unidentified polar compounds. The surfactant X-100 extracted only 1% of TRR and was not analysed due to low radioactivity. In summary, wheat plants metabolised imazamox to the hydroxymethyl metabolite CL 263284, which was further metabolised to the glucose conjugate CL 189215 and the dicarboxylic acid compound CL 312622. These results show that low concentrations of imazamox-derived residues may be expected after treatment with imazamox in all wheat tissues studied.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	^{14}C-pyridine-labelled (6 position) imazamox (imazamox) ^{13}C-pyridine-labelled (6 position) imazamox Unlabelled imazamox
Lot/Batch #:	^{14}C-pyridine-label: AC 8873:23 ^{13}C-pyridine-label: AC-GL0090-1 Unlabelled AC 7963:33
Purity:	Radiochemical purity: 96.3% (^{14}C) Radiochemical purity of mixture: 95.7% Specific activity: 80.6 μCi/mg (^{14}C) Specific activity of mixture: 17.9 µCi/mg
CAS#:	114311-32-9
Stability of test compound:	The test item was stable over the test period.

2. Test Commodity:

Crop:	Wheat
Type:	Cereals
Variety:	Fidel selection number 2
Botanical name:	*Triticum aestivum* L.
Crop parts(s) or processed commodity:	Foliage, straw, grain
Sample size:	Not relevant

3. Soil:

A sandy loam was used.

B. STUDY DESIGN

The imidazolinone-tolerant wheat used in this study was grown at a field test site located within American Cyanamid Company Agricultural Products Research Division, Princeton, New Jersey, USA.

1. Test procedure

Wheat (imidazolinone-tolerant; variety: Fidel selection number 2; Lot 92:1214) was planted between 1.3 cm to 2.5 cm (0.5 inch and 1 inch) deep in either five (treatment plot) rows or three (control plot) rows. Rows were spaced 20 cm (8 inches) apart and were 1.8 m (6 feet) long. Six grams of seed (approximately 175 seeds) were used for each row. Emergence was noted seven days after sowing. On the day of application, about six months after sowing, the blank formulation and the [6-pyridine-^{14}C]-imazamox preparation were diluted to 100 mL with water. The blank formulation solution was poured into the sprayer and the container rinsed twice with 50 mL water and the rinses poured into the sprayer. The sprayer was pressurized and the solution was applied to the control plot using a side-to-side motion perpendicular to and parallel to the wheat rows. One hundred mL of water was poured into the sprayer and applied to the control plot (Plot B). The same procedure was used to spray the treatment plot (Plot A), except the [6-pyridine-^{14}C]-imazamox solution was used and three 100-µL samples were removed from the 200 mL of application solution and assayed by liquid scintillation counting (LSC). Wheat tillers (immature wheat foliage cut at ground level) were sampled the day prior to treatment (-1 DAT), approximately 4 hours after treatment (0 DAT), 28 DAT (representing wheat at the forage stage) and 45 DAT (representing wheat at the hay stage). Approximately 5% of the tillers were removed at -1 DAT and 0 DAT; approximately 15% of the tillers were removed at 28 DAT and 45 DAT. At harvest (70 DAT) all ears were removed and threshed using a wheat head thresher to separate the grain from the chaff. Remaining wheat straw was removed by cutting at ground level, and was combined with the chaff separated from the grain. Harvest wheat grain and straw plus chaff were bagged separately. Each plot was divided into four quadrants. Soil cores were taken to approximately 30 cm (12 inches) the day prior to test substance application (-1 DAT) and approximately 3 hours (0 DAT) after application; soil cores were taken to approximately 46 cm (18 inches) at wheat harvest (70 DAT). Soil cores were taken from the control plot and treatment plot. Four soil cores were taken from each plot, one core per plot quadrant. Plant samples (-1 DAT, 0 DAT, 28 DAT and 45 DAT) and soil samples (-1 DAT and 0 DAT) were taken from the field within approximately one hour of sampling and stored in a freezer (approximately -20°C). Harvest wheat grain and wheat straw, including soil samples taken at harvest were taken from the field within approximately four hours of sampling and stored in a freezer (-20°C).

2. Description of analytical procedures

All samples were stored in a freezer at -18°C or below. Extracts were stored in a refrigerator or in a freezer.
Sample preparation: Plant: Immature wheat foliage samples (-1 DAT, 0 DAT, 28 DAT and 45 DAT) as well as harvest wheat grain and wheat straw samples (70 DAT) were ground with dry ice. The ground samples were stored in a freezer and in order to allow the dry ice to sublime. Soil: Soil cores were thawed and cut with a razor into 0 to 8 cm, 8 to 15 cm, 15 to 30 cm (0 to 3, 3 to 6, and 6 to 12 inch) sections for the -1 DAT and 0 DAT samples. For the 70 DAT (wheat harvest) 0-8, 8-15, 15-30 and 30-46 cm sections were cut. The soil cores were pushed from the plastic liners onto aluminum trays. Cores from quadrants 1 and 3 and from quadrants 2 and 4 were combined into two replicate samples (at each depth) for each sampling time. The soil was weighed and air-dried overnight. After air drying, the soil was reweighed and ground in dry ice. The ground soil was placed in a fume hood to allow the dry ice to sublime.
Radioanalysis: Samples were analysed for total radioactive residues (TRR) by oxidation followed by LSC. Subsamples of soil, immature wheat foliage (28 and 45 DAT) and harvest wheat grain or harvest straw were weighed and combusted. Liquid scintillation cocktails used were an absorber and a scintillator. The ^{14}C-residues in the various samples were quantitated by liquid scintillation counting (LSC).
Extraction: Carbon-14 was extracted from immature wheat foliage (0 DAT, 28 DAT (representing forage stage) and 45 DAT (representing hay stage)), harvest wheat grain and harvest wheat straw using the procedures described below. Some subtle modifications to the cleanup procedure were required due to the variable nature of the samples. Prior to extraction, all samples were further ground with dry ice. All samples were stored frozen until extraction and dry ice was allowed to sublime. Extraction of the radioactive residues from the various tissues was accomplished using a homogeniser. Residual radioactive residues (RRR) were also analysed as described above for radioanalysis of plant tissue. Immature wheat **foliage** samples taken 28 DAT represent wheat at the forage stage while samples taken 45 DAT represent wheat at the hay stage. Ground samples of 0 DAT, 28 DAT, or 45 DAT were homogenised with methanol/water (8:2, v/v). The suspensions were centrifuged and supernatants filtered and combined. After drying overnight, the RRR was assayed for radioactive residues by combustion followed by LSC. The RRR samples were stored frozen pending further analysis. The methanol was removed from the filtered supernatants in vaccuo and the aqueous phase was partitioned three times with methylene chloride. The methylene chloride phases were removed and combined and evaporated to dryness. The residue was reconstituted in acetonitrile and filtered. For convenience this isolate was identified as the acetonitrile fraction. The aqueous phase was filtered, and this isolate was identified as the aqueous fraction. Solutions were stored refrigerated pending further analysis.

Ground samples of harvest wheat **grain** and wheat **straw** were homogenised three times with methanol/water (8/2; v/v). The suspensions were centrifuged, supernatants filtered and combined. After drying overnight, the RRR-1 was assayed for radioactive residues by combustion followed by LSC. These samples were stored frozen pending further analysis. The methanol was removed from the filtered supernatants in vaccuo and the aqueous phase was filtered. Solutions were stored refrigerated pending further analysis. Further characterisation of the carbon-14 residues associated with the PES of the harvest wheat straw was necessary. The straw RRR-1 was further extracted using the procedure steps described below. The RRR-1 was extracted with a mixture of methanol/water/concentrated HCl (8:2:0.2, v/v/v). The suspensions were centrifuged, supernatants filtered and combined. After drying overnight, the RRR-2 was assayed for radioactive residues by combustion and subsequent LSC. The methanol was removed from the filtered supernatants in vaccuo and the aqueous portion filtered. The solution (Extract-2) was stored refrigerated pending assay by combustion and subsequent LSC. The RRR-2 remaining from the dilute acid extraction was further extracted with 0.5% Triton X-100 in water. A sample of this solution was added to RRR-2 and this mixture was homogenised once. The homogenate and another part of the Triton X-100 were transferred to a flask. After stirring overnight the slurry was removed and homogenised again. The homogenate was centrifuged and filtered. The solid residue (RRR-3) was allowed to dry and assayed for radioactive residue by combustion followed by LSC. An aliquot of the filtered supernatant (Extract-3) was removed and mixed with 2,2-dimethoxy-propane. A small amount of concentrated HCl was added and the water in the supernatant allowed to react with the 2,2-dimethoxypropane to form methanol and acetone. The solution was neutralized with 7 N NaOH. The methanol and acetone were evaporated in vaccuo. Further 2,2-dimethoxypropane and a small amount of concentrated HCl was again added to remove any residual water. This solution was centrifuged, the supernatant removed and evaporated to dryness. The residue was reconstituted in methanol (Extract-4) and stored refrigerated pending assay by combustion and subsequent LSC. After drying, the RRR-3 was assayed for radioactive residues by combustion and subsequent LSC. A sample of the remaining residue (RRR-3) was further extracted by refluxing with 6 N HCl. The refluxed marc and acid solution were transferred to centrifuge tubes and the round-bottom flask was washed with water and this was centrifuged and the supernatant removed. Solid residue was dried and assayed for radioactivity by combustion followed by LSC. An aliquot of the supernatant was reacted with 2,2-dimethoxypropane to convert the water to acetone and methanol. This was centrifuged and the volume was reduced in vacuo and made up with methanol. This is Extract-4. Samples were stored in a refrigerator and assayed by combustion followed by LSC.
HPLC: Extracts were concentrated, assayed by LSC and characterised/identified by HPLC.

3. Identification of metabolites

The initial identification of radioactive components in the extracts from the wheat samples was done by co-chromatography with analytical standards using reversed-phase HPLC with a C-8 column and isocratic aqueous acetonitrile with H_3PO_4 mobile phase.
A second HPLC system was used with a cyano column and a gradient of aqueous methanol with trifluoroacetic acid mobile phase. Retention times of radioactive peaks in the extracts were compared to analytical standards in this system.
Identification of metabolites was also done by gas chromatography-negative ion chemical ionization mass spectrometric (GC-NICIMS) analysis. In order to perform the mass spectrometric analysis with gas chromatrography, the metabolites had to first be derivatized by methylation. Methylation was done in the GC injection port by the methylating agent trimethylanalinium hydroxide (TMAH). Imazamox, CL 263284 and CL 312622 were analysed in this way. Because carbon-13 was used in the spray solution, doublets with molecular weights corresponding to the methylated carbon-12 and carbon-13 molecules appear in the mass spectrograms.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The ^{14}C-imazamox soil data are presented in Table 6.2.1-10. The TRR in the top 8 cm of soil immediately after treatment (0 DAT) averaged 0.10 mg/kg ^{14}C-imazamox equivalents. Soil sampled at wheat harvest (70 DAT) averaged 0.050 mg/kg ^{14}C-imazamox equivalents in the 0 to 8 cm layer; 0.014 mg/kg ^{14}C-imazamox equivalents in the 8 to 15 cm layer and <0.01 (0.007) mg/kg ^{14}C-imazamox equivalents in the 15 to 30 cm layer. At 0 DAT, the entire recovered radioactivity in the soil cores was located in the top 8 cm of soil at all sample intervals.

The carbon-14 residue data for various wheat matrices are presented in Table 6.2.1-10. The TRR found in 0 DAT immature wheat foliage was approximately 5.6 mg/kg ^{14}C-imazamox equivalents. Carbon-14 residues in 28 DAT (representing forage-stage wheat) and 45 DAT (representing wheat at hay stage) were approximately 0.10 and 0.09 mg/kg ^{14}C-imazamox equivalents, respectively. Most likely plant growth dilution resulted in the rapid decline in residue from the time of application.
Carbon-14 residues in harvest wheat straw and harvest wheat grain (70 DAT) were approximately 0.16 and 0.07 mg/kg ^{14}C-imazamox equivalents, respectively. This increase in residue in straw most probably is a result of dessication of the plants at maturity.

Table 6.2.1-10: Total radioactive residues (TRRs) in wheat foliage, grain, straw and soil samples after application of [^{14}C] imazamox

TRRs in treated wheat foliage, straw, grain and soil			
Matrix	**DAT/ Soil depth [cm]**		**TRR determined by direct combustion [mg/kg]**
Foliage	0		5.57
	28		0.102
	45		0.087
Straw	70		0.157
Grain	70		0.067
Soil*	-1 DAT	0-8	<0.002
		8-15	<0.002
		15-30	<0.002
	0 DAT	0-8	0.101
		8-15	<0.002
		15-30	<0.002
	70 DAT	0-8	0.050
		8-15	0.014
		15-30	0.007
		30-46	0.002

DAT = Days after treatment
* Mean of duplicate values

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The extractabilities of radioactive residues from wheat foliage, straw and grain are summarized in Table 6.2.1-11.

Table 6.2.1-11: Extraction efficiency of residues of [^{14}C]-imazamox in wheat samples

Matrix	DAT	TRR calc. *	Distribution of Radioactive Residues				ERR [1]		RRR [2]	
			Organic Extract		Aqueous Extract					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Plant	0	5.57	5.15	92.5	0.27	4.9	5.42	97.4	0.14	2.6
Forage	28	0.102	0.026	25.6	0.058	56.7	1.084	82.3	0.018	17.7
Hay	45	0.087	0.017	19.6	0.049	56.0	0.066	75.6	0.021	24.4
Grain	70	0.067	0.050**	75.2**	n.d.		0.050	75.2	0.017	24.8
Straw	70	0.157	0.083 [3]	52.5 [3]	0.062 [4]	39.0 [4]	0.145	91.5	0.008	5.0

DAT = Days after treatment
* TRR was determined after combustion
** Aqueos methanol
[1] ERR = extractable radioactive residue
[2] RRR = residual radioactive residue (after solvent extraction)
[3] Sum of aqueous methanol and aqueous methanol, dilute HCl
[4] Sum of X-100 and 6N aqueous HCl, reflux

1. Extraction and characterisation of residues in wheat

The extractability data are calculated as the radioactive residue recovered from the extracts divided by the sum of the radioactive residue in the extracts and from the residual radioactive residues. The radioactive residues found in the 0 DAT, 28 DAT and 45 DAT immature wheat foliage as well as the harvest wheat grain were readily extractable, with approximately 97, 82, 76 and 75% of TRR extracted into methanol/water, respectively. However, only approximately 46% of TRR was extractable from the harvest wheat straw sample.
The RRR from the 28 DAT and 45 DAT immature wheat foliage samples as well as the harvest wheat grain samples contained less than 0.05 mg/kg ^{14}C-imazamox equivalents. No further characterisation was required to be performed on these samples.
Subsequent extraction of the harvest wheat straw RRR with aqueous methanol plus dilute acid released approximately 6% of TRR. An additional 1% of the TRR was released by surfactant extraction. Acid reflux released another 38% of the TRR. The resulting RRR contained only 5.0% of the TRR (<0.05 mg/kg ^{14}C-imazamox) and therefore was not characterised further.
The methanol/water extracts from the 0 DAT, 28 DAT and 45 DAT immature wheat foliage samples as well as the harvest wheat grain and straw samples were examined by HPLC.

The methanol/water extracts from the 0 DAT, 28 DAT and 45 DAT immature wheat foliage and the harvest wheat straw and harvest wheat grain (as well as the extracts isolated from the sequential extraction of the wheat straw RRR) samples were analysed by HPLC to establish the metabolic profile of imazamox. Characterisation of the residues was determined based on comparing the retention times of the residue components to retention times of reference standards. The reference standards used were imazamox, CL 263284, CL 312622 and CL 189215.
Extractability of radioactivity in wheat tissues fortified with [6-pyridine-^{14}C]-imazamox ranged from approximately 86 to 93%. Intact imazamox accounted for approximately 89, 82, 88 and 78% of the extractable radioactivity for 28 and 45 DAT immature wheat foliage, 70-DAT harvest wheat grain, and 70-DAT harvest wheat straw, respectively.
The imazamox peak is the only major peak. There were other peaks at low levels near background. This demonstrates the stability of [6-pyridine-^{14}C]-imazamox during the extraction procedures.

Table 6.2.1-12: Metabolites detected in wheat matrices following foliar application of [^{14}C]-imazamox

Components*	Foliage						Grain (70 DAT)		Straw (70 DAT)	
	0 DAT		28 DAT		45 DAT					
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Imazamox	5.11	91.8	0.042	41.6	0.035	39.9	0.027	40.3	0.015	9.5
CL 263284	n.d.		0.010	9.8	0.008	9.7	0.007	10.3	0.020	13.0
CL 312622	n.d.		0.006	6.2	0.004	5.1	n.d.		0.009	5.7
CL 189215	n.d.		0.004	3.5	0.002	2.3	0.002	3.70	0.004	2.8
Total identified from ERR	5.11	91.8	0.062	61.1	0.049	57.0	0.036	54.3	0.048	31.0
Total characterised from ERR [1)]	0.31	5.6	0.021	21.4	0.015	18.6	0.014	20.9	0.096	60.5**
Total identified and/or characterised from ERR [1)]	5.41	97.4	0.083	82.5	0.064	75.6	0.05	75.2	0.144	91.5
Unextractable (RRR) [2)]	0.14	2.6	0.018	17.7	0.021	24.4	0.017	24.8	0.008	5.0
Grand total [3)]	5.55	100	0.101	100.2	0.085	100	0.067	100	0.152	96.5

* The extracts (methanol + aqueous) for these samples were combined and analysed by HPLC

** For this sample, the unknown component is the sum of the radioactivity not accounted for by the identified compounds in the initial extract and the radioactivity in the dilute acid, X-100 and 6 N acid axtracts of the RRR from the initial extract.
n.d. = not detected

1 ERR = extractable radioactive residue

2 RRR = residual radioactive residue, remaining after extraction with solvents like methanol and water

3 Grand total = total identified and/or characterised from ERR + RRR

2. Identification and quantification of extractable residues in wheat

0 DAT Foliage: HPLC analysis of the organic and aqueous phases of the methanol/water extract showed the predominant compound present had the same retention time as the unaltered parent imazamox. Imazamox accounted for approximately 92% of TRR. Several minor components present at levels near background radioactivity accounted for the remaining radioactivity. No known metabolites (CL 263284, CL 312622, CL 189215) were detected. This again demonstrates the stability of [6-pyridine-^{14}C]-imazamox during the extraction procedures.

28 DAT Foliage: HPLC analysis of the organic and aqueous phases of the 28 DAT immature wheat foliage sample methanol/water extract showed unaltered parent imazamox was present in both phases. There was only one major radioactive peak (at the HPLC retention time of imazamox) in the organic phase. In the aqueous phase, there were four peaks with HPLC retention times corresponding to imazamox, CL 263284, CL 312622 and CL 189215. Combining both the organic and aqueous phases, the methanol/water extract contained 0.042 mg/kg (approximately 42% of the TRR) imazamox. CL 263284 was present at 0.01 mg/kg and accounted for approximately 10% of the TRR. The other two metabolites identified (CL 312622 and CL 189215) were both less than 0.01 mg/kg and each accounted for less than 10% of TRR. Several minor components present at levels near background radioactivity accounted for the remaining radioactivity.

45 DAT Foliage: HPLC analysis of the organic and aqueous phases of the 45 DAT immature wheat foliage sample extract showed unaltered parent imazamox was present in both phases. There was only one major radioactive peak (at the HPLC retention time of imazamox) in the organic phase. In the aqueous phase, there were four peaks with HPLC retention times corresponding to imazamox, CL 263284, CL 312622 and CL 189215. Combining both the organic and aqueous phases, the methanol/water extract contained 0.035 mg/kg (approximately 40% of the TRR) imazamox. The other three metabolites (CL 263284, CL 312622, CL 189215) were all less than 0.01 mg/kg and each accounted for less than 10% of TRR. Several minor components present at levels near background radioactivity accounted for the remaining radioactivity.

70 DAT Grain: HPLC analysis of the extract from the 70 DAT harvest wheat grain sample showed imazamox was present at 0.027 mg/kg, accounting for approximately 40% of the TRR. Two other components (CL 263284 and CL 189215) were also present at less than 0.01 mg/kg; the di-acid metabolite (CL 312622) was not observed. Several minor components present at levels near background radioactivity accounted for the remaining radioactivity.
70 DAT Straw: HPLC analysis of extract from wheat straw showed imazamox, CL 263284, CL 312622 and CL 189215 were present. The metabolite CL 263284 was present in the largest quantity, at 0.020 mg/kg (13.0% of TRR), followed by imazamox at 0.015 mg/kg (9.5% of TRR), and CL 312622 and CL 189215 at less than 0.01 mg/kg (<10% of TRR) each. The dilute acid extract of the RRR from the first extract contained 0.01 mg/kg (6.3% of TRR), and HPLC analysis of this extract showed several minor components at near background levels of radioactivity. One peak was of a polar compound(s) that eluted at the void volume of the column. The X-100 extract contained 1.0% of TRR (<0.01 mg/kg) and was not analysed due to low radioactivity. The 6 N acid reflux extract contained 0.06 mg/kg (38.0% of TRR). HPLC analysis of this extract showed several minor components present at near background levels of radioactivity. One peak was of a polar compound(s) that eluted at the void volume of the column. The total unknowns in the straw sample accounted for 0.096 mg/kg (60.5% of TRR). These were all minor compounds at near background levels of radioactivity present in the extracts.
28 DAT Foliage: HPLC analysis of the aqueous fraction of the 28 DAT immature wheat foliage extract showed radioactive peaks at the retention times of imazamox, CL 263284 and CL 312622 analytical standards. Radioactivity was also detected at the retention time of CL 189215, but this was not resolved adequately from the CL 263284 peak. This confirms the initial identification of the metabolites in this sample.
45 DAT Foliage: HPLC analysis of the aqueous fraction of the 45 DAT immature wheat foliage extract showed radioactive peaks at the retention times of CL 263284, CL 312622 and CL 189215 analytical standards. This confirms the initial identification of the metabolites in this sample. No imazamox was detected in this extract partition. This is from a different extraction of the 45 DAT sample than was used for HPLC with a C-8 column.
70 DAT Grain: HPLC analysis of the 70 DAT harvest wheat grain extract showed radioactive peaks at the retention times of imazamox, CL 263284 and CL 189215 analytical standards. This confirms the initial identification of the metabolites in this sample.
70 DAT Straw: HPLC analysis of the 70 DAT harvest wheat straw extract showed radioactive peaks at the retention times of imazamox, CL 263284 and CL 312622 analytical standards. This confirms the initial identification of the metabolites in this sample. A small amount of radioactivity was detected at the retention time of CL 189215, but this is not resolved from a larger unknown radioactive peak that eluted approximately 1 minute earlier. This is from a different extraction of the straw sample than was used for HPLC with a C-8 column.

3. Proposed metabolic pathway

The proposed metabolic pathway of imazamox in wheat is shown in Figure 6.2.1-2. Wheat plants metabolised imazamox to the hydroxymethyl metabolite CL 263284, which was further metabolised to the glucose conjugate CL 189215 and to the dicarboxylic acid compound CL 312622.

4. Storage stability

Soil, immature wheat foliage, harvest wheat straw and harvest wheat grain samples were removed from the freezer for the combustion and HPLC analyses at times ranging from 4 to 39 days after sampling, except for the 0 DAT plant sample, which was analysed by HPLC 423 days after sampling.
The residue components in the samples appear to be stable for more than 14 months when stored frozen. The 0 DAT sample of treatment plot A wheat foliage showed the only significant component was the parent compound imazamox when extracted and analysed 423 days after the sample was taken.

III. CONCLUSION

This study shows that applied imazamox as post-emergence at 0.14 kg a.s./ha (0.125 lb a.s./A) to wheat will result in residues in wheat forage, hay, grain and straw. Generally, the imazamox-derived residues were low and decreased as time from treatment increased, with 0.102 and 0.087 mg/kg TRR detected in the immature wheat foliage samples taken 28 and 45 DAT, respectively, and 0.067 mg/kg TRR detected in the 70 DAT harvest wheat grain sample. An exception was for the 70 DAT harvest wheat straw sample, where 0.157 mg/kg TRR was detected. This was probably due to the desiccation of the plants before harvest. Imazamox was metabolised in all tissues to the compounds CL 263284, CL 312622 and CL 189215, except for wheat grain, which did not contain CL 312622. Imazamox accounted for approximately 40% of TRR in wheat forage (28 DAT), hay (45 DAT) and grain samples. CL 263284, CL 312622 and CL 189215 each accounted for less than 10% of TRR in these samples, which corresponds to 0.01 mg/kg or less. In the straw sample, imazamox accounted for approximately 9.5% of TRR (0.015 mg/kg), and CL 263284 accounted for 13.0% of TRR (0.020 mg/kg). The other metabolites (CL 312622 and CL 189215) accounted for less than 6% of TRR (<0.01 mg/kg). In summary, wheat plants metabolised imazamox to the hydroxymethyl metabolite CL 263284, which was further metabolised to the glucose conjugate CL 189215 and to the dicarboxylic acid compound CL 312622.

Report:	CA 6.2.1/4 Grosshans F. et al., 2012a Metabolism of 14C-Imazamox in wheat 2012/1064722
Guidelines:	EPA 860.1300: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1000: Background - PMRA Section 97.13 (Canada): Residue Chemistry Guidelines Confined Accumulation in Rotational Crops (June 1997), JMAFF 59 NohSan No 4200, BBA IV 3-2, Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft), OECD 501
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The objective of the present study in spring wheat was to investigate the amount of imazamox (BAS 720 H) residues and the nature of its degradation products after foliar application. The wheat was treated once with ^{14}C,^{15}N-labelled imazamox (labelled in the imidazolinone ring) at growth stage BBCH 13-24 with a nominal application rate of 75 g a.s./ha, in combination with DASH (adjuvant). Forage and hay were sampled 8 days after treatment, while straw and grain samples were harvested 62 days after treatment. The highest levels of the calculated total radioactive residues (TRR) were found in wheat hay accounting for 8.011 mg/kg followed by straw with 3.199 mg/kg. Lower levels of 1.602 mg/kg and 1.412 mg/kg were detected in forage and grain, respectively. For the investigations of metabolic patterns, subsamples of the four matrices forage, hay, straw and grain were extracted with methanol and water. The extractability of the radioactive residues was very high for wheat forage and hay (>91% TRR) and high for straw and grain (>79% TRR). For wheat forage, hay and straw, the predominant part of the radioactive residues was extracted with methanol, and only small portions were extracted with water. For wheat grain, similar amounts of residues were extracted with methanol and water. The residual radioactive residues after solvent extraction (RRR) were below 10% of the TRR for forage and hay, about 20% of the TRR for straw and 13% TRR for grain. The residues after solvent extraction were further solubilised, whereby 4% to 14% TRR were additionally released. Identification of metabolites was mainly based on HPLC-MS investigations of isolated fractions from wheat straw methanol extract. The peak assignment of wheat straw and the other matrices was based on comparison of the retention times of the isolated components with the ^{14}C-signals of the quantitative and confirmatory HPLC analyses. Moreover, the isolated fractions were used for co-chromatography experiments with solvent extracts of wheat forage and grain in order to support the peak assignment. In wheat forage, hay and grain imazamox was the main component identified (62.4 to 76.2% TRR) whereas in wheat straw it represented only 9.3% TRR. In wheat straw metabolite CL 263284, which resulted from a demethylation from the parent compound, was the main constituent (41.0% TRR). CL 263284 was generally the metabolite with the highest concentration (18.6% TRR in forage, 24.1% TRR in hay and 6.5% TRR in grain). The glycosylated form of CL 263284, metabolite CL 189215, was also found in all four matrices (from 1.7 to 15.2% TRR).

The dicarboxylic acid CL 312622, which resulted from oxidation of CL 263284, was found at low levels in forage and hay (up to 7% TRR) and at slightly higher levels in straw (17.5% TRR). In the present study, polar fractions of wheat straw methanol extracts were analysed by HPLC-MS in order to identify putative cleavage of the imidazoline moiety from the parent molecule. However, no such cleavage products were observed. The enantiomer ratio of the parent compound and its metabolite CL 263284 in the methanol extracts remained at approximately 1:1, as it was determined for the radiolabelled test item imazamox. Storage stability investigations were performed in the wheat matrices at the beginning and at the end of the study. For all four matrices a re-analysis of stored extracts was performed. For straw and grain stored samples were additionally re-extracted. Initial analyses of wheat forage, hay, straw and grain for quantification were carried out within a maximum of 160 days after sampling. Nonetheless, the stored extracts were re-analysed approximately one year after quantitative analysis. They were stored 323 to 328 days prior to measurement. In all cases, the later chromatograms showed similar peak patterns as the initial analyses. Additionally, a re-extraction of stored aliquots from straw and grain was carried out after approximately one year (362 days of storage). The resulting extracts were HLPC measured within 9 days. The metabolite patterns were qualitatively and quantitatively comparable to the initial extracts.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	5-^{14}C, 3-^{15}N-imidazolinone-labelled imazamox Unlabelled imazamox
Lot/Batch #:	^{14}C, ^{15}N imazamox: 1003-1001 Unlabelled AC12820-7
Purity:	Radiochemical purity: 98.7% Chemical purity: 95.3% Specific activity: 7.0 MBq/mg
CAS#:	114311-32-9
Stability of test compound:	The test item was stable over the test period.

2. Test Commodity:

Crop:	Spring wheat
Type:	Cereals
Variety:	AP603 CL
Botanical name:	*Triticum aestivum* L.
Crop parts(s) or processed commodity:	Forage, hay, straw, grain
Sample size:	Not relevant

3. Soil: A sandy loam was used. The soil physicochemical properties are described below (see Table 6.2.1-13).

Table 6.2.1-13: Soil physicochemical properties

Soil Series	Soil Type	pH	OM %	Sand %	Silt %	Clay %	Maximal Water holding capacity	CEC[1] cmol/kg
Bruch West	*Sandy Loam	**7.1	***2.5	*67.1	*22.1	*10.8	28.8	12.6

NA not applicable * USDA scheme ** ($CaCl_2$) ***Organic matter calculated as 1.72 x percent organic carbon
1) Cation exchange capacity

B. STUDY DESIGN

The study was carried out at the Agricultural Research Centre of BASF in Limburgerhof, Germany. The plant uptake part of the study was conducted in plastic containers located in climatic chambers. The phytotrons simulated the natural climatic conditions for a typical spring wheat growing area. Initially, the virtual starting date was May 01, 2010. Six days after sawing, the plants were shifted into another phytotron, what resulted in a virtual starting date of May 07, 2010.

1. Test procedure

Spring wheat (imidazolinone herbicide tolerant variety: AP603 CL) was sowed into ten containers filled with loamy sand soil. The maintenance of the crop was performed in accordance with normal agricultural practice; fertilizers and additional pesticides were applied to achieve an adequate plant growth. According to the study protocol, the crop was treated once with ^{14}C, ^{15}N-imazamox at a nominal rate of 75 g a.s./ha (approximately 0.067 Ib/A) at growth stage BBCH 13-24. Samples of wheat forage and hay were taken 8 days after application (8 DAT). Harvest of wheat plants and sampling of straw and grain was accomplished 62 days after application (62 DAT). For the preparation of the application formulation, calculated amounts of ^{14}C, ^{15}N-imazamox and unlabelled imazamox (2:1 ratio) were weighed. The test item was dissolved in a mixture of blank formulation BAS 720 AC H, the adjuvant DASH (BAS 160 00 S) and water. The purity of the application solution was confirmed using HPLC and the isotope pattern was analysed by HPLC-MS analysis. The application formulation was applied with an automatic spray track at an actual application rate of 75.7 g a.s./ha (corresponding to a spray volume of 219.12 L/ha). Considering the loss recovered in the washing liquids of the spray track system, 99.88% of the application solution was actually applied. Eight days after the application (DALA), 80 wheat plants (BBCH growth stage 39) were thinned out. One half of the plants was minced and frozen as forage, the other half was minced and dried at room temperature. At 62 DALA the ears and straw (BBCH growth stage 89) were cut off and the straw was minced. Afterwards, ears were separated into chaff and grain using a thresher. The chaff was mixed to the straw.

2. Description of analytical procedures

All samples were stored in a freezer at -18°C or below. Extracts were stored in a refrigerator or in a freezer.
Combustion: Homogenised solid plant samples were weighed and combusted by means of an automatic sample oxidizer. The $^{14}CO_2$ was trapped by an absorption and scintillation liquid, and the collected radioactivity was measured by liquid scintillation counting. ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly. In order to determine the background radioactivity, aliquots of untreated samples of wheat grain, straw and whole plant were combusted under the same conditions. The limit of quantitation in mg/kg was calculated from the twofold background radioactivity level (dpm/g matrix) divided by the corresponding specific radioactivity.
Homogenization / solvent extraction: All samples (forage, hay, straw and grain) were homogenised with dry ice. After sublimation of the dry ice, the samples were weighed, mixed, divided into aliquots and radioassayed. The samples were extracted with methanol and water. After centrifugation, the supernatant was filtered. The residue after solvent extraction was dried under a fume hood, homogenised and radioassayed.
Solubilisation of the RRR: The residues after solvent extraction were extracted twice with 1% ammonia, the samples were centrifuged and the supernatants filtered. After ammonia extraction, the samples were solubilised with enzymes and HCl (only straw and hay), whereby the following protocols were applied: For macerozyme incubations, the sample was resuspended in a sodium acetate buffer (pH 7), suitable amounts of macerozyme R-10 and cellulase were added and the mixture was incubated. After centrifugation, the residue was washed with water and the supernatants were pooled. For amylase incubations, the sample was resuspended in phosphate buffer (pH 6), suitable amounts of α-amylase, β-amylase and amyloglucosidase were added and the mixture was incubated. The sample was mixed with acetonitrile, chilled, centrifuged, the residue was washed with water and the supernatants were pooled. For tyrosinase incubations, the sample was resuspended in phosphate buffer (pH 6), suitable amounts of tyrosinase and laccase were added and the mixture was incubated. After centrifugation, the residue was washed with water and the supernatants were pooled. For the solubilisation with HCl, the sample was extracted once with 1 N HCl and centrifuged. Thereafter, 6 N HCl was added to the sample and the mixture was heated for 4 h under reflux in a water bath. The sample was chilled, centrifuged and the extract neutralised with NaOH. The residue was dried.
Extraction: In addition to the solvent extractions (methanol/water), all matrices were also extracted with the following alternative extraction protocols:
Extraction protocol of the residue analysis methods: the samples were mixed with methanol, water and 1 N HCl (60/39/1), extracted with a homogeniser, centrifuged and filtered. The extracts were analysed using HPLC.
QuEChERS (only straw and grain): the samples (straw and grain) were resuspended in water/acetonitrile (1:1) and the QuEChERS chemicals were added ($MgSO_4$, NaCl and di- and trisodium citrate). The mixture was strongly shaken and centrifuged. The extracts were analysed using HPLC.
S19 (only grain): the sample was mixed with water at 40°C, acetone was added and after 30 min, the mixture was extracted with a homogeniser. After centrifugation, NaCl was added to the pellet and the sample was extracted with cyclohexane/ethyl acetate (1:1). The sample was centrifuged and the phases were separated.
HPLC: Extracts were concentrated, assayed by LSC and characterised by HPLC.

3. Identification of metabolites

Work-up for enantiomer specific analysis: An aliquot of wheat forage, hay or grain was extracted with methanol and water: The methanol extract was concentrated to dryness, redissolved in water upon sonication and acidified to pH 3 with formic acid. The sample was extracted with ethyl acetate. The ethyl acetate phase was dried with Na_2SO_4, concentrated and subsequently an ethyl acetate/water (acidified with formic acid to pH 2) partition was performed. The water phase was concentrated and water and acetonitrile were added. The sample was fractionated using HPLC, whereby two fractions were cut. Fraction 1 was concentrated and subjected to enantiomer-specific analysis using HPLC, while fraction 2 was subjected to enantiomer-specific analysis using HPLC. In order to obtain a better resolution with HPLC, an adequate amount of hydroxy reference item CL 263284 was spiked into the samples prior analysis.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

In the present study, the TRR was calculated by summarizing the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) after solvent extraction. The calculated TRR of wheat forage sampled 8 DAT was 1.602 mg/kg. In wheat hay, due to a lower water content, the TRR amounted to 8.011 mg/kg, which is approximately 5 times higher compared to forage. The TRR in wheat straw and grain sampled 62 days after the application was calculated to be 3.199 mg/kg and 1.412 mg/kg, respectively. The calculated TRR values were set to 100% TRR for all matrices. Additionally, the TRR was measured by direct combustion analysis followed by LSC. The measured TRR of all four matrices showed no major differences to the calculated TRR values.

Table 6.2.1-14: Total radioactive residues (TRRs) in wheat samples following foliar application of [^{14}C]imazamox

TRRs in Treated Wheat			
Matrix	**DAT**	**TRR Determined by Direct Combustion [mg/kg]**	**TRR Calculated [mg/kg] ***
Forage	8	1.819	1.602
Hay	8	10.091	8.011
Straw	62	3.493	3.199
Grain	62	1.374	1.412

DAT = Days after treatment
* Sum of ERR (methanol extract and water extract) and RRR (extraction residue)

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The extractabilities of radioactive residues from wheat plant, hay, straw and grain are summarized in Table 6.2.1-15.

Table 6.2.1-15: Extraction efficiency of residues of [^{14}C]imazamox in wheat samples

Matrix	DAT	TRR calc. *	Distribution of Radioactive Residues				ERR [1]		RRR [2]	
			Combined Methanol Extract		Combined Aqueous Extract					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Forage	8	1.602	1.427	89.0	0.055	3.4	1.482	92.5	0.121	7.5
Hay	8	8.011	6.008	75.0	1.329	16.6	7.337	91.6	0.674	8.4
Straw	62	3.199	1.962	61.3	0.586	18.3	2.548	79.6	0.651	20.4
Grain	62	1.412	0.618	43.8	0.608	43.1	1.227	86.9	0.185	13.1

DAT = Days after treatment
* TRR was calculated as the sum of ERR + RRR
1 ERR = extractable radioactive residue
2 RRR = residual radioactive residue (after solvent extraction)

1. Extraction and characterisation of residues in wheat

The extractabilities of the wheat matrices with methanol and water are summarized in Table 6.2.1-15. The extractability of wheat forage and hay with methanol and water was very high and accounted for 92.5% and 91.6% of the TRR. The major part of the residues was extracted with methanol (forage: 89.0% TRR, hay: 75.0% TRR). The extractability of wheat straw and grain with methanol and water was high and accounted for 79.6% and 86.9% of the TRR. For straw, the major part of the residues was extracted with methanol (61.3% TRR), whereas for grain virtually equal amounts were extracted with methanol and water (methanol: 43.8% TRR, water: 43.1% TRR).

Table 6.2.1-16: Metabolites detected in wheat matrices following foliar application of [^{14}C]imazamox

Components*	Forage (8 DAT)		Hay (8 DAT)		Straw (62 DAT)		Grain (62 DAT)	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Imazamox	1.079	67.3	4.966	62.0	0.251	7.8	1.050	74.4
CL 189215	0.027	1.7	0.411	5.1	0.486	15.2	0.036	2.5
CL 263284	0.295	18.4	1.914	23.9	1.213	37.9	0.092	6.5
CL 312622	0.113	7.0	0.399	5.0	0.544	17.0	n.d.	
Total identified from ERR	1.514	94.5	7.690	96.0	2.494	78.0	1.177	83.4
Total characterised from ERR 1)	0.066	4.1	0.077	1.0	0.337	10.5	0.077	5.5
Total identified and/or characterised from ERR 1)	1.580	98.6	7.767	97.0	2.831	88.5	1.255	88.9
Unextractable (RRR) 2)	0.062	3.9	0.523	6.5	0.439	13.7	0.143	10.1
Grand total 3)	1.688	105.3	8.392	104.7	3.336	104.3	1.416	100.3

* The extracts (methanol + aqueous) for these samples were combined and analysed by HPLC
n.d. = not detected
1 ERR = extractable radioactive residue
2 RRR = residual radioactive residue, remaining after extraction with solvents like methanol and water
3 Grand total = (total identified and/or characterised from ERR + RRR) * 100% / calculated TRR

2. Identification and quantification of extractable residues in wheat

Wheat forage: Analysis of the methanol extract of wheat forage using HPLC resulted in a pattern of six peaks, of which four were identified. The main peak was identified as the parent compound imazamox and accounted for 1.046 mg/kg or 65.3% TRR. Metabolite CL 263284 was the second most abundant component and accounted for 0.286 mg/kg or 17.9% TRR. The metabolites CL 312622 and CL 189215 were identified at levels of up to 6.7% TRR. The metabolite pattern was confirmed with a similar methanol extract using HPLC. Additionally, co-chromatography experiments with a similar methanol extract and the reference items CL 189215 and imazamox and a MS sample containing CL 263284 and CL 312622 confirmed the peak identities. In the water extract the same components as in the methanol extract were identified with HPLC. Likewise, the parent compound imazamox was the main constituent at 0.033 mg/kg or 2.1% TRR. The remaining components CL 263284, CL 312622 and CL 189215 were identified at levels of up to 0.6% TRR. Analysis of the same water extract using HPLC confirmed the presence of the parent compound and metabolite CL 263284. No signals corresponding to the reference items Reg. No. 60967 and Reg. No. 199553 (putative cleavage products of imazamox, imidazoline moiety) were detected in the solvent extracts using HPLC. The residue after solvent extraction was further solubilised with ammonia and different enzyme treatments. Analysis of the ammonia solubilisate using HPLC resulted in the identification of imazamox and metabolite CL 263284, which accounted for up to 0.4% of the TRR. In the ERR, 94.5% of the total radioactive residues were identified and 4.1% were characterised by HPLC. In the RRR, additional 3.9% of the TRR were identified and characterised.
Wheat hay: Analysis of the methanol extract of wheat hay using HPLC resulted in a pattern of five peaks, of which four were identified. The parent compound imazamox represented the main peak and accounted for 4.056 mg/kg or 50.6% TRR. The second most abundant component was metabolite CL 263284, which accounted for 1.689 mg/kg or 21.1%. The metabolites CL 312622 and CL 189215 were identified at levels of up to 4.2% TRR. The metabolite pattern was confirmed using HPLC. In the water extract the same components as in the methanol extract were identified with HPLC. The parent compound imazamox was again the main constituent, accounting for 0.910 mg/kg or 11.4% TRR. The remaining components CL 263284, CL 312622 and CL 189215 were identified at levels of up to 2.8% TRR. Analysis of the same water extract using HPLC confirmed the metabolite pattern of the quantitative analysis. No signals corresponding to the reference items Reg. No. 60967 and Reg. No. 199553 (putative cleavage products of imazamox, imidazoline moiety) were detected in the solvent extracts using HPLC. The residue after solvent extraction was further solubilised with ammonia, different enzyme treatments and incubations with HCl. The ammonia solubilisate was analysed using HPLC. This resulted in the identification of imazamox and metabolite CL 263284, which accounted for up to 0.4% of the TRR. In the ERR, 96.0% of the total radioactive residues were identified and 1.0% were characterised by HPLC. In the RRR, additional 6.5% of the TRR were identified and characterised.
Wheat straw: Analysis of the methanol extract of wheat straw using HPLC resulted in a pattern of eleven peaks, of which four were identified. The main peak was identified as metabolite CL 263284 and accounted for 1.041 mg/kg or 32.6% TRR. The metabolites CL 189215 and CL 312622 were identified at levels of 0.394 mg/kg or 12.3% TRR and 0.396 mg/kg or 12.4% TRR, respectively. The parent compound imazamox accounted for 6.1% TRR. The metabolite pattern was confirmed with the same methanol extract using HPLC.

In the water extract of a similar extraction the same components as in the methanol extract were identified with HPLC. Likewise, the main constituent was CL 263284 at 0.172 mg/kg or 5.4% TRR. The remaining components imazamox, CL 189215 and CL 312622 were identified at levels of up to 4.6% TRR. Analysis of the same water extract using HPLC confirmed the metabolite pattern of the quantitative analysis. In the solvent extracts up to four non-identified peaks (up to approximately 1% TRR) eluted prior to metabolite CL 189215 using HPLC. It was not possible to assign the peaks to putative cleavage products of imazamox (imidazoline moiety) by HPLC-MS experiments. The residue after solvent extraction was further solubilised with ammonia, different enzyme treatments and incubations with HCl. Analysis of the ammonia solubilisate using HPLC resulted in the identification of imazamox, metabolite CL 263284 and CL 312622, which accounted for up to 1.2% of the TRR. In the ERR, 78.0% of the total radioactive residues were identified and 10.5% were characterised by HPLC. In the RRR, additional 13.7% of the TRR were identified and characterised.

Wheat grain: Analysis of the methanol extract of wheat grain using HPLC resulted in a pattern of eleven peaks, of which three were identified. The main peak was identified as the parent compound imazamox and accounted for 0.577 mg/kg or 40.8% TRR. The metabolites CL 263284 and CL 189215 were identified at levels of up to 3.5% TRR. The metabolite pattern was confirmed with a similar methanol extract using HPLC. In the water extract the same components as in the methanol extract were identified with HPLC. The parent compound imazamox was the main constituent and accounted for 0.473 mg/kg or 33.5% TRR. The remaining components CL 263284 and CL 189215 were identified at levels of up to 3.0% TRR. Analysis of a similar water extract using HPLC confirmed the metabolite pattern of the quantitative analysis. Additionally, co-chromatography experiments with similar methanol and water extracts, the reference items CL 189215 and imazamox and a MS sample containing CL 263284 and CL 312622 confirmed the peak identities. In the solvent extracts up to four non-identified peaks (up to approximately 1% TRR) eluted prior to metabolite CL 189215 using HPLC. For wheat grain, it was not possible to assign similar peaks to putative cleavage products of imazamox (imidazoline moiety) by HPLC-MS experiments. The residue after solvent extraction was further solubilised with ammonia and different enzyme treatments. The ammonia solubilisate was analysed using HPLC. One peak was identified as the parent compound imazamox and accounted for 0.026 mg/kg or 1.8% TRR. In the ERR, 83.4% of the total radioactive residues were identified and 5.5% were characterised by HPLC. In the RRR, additional 10.1% of the TRR were identified and characterised.

Enantiomer ratio of imazamox and CL 263284: In order to analyse if one enantiomer of imazamox was preferably metabolised in wheat, enantiomer specific analyses were performed in all four matrices (forage, hay, straw and grain). For the test item the ratio of the two enantiomers was found to be approximately 1:1. For the determination of the enantiomer ratio in the different matrices, the parent compound and metabolite CL 263284 were isolated from methanol extracts and analysed using HPLC. The enantiomer ratio of imazamox and its metabolite CL 263284 were found to be approximately 1:1 in all matrices, thus remained unchanged during metabolism of imazamox in wheat.

3. Proposed metabolic pathway

The proposed metabolic pathway of imazamox in wheat is shown in Figure 6.2.1-2. The key step of the metabolism of imazamox in spring wheat after foliar application is the cleavage of the methyl ether group (demethylation) resulting in metabolite CL 263284. A subsequent glycosylation of the hydroxyl group resulted in the formation of metabolite CL 189215. Alternatively, the hydroxyl group of CL 263284 was oxidized to a carboxyl group, which resulted in CL 312622, a dicarboxylic acid.

4. Extractability of residues according to residue analytical methods

Additionally to the methanol and water extractions of the metabolism investigations samples were extracted following the protocol of the residue methods (all matrices), QuEChERS method (straw and grain) and S19 method (grain). The extractabilities of the alternative extraction methods demonstrate the ability of the internal residue method to capture the residues of imazamox in the most appropriate way. The extracts of the residue methods were analysed using HPLC and the extracts of the QuEChERS method were analysed.

5. Storage stability

Storage stability investigations were performed in wheat matrices at the beginning and at the end of the study. For all four matrices a re-analysis of stored extracts was performed. For straw and grain stored samples were additionally re-extracted. Initial analyses of wheat forage, hay, straw and grain for quantification and confirmation were carried out within a maximum of 160 days after sampling (except confirmatory analyses of the grain MeOH extract, 335 days after sampling). Nonetheless, the stored extracts were re-analysed approximately 11 months after quantitative analysis using HPLC. They were stored 323 to 328 days prior to measurement. In all cases, the later chromatograms showed similar peak patterns as the initial analyses. Additionally, a re-extraction of stored aliquots from straw and grain was carried out after approximately one year (362 days of storage). The resulting extracts were HLPC measured within 9 days. The metabolite patterns were qualitatively and quantitatively comparable to the initial extracts. Summarizing the results, the storage stability was confirmed in the matrices and in solvent extracts (methanol and water) over a storage interval of at least 323 days after sampling.
Wheat forage: The methanol and water extracts of wheat forage were initially HPLC analysed within 102 to 126 days after sampling. A re-analysis of stored methanol and water extracts (storage time 324 days) using HPLC largely confirmed the metabolic patterns of the initial analyses. The methanol extract of the re-analysis was used for co-chromatography experiments, resulting in a storage time of 340 to 344 days after extraction. However, the peak patterns of the unspiked methanol extract were identical to those of the initial analyses using HPLC.
Wheat hay: The methanol and water extracts of wheat hay were initially HPLC analysed within 102 to 129 days after sampling. A re-analysis of stored methanol and water extracts (storage time approximately 323 days) using HPLC confirmed the metabolic patterns of the initial analyses.
Wheat straw: The methanol and water extracts of wheat straw were initially HPLC analysed within 47 to 75 days after sampling. A re-analysis of stored methanol and water extracts (storage time 326 days) using HPLC confirmed the metabolic patterns of the initial analyses.

For the isolation of metabolites by fractionation, stored aliquots of wheat straw were extracted 201 to 231 days after sampling and HPLC analysed within the next 21 days. In order to demonstrate the stability of the components in straw during storage, a re-extraction was performed 362 days after sampling and HPLC analysed within 8 days. The resulting chromatograms agreed well with the peak patterns obtained after the initial extractions.

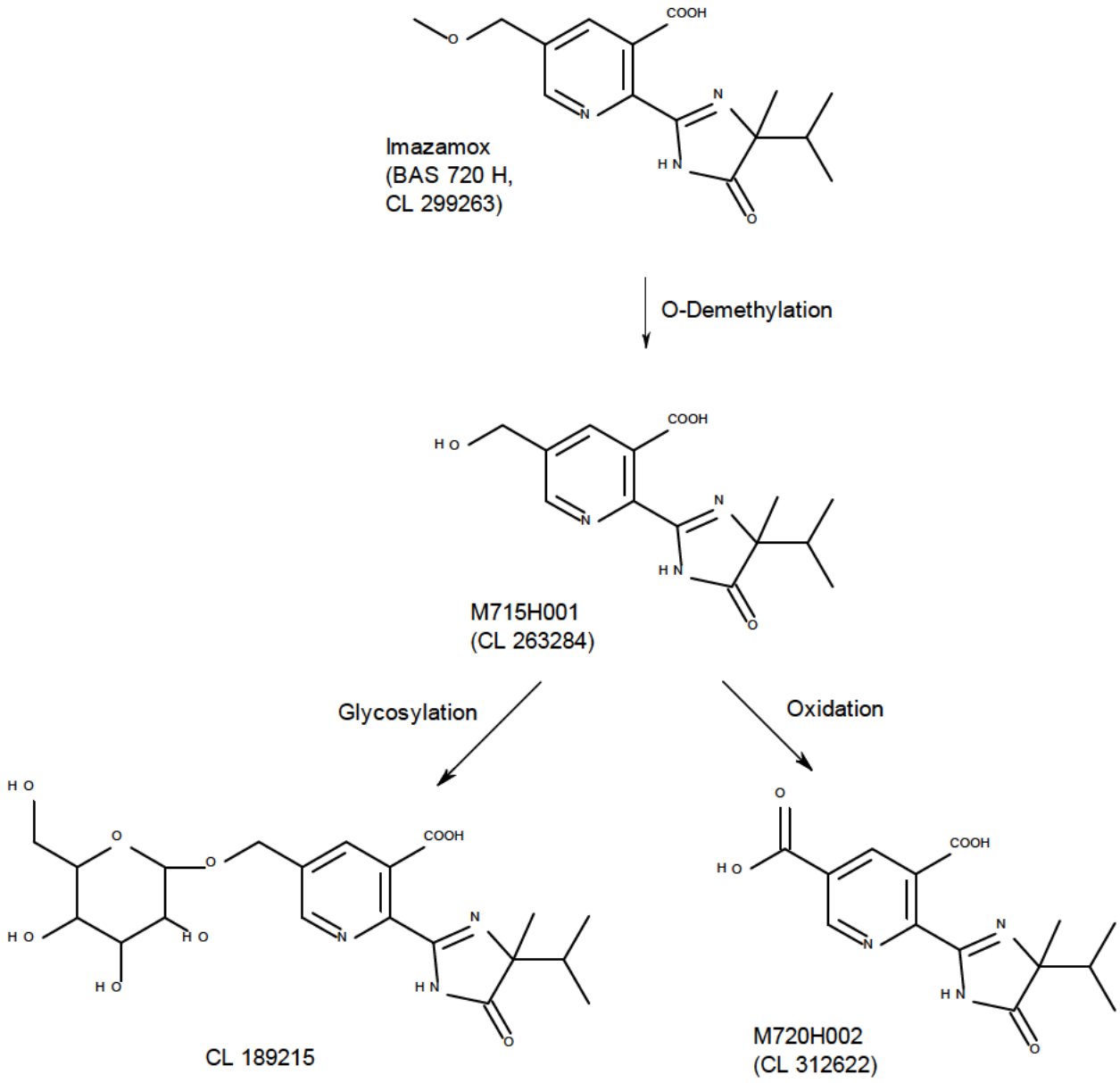
Wheat grain: The methanol and water extracts of wheat grain were initially HPLC analysed within 47 to 160 days after sampling, except the confirmatory analysis of the MeOH extract, which was analysed 335 days after sampling. A re-analysis of stored methanol and water extracts (storage time approximately 327 days) using HPLC confirmed the metabolic patterns of the initial analyses. In order to demonstrate the stability of the components in grain during storage, a re-extraction was performed 362 days after sampling and HPLC analysed within 9 days. The obtained chromatograms agreed well with the peak patterns obtained after the initial extractions. The methanol and water extracts of the re-analysis were used for co-chromatography experiments, resulting in a storage time of 343 to 357 days after extraction. However, the peak patterns of the unspiked extracts were identical to those of the initial analyses using HPLC.

III. CONCLUSION

$^{14}C,^{15}N$-labelled imazamox (BAS 720 H) was applied to inidazolinone herbicide tolerant wheat by one foliar application at a rate of 75.7 g a.s./ha at growth stage BBCH 13-24. Forage and hay were sampled 8 days after treatment, while straw and grain were harvested 62 days after treatment. The highest levels of total radioactive residues (TRR) were found in wheat hay accounting for 8.011 mg/kg followed by straw with 3.199 mg/kg. Lower levels of 1.602 mg/kg and 1.412 mg/kg were detected in forage and grain, respectively. The extractability of the radioactive residues with methanol and water was very high for wheat forage and hay (>91% TRR) and high for straw and grain (>79% TRR). For wheat forage, hay and straw, the predominant part of the radioactive residues was extracted with methanol, and only small portions were extracted with water. For wheat grain, similar amounts of residues were extracted with methanol and water. The residual radioactive residues after solvent extraction (RRR) were below 10% of the TRR for forage and hay, about 20% of the TRR for straw and 13% TRR for grain. The residues after solvent extraction were further solubilised, whereby 4% to 14% TRR were additionally released. Identification of metabolites was mainly based on HPLC-MS investigations of isolated fractions from wheat straw methanol extract. The isolated fractions, which contained metabolites CL 263284, CL 189215 and CL 312622, were used for the determination of retention times and for co-chromatography experiments. In wheat forage, hay and grain imazamox was the main component identified (>60% TRR) whereas in wheat straw it represented only about 9% TRR. In wheat straw metabolite CL 263284, which resulted from an ether bond cleavage from the parent compound, was the main constituent (>40% TRR). CL 263284 was generally the metabolite with the highest concentration. The glycosylated form of CL 263284, metabolite CL 189215, was also found in all four matrices (from approximately 2.0 to 15% TRR). The dicarboxylic acid CL 312622, which resulted from oxidation of CL 263284, was found at low levels in forage and hay (up to 7% TRR) and at slightly higher levels in straw (17.5% TRR).

In the present study, polar fractions of wheat straw methanol extracts were analysed by HPLC-MS in order to identify putative cleavage of the imidazolinone moiety from the parent molecule. However, no such cleavage products were observed. The enantiomer ratios of imazamox and its metabolite CL 263284 in methanol extracts were approximately 1:1, thus remaining unchanged as compared to the ratio in the applied test item. Storage stability investigations were performed in extracts of all four wheat matrices showing that there was no relevant change in the peak patterns during the storage of sample extracts over a period of approximately one year. Additionally, analysis of re-extractions from straw and grain samples after approximately one year of storage confirmed the stability of the metabolic pattern over this time interval.

Figure 6.2.1-2: Proposed Metabolic Pathway of Imazamox in (IMI-tolerant) Wheat



Report:	CA 6.2.1/5 Thiaener J. et al., 2014a Metabolism of 14C-Imazamox in rice 2013/1307620
Guidelines:	EPA 860.1300: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1000: Background - PMRA Section 97.2 (Canada): Residue Chemistry Guidelines: Plants and Lifestock (June 1997), JMAFF 59 NohSan No 4200, BBA IV 3-2, Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft), OECD 501 - Metabolism in crops (adopted January 8 2007)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The objective of the present study was to investigate the magnitude and nature of residues of imazamox (BAS 720 H) and its degradation products after foliar application to paddy rice. The rice (imidazolinone herbicide resistant variety) was treated once with ^{14}C, ^{15}N-labelled imazamox (BAS 720 H) with an nominal application rate of 75 g a.s./ha in combination with the adjuvant Dash. Forage was sampled 42 days after treatment, while straw and grain samples were harvested 182 days after treatment. The highest levels of the calculated total radioactive residues (TRR) were found in rice forage accounting for 0.257 mg followed by straw with 0.207 mg/kg. Lower levels of 0.108 mg/kg were detected in grain. For the investigations of metabolic patterns, subsamples of the three matrices forage, straw and grain were extracted with methanol and water.
The extractability of the radioactive residues with methanol and water was high for the three rice matrices (77.9 to 87.1% TRR). For forage and straw, the predominant part of the radioactive residues was extracted with methanol, while for rice grain similar amounts of residues were extracted with methanol and water. The residual radioactive residues after solvent extraction (RRR) were 12.0% of the TRR for forage, about 25.2% of the TRR for straw and 22.5% TRR for grain. The residues after solvent extraction were further solubilized using an individual combination of sequential solubilization steps, whereby 4.9 to 11.8% TRR were additionally released. The most effective solubilization steps were the treatment with aqueous ammonia and incubation with macerozyme.
Identification of imazamox and its metabolites was based on retention time comparison and co-chromatography experiments with the reference items. As reference items isolated fractions from a wheat metabolism study, which contained metabolites CL 263284, CL 189215 and CL 312622, were used for the determination of retention times and for co-chromatography with a processed methanol extract sample of rice forage. Likewise, the active substance was successfully identified. For rice straw and grain peak assignment was based on comparison of the retention times of the reference items with the 14C-signals of the HPLC analyses.
In the ERR, the parent compound imazamox accounted for 18.1% TRR in forage and was detected in lower amounts in straw and grain (up to 4.6% TRR). In all rice matrices the metabolite CL 263284, which resulted from a demethylation of the parent compound, was the main component identified (up to 48% TRR). The glycosylated form of CL 263284, metabolite CL 189215, was also found in all three matrices (from 7.5 to 25.5% TRR). The dicarboxylic acid CL 312622, which resulted from oxidation of CL 263284, was found at lower levels (up to 4.8% TRR).

The enantiomer ratio of imazamox and its metabolite M715H001 were found to be approximately 1:1 in rice straw, while in forage and grain higher ratios for enantiomer 2 of imazamox than for enantiomer 1were detected (70.0 and 76.1%, respectively).
Storage stability investigations were performed in the rice matrices at the beginning and at the end of the study. For all three matrices a re-analysis of stored extracts and a re-extraction of homogenized subsamples was performed. From the obtained results it can be concluded that the identified components in the matrices and extracts are stable over the period of investigation.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: 3-^{14}C-pyridine, 3-^{15}N-imidazolinone-labelled imazamox
Unlabelled imazamox, Dash (BAS 160 00 S)

Lot/Batch #: ^{14}C, ^{15}N imazamox: 1004-1101
Unlabelled AC12820-7
Dash: 48316668E0

Purity: Radiochemical purity: 98.6%
Chemical purity: 97.2%
Specific activity: 6.65 MBq/mg

CAS#: 114311-32-9

Stability of test compound: The test compound is stable over the investigated time period

2. Test Commodity:

Crop: Paddy rice
Type: Cereals
Variety: CL 12 Japonica Clearfield
Botanical name: *Oryza sativa*
Crop parts(s) or processed commodity: Forage, straw, grain
Sample size: Not relevant

3. Soil: A sandy loam was used. The soil physicochemical properties are described below (see Table 6.2.1-17).

Table 6.2.1-17: Soil physicochemical properties

Soil Series	Soil Type	pH	OM %	Sand %	Silt %	Clay %	Maximal Water holding capacity[2]	CEC[1] cmol/kg
Bruch West	*Sandy Loam	**7.2	***4.7	*75.1	*15.3	*9.6	26.4	11.4

* USDA scheme ** ($CaCl_2$) ***Organic matter calculated as 1.72 x percent organic carbon
1) Cation exchange capacity 2) g/100 g dry soil

B. STUDY DESIGN

The study was carried out at the Agricultural Research Centre of BASF in Limburgerhof, Germany. The plant uptake part of the study was conducted in plastic containers located in climatic chambers. The phytotrons simulated the natural climatic conditions of a typical rice growing area.

1. Test procedure

Paddy rice (*Oryza sativa*, imidazolinone herbicide tolerant variety: CL 12 Japonica Clearfield) was sowed into multicells filled with sandy loam soil. After germination, the young plants were transferred into plastic containers filled with sandy loam soil. The maintenance of the crop was performed in accordance with normal agricultural practice; fertilizers and additional pesticides were applied to achieve an adequate plant growth. According to the study protocol, the crop was treated once with ^{14}C, ^{15}N-labelled imazamox at a nominal rate of 75 g a.s./ha (approximately 0.067 lb/A) at BBCH growth stage 25. For the preparation of the application formulation, calculated amounts of ^{14}C, ^{15}N-imazamox and unlabelled imazamox (2:1 ratio) were weighed. The test item was dissolved in a mixture of blank formulation BAS 720 AC H, the adjuvant Dash (BAS 160 00 S) and water. Thereafter, the mixture was sonicated to generate a homogenous emulsion. The purity of the application solution was confirmed using HPLC and the isotope pattern was analysed by HPLC-MS analysis. The application formulation was applied with an automatic spray track at an actual application rate of 75.7 g a.s./ha (corresponding to a spray volume of approximately 222 L/ha). Considering the loss recovered in the washing liquids of the spray track system, 99.7% of the application solution was actually applied. Samples of rice forage were taken 42 days after application (42 DAT) at BBCH growth stage 49, minced and frozen. Harvest of rice plants and sampling of straw and grain was accomplished 182 days after application (182 DAT) at BBCH growth stage 89. Panicles and straw were cut off and the straw was minced. Afterwards, panicles were separated into chaff and grain using a thresher. The chaff was mixed to the straw.

2. Description of analytical procedures

All samples were stored in a freezer at -18°C or below. Extracts were stored in a refrigerator or, for longer periods, in a freezer.
Combustion: Homogenised solid plant samples were weighed and combusted by means of an automatic sample oxidizer. The $^{14}CO_2$ was trapped by an absorption and scintillation liquid, and the collected radioactivity was measured by liquid scintillation counting. ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly. In order to determine the background radioactivity, aliquots of untreated samples of rice grain, straw and whole plant were combusted under the same conditions. The limit of quantitation in mg/kg was calculated from the twofold background radioactivity level (dpm/g matrix) divided by the corresponding specific radioactivity.

Homogenization / solvent extraction: All samples (forage, straw and grain) were homogenised with dry ice. After sublimation of the dry ice, the samples were weighed and radioassayed. The samples were extracted with methanol and water. After centrifugation, the supernatant was filtered. The residue after solvent extraction was dried, homogenised and radioassayed.
Solubilisation of the RRR: For homogenisation of residual radioactive residues after extraction with methanol and water (RRR) the analytical mill was used. The residues with a sufficient level of radioactivity were subsequently extracted twice with 1% ammonia, the samples were centrifuged and the supernatants decanted from the residue. After ammonia extraction, the residues were dried, ground and subsequently solubilized with different enzymes.
For **macerozyme** incubations, the sample was resuspended in a sodium acetate buffer (pH 5), suitable amounts of macerozyme R-10 and cellulase were added and the mixture was incubated on a shaker at 37°C for about 20 hours. After centrifugation, the supernatant was decanted from the residue and the reaction was quenched by mixing the sample with acetonitrile.
For **glucosidase** incubations, the sample was resuspended in a sodium acetate buffer (pH 5), suitable amounts of β-glucosidase and hesperidinase were added and the mixture was incubated on a shaker at 37°C for about 20 hours. After centrifugation, the supernatant was decanted from the residue and the reaction was quenched by mixing the sample with acetonitrile.
For **amylase** incubations, the sample was resuspended in phosphate buffer (pH 6), suitable amounts of α-amylase, β-amylase and amyloglucosidase were added and the mixture was incubated on a shaker at 37°C for about 90 hours. The sample was mixed with acetonitrile, centrifuged and the supernatant was decanted from the residue.
For **tyrosinase** incubations, the sample was resuspended in phosphate buffer (pH 6), suitable amounts of tyrosinase and laccase were added and the mixture was incubated on a shaker at 37°C for about 20 hours. After centrifugation, the supernatant was decanted from the residue and the reaction was quenched by mixing the sample with acetonitrile.
SPE clean-up: Several samples were cleaned-up on an SPE column.
HPLC: Extracts were concentrated, assayed by LSC (liquid scintillation counter for the quantitation of radioactivity in liquid samples) and characterised by HPLC.
LSC measurement of liquid samples: For the quantitation of radioactivity in liquid samples a liquid scintillation counter (LSC) was used. Aliquots of liquid samples were mixed with a sufficient volume of a suitable scintillator prior to measurement. All data were corrected using appropriate quench curves and are expressed in decays per minute (dpm).
Enantiomer-specific analysis: Metabolites were isolated from methanol extracts of forage, straw and grain, purified via HPLC and a purified fraction was subjected to enantiomer-specific HPLC analysis.

3. Identification of metabolites

The peak assignment for imazamox and its metabolites CL 263284, CL 189215 and CL 312622 was based on co-chromatography experiments and comparison of the retention times of the components with the ^{14}C-signals of the HPLC analyses.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The TRR was calculated by summarizing the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) after solvent extraction. The calculated TRR of rice forage sampled 42 DAT was 0.255 mg/kg. The TRR in rice straw and grain sampled 182 days after the application was calculated to be 0.213 mg/kg and 0.111 mg/kg, respectively. Additionally, the TRR was measured by direct combustion analysis followed by LSC. The measured TRR of all three matrices showed no major differences to the calculated TRR values.

Table 6.2.1-18: Total radioactive residues (TRRs) in rice samples following foliar application of [^{14}C]imazamox

TRRs in Treated Rice			
Matrix	**DAT***	**TRR Determined by Direct Combustion [mg/kg]**	**TRR Calculated [mg/kg]****
Forage	42	0.257	0.255
Straw	182	0.207	0.213
Grain	182	0.108	0.111

* DAT = Days after treatment
** Sum of ERR (extraction with methanol and water) and RRR (extraction residue)

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The extractabilities of radioactive residues from rice plant, straw and grain are summarized in Table 6.2.1-19.

Table 6.2.1-19: Extraction efficiency of residues of [^{14}C]imazamox in paddy rice samples

Matrix	DAT*	TRR combusted	Distribution of Radioactive Residues				ERR [1]		RRR [2]	
			Combined Methanol Extract		Combined Aqueous Extract					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Forage	42	0.257	0.211	82.3	0.013	4.9	0.224	87.1	0.031	12.0
Straw	182	0.207	0.117	56.6	0.044	21.4	0.161	77.9	0.052	25.2
Grain	182	0.108	0.043	39.5	0.044	40.7	0.087	80.2	0.024	22.5

* DAT = Days after treatment
[1] ERR = extractable radioactive residue
[2] RRR = residual radioactive residue (after solvent extraction)

1. Extraction and characterisation of residues in rice

The extractability of rice forage with methanol and water was high and accounted for 87.1% of the TRR. The major part of the residues was extracted with methanol (82.3% TRR). The extractability of rice straw and grain with methanol and water accounted for 77.9% and 80.2% of the TRR. For straw, the major part of the residues was extracted with methanol (56.6% TRR), whereas for grain virtually equal amounts were extracted with methanol and water (methanol: 39.5% TRR, water: 40.7% TRR).

Table 6.2.1-20: Metabolites detected in paddy rice matrices following foliar application of [^{14}C]imazamox

Components*	Forage (42 DAT)		Straw (182 DAT)		Grain (182 DAT)	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Imazamox	0.046	18.1	0.010	4.6	0.005	4.3
CL 189215	0.019	7.5	0.020	9.5	0.028	25.5
CL 263284	0.123	48.0	0.052	25.2	0.036	32.9
CL 312622	0.011	4.5	0.010	4.8	0.003	2.9
Total identified from ERR	0.200	78.0	0.091	44.1	0.071	65.6
Total characterised from ERR [1)]	0.018	7.1	0.049	23.6	0.011	10.4
Total identified and/or characterised from ERR [1)]	0.219	85.1	0.140	67.7	0.082	75.9
Unextractable (RRR) [2)]	0.031	12.0	0.052	25.2	0.024	22.5
Ammonia solubilizates	0.006	2.2	0.015	7.5	0.003	2.8
Macerozyme solubilizate	0.005	1.8	0.006	2.7	0.002	1.5
Tyrosinase solubilizate	0.001	0.4	0.002	0.8	0.005	5.0
Amylase solubilizate	0.001	0.6	0.002	0.9	0.002	2.1
Total characterised from RRR	0.013	4.9	0.024	11.8	0.012	11.4
Total identified and characterised	0.231	90.1	0.165	79.5	0.095	87.3
Final residue	0.019	7.3	0.026	12.6	0.013	12.2
Grand total [3)]	0.250	97.4	0.191	92.2	0.108	99.5

* The extracts (methanol + aqueous) for these samples were combined and analysed by HPLC
1 ERR = extractable radioactive residue
2 RRR = residual radioactive residue, remaining after extraction with solvents like methanol and water
3 Grand total = (total identified and/or characterised from ERR + RRR) * 100% / calculated TRR

2. Identification and quantification of extractable residues in rice

Rice forage: Analysis of the methanol extract of rice forage using HPLC resulted in a pattern of nine peaks, of which four were identified. The main peak was identified as the metabolite CL 263284 and accounted for 0.119 mg/kg or 46.5% TRR. The parent compound imazamox was the second most abundant component and accounted for 0.045 mg/kg or 17.5% TRR. The metabolites CL 312622 and CL 189215 were identified at levels of up to 7.1% TRR. The remaining peaks were characterised by their chromatographic properties (each below or equal to 0.007 mg/kg or 2.8% TRR). The metabolite pattern was confirmed using a similar HPLC method. Additionally, co-chromatography experiments with the processed methanol extract and the reference items imazamox, CL 263284, CL 189215 and CL 312622 confirmed the peak identities. In the water extract the same components as in the methanol extract were identified with HPLC. The metabolite CL 263284 was the main constituent at 0.004 mg/kg or 1.4% TRR. The remaining components CL 189215, CL 312622 and imazamox were identified at levels of up to 0.6% TRR. The remaining peaks were characterised by their chromatographic properties (each below or equal to 0.001 mg/kg or 0.5% TRR). Analysis of the same water extract using a second HPLC method confirmed the metabolite pattern.

In the ERR, 78.0% of the total radioactive residues were identified and 7.1% were characterised by HPLC. In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of rice forage with methanol and water (0.031 mg/kg or 12.0% TRR) were subjected to a sequential solubilization procedure. The residue after solvent extraction was consecutively incubated with ammonia solution, macerozyme / cellulase, tyrosinase / laccase and amylases / amyloglucosidase. These solubilization steps released portions of 0.006 mg/kg or 2.2% TRR (sum of two ammonia solubilizates), 0.005 mg/kg or 1.8% TRR (macerozyme), 0.001 mg/kg or 0.4% TRR (tyrosinase) and 0.001 mg/kg or 0.6% TRR (amylase). The final residue accounted for 0.019 mg/kg or 7.3% TRR.
During the sequential solubilization procedure, 0.013 mg/kg or 4.9% TRR were released being thereby characterised as soluble with ammonia, macerozyme, tyrosinase and amylase.
In total, 85.1 % of the TRR were identified or characterised from the ERR and additional 4.9% of the TRR were characterised by solubilization from the RRR. Summarised with the final residue, the radioactive residues accounted for a Grand Total of 0.250 mg/kg or 97.4 % TRR.

Rice straw: Analysis of the methanol extract of rice straw using HPLC resulted in a pattern of 15 peaks, of which four were identified. The main peak was identified as the metabolite CL 263284 and accounted for 0.047 mg/kg or 22.5% TRR. The metabolite CL 189215 was the second most abundant component and accounted for 0.017 mg/kg or 8.1% TRR. The metabolite CL 312622 and the parent compound imazamox were identified at levels of up to 4.0% TRR. The remaining peaks were characterised by their chromatographic properties (each below or equal to 0.006 mg/kg or 2.9% TRR). The metabolite pattern was confirmed using HPLC.
In the water extract the same components as in the methanol extract were identified with HPLC. The metabolite CL 263284 accounted for 0.006 mg/kg or 2.7% TRR. The remaining components CL 189215, CL 312622 and imazamox were identified at levels of up to 1.4% TRR. The main component was a polar peak, characterised by its chromatographic properties (0.007 mg/kg or 3.2% TRR). The remaining peaks accounted for up to 1.1% TRR. Analysis of an identical water extract using HPLC largely confirmed the metabolite pattern. Additionally, 0.004 mg/kg or 1.9% TRR were characterised by their solubility in water (additional extraction of solid residue).
In the ERR, 44.1% of the total radioactive residues were identified and 23.6% were characterised. In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of rice straw with methanol and water (0.052 mg/kg or 25.2% TRR) were subjected to a sequential solubilization procedure. The residue after solvent extraction was consecutively incubated with ammonia solution, macerozyme / cellulase, tyrosinase / laccase and amylases / amyloglucosidase. These solubilization steps released portions of 0.015 mg/kg or 7.5% TRR (pooled sample of two ammonia solubilizates), 0.006 mg/kg or 2.7% TRR (macerozyme), 0.002 mg/kg or 0.8% TRR (tyrosinase) and 0.002 mg/kg or 0.9% TRR (amylase). The final residue accounted for 0.026 mg/kg or 12.6% TRR and was after the different enzymatic treatments regarded as not bioavailable.
During the sequential solubilization procedure, 0.024 mg/kg or 11.8% TRR were released being thereby characterised as soluble with ammonia, macerozyme, tyrosinase and amylase.
In total, 67.7% of the TRR were identified or characterised from the ERR and additional 11.8% of the TRR were characterised by solubilization from the RRR. Summarised with the final residue, the radioactive residues accounted for a Grand Total of 0.191 mg/kg or 92.2% TRR.

Rice grain: Analysis of the methanol extract of rice grain using HPLC resulted in a pattern of nine peaks, of which four were identified. The main peak was identified as the metabolite CL 263284 and accounted for 0.023 mg/kg or 21.7% TRR. The metabolite CL 189215 was the second most abundant component and accounted for 0.013 mg/kg or 12.4% TRR. The metabolite CL 312622 and the parent compound imazamox were identified at levels of up to 3.1% TRR. The remaining peaks were characterised by their chromatographic properties (each below or equal to 0.001 mg/kg or 0.5% TRR). The metabolite pattern was confirmed using HPLC.
In the water extract the same components as in the methanol extract were identified with HPLC. The metabolites CL 263284 and CL 189215 accounted for 0.012 mg/kg or 11.2% TRR and 0.014 mg/kg or 13.1% TRR, respectively. The metabolite CL 312622 and imazamox were identified at levels of up to 2.1% TRR. The remaining peaks were characterised by their chromatographic properties and accounted for up to 1.7% TRR. Analysis of an identical water extract using HPLC confirmed the metabolite pattern. Additionally, 0.006 mg/kg or 5.8% TRR were characterised by their solubility in water (additional extraction of solid residue). In the ERR, 65.6% of the total radioactive residues were identified and 10.4% were characterised.
In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of rice grain with methanol and water (0.024 mg/kg or 22.5% TRR) were subjected to a sequential solubilization procedure. The residue after solvent extraction was consecutively incubated with ammonia solution, glucosidase / hesperidinase, macerozyme / cellulase and amylases / amyloglucosidase. These solubilization steps released portions of 0.003 mg/kg or 2.8% TRR (pooled sample of two ammonia solubilizates), 0.002 mg/kg or 1.5% TRR (glucosidase), 0.005 mg/kg or 5.0% TRR (macerozyme) and 0.002 mg/kg or 2.1% TRR (amylase). The final residue accounted for 0.013 mg/kg or 12.2% TRR and was after the different enzymatic treatments regarded as not bioavailable.
During the sequential solubilization procedure, 0.012 mg/kg or 11.4% TRR were released being thereby characterised as soluble with ammonia, glucosidase, macerozyme and amylase.
In total, 75.9% of the TRR were identified or characterised from the ERR and additional 11.4% of the TRR were characterised by solubilization from the RRR. Summarised with the final residue, the radioactive residues accounted for a Grand Total of 0.108 mg/kg or 99.5% TRR.

Enantiomer ratio of imazamox: In order to analyse if one enantiomer of imazamox was preferably metabolised in rice, enantiomer-specific analyses were performed in all three matrices (forage, straw and grain). For the test item (diluted stock solution) the ratio of enantiomer 1 to enantiomer 2 was found to be approximately 1:1. For the determination of the enantiomer ratio in the different matrices, the parent compound and metabolite CL 263284 were isolated from methanol extracts and analysed using different HPLC methods for the enantiomer-specific analysis of imazamox and CL 263284. Despite several efforts, no reliable results for the enantiomer-specific analyses of CL 263284 could be obtained in forage and grain since no separation of the enantiomers was achieved in these matrices. The enantiomer ratio of imazamox and its metabolite CL 263284 were found to be approximately 1:1 in rice straw, while for forage and grain higher ratios for enantiomer 2 of imazamox than for enantiomer 1 were detected (70.0 and 76.1%, respectively).

3. Proposed metabolic pathway

The proposed metabolic pathway of imazamox in paddy rice is shown in Figure 6.2.1-3. The key step of the metabolism of imazamox in rice after foliar application is the cleavage of the methyl ether group (demethylation) resulting in metabolite CL 263284. A subsequent glycosylation of the hydroxyl group resulted in the formation of metabolite CL 189215. Alternatively, the hydroxyl group of CL 263284 was oxidised to a carboxyl group, which resulted in CL 312622, a dicarboxylic acid.

4. Storage stability

Storage stability investigations were performed in the rice matrices at the beginning and at the end of the study. For all three matrices a re-analysis of stored extracts and a re-extraction of stored samples was performed.
Methanol and water extracts of forage, straw and grain used for quantitative metabolite investigations were prepared within periods of 17 to 157 days after sampling.
Rice forage: The methanol extract of rice forage was initially HPLC analysed 189 days after sampling. The analyses for quantitative evaluation and confirmatory analyses of the methanol and water extracts were performed within 241 to 276 days after sampling.
A re-analysis of stored methanol extract (storage time of extract 263 days) using HPLC confirmed the metabolic patterns of the initial analysis. In order to demonstrate the stability of the components in homogenised forage during storage, a re-extraction was performed 407 days after sampling and HPLC analysed within 13 days. The obtained chromatogram agreed well with the peak patterns obtained after the initial extraction.
Rice straw: The methanol extract of rice straw was initially HPLC analysed 49 days after sampling. The analyses for quantitative evaluation and confirmatory analyses of the methanol and water extracts were performed within 107 to 134 days after sampling.
A re-analysis of stored methanol extract (storage time of extract 263 days) using HPLC confirmed the metabolic patterns of the initial analysis. In order to demonstrate the stability of the components in homogenised straw during storage, a re-extraction was performed 267 days after sampling and HPLC analysed within 13 days. The obtained chromatogram agreed well with the peak patterns obtained after the initial extraction.
Rice grain: The methanol extract of rice grain was initially HPLC analysed 49 days after sampling. The analyses for quantitative evaluation and confirmatory analyses of the methanol and water extracts were performed within 106 to 135 days after sampling. A re-analysis of stored methanol extract (storage time of extract 263 days) using HPLC confirmed the metabolic pattems of the initial analysis. In order to demonstrate the stability of the components in homogenised grain during storage, a re-extraction was performed 267 days after sampling and HPLC analysed within 14 days. The obtained chromatogram agreed well with the peak patterns obtained after the initial extraction.
Summary:
In all cases, the chromatograms of re-analysed extracts and of re-extracted matrix showed similar peak patterns as the initial analyses. Summarizing the results, the storage stability was confirmed in the matrices over a storage interval of at least 280 days and in methanol extracts over a storage interval of at least 263 days after sampling.

III. CONCLUSION

^{14}C,^{15}N-labelled imazamox (BAS 720 H, Reg. No. 4096483, CL299263) was applied to imidazolinone herbicide resistant paddy rice by one foliar application at a rate of 75 g a.s./ha, in combination with the adjuvant Dash. Forage was sampled 42 days after treatment, while straw and grain were harvested 182 days after treatment.

The highest levels of total radioactive residues (TRR) were found in rice forage accounting for 0.257 mg/kg followed by straw with 0.207 mg/kg. Lower levels of 0.108 mg/kg were detected in grain.

The extractability of the radioactive residues with methanol and water was high for the three rice matrices (77.9 to 87.1% TRR). For forage and straw, the predominant part of the radioactive residues was extracted with methanol, while for rice grain similar amounts of residues were extracted with methanol and water. The residual radioactive residues after solvent extraction (RRR) were 12.0% of the TRR for forage, about 25.2% of the TRR for straw and 22.5% TRR for grain. The residues after solvent extraction were further solubilised using an individual combination of sequential solubilization steps, whereby 4.9 to 11.8% TRR were additionally released.

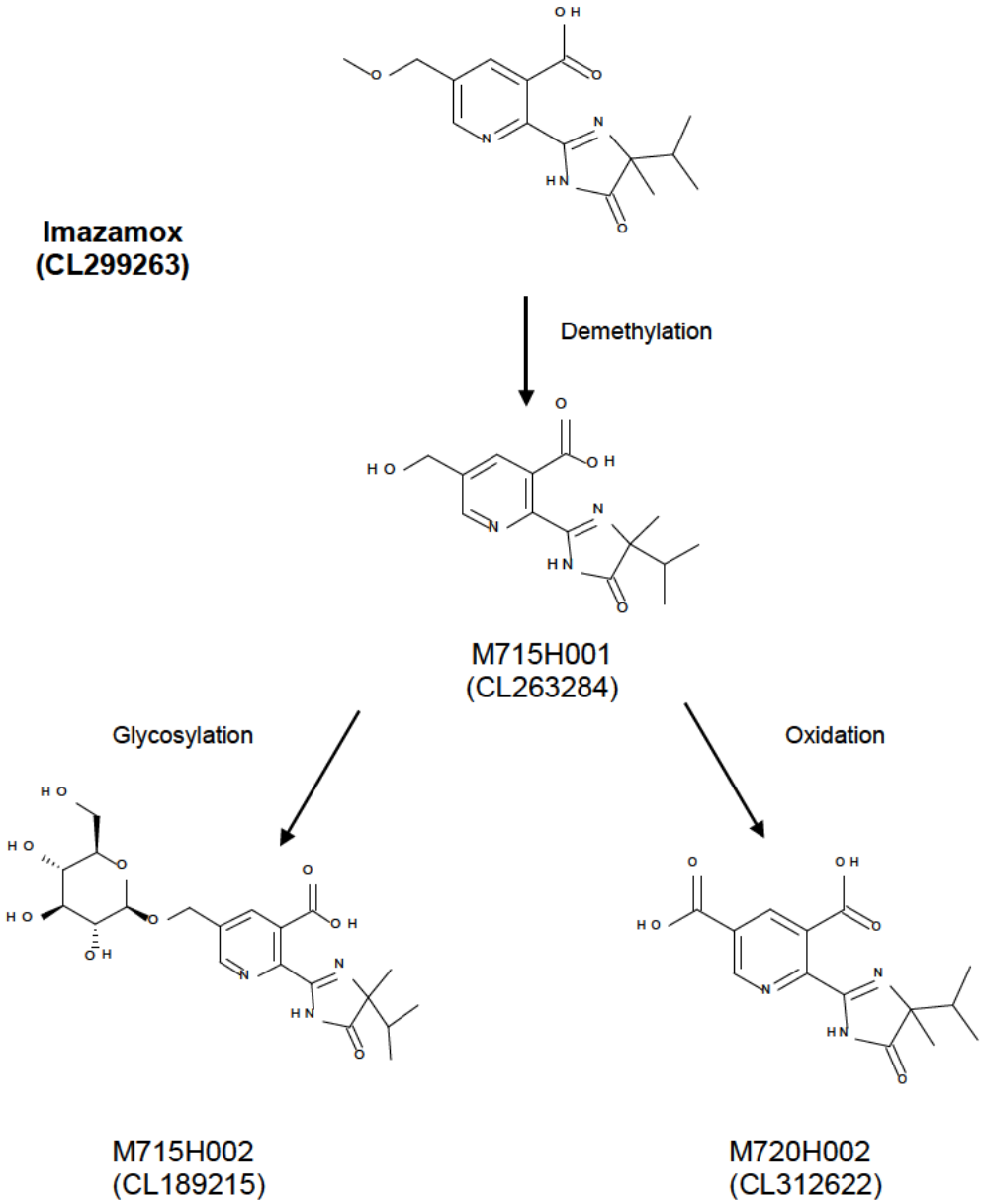
Identification of imazamox and its metabolites was based retention time comparison and co-chromatography experiments with the reference items. The isolated fractions from a wheat metabolism study, which contained metabolites CL 263284, CL 189215 and CL 312622, were used for the determination of retention times and for co-chromatography with a processed methanol extract sample of rice forage. Likewise, the active substance was successfully identified.

In the ERR, the parent compound imazamox accounted for 18.1% TRR in forage and was detected in lower amounts in straw and grain (up to 4.6% TRR). In all rice matrices the metabolite CL263284 (M715H001), which resulted from cleavage demethylation of the parent compound, was the main component identified (up to 48.0% TRR). The glycosylated form of M715H001, metabolite CL 189215 (M715H002), was also found in all three matrices (from 7.5 to 25.5% TRR). The dicarboxylic acid CL 312622 (M720H002), which resulted from oxidation of M715H001, was found at lower levels (up to 4.8% TRR).

The enantiomer ratio of imazamox and its metabolite M715H001 were found to be approximately 1:1 in rice straw, while for forage and grain higher ratios for enantiomer 2 of imazamox than for enantiomer 1were detected.

Storage stability investigations were performed in extracts of all three rice matrices (forage, straw and grain) showing that there was no relevant change in the peak patterns during the storage of sample extracts and homogenised matrices.

Figure 6.2.1-3: Proposed Metabolic Pathway of Imazamox in (IMI-tolerant) Paddy Rice



Report:	CA 6.2.1/6 Wu S.-S. 1998(a) CL 299263: Metabolism of [Pyridine-6-14C] CL 299263 in alfalfa under field conditions BASF DocID ID-640-008
Guidelines:	EPA 40 CFR 158.240; EPA 860.1300; EEC 91/414 Annex II (Part A Section 6.1)
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

Executive Summary

The [^{14}C]imazamox was applied once post-emergence to established and newly seeded alfalfa at the trifoliate stage, and to newly seeded alfalfa 5 days after the first cut and at the early bud stage at an application rate of 134.5 g a.s./ha. The total radioactive residues (TRR) declined significantly from 10.28-15.28 mg/kg at 0 DAT to 0.18 to 0.67 mg/kg for forage and 0.83 mg/kg for hay at 28 DAT, and to <0.01 to 0.02 mg/kg at the third cut for the forage samples collected from all the treatment plots, except for the seed production plot. The TRRs in hay were higher than those observed in forage at the same time intervals due to the dehydration of the samples. The total radioactive residues (TRR) in forage was 6.51 mg/kg at 0 DAT after a late treatment of the newly seeded alfalfa with [^{14}C] labeled imazamox at early bud stage; at harvest, the residue level in the alfalfa seed was 0.02 mg/kg.

The incurred radioactive residues in forage and hay were mostly (64.6 to 98.8%) extractable with aqueous acetone-methanol mixture. An additional 1.2 to 14.2% of the TRR were extracted into acidic aqueous methanol. Cellulase treatment of the dry RRR solubilized another 1.0-7.1% of the TRR. This suggested the presence of glycosidic linkages of the residue with endocons in the alfalfa samples. All forage and hay samples analyzed gave a similar metabolite profile at a comparable post-application sampling period, regardless of the type of crop (established versus newly seeded) and application timing. At 0 DAT, the unchanged parent compound accounted for the majority of the TRR. At later sampling periods, imazamox was extensively metabolized by the alfalfa plants and declined to a very low level, whereas its oxidation products (CL 263284 and CL 312622) and a glucose conjugate (CL 189215) of CL 263284 became the most prominent terminal residues.

Total radioactive residue in the alfalfa seed was 0.02 mg/kg, of which nearly half was extractable with aqueous acetone-methanol mixture. An additional 15 and 6.4% of TRR was extracted into acidic aqueous methanol and solubilized by cellulase, respectively. HPLC analysis of the extractable residue showed that imazamox was extensively metabolized into a number of metabolites. Metabolites CL 189215, CL 263284, CL 312622 as well as other multiple components were observed in the methanol/acetone/water extract of the alfalfa seed with none of the components exceeding a concentration of >0.01 mg/kg.

In summary, it appears that the metabolism of imazamox in alfalfa is not affected by the age of the crop, timing of application, nor the environmental factors of different geographic locations. Imazamox was rapidly metabolized in alfalfa via O-dealkylation of the methoxy-methyl side chain to form the 5-hydroxy-methyl metabolite CL 263284. Subsequently, the hydroxyl-methyl metabolite was either rapidly conjugated with glucose to produce the glucoside metabolite (CL 189215) in alfalfa or further metabolized to form a 3,5-dicarboxylic acid derivative (CL 312622) plus other minor unknown metabolites. The metabolic route of imazamox in alfalfa was thus consistent with those found in pea, soybean, wheat, and rat.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	^{14}C-pyridine-labeled (6 position) imazamox ^{13}C-pyridine-labeled (6 position) imazamox Unlabeled [^{12}C] imazamox
Lot/Batch #:	^{14}C + ^{13}C + ^{12}C imazamox: AC10727-92 Unlabeled [^{12}C] imazamox: AC 9745-124A
Purity:	Radiochemical purity: 98.6% of isotopic mix Specific activity of mixture: 20.0 μCi/mg
CAS#:	114311-32-9
Stability of test compound:	The test item was stable over the test period.

2. Test Commodity:

Crop:	Alfalfa
Type:	Legume vegetables fresh
Variety:	WL414, Avalanche (imidazolinone tolerant)
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Forage, hay
Sample size:	34-2499 g

3. Soil: A loamy sand was used. The soil physicochemical properties are described below (see Table 6.2.1-21).

Table 6.2.1-21: Soil physicochemical properties

Soil series	Soil type	pH	OM %	Sand %	Silt %	Clay %	Moisture at 1/3 bar %	CEC cmol/kg
Not reported	Loamy sand*	7.6	0.5***	77-83*	9-17*	6-8*	7.3-14.7	4.7-11.5

* USDA scheme ***Organic matter

B. STUDY DESIGN

A metabolism study in alfalfa was conducted with [^{14}C]imazamox for American Cyanamid in 1997. The field phase of the study was performed by Qualls Agricultural Laboratory, at the Ephrata, North Carolina test facility and at EXCEL Research Services, Inc. (EXCEL), Madera, California.

1. Test procedure

Field trials

The experiment consisted of four treatment plots and four control plots. The control Plots A, C, E and G were sprayed with a formulation blank solution. On the treated plots ^{14}C labeled imazamox (formulated as an ammonium salt) was applied once at an exaggerated rate of 134.5 g a.s./ha (3X) as a post-emergence treatment. The formulated solution was sprayed onto established alfalfa at the 3rd trifoliate stage (plot B, Ephrata, WA), newly seeded alfalfa plants at the 3rd trifoliate stage (plot D, Madera, CA); newly seeded alfalfa plants 5 days after the 1st cut (plot F, Madera, CA) and newly seeded alfalfa plants at early bud stage (plot H, Madera, CA).

Harvest

The alfalfa forage and hay samples were taken from all four plots at the day of the application (0 DAT), 26 DAT (plots B, D and F), 45 or 74 DAT (1st cut; plots B and D, respectively); 87, 111 or 25 DAT (2nd cut, plots B, D and F, respectively) and 87, 157 or 53 DAT (3rd cut, plots B, D and F, respectively). Mature alfalfa seeds were sampled 74 DAT from plot H. Samples of plants were taken by cutting the plants just above the soil surface with shears. Soil samples were taken at 0 DAT (30 cm depth) and at the last plant sampling event from all plots (46 cm depth).

2. Description of analytical procedures

All samples were shipped on dry ice. All samples were immediately stored in a freezer at approximately -20°C.

Plant:

Whole plant samples were ground separately with dry ice in an appropriate grinder or mill. The mixture was transferred to loosely covered containers and placed in a freezer to allow for complete dissipation of carbon dioxide from the samples. The total radioactive residue (TRR) in green plants, hay and seeds was determined by combustion of nominal 0.5 g samples in triplicate except the plant samples collected on the day of treatment (0 DAT). Because of very small sample size and the expected high radioactivity, the TRR in the 0 DAT plant samples was determined by solvent extraction followed by combustion. Background values were determined from control samples taken at the same sampling intervals.

Soil:

Soil cores were thawed and cut into sections of 0-8 cm, 8-15 cm, 15-30 cm and 30-46 cm. The soil was dislodged from each section, common depths composited, weighed and air dried. When dry, the soil was weighed, ground and stored frozen until analysis. Subsamples of each composite sample were combusted in triplicate to determine the total ^{14}C residue. Background values were determined using control samples taken at the corresponding interval.

Radioanalysis:
Plant tissue and soil samples were analyzed for total radioactive residues (TRR) using an oxidizer. Triplicate subsamples of plant tissues or RRR were weighed for combustion. The soil samples were combusted using the same procedures. Radiolabeled carbon dioxide from each combusted sample was trapped in a mixture of absorber and scintillant. The ^{14}C residues in samples were determined by liquid scintillation counting (LSC).

Extraction:
Ground alfalfa samples were extracted with methanol/acetone/water (1:1:1, v/v/v), shaken overnight and then extracted (0 DAT forage samples were homogenized immediately with extraction solvent). The homogenate was centrifuged and the RRR were extracted two more times using the same extraction procedure. After drying, aliquots of the RRR were combusted in triplicate using a sample oxidizer to determine the extent of RRR. The combined methanol/acetone/water extracts were concentrated, re-dissolved in extraction solution and assayed in triplicate by LSC or by combustion to determine the extractability of the ^{14}C residue. A subsample of the methanol/acetone/water fraction was further concentrated, reconstituted in water, filtered and analyzed for the radioactive components by HPLC to determine the metabolite profiles.

Cold Methanol Precipitation
Due to the low total radioactive residue in the forage and hay samples from late sampling intervals (2^{nd} and/or 3^{rd} cut), the extracted radioactive residues of these samples frequently required additional cleanup by cold methanol precipitation before HPLC analysis. An aliquot of the methanol/acetone/water extract was reduced to a small volume of non-viscous residuum, methanol was added until a precipitate began to form. The extract was chilled, filtered, and the filtrate reconstituted in water for LSC.

Solvent Partition
The concentrated methanol/acetone/water extract of alfalfa seed could not be analyzed directly by HPLC because of the low radiocarbon content and the complex nature of the matrix. The extract was subjected to hexane partition and followed by cold methanol precipitation to remove the interfering matrices before HPLC analysis.

Residual readioactive residues
RRR of the extracted alfalfa forage, hay and seed containing greater than 0.05 mg/kg or 10% of the recovered radioactivity were re-extracted three times with a mild acidic solvent. Exceptions were: the 0 DAT forage samples which had extractabilities ranging 98.2 to 98.8%; the forage sampled at 111 DAT from plot D, which contained a very low residue level (0.003 mg/kg) in the RRR; and one of the two repeatedly analyzed samples (i.e. 26 DAT forage from plot D and 55 DAT hay from plot F). Dry RRR I (2-60 g) from the methanol/acetone/water extraction were soaked and shaken overnight with the acidic extraction solvent (methanol/water/HCl, 40:9:1, v/v/v). The mixture was then homogenized and centrifuged to separate the extract from the solid matrix. The procedure was repeated twice. The combined extracts were evaporated to dryness and reconstituted in aqueous methanol (methanol/water = 4:1, v/v). Triplicate aliquots of the extract were assayed for radioactivity by direct LSC or by combustion. After air-drying, aliquotes of the RRR (designated as RRR II) were combusted in an oxidizer to determine the radioactivity content.

The extracted residues (if >0.01 mg/kg) from the alfalfa samples were further cleaned up by cold methanol precipitation, if necessary, and analyzed by HPLC to determine the metabolite profile.

Enzymatic treatment
The methanol/acetone/water extract of the alfalfa hay was first partially purified on solid phase extraction cartridges (SPE). An aliquot of the methanol eluate was evaporated to dryness and reconstituted in methanol. The prepared sample was purified by repeated HPLC and HPLC fractions were collected. Aliquots of fractions which contained the major portion of the radioactivity was analyzed by LSC to locate metabolite CL 189215 (M2). Solvent in fraction 20 (metabolite CL 189215) was evaporated to dryness and the residuum was taken up in methanol. A portion of this methanol solution was evaporated to dryness and the residuum containing metabolite CL 189215 was incubated with β-glucosidase. The incubation was quenched by adding methanol, centrifuged and the concentrated supernatant was analyzed by HPLC.

The dry RRR II derived from the acidic methanol/water extraction of the alfalfa samples (forage, hay or seed) containing radioactivity greater than 10% of the TRR and/or significant ^{14}C residue were treated with cellulose. The mixture was centrifuged to separate the extract from the solid precipitate. The extract was set aside. To the solid precipitate, an additional fresh cellulase was added. The mixture was further incubated, centrifuged, the supernatants were combined, reduced to dryness, reconstituted in water and then assayed for radioactivity by direct LSC or by combustion. The undigested solids (designated as RRR III) were allowed to air dry and stored in a refrigerator until further analysis.

Base hydrolysis
The methanol/acetone/water extract of the alfalfa hay was partially purified on SPE cartridges to prepare the sample for base hydrolysis. An aliquot of the methanol eluate was evaporated to dryness, reconstituted in 1 N NaOH and the mixture incubated in a water bath and diluted. The pH of the solution was adjusted to about 7 before analysis by HPLC.

3. Identification of metabolites

HPLC: High performance liquid chromatography was performed to verify the purity of ^{14}C labeled imazamox and the stability of the test substance, to establish the profiles of the radiocomponents in the sample extracts and fractions, and to purify radioactive residue components. The reversed-phase HPLC system was equipped with a variable wavelength UV detector. Extracts were concentrated, assayed by LSC and characterized by HPLC. Structural characterization of the parent compound and the major metabolites was performed by Electrospray Ionization Liquid Chroma-tography/Mass Spectrometry (LC-ESI/MS).

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residues (TRR) in the forage taken 0 DAT ranged from 6.51 mg/kg in alfalfa treated at the early bud stage to 10.28 mg/kg in alfalfa treated at 5 days after first cut, to 14.00-15.28 mg/kg in the newly seeded alfalfa and alfalfa just breaking dormancy treated at third trifoliate stage. Because of the rapid growth that increased plant mass, the TRR in the forage declined significantly from 0 DAT to 26 DAT (proposed pre-harvest interval) irrespective of timings of treatment and geographic locations. Forage samples taken at 26 DAT contained radioactive residue of 0.34 mg/kg in the established alfalfa treated at third trifoliate (Plot B), 0.67 mg/kg in the newly seeded alfalfa treated at third trifoliate (Plot D), and 0.18 mg/kg for the newly seeded alfalfa treated at five days after the first cut (Plot F). The total radioactive residue level in the forage continued to decline to levels between <0.01 mg/kg and 0.02 mg/kg at the third cut. The TRRs in the hay were higher than those observed in the forage at the same time intervals due to the dehydration of the samples. TRR in the alfalfa hay was three to five times higher than the TRR found in forage sampled at the same sampling intervals. At harvest, residue levels in alfalfa seed were 0.02 mg/kg.

Total ^{14}C residues in soil samples, expressed on dry soil weight, are summarized in Table 6.2.1-23. ^{14}C residues in soil (0-8 cm layer) at 0 DAT in soil of Plot B ranged from 0.04 mg/kg to 0.15 mg/kg. The TRR in the 3-6 and 15-30 cm layers was <0.01 mg/kg. Similarly, ^{14}C residues in soil (0-8 cm layer) at 0 DAT in soil of Plots D, F and H ranged from 0.06 mg/kg to 0.09 mg/kg. The TRR in the 8-15 and 15-30 cm layers was <0.01 mg/kg except for one soil fraction (0.01 mg/kg, 8-15 cm from all four soil cores in plot F). At harvest, the ^{14}C residues in soil (0-8 cm layer) from all the treatment plots had declined to 0.01-0.02 mg/kg. The TRR in the 8-15 cm layer of soil ranged from 0.01 to 0.03 mg/kg. Total radioactive residue in the 15-30 and 30-46 cm layers was <0.01 mg/kg except for one sample (0.01 mg/kg, 15-30 cm from plot F). However, the TRR in the 6-12 and 30-46 cm layers of soil collected from Plot B ranged from 0.01 to 0.05 mg/kg. The purpose of analyzing soil samples was to demonstrate deposition of [^{14}C] imazamox on the soil. No attempts were made to further characterize the nature of radioactive residues in the soil.

Table 6.2.1-22: Total radioactive residues (TRRs) in various alfalfa matrices after application of [^{14}C]imazamox

TRRs in treated alfalfa matrices			
Plot	**Matrix plant**	**DAT plant**	**TRR (combustion) [mg/kg]**
Plot B (breaking dormancy, at 3rd trifoliate stage)	Forage	0	15.28
	Forage	26	0.34
	Forage	45 - 1st cut	0.21
	Hay	48 - 1st cut	0.63
	Forage	87 - 2nd cut	0.03
	Hay	89 - 2nd cut	0.12
	Forage	129 - 3rd cut	0.02
	Hay	131 - 3rd cut	0.06
Plot D (newly seeded crop, at 3rd trifoliate stage)	Forage	0	14.00
	Forage	26	0.67
	Forage	76 - 1st cut	0.07
	Hay	78 - 1st cut	0.35
	Forage	111 - 2nd cut	0.01
	Hay	113 - 2nd cut	0.04
	Forage	157 - 3rd cut	<0.01
	Hay	160 - 3rd cut	0.02
Plot F (newly seeded crop, late treatment at 5 days after 1st cut)	Forage	0	10.28
	Forage	26 - 2nd cut	0.18
	Hay	28 - 2nd cut	0.83
	Forage	53 - 3rd cut	0.02
	Hay	55 - 3rd cut	0.08
Plot H (newly seeded crop, late treatm. At early bud stage)	Forage	0	6.51
	Seed	74	0.02

DAT days after treatment

Table 6.2.1-23: Total radioactive residues (TRRs) in various soil samples after application of [^{14}C]imazamox

Soil	DAT soil	Fraction* [cm]	TRR (combustion) [mg/kg]
Plot B, WA (established crop at 3rd trifoliate stage)	0	0-8	0.15 (0.04)
		8-15	<0.01
		15-30	<0.01
		30-46	NS
	129	0-8	0.02
		8-15	0.03 (0.01)
		15-30	0.05 (0.01)
		30-46	0.02 (0.01)
Plot B, WA (newly seeded crop, at 3rd trifoliate stage)	0	0-8	0.07 (0.06)
		8-15	<0.01
		15-30	<0.01
		30-46	NS
	157	0-8	0.01
		8-15	0.01
		15-30	<0.01
		30-46	<0.01
Plot B, WA (late treatment 5 days after 1st cut)	0	0-8	0.09
		8-15	0.01
		15-30	<0.01
		30-46	NS
	53	0-8	0.01
		8-15	0.01
		15-30	0.01 (<0.01)
		30-46	<0.01
Plot B, WA (late treatment at early bud stage)	0	0-8	0.09 (0.08)
		8-15	<0.01
		15-30	<0.01
		30-46	NS
	73	0-8	0.01
		8-15	0.02 (0.01)
		15-30	<0.01
		30-46	<0.01

DAT days after treatment
NS not sampled
* higher (lower) value of two replicate samples for each soil depth
The TRR in soil are rounded to the nearest 0.01 mg/kg

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

The extractabilities of radioactive residues from alfalfa plant, hay and seed are summarized in Table 6.2.1-24.

Table 6.2.1-24: Extraction efficiency of residues of [^{14}C]imazamox in alfalfa matrices

TRRs in Treated Alfalfa Matrices							
Treatment/Plot	Matrix	Sampling Interval	TRR (combusted) [mg/kg]	ERR (Methanol/Acetone/Water + Acidic Methanol/Water)		RRR II[1]	
				% TRR	mg/kg	% TRR	mg/kg
Plot B*	Forage	0 DAT	15.28	98.8	15.097	1.2	0.183
	Forage	26 DAT	0.34	90.5	0.308	9.5	0.032
	Forage	45 - 1st cut	0.21	94.9	0.199	5.1	0.011
	Hay	48 - 1st cut	0.63	95.4	0.601	4.6	0.029
	Forage	87 - 2nd cut	0.03	91.4	0.027	8.6	0.003
	Hay	89 - 2nd cut	0.12	93.6	0.112	6.4	0.008
	Forage	129 - 3rd cut	0.02	96.4	0.019	3.6	0.001
	Hay	131 - 3rd cut	0.06	94.0	0.056	6.0	0.004
Plot D**	Forage	0 DAT	14.00	98.2	13.748	1.8	0.252
	Forage	26 DAT	0.67	83.4	0.559	16.6	0.111
	Forage	76 - 1st cut	0.07	83.8	0.059	16.2	0.011
	Hay	78 - 1st cut	0.35	78.5	0.274	21.5	0.075
	Forage	111 - 2nd cut	0.01	74.9	0.007	25.1	0.003
	Hay	113 - 2nd cut	0.04	81.2	0.032	18.8	0.008
	Forage	157 - 3rd cut	<0.01	n.e.	n.e.	n.e.	n.e.
	Hay	160 - 3rd cut	0.02	79.6	0.016	20.4	0.004
Plot F***	Forage	0 DAT	10.28	98.8	10.157	1.2	0.123
	Forage	26 - 2nd cut	0.18	90.9	0.164	9.1	0.016
	Hay	28 - 2nd cut	0.83	91.5	0.760	8.5	0.071
	Forage	53 - 3rd cut	0.02	86.1	0.017	13.9	0.003
	Hay	55 - 3rd cut	0.08	78.8	0.063	21.2	0.017
Plot H****	Forage	0 DAT	6.51	98.2	6.393	1.8	0.117
	Seed	74 DAT	0.02	72.7 66.6[2]	0.015 0.013[2]	30.0	0.006

* breaking dormancy, at 3rd trifoliate stage
** newly seeded crop, at 3rd trifoliate stage
*** newly seeded crop, late treatment at 5 days after 1st cut
**** newly seeded crop, late treatment at early bud stage
1 residual radioactive residues after methanol/acetone/water extraction followed by acidic methanol/water extraction
2 Two separate alfalfa samples were extracted with methanol/acetone/water at two different times
n.e. not extracted

Table 6.2.1-25: Distribution of TRR following solvent partitioning and extraction of residues of [^{14}C]imazamox in alfalfa matrices

Treatment/ Plot	Matrix	Sampling Interval	TRR (combusted) [mg/kg]	Distribution of Radioactive Residues				ERR		RRR II[1]	
				Methanol/ Acetone/ Water		Acidic Methanol/ Water					
				% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Plot B*	Forage	0 DAT	15.28	98.8	15.097	n.e.	n.e.	98.8	15.097	1.2	0.183
	Forage	26 DAT	0.34	89.3	0.304	1.2	0.004	90.5	0.308	9.5	0.032
	Forage	45 - 1st cut	0.21	94.9	0.199	n.e.	n.e.	94.9	0.199	5.1	0.011
	Hay	48 - 1st cut	0.63	91.5	0.576	3.9	0.025	95.4	0.601	4.6	0.029
	Forage	87 - 2nd cut	0.03	91.4	0.027	n.e.	n.e.	91.4	0.027	8.6	0.003
	Hay	89 - 2nd cut	0.12	88.6	0.106	5.0	0.006	93.6	0.112	6.4	0.008
	Forage	129 - 3rd cut	0.02	96.4	0.019	n.e.	n.e.	96.4	0.019	3.6	0.001
	Hay	131 - 3rd cut	0.06	86.7	0.052	7.3	0.004	94.0	0.056	6.0	0.004
Plot D**	Forage	0 DAT	14.00	98.2	13.748	n.e.	n.e.	98.2	13.748	1.8	0.252
	Forage	26 DAT	0.67	77.3 77.0[2]	0.518 0.516[2]	6.4	0.043	83.4	0.559	16.6	0.111
	Forage	76 - 1st cut	0.07	74.1	0.052	9.7	0.007	83.8	0.059	16.2	0.011
	Hay	78 - 1st cut	0.35	64.7	0.226	13.8	0.048	78.5	0.274	21.5	0.075
	Forage	111 - 2nd cut	0.01	74.9	0.007	n.e.	n.e.	74.9	0.007	25.1	0.003
	Hay	113 - 2nd cut	0.04	70.5	0.028	10.7	0.004	81.2	0.032	18.8	0.008
	Forage	157 - 3rd cut	<0.01	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
	Hay	160 - 3rd cut	0.02	68.5	0.014	11.1	0.002	79.6	0.016	20.4	0.004
Plot F***	Forage	0 DAT	10.28	98.8	10.157	n.e.	n.e.	98.8	10.157	1.2	0.123
	Forage	26 - 2nd cut	0.18	85.9	0.155	5.0	0.009	90.9	0.164	9.1	0.016
	Hay	28 - 2nd cut	0.83	80.7	0.670	10.8	0.090	91.5	0.760	8.5	0.071
	Forage	53 - 3rd cut	0.02	80.2	0.016	5.9	0.001	86.1	0.017	13.9	0.003
	Hay	55 - 3rd cut	0.08	65.3 64.6[2]	0.052 0.052[2]	14.2	0.011	78.8	0.063	21.2	0.017

Table 6.2.1-25: Distribution of TRR following solvent partitioning and extraction of residues of [^{14}C]imazamox in alfalfa matrices

Treatment/ Plot	Matrix	Sampling Interval	TRR (combusted) [mg/kg]	Distribution of Radioactive Residues				ERR		RRR II[1]	
				Methanol/ Acetone/ Water		Acidic Methanol/ Water					
				% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Plot H****	Forage	0 DAT	6.51	98.2	6.393	n.e.	n.e.	98.2	6.393	1.8	0.117
	Seed	74 DAT	0.02	57.7 51.6[2]	0.012 0.010[2]	15.0	0.003	72.7 66.6[2]	0.015 0.013[2]	30.0	0.006

* breaking dormancy, at 3rd trifoliate stage
** newly seeded crop, at 3rd trifoliate stage
*** newly seeded crop, late treatment at 5 days after 1st cut
***** newly seeded crop, late treatment at early bud stage
1 residual radioactive residues after methanol/acetone/water extraction followed by acidic methanol/water extraction
2 Two separate alfalfa samples were extracted with methanol/acetone/water at two different times
n.e. not extracted

1. Extraction and characterization of residues in alfalfa

Overall, the extracted residues from all forage and hay samples ranged from 64.7 to 98.8% of the TRR. The extractability of the incurred ^{14}C residues in the samples taken from the established alfalfa ranged from 86.7% for the third cut sample (131 DAT) to 98.8% for the 0 DAT sample. However, for the newly seeded alfalfa, the extractability significantly decreased for the forage and hay samples at the later growth stages. For the alfalfa seeded in 1997, the extractability for the forage and hay samples decreased from 77.0 to 85.9% at 26 DAT to 64.6 to 68.5% at the third cuts (55 DAT to 160 DAT). The unextracted radioactivity in the RRR I accounted for 1.2 to 35.4% of the TRR. The residue levels in these solids ranged from 0.001 to 0.252 mg/kg. More than half (51.6 to 57.7%; 0.01 mg/kg) of the TRR in the alfalfa seed was extracted with the same solvent mixture. The radioactive residue concentration in the RRR I of the alfalfa seed was very low at <0.01 mg/kg (or 42.3-48.4% of the TRR in the seed). Following extraction with the solvent mixture of aqueous methanol and acetone, RRR I containing greater than 0.05 mg/kg or 10% TRR were re-extracted using acidified aqueous methanol (2% HCl in 80% methanol). The acidified solvent released an additional 1.2 to 15.0% of the TRR in the original alfalfa samples. The residue levels in these extracts ranged from 0.001 to 0.090 mg/kg. The unextracted fraction was designated as RRR II.

Attempts were made to release the remaining significant level of the bound radioactive residues in the RRR II by enzyme and acid hydrolyses. The results suggested the presence of glycosidic linkages of the residue with endocons in the alfalfa samples. After cellulase hydrolysis, the solid plant matrices (RRR III) that contained greater than 0.05 mg/kg radioactive residue were subjected to acid hydrolysis (6 N HCl reflux). The acid hydrolysis step released about 60% of the radioactivity in the plant matrices RRR III (equivalent to 8-9% of TRR or 0.033-0.056 mg/kg) while about 40% (equivalent to 6% of TRR or 0.022-0.039 mg/kg) of the radioactivity remained bound (data not shown). The results suggested that the labeled carbon has been incorporated into undefined plant constituents.

Table 6.2.1-26: Metabolites detected in alfalfa matrices following foliar application of [^{14}C]imazamox

Plot	Matrix	Sampling Interval (DAT)	TRR[2] [mg/kg]	Components							
				CL 189215		CL 263284		CL 312622		Imazamox	
				% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Plot B*	Forage	0	15.28	1.24	0.189	0.94	0.144	1.60	0.244	84.96	12.98
	Forage	26	0.34	13.98	0.048	23.15	0.079	22.11	0.075	5.52	0.019
	Forage	45-1st cut	0.21	25.66	0.054	18.26	0.038	26.67	0.056	2.65	0.006
	Hay	48-1st cut	0.63	23.85	0.150	14.48	0.091	26.05	0.164	5.52	0.034
	Forage	87-2nd cut	0.03	25.84	0.008	21.08	0.006	13.79	0.004	4.51	0.001
	Hay	89-2nd cut	0.12	30.81	0.037	11.28	0.014	21.67	0.026	1.53	0.002
	Forage	129-3rd cut	0.02	20.15	0.004	24.67	0.005	13.14	0.003	6.40	0.001
	Hay	131-3rd cut	0.06	21.90	0.013	25.06	0.015	20.64	0.012	2.63	0.002
Plot D**	Forage	0	14.00	0.93	0.130	1.23	0.172	1.56	0.218	82.92	11.609
	Forage	26	0.67	19.80	0.132	14.11	0.095	17.80	0.119	3.46	0.023
	Forage	76-1st cut	0.07	16.96	0.012	9.49	0.007	13.91	0.010	4.36	0.003
	Hay	78-1st cut	0.35	19.62	0.069	14.73	0.051	20.13	0.070	3.60	0.013
	Forage	111-2nd cut	0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Hay	113-2ndcut	0.04	11.83	0.005	14.90	0.006	3.96	0.002	2.73	0.001
	Forage	157-3rd cut	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Hay	160-3rd cut	0.02	16.80	0.003	15.89	0.003	0.71	<0.001	2.43	<0.001
Plot F***	Forage	0	10.28	1.50	0.154	1.93	0.198	1.97	0.203	79.66	8.189
	Forage	26-2nd cut	0.18	25.84	0.047	14.24	0.026	17.98	0.032	4.20	0.008
	Hay	28-2nd cut	0.83	25.02	0.208	12.72	0.105	22.48	0.186	4.36	0.036
	Forage	53-3rd cut	0.02	15.69	0.003	16.53	0.003	7.03	0.001	1.57	<0.001
	Hay	57-3rd cut	0.08	14.07	0.011	18.53	0.015	7.81	0.007	3.62	0.003
Plot H****	Forage	0	6.51	0.70	0.046	1.02	0.066	0.67	0.044	86.63	5.640
	Seed	74	0.02	3.00	0.001	7.21	0.001	2.05	<0.001	2.32	<0.001

* breaking dormancy, at 3rd trifoliate stage
** newly seeded crop, at 3rd trifoliate stage
*** newly seeded crop, late treatment at 5 days after 1st cut
**** newly seeded crop, late treatment at early bud stage

Table 6.2.1-27: Balance of identified, characterized and unectractable radioactive residues in alfalfa matrices following foliar application of [^{14}C]imazamox

Plot	Matrix	Sampling Interval (DAT)	Total Identified		Total Characterized		Total Identified and/or Characterized		Unextractable (RRR)		Grand Total	
			% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Plot	Forage	0	88.74	13.557	12.95	1.981	101.7	15.538	1.2	0.183	102.9	15.721
B*	Forage	26	64.76	0.221	28.74	0.097	93.5	0.318	6.5	0.022	100.0	0.340
	Forage	45-1st cut	73.24	0.154	21.66	0.046	94.9	0.200	5.1	0.011	100.0	0.211
	Hay	48-1st cut	69.90	0.439	25.50	0.160	95.4	0.599	4.6	0.029	100.0	0.628
	Forage	87-2nd cut	65.22	0.019	26.18	0.008	91.4	0.027	8.6	0.003	100.0	0.030
	Hay	89-2nd cut	65.29	0.079	28.31	0.034	93.6	0.113	6.4	0.008	100.0	0.121
	Forage	129-3rd cut	64.36	0.013	32.04	0.007	96.4	0.020	3.6	0.001	100.0	0.021
	Hay	131-3rd cut	70.23	0.042	23.76	0.014	94.0	0.056	6.0	0.004	100.0	0.060
Plot	Forage	0	86.64	12.129	11.55	1.617	98.2	13.746	1.8	0.252	100.0	13.998
D**	Forage	26	55.17	0.369	39.02	0.262	94.2	0.631	5.8	0.039	100.0	0.670
	Forage	76-1st cut	44.71	0.032	43.88	0.030	88.6	0.062	11.4	0.008	100.0	0.070
	Hay	78-1st cut	58.08	0.203	35.53	0.125	94.3	0.328	6.4	0.022	100.7	0.350
	Forage	111-2nd cut	n.a.	n.a.	74.9	0.007	74.9	0.007	25.1	0.003	100.0	0.010
	Hay	113-2ndcut	33.42	0.014	50.19	0.020	83.6	0.034	16.4	0.007	100.0	0.041
	Forage	157-3rd cut	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Hay	160-3rd cut	35.83	0.008	44.78	0.009	80.6	0.017	19.4	0.004	100.0	0.021
Plot	Forage	0	85.66	8.744	13.75	1.414	99.4	10.158	1.2	0.123	100.6	10.281
F***	Forage	26-2nd cut	62.26	0.113	28.64	0.052	90.9	0.165	9.1	0.016	100.0	0.181
	Hay	28-2nd cut	64.58	0.535	30.00	0.249	94.6	0.784	5.4	0.045	100.0	0.829
	Forage	53-3rd cut	40.82	0.008	45.28	0.009	86.1	0.017	13.9	0.003	100.0	0.020
	Hay	57-3rd cut	44.03	0.036	41.86	0.034	85.9	0.070	14.1	0.011	100.0	0.081
Plot	Forage	0	89.02	5.796	9.19	0.598	98.2	6.394	1.8	0.117	100.0	6.511
H****	Seed	74	14.58	0.004	56.11	0.012	70.7	0.016	25.9	0.005	96.6	0.021

* breaking dormancy, at 3rd trifoliate stage
** newly seeded crop, at 3rd trifoliate stage
*** newly seeded crop, late treatment at 5 days after 1st cut
**** newly seeded crop, late treatment at early bud stage

2. Identification and quantitation of extractable residues in alfalfa

The nature of the radioactive components in the extracts of alfalfa samples was first characterized by matching the HPLC profile with the available synthetic reference compounds. Identification of metabolites was then accomplished by isolating the main radiocomponents from the alfalfa samples for mass spectrometry, if possible.

Characterization of extractable residues: At 0 DAT, the unchanged imazamox parent accounted for the majority of the TRR (79.7 to 86.6%) in the forage extracts from all treatment plots (see Table 6.2.1-26). All other components, including CL 189215 (M2; glucoside), CL 263284 (M3; 5-hydroxymethyl) and CL 312622 (M4; 3,5-di-acid) were found to be minor (each at <5% of the total residue). On 0 DAT an additional minor metabolite (M6, 2.8-3.3% TRR) was eluted that proved to correspond with an impurity in the ^{14}C-labeled test material. The radioprofiles are qualitatively similar for the extracts from green plants and hay collected at 26 DAT (approximate proposed pre-harvest interval) from both the established and the newly seeded alfalfa following treatment with ^{14}C-mazamox at different growth stages. Imazamox was extensively metabolized in alfalfa and accounted for only 3-6% of the TRR at 26 DAT. The metabolites included CL 189215 (14-26% TRR), CL 263284 (14-23% TRR) and CL 312622 (18-22% TRR), a very polar peak/region (M1, 5-7%) as well as many other very minor components which were separated by reversed-phase HPLC. The polar radiocomponent (designated M1) observed in the alfalfa hay extracts was also observed in alfalfa forage extracts at various concentration levels. This radiocomponent (Fractions 3-5, 0.002-0.055 mg/kg) was isolated from hay extracts and identified by HPLC as consisting of multiple components with concentrations of <0.01 mg/kg each. Following extractions of the alfalfa forage, hay and seed samples with the aqueous methanol acetone mixture, the PES that contained 10% or more of the TRR were re-extracted with a mild acidic solvent. The results showed an additional 1.2 to 14.2% (0.002-0.090 mg/kg) of the total radioactive residues were extracted into the acidic aqueous methanol (data not shown). The HPLC profile of the acidic aqueous methanol extracts containing >0.01 mg/kg radiocarbon showed that in addition to the main radio-metabolites found in the initial methanol/water/acetone extract, a number of more polar components were present in the extracts. The percent contribution of these radio-components, however, were low and their concentrations were all <0.01 mg/kg, except for one peak region (0.01 mg/kg) observed in the extract of the 28 DAT alfalfa hay collected from the late treatment plot F.

Characterization of bound residues: Following extraction of the alfalfa samples with 2% HCl-methanol/water, the dry RRR II that contained more than 10% of the TRR or significant residue was treated enzymatically. HPLC analysis of the cellulase hydrolyzate of the 28-DAT alfalfa hay RRR II showed that the enzyme hydrolyzate contained multiple minor unknowns and a polar region eluting at 3-7 minutes. The results suggested the presence of glycosidic linkages of the residue with endocons in the alfalfa samples. Because of the very low concentration of these radiocomponents, no attempt was made to further characterize these unknowns.

Hydrolysis of the alfalfa RRR (26 DAT forage and 78 DAT hay from plot D) via 8 hour reflux with 6 N HCl released about 60% of the radioactivity (8-9% of TRR, 0.033-0.056 mg/kg) and about 40% (6% of TRR, 0.022-0.039 mg/kg) remained unhydrolyzed The radioactivity released from the alfalfa RRR by acid hydrolysis was predominantly water soluble (97.8%). Very little radioactivity (2.2%, <0.01 mg/kg) was extracted into ethyl acetate. This suggests that the radiocomponents of 6 N HCl digest are highly hydrophilic. Due to the complexity and low radioactivity of the digest, the sample from the alfalfa hay was found not to be amenable to HPLC analysis. HPLC analysis of the digest from the alfalfa forage sample showed that the HCl hydrolyzate contained multiple undefined components and a polar region eluting at 4-6 minutes.

Characterization of the ^{14}C residues in the alfalfa seed: The total radioactive residue (TRR) in the alfalfa seed was 0.02 mg/kg, of which nearly 50% (0.01 mg/kg) was extracted with methanol/acetone/water (1:1:1). The extractable ^{14}C residue (ca. 50% of TRR, 0.01 mg/kg) was analyzed by HPLC. The radioprofile showed that imazamox was extensively metabolized to form a number of metabolites. Among these very low level metabolites, four radio-components appeared to coincide with those found in the alfalfa forage and hay extracts, i. e. CL 189215, CL 263284, CL 312622 and a very polar radioactive peak/region (M1), which has been shown to comprise multiple components. Following extraction with methanol/water/acetone, the RRR of the alfalfa seed was re-extracted by a mild acidic solvent followed by the enzyme treatment with cellulase. Due to the very low residue levels in either the acidic solvent extract (15% TRR, 0.003 mg/kg) or the cellulase hydrolyzate (6.4% TRR, 0.001 mg/kg), no attempts were made to further characterize the nature of the residues.

3. Proposed metabolic pathway

Characterization and identification of ^{14}C residues in the various substrates indicates that imazamox was rapidly metabolized in alfalfa via O-dealkylation of the methoxy-methyl side chain to form the 5-hydroxy-methyl metabolite CL 263284. Subsequently, the hydroxyl-methyl metabolite was either rapidly conjugated with glucose to produce the glucoside metabolite (CL 189215) in alfalfa or further metabolized to form a 3,5- dicarboxylic acid derivative (CL 312622) plus other polar minor unknown metabolites. The proposed metabolic pathway of imazamox in alfalfa is shown in Figure 6.2.1-4.

4. Storage stability

The metabolite profile of the alfalfa hay based on HPLC analysis shows that the incurred ^{14}C-imazamox-derived residues in the alfalfa hay are stable when stored frozen for about 13 months. The quantitative distribution of imazamox and metabolites CL 189215, CL 263284, CL 312622 and the very polar peak/region (M1) in the sample analyzed within one month after sampling is similar to those found in the sample analyzed at about 13 months after sampling. The slightly lowered peak height for metabolite CL 312622 in the later sample was due to the peak being split into two chromatographic fractions by collecting at a smaller fraction size.

III. CONCLUSION

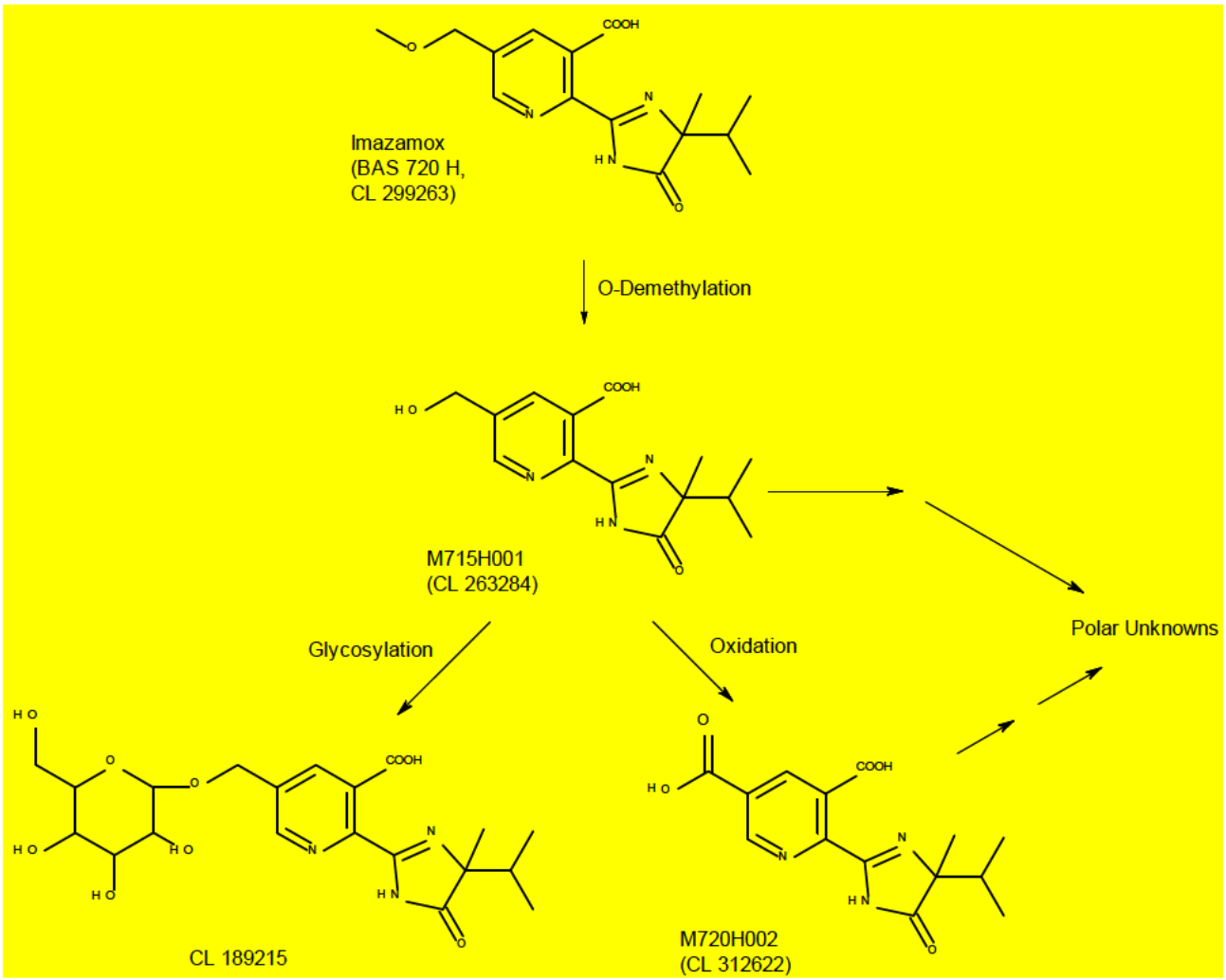
The [^{14}C]imazamox was applied once post-emergence to established and newly seeded alfalfa at the trifoliate stage, and to newly seeded alfalfa 5 days after the first cut and at the early bud stage at an application rate of 134.5 g a.s./ha. The total radioactive residues (TRR) declined significantly from 10.28-15.28 mg/kg at 0 DAT to 0.18 to 0.67 mg/kg for forage and 0.83 mg/kg for hay at 28 DAT, and to <0.01 to 0.02 mg/kg at the third cut for the forage samples collected from all the treatment plots, except for the seed production plot. The TRRs in hay were higher than those observed in forage at the same time intervals due to the dehydration of the samples. The total radioactive residues (TRR) in forage was 6.51 mg/kg at 0 DAT after a late treatment of the newly seeded alfalfa with [^{14}C] labeled imazamox at early bud stage; at harvest, the residue level in the alfalfa seed was 0.02 mg/kg.

The incurred radioactive residues in forage and hay were mostly (64.6 to 98.8%) extractable with aqueous acetone-methanol mixture. An additional 1.2 to 14.2% of the TRR were extracted into acidic aqueous methanol. Cellulase treatment of the dry RRR solubilized another 1.0-7.1% of the TRR. This suggested the presence of glycosidic linkages of the residue with endocons in the alfalfa samples. All forage and hay samples analyzed gave a similar metabolite profile at a comparable post-application sampling period, regardless of the type of crop (established versus newly seeded) and application timing. At 0 DAT, the unchanged parent compound accounted for the majority of the TRR. At later sampling periods, imazamox was extensively metabolized by the alfalfa plants and declined to a very low level, whereas its oxidation products (CL 263284 and CL 312622) and a glucose conjugate (CL 189215) of CL 263284 became the most prominent terminal residues.

Total radioactive residue in the alfalfa seed was 0.02 mg/kg, of which nearly half was extractable with aqueous acetone-methanol mixture. An additional 15 and 6.4% of TRR was extracted into acidic aqueous methanol and solubilized by cellulase, respectively. HPLC analysis of the extractable residue showed that imazamox was extensively metabolized into a number of metabolites. Metabolites CL 189215, CL 263284, CL 312622 as well as other multiple components were observed in the methanol/acetone/water extract of the alfalfa seed with none of the components exceeding a concentration of >0.01 mg/kg.

In summary, it appears that the metabolism of imazamox in alfalfa is not affected by the age of the crop, timing of application, nor the environmental factors of different geographic locations. Imazamox was rapidly metabolized in alfalfa via O-dealkylation of the methoxy-methyl side chain to form the 5-hydroxy-methyl metabolite CL 263284. Subsequently, the hydroxyl-methyl metabolite was either rapidly conjugated with glucose to produce the glucoside metabolite (CL 189215) in alfalfa or further metabolized to form a 3,5-dicarboxylic acid derivative (CL 312622) plus other minor unknown metabolites. The proposed metabolic pathway of imazamox in alfalfa is shown in Figure 6.2.1-4. Thus, the metabolic route of imazamox in alfalfa was consistent with those found in pea, soybean, wheat, and rat.

Figure 6.2.1-4: Proposed Metabolic Pathway of Imazamox in (IMI-tolerant) Afalfa



Overall Summary Plant Metabolism

The present Annex I renewal dossier provides metabolism studies in imidazolinone herbicide resistant oilseed rape, wheat, paddy rice and conventional alfalfa. For oilseed rape and wheat, studies with the radiolabel both in the pyridine ring and in the imidazolinone moiety are submitted, giving a complete picture of the metabolism of imazamox and allowing to compare the metabolic fate of both labels. The studies with the ^{14}C, ^{15}N-labelled imazamox (imidazolinone label) in oilseed rape and wheat qualitatively confirmed the results of the metabolism studies with the [^{14}C]imazamox (pyridine label). It was demonstrated that no cleavage of the imidazoline moiety from the parent molecule occurred. Enantiomer specific HPLC analyses were performed to determine whether one enantiomer of the parent compound imazamox was preferentially metabolised. No significant trend for a stereospecific metabolism was found. Both new studies confirm the previous understanding of the metabolism of imazamox in plants. Furthermore, the extraction efficiency of relevant data generation methods and multi-residue methods was investigated. It was demonstrated that an acidic methanol water mixture as extraction solvent (as used with data generation method M 3519, identical with the extraction solvent used in the new method L0188/01) has the capability to extract the relevant residues of imazamox in the most appropriate way. Storage stability was demonstrated for extracts of all oilseed rape and wheat matrices over a period of at least 11 months and analysis of re-extractions from straw and grain samples after approximately one year of storage confirmed the stability of the metabolic pattern over this time interval. The results of the new study in paddy rice (performed with the radiolabel in the pyridine ring, since no cleavage of the molecule was expected) confirm the metabolic pathway as derived from the new oilseed rape and wheat study as well as from all previous plant metabolism studies.

The proposed metabolic pathway of imazamox in oilseed rape (see Figure 6.2.1-1), wheat (Figure 6.2.1-2), rice (Figure 6.2.1-3) and alfalfa (Figure 6.2.1-4) is similar and was confirmed as involving O-demethylation of the methoxymethyl group to form the hydroxymethyl metabolite (CL 263284) which undergoes further metabolism via oxidation and/or glucose conjugation to yield minor amounts of the dicarboxylic acid metabolite (CL 312622), and the glucose conjugate (CL 189215).

CA 6.2.2 Poultry

A metabolism study with parent compound imazamox in laying hens was included in the previous submission, demonstrating a rapid and efficient excretion of mainly unchanged imazamox without retention or accumulation in the eggs and tissues. The two studies summarized below show the metabolic fate of CL 263284 and CL 312622 and were included in this dossier since these metabolites might occur in detectable amounts in crops used as animal feed. These studies, like the study with parent imazamox, were performed with the pyridine ring label only. Since the new rat metabolism study as well as the two new plant metabolism studies with the imidazolinone label proved that no cleavage between the rings occur, the below provided studies are believed to sufficiently describe the metabolic fate of the imazamox metabolites in laying hens. Both studies were not part of the previous evaluation.
Metabolism of the plant metabolite glucoside CL 189215 was not investigated in a separate study since O-glucosides are known to be easily cleaved under the conditions in the gastrointestinal tract yielding the aglycon CL 263284 in this case. As the study with CL 263284 confirms a rapid excretion and virtually no transfer of residues from diet into tissues and eggs, an additional poultry study with CL 189215 seems not justified at this point.

Report:	CA 6.2.2/1 [redacted] 1994a CL 263,284: Metabolic fate of [14C] CL 263,284 in tissues and eggs of the laying hen IA-440-002
Guidelines:	EPA 171-4(b), EPA 40 CFR 158.240, FIFRA Office of Pesticides Programmes. Pesticide Assessment Guidelines. Subdivision O. Nature of the Residues in Livestock (EPA October 1982)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

A poultry metabolism study was conducted with the radiolabelled imazamox metabolite CL 263284 (6-pyridine label; 4.13 µCi/mg; radiochemical purity 97.0%). Hens were dosed orally with feeding levels for each group at 2.14 or 10.9 mg/kg feed daily for seven days, equivalent to 0.148 and 0.786 mg/kg-bw/day. Eggs and excreta were collected daily. After seven days of dosing, the hens were sacrificed and the tissues (liver, kidney, muscle and skin with adhering fat) and blood were collected for analysis approximately 22 hours after the last dose. Total recovery of radioactivity in excreta (urine and feces) was 85.3% and 88.6% for the low and high dose, respectively. Residues in all tissues, blood and eggs were less than 0.01 mg/kg, the validated detection limit. In summary, orally administered CL 263284 was mainly eliminated from the hen through urine and feces. No detectable (<0.01 mg/kg) ^{14}C-CL 263284-derived residues were found in eggs or edible tissue.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	^{14}C-pyridine-labelled (6 position) CL 263284 Unlabelled CL 263284
Lot/Batch #:	^{14}C-CL 263284: AC 8877:52-71 Unlabelled CL 263284: AC 8187-59A
Purity:	Radiochemical purity: 97.0% Specific activity: 4.13 μCi/mg (^{14}C-CL 263284)
CAS#:	81335-78-6

B. STUDY DESIGN AND METHODS

Experimental Conditions

Carbon-14 labelled CL 263284 or blank gelatin capsules were administered orally to laying hen weighing approximately 1.5 kg. Hens were separated into three groups: group A (control), group B (low dose) and group C (high dose) each containing of 8 animals. Hens were dosed for seven consecutive days with the nominal dietary equivalent of 0 mg/kg, 2 mg/kg (actual: 2.14 mg/kg, 0.148 mg/kg-bw/day) and 10 mg/kg (actual: 10.9 mg/kg, 0.786 mg/kg-bw/day) [^{14}C]-CL 263284, respectively.
The tracer preparation used in this study was a mixture of [pyridine-6-^{14}C]-CL 263284 and non-labelled CL 263284 material.

Sampling

Eggs were collected twice daily and pooled; excreta samples were collected daily and pooled. Egg and excreta samples were collected one day prior to treatment and continued until the time of sacrifice. Samples were either refrigerated (eggs) or stored frozen (excreta). After seven days of dosing, the hens were sacrificed (approximately 22 hours after the last dose) and the following tissue samples were collected: blood, liver, kidney, muscle and skin with adhering fat and were stored frozen.

Analytical Determinations

The total radioactive residues (TRR) in the eggs, tissues, blood and combined seven day excreta samples were determined by combustion followed by LSC. The specific radioactivity afforded a validated detection limit of 0.01 mg/kg CL 263284 equivalents in the tissues, eggs and excreta when 0.5 g (nominal) sample aliquots were analysed by combustion and liquid scintillation counting (LSC) or by direct LSC.

II. RESULTS AND DISCUSSION

TRR levels in all control samples, tissue samples (muscle, skin, liver, kidney), blood and eggs from both dose groups were non-detectable (<0.01 mg/kg).
Elimination of carbon-14 radioactivity via excreta accounted for 85.3% and 88.6% of the total cumulative dose for the low dose and the high dose groups, respectively. The results are presented in the table below.

Table 6.2.2-1: Total radioactive residues (TRRs) in hen treated with ^{14}C-CL 263284

Sample	TRR			
	2 mg/kg Treatment		10 mg/kg Treatment	
	%	mg/kg	%	mg/kg
Egg (day 1-7)	n.r.	<0.01	n r.	<0.01
Muscle	n.r.	<0.01	n r.	<0.01
Skin with adhering fat	n.r.	<0.01	n r.	<0.01
Kidney	n.r.	<0.01	n r.	<0.01
Liver	n.r.	<0.01	n r.	<0.01
Blood	n.r.	<0.01	n r.	<0.01
Excreta (day 1-7)	**85.3**	n r.	**88.6**	n r.

TRR = total radioactive residue
n r. = not reported

III. CONCLUSION

The results of this laying hen study at the actual dose levels of 2.14 and 10.9 mg/kg (0.148 and 0.786 mg/kg-bw/day) of orally ingested CL 263284 showed that the compound or derived residues are excreted without retention or accumulation in eggs and edible poultry tissues. All residues in tissues and eggs were <0.01 mg/kg, the validated detection limit of the radioassay, showing a very low transfer of CL 263,284-derived residues from poultry feed into poultry tissues and eggs.

Report: CA 6.2.2/2
[redacted] 1999a
CL 312, 622: Metabolic fate of [14C] CL 312, 622 in tissues and eggs of the laying hen
ID-440-005
Guidelines: EPA 40 CFR 158.240, EPA 860.1300
GLP: <none>

Executive Summary

Laying hens were orally treated with imazamox metabolite CL 312622, a mixture of ^{13}C- and ^{14}C-CL 312622 labelled in the pyridine ring. The hens were dosed for five consecutive days with the nominal dietary equivalent of 0 mg/kg (control), 0.1 mg/kg (low dose) and 11 mg/kg (high dose) [^{14}C]-CL 312622 to determine total [^{14}C]-CL 312622 derived residues in daily eggs, bile and in the edible tissues (muscle, liver and skin with adhering fat) at sacrifice. The actual administered doses were 0.13 mg/kg (0.0087 mg/kg-bw/day) for the low dose and 10.5 mg/kg (0.733 mg/kg-bw/day) for the high dose. Daily radioactivity in excreta was determined to assess the extent of elimination and recovery of administered radioactive doses.
In summary, the results of this laying hen study at the actual dosages of 0.13 mg/kg and 10.5 mg/kg of orally ingested CL 312622 showed that the parent compound or derived residues as well as CL 152795 (present as an impurity in the dosing solution) were excreted without retention or accumulation in eggs and edible poultry tissues. All residues in tissues and eggs were <0.006 mg/kg, the validated detection limit of the radioassay. Minimal retention by the bile at the high dose of 10.5 mg/kg in the feed was observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: ^{14}C-pyridine-labelled (6 position) CL 312622
^{13}C-pyridine-labelled (6 position) CL 312622
Unlabelled CL 312622

Lot/Batch #: ^{14}C-CL 312622: AC 11662-34B
^{13}C- CL 312622: AC 11662-23
Unlabelled CL 312622: AC 10194-40A

Purity: Radiochemical purity: 96.6% with 7.2-7.3% impurity (CL 152795)
Specific activity: 6.68 μCi/mg (^{14}C-CL 312622)

CAS#: -

B. STUDY DESIGN AND METHODS

Experimental Conditions

Carbon-14 labelled CL 312622 was administered orally to Hy Line W-36, White Leghorn laying hens once daily via gelatin capsule for five consecutive days at nominal dietary feed intake equivalents of 0.1 and 11 mg/kg. The actual doses based on the concentration of the encapsulated active substance and average feed consumption were 0.13 and 10.5 mg/kg, equivalent to 0.0087 and 0.733 mg/kg-bw/day. There were one control and two treatment groups, each containing eight hens. The animals weighed between 1.2 and 1.9 kg and were in good egg production.
The tracer preparation used in this study was a mixture of [pyridine-6-^{14}C]-CL 312622 and [pyridine-6-^{13}C]-CL 312622 as well as non-labelled CL 312622 material.

Sampling

Eggs were collected twice daily and kept refrigerated. Collection began one day prior to treatment. Sacrifice occurred approximately 22 hours after the last dose. Skin with adhering fat, muscle, bile and liver were taken in the order given. Bile and tissue from each group were pooled. Bile samples were refrigerated and tissue samples were stored frozen.
The excreta from all hens were pooled daily. Excreta collection began one day before treatment and continued until the time of sacrifice. The excreta samples were kept frozen.

Analytical Determinations

The total radioactive residues (TRR) in the egg and skin with adhering fat samples were determined by direct scintillation counting (LSC). TRR in the liver, muscle and excreta samples were determined by combustion followed by LSC. The specific radioactivity afforded a validated detection limit of 0.006 mg/kg CL 312622 equivalents in the tissues, eggs and excreta.
Detection of CL 312622-related residues in excreta extract was accomplished by HPLC-UV. The radioactivity was quantitated by LSC.

II. RESULTS AND DISCUSSION

At sacrifice, approximately 22 hours after the last dose, total radioactive residues (TRR) were determined in tissues (liver, muscle and skin with adhering fat) and bile. Residues in eggs were determined daily. Whole eggs from hens in each of the treatment groups were composited daily for analysis. The TRR was determined on composite tissue and bile samples from hens in each of the two treatment groups.
With the exception of high dose composite bile sample (0.018 mg/kg), residue in tissues (liver, muscle and skin with adhering fat), eggs and bile were all less than or equal to the validated detection limit (0.006 mg/kg) of the radioassay. Recovery of the carbon-14 in excreta collected over the five day treatment period averaged 87.5% and 90.9% of the total administered dose from the low dose (0.13 mg/kg) and high dose (10.5 mg/kg), respectively.

Table 6.2.2-2: Total radioactive residues (TRRs) in hen treated with ^{14}C-CL 312622

Sample	TRR			
	0.13 mg/kg Treatment		10.5 mg/kg Treatment	
	%	mg/kg	%	mg/kg
Eggs (day 1-5)	n r.	<0.006	n r.	<0.006
Bile	n r.	0.006	n r.	0.018
Liver	n r.	<0.006	n r.	<0.006
Muscle	n r.	<0.006	n r.	<0.006
Skin with adhering fat	n r.	<0.006	n r.	<0.006
Excreta (day 1)	n r.	0.094	n r.	7.3
Excreta (day 2)	n r.	0.108	n r.	9.2
Excreta (day 3)	n r.	0.104	n r.	8.5
Excreta (day 4)	n r.	0.094	n r.	7.3
Excreta (day 5)	n r.	0.097	n r.	5.3
Excreta (total)	**87.5**	n r.	**90.9**	n r.

TRR = total radioactive residue
n r. = not reported

The extremely low residues in eggs and edible tissues precluded further characterisation. In an attempt to elucidate the nature of the residue, the high dose Day-2 excreta sample was extracted with methanol. The extractability of the carbon-14 residue was 91.2%. The extractable carbon-14 residue was analysed by HPLC. The major component of the extractable residue was unchanged parent, accounting for 76.7% of the extractable radioactive residue (ERR). The polar dosing solution impurity (CL 152795) accounted for approximately 6.5% of the ERR. All other components were insignificant and no single component exceeded 5% of the TRR.
Data on the distribution of the recovered radioactivity from the excreta, as determined by HPLC, are summarized in Table 6.2.2-3 below.

Table 6.2.2-3: Distribution of radioactivity in high dose hen excreta (Day 2)

Compound	Excreta (High Dose)	
	% ERR	mg/kg
CL 312622	76.7	7.0
CL 152795	6.5	0.6
Non-polar unknowns / methylester	2.2	0.2

ERR = extractable radioactive residue

III. CONCLUSION

In summary, the results of this laying hen study at the actual dosages of 0.13 mg/kg (0.0087 mg/kg-bw/day) and 10.5 mg/kg (0.733 mg/kg-bw/day) of orally ingested CL 312622 showed that the compound or derived residues as well as CL 152795 (present as an impurity in the dosing solution) were excreted without retention or accumulation in eggs and edible poultry tissues. All residues in tissues and eggs were <0.006 mg/kg, the validated detection limit of the radioassay. Minimal retention by the bile at the high dose of 10.5 mg/kg in the feed was observed.

CA 6.2.3 Lactating ruminants

A metabolism study with parent imazamox (labelled in the pyridine ring) in lactating goats has been part of the previous submission and evaluation. A short summary is shown below (taken from the Monograph on imazamox compiled during the last EU review by the RMS France).
The information is presented here for the sake of completeness and to present the residues in tissues and milk which have been used for the derivation of MRLs for animal products in chapter 6.7 of this dossier.

Carbon-14 residue in tissues, milk and blood in the low dose (2.08 mg/kg) and high dose (11.6 mg/kg) lactating goats during the 7-day treatment period are shown in Table 6.2.3-1. The percent of the cumulative dose of the ^{14}C-CL 299263-derived radioactivity in urine was 91.2% for the low dose goat and 64.8% for the high dose goat. The cumulative percent of the ^{14}C-CL 299263-derived radioactivity in feces was 15.0% for the low dose goat and 24.0% for the high dose goat. These results yield a total recovery of approximately 106% and 89% of the total administered dose in the low dose goat and the high dose goat, respectively.

Table 6.2.3-1: Total radioactive residues of imazamox in lactating goats

Matrix	Total radioactive residues (mg/kg Imazamox equivalents)	
	low dose goat (2.08 mg/kg feed)	high dose goat (11.6 mg/kg feed)
Milk	< 0.01	< 0.01
Blood	< 0.01	< 0.01
Leg Muscle	< 0.01	< 0.01
Loin Muscle	< 0.01	< 0.01
Liver	< 0.01	< 0.01
Kidney	0.02	0.06
Omental Fat	< 0.01	< 0.01

Orally administered imazamox in the goat is mainly excreted in urine. Carbon-14 residue in tissues, milk and blood in the low dose (2.08 mg/kg) and high dose (11.6 mg/kg) lactating goats during the 7-day treatment period are shown in Table 6.2.3-1. The data showed that residues in all tissues, except the kidney, taken approximately 20 hours after the last of seven consecutive daily doses were all below the validated detection limit of 0.01 mg/kg ^{14}C-CL 299263 equivalents in both the low dose (2.08 mg/kg feed) and high dose (11.6 mg/kg feed) goats. The kidney from the low dose goat contained 0.02 mg/kg ^{14}C-CL 299263 equivalents; the kidney from the high dose goat contained 0.06 mg/kg ^{14}C-CL 299263 equivalents. This was mostly imazamox. Unaltered parent accounted for most of the excreted residue.

The results of this lactating goat study conducted at the actual dose levels of 2.08 and 11.6 mg/kg feed clearly demonstrated that the parent was excreted without retention or accumulation in the milk or edible tissues. Only the kidney showed detectable residues (probably due to residual urine) and the residue was identified as the unchanged parent compound imazamox, CL 299263.

It was concluded that ruminants fed commodities containing residues of imazamox would not produce secondary metabolites as tissue residues, and elimination of the CL 299263 via the urine and feces is rapid and efficient.

The two studies summarized below show the metabolic fate of CL 263284 and CL 312622 in lactating goats and were included in this dossier since these metabolites might occur in detectable amounts in crops used as animal feed. These studies, like the study with parent imazamox, were performed with the pyridine ring label only. Since the new rat metabolism study as well as the two new plant metabolism studies with the imidazolinone label proved that no cleavage between the rings occur, the below provided studies are believed to sufficiently describe the metabolic fate of the imazamox metabolites in lactating goats. Both studies were not part of the last evaluation.
Metabolism of the plant metabolite glucoside CL 189215 was not investigated in a separate study since O-glucosides are known to be easily cleaved under the conditions in the gastrointestinal tract yielding the aglycon CL 263284. As the study with CL 263284 confirms a rapid excretion and virtually no transfer of residues from diet into tissues and milk, an additional cattle study with CL 189215 seems not justified at this point.

Report:	CA 6.2.3/1 [redacted] 1994a CL 263,284: Metabolism of [14C] CL 263,284 in the lactating goat IA-440-001
Guidelines:	EPA 171-4(b)
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

A ruminant metabolism study was conducted with the radiolabelled imazamox metabolite CL 263284 (6-pyridine label; 4.13 µCi/mg; radiochemical purity 97.0%). Dose levels for the goats were 2.33, or 14.5 mg/kg (0.0846 and 0.308 mg/kg-bw/day) feed daily for seven days. Samples of blood, milk and excreta were collected daily. After seven days of dosing, the goats were sacrificed and the tissues kidney, liver, muscle and fat were collected. During treatment, TRR in the daily blood and milk samples were less than 0.01 mg/kg, regardless of the treatment dose levels. The TRR in liver, muscle, and omental fat were less than 0.01 mg/kg, regardless of the dose level. Analysis of the kidney extract showed that 9% (<0.01 mg/kg) of the extractable TRR was CL 263284, and the remaining residue was predominantly a labile component M1 (78% TRR, 0.02 mg/kg), possibly a salt of CL 263284, which converted to CL 263284 on exposure to aqueous buffer.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	^{14}C-pyridine-labelled (6 position) CL 263284 Unlabelled CL 263284
Lot/Batch #:	^{14}C-CL 263284: AC 8877:71 Unlabelled CL 263284: AC 8187-59A
Purity:	Radiochemical purity: 97.0% Specific activity: 4.13 μCi/mg (^{14}C-CL 263284); 15.7 µCi (low dose); 77.7 µCi (high dose)
CAS#:	81335-78-6

B. STUDY DESIGN AND METHODS

Experimental Conditions

Carbon-14 labelled CL 263284 or blank gelatin capsules were administered orally to lactating goats weighing 45 and 62 kg that were 2-3 years of age. The three goats were dosed for seven consecutive days with the dietary equivalent of 0 mg/kg, 2.33 mg/kg and 14.5 mg/kg [^{14}C]-CL 263284, equivalent to 0.0846 and 0.308 mg/kg-bw/day, as groups A (control), B (low dose), and C (high dose), respectively.
The tracer preparation used in this study was a mixture of [pyridine-6-^{14}C]-CL 263284 and non-labelled CL 263284 material.

Sampling

Milk, urine, blood and feces samples were collected daily starting one day before first dosing and either refrigerated (milk, urine, blood) or stored frozen (feces). After seven days of dosing, the goats were sacrificed (approximately 20 hours after the last dose) and the following tissue samples were collected: muscle, fat (omental), liver and kidney. Tissue samples were stored frozen.

Analytical Determinations

The total radioactive residues (TRR) in the milk and the urine samples were determined by direct scintillation counting (LSC). TRR in the tissues and excreta were determined by combustion followed by LSC. Radioactivity in the extracts of the tissues, in the feces samples and in liquid chromatographic fractions was determined by LSC. Radioactivity in the extract and marc generated after kidney and feces extraction was determined by combustion followed by LSC.
Kidney, urine and feces samples were additionally analysed via HPLC-MS. Retention times were compared with the reference standard CL 263284.

II. RESULTS AND DISCUSSION

TRR levels in most control samples and most tissue samples (muscle, fat, liver) from the low and high dose treated goat and from all milk samples were non-detectable (<0.01 mg/kg). The control sample from kidney showed a residue of 0.01 mg/kg and the kidney sample from the high dose goat was 0.03 mg/kg. The TRR values obtained for the tissues, milk, urine and feces are included in Table 6.2.3-2.
Elimination of carbon-14 radioactivity via urine accounted for 14.6% and 18.2% of the total cumulative dose for the low dose and the high dose groups, respectively. The radioactivity excreted in feces represented 81.7% and 67.8% of the total cumulative dose for the low dose and the high dose groups, respectively. The recovery of radioactivity in urine and feces totaled 96.3% and 86% for the low and the high dose groups, respectively.

Table 6.2.3-2: Total radioactive residues (TRRs) in goat treated with ^{14}C-CL 263284

Sample	TRR			
	2.33 mg/kg Treatment		14.5 mg/kg Treatment	
	%	mg/kg	%	mg/kg
Milk (day 1-7)	n.r.	<0.01	n r.	<0.01
Muscle	n.r.	<0.01	n r.	<0.01
Fat	n.r.	<0.01	n r.	<0.01
Kidney*	n.r.	<0.01	n r.	0.03
Liver	n.r.	<0.01	n r.	<0.01
Blood (day 1-7)	n.r.	<0.01	n r.	<0.01
Urine (day 1)	9.8	n r.	11.1	n r.
Urine (day 2)	12.1	n r.	23.3	n r.
Urine (day 3)	10.6	n r.	21.4	n r.
Urine (day 4)	11.3	n r.	16.4	n r.
Urine (day 5)	11.6	n r.	16.1	n r.
Urine (day 6)	34.9	n r.	19.7	n r.
Urine (day 7)	12.1	n r.	19.3	n r.
Total	14.6	n r.	18.2	n r.
Feces (day 1)	53.7	n r.	14.6	n r.
Feces (day 2)	93.4	n r.	79.4	n r.
Feces (day 3)	85.3	n r.	75.4	n r.
Feces (day 4)	97.5	n r.	35.7	n r.
Feces (day 5)	79.2	n r.	93.1	n r.
Feces (day 6)	84.3	n r.	86.8	n r.
Feces (day 7)	78.5	n r.	89.6	n r.
Total	81.7	n r.	67.8	n r.
Total excreta	96.3	n r.	86.0	n r.

TRR = total radioactive residue
n r. = not reported
*control goat had a residue of 0.01 mg/kg

As shown in Table 6.2.3-2, all milk and all tissue samples contained less than 0.01 mg/kg of TRR, the detection limit, except the kidney samples from goat treated at the high dose rate. The kidney and the feces samples were used to characterise the distribution of radioactivity.
Kidney: Radioactive residues were extracted from kidney using methanol as solvent. The overall recovery in the extractable portion was 100%, main radioactivity was observed in the extract (91%) and only minor amounts were detected in the marc (9%). The concentrated extract was analysed by HPLC to determine the nature of the radioactive residue. The parent compound (CL 263284) was the minor radioactive residue (9%). One further peak was present in the chromatogram (M1; 78%). M1 was further characterised by treatment with either β-glucuronidase, sulfatase or buffer alone.
M1 showed to be a very labile component and was easily degraded to CL 263284 under non-enzymatic or enzymatic hydrolysis. Because M1 was converted to CL 263284 in buffer alone, it is likely that M1 was the parent compound weakly coupled with kidney endogenous components. It is suspected that M1 is a salt of CL 263284 that is readily dissociated in aqueous solutions such as buffer.
Feces: HPLC analysis of the feces extract of goat C (high dose) showed that 96% of the radioactivity had a retention tune corresponding to that of CL 263284 reference standard. HPLC analysis of the feces extract of goat B (low dose) showed that the major component had a retention time at 9 minutes which is the same as the M1 component found in the high dose kidney. The M1 component was very labile and was easily converted to CL 263284 under aqueous, acidic conditions or incubated with β-glucuronidase and sulfatase.

III. CONCLUSION

The results of this lactating goat study conducted at the actual dose levels of 2.33 and 14.5 mg/kg feed equivalents of CL 263284 (0.0846 and 0.308 mg/kg-bw/day) showed that the metabolite compound was excreted without retention or accumulation in milk. Of the edible tissues only kidney showed detectable residues and these were identified as the compound CL 263284 and a labile metabolite that converted to CL 263284 in aqueous solution. This shows a very low transfer of CL 263284-derived residues from animal feed into tissues and milk of lactating ruminants.

Report: CA 6.2.3/2
[redacted] 1999a
CL 312, 622: Metabolism of [14C]-CL 312, 622 in the lactating goat
ID-440-006
Guidelines: EPA 40 CFR 158.240, EPA 860.1300
GLP: <none>

Executive Summary

Lactating goats were orally treated with imazamox metabolite CL 312622, a mixture of ^{13}C- and ^{14}C-CL 312622 labelled at carbon 6 of the pyridine ring. The three goats were dosed for five consecutive days with the nominal dietary equivalent of 0 mg/kg (control), 3 mg/kg (low dose) and 30 mg/kg (high dose) [^{14}C]-CL 312622 to determine total [^{14}C]-CL 312622 derived residues in daily milk and in the edible tissues (muscle, fat, liver and kidney) at sacrifice. The actual administered doses were 3.14 mg/kg (0.753 mg/kg-bw/day) for the low dose and 33.4 mg/kg (6.77 mg/kg-bw/day) for the high dose. Daily urinary and fecal radioactivity was determined to assess the extent of elimination and recovery of administered radioactive doses.
The results of this lactating goat study showed that the major route of elimination of CL 312622 was via feces and to a lesser extent via urine. At the end of the 5-day dosing period, 98.9% (8.5% in urine and 90.4% in feces) of the administered cumulative low dose was recovered and 97.0% (7.0% in urine and 90.0% in feces) of the administered cumulative dose was recovered in the high dose treatment group.
The results of this CL 312622 study showed that CL 312622 and CL 152795 (present as an impurity in the dosing solution) were excreted with minimal retention by the kidney and the liver at the exaggerated dose of 33.4 mg/kg in the feed. There was no accumulation in any other edible goat tissue or in the milk. There were no residues found in the tissues or milk of the low dose treated goat.

I. MATERIAL AND METHODS

A. MATERIALS

2. Test Material:

Description: ^{14}C-pyridine-labelled (6 position) CL 312622
^{13}C-pyridine-labelled (6 position) CL 312622
Unlabelled CL 312622

Lot/Batch #: $^{12}C/^{13}C/^{14}C$-CL 312622 (isotopic mixture): AC 11662-34B
Unlabelled CL 312622: AC 10194-40A

Purity: Radiochemical purity (average): 88.6% (low dose); 87.4% (high dose); 8.4% impurity (CL 152795)
Specific activity: 6.68 μCi/mg ($^{12}C/^{13}C/^{14}C$-CL 312622 isotopic mixture)

CAS#: -

B. STUDY DESIGN AND METHODS

Experimental Conditions

Carbon-14 labelled CL 312622 or blank gelatin capsules were administered orally to lactating goats weighing between 33 and 70 kg (at delivery) that were less than 3 years of age. The three goats were dosed orally by capsule for five consecutive days with the nominal dietary equivalent of 0 mg/kg, 3 mg/kg (0.753 mg/kg-bw/day) and 30 mg/kg (6.77 mg/kg-bw/day) [^{14}C]-CL 312622 as groups A (control), B (low dose), and C (high dose), respectively.
The tracer preparation used in this study was a mixture of [pyridine-6-^{14}C]-CL 312622 and [pyridine-6-^{13}C]-CL 312622 as well as non-labelled CL 312622 material.

Sampling

Milk, urine and feces samples were collected daily and either refrigerated (milk, urine) or stored frozen (feces). After five days of dosing, the goats were sacrificed (approximately 22 hours after the last dose) and the following tissue samples were collected: muscle (leg and loin), fat (omental), liver and kidney. Tissue samples were stored frozen.

Analytical Determinations

Total radioactive residues (TRR) in milk and urine samples were determined by direct scintillation counting (LSC). TRR in tissues and excreta were determined by combustion followed by LSC. Radioactivity in the extracts of tissues, in feces samples and in liquid chromatographic fractions was determined by LSC. Radioactivity in the post-extraction solids (PES) generated after tissue extraction was determined by combustion followed by LSC. The specific radioactivity afforded a validated detection limit of 0.006 mg/kg CL 312622 equivalents in the tissues, milk, urine and feces when 0.5 g or 0.5 mL (nominal) sample aliquots were analysed by combustion and liquid scintillation counting (LSC) or by direct LSC.

II. RESULTS AND DISCUSSION

TRR levels in all control samples, pretreatment samples, tissue samples (muscle, fat, liver, kidney) from the low dose treated goat and from all milk samples were non-detectable (<0.006 mg/kg). TRR levels in the muscle and fat samples of the high dose goat were also non-detectable. The TRR values obtained for the kidney (0.025 mg/kg) and the liver (0.009 mg/kg) of the high dose goat are included in Table 6.2.3-3 which also summarizes the TRR values in muscle and fat (at sacrifice) and daily milk (<0.006 mg/kg in all samples).
Elimination of carbon-14 radioactivity via urine accounted for 8.5% and 7.0% of the total cumulative dose for the low dose and the high dose groups, respectively. The radioactivity excreted in feces represented 90.4% and 90.0% of the total cumulative dose for the low dose and the high dose groups, respectively. The recovery of radioactivity in urine and feces totaled 98.9% and 97.0% for the low and the high dose groups, respectively.

Table 6.2.3-3: Total radioactive residues (TRRs) in goat treated with ^{14}C-CL 312622

Sample	TRR			
	3.14 mg/kg Treatment		33.4 mg/kg Treatment	
	%	mg/kg	%	mg/kg
Milk (daily)	n.r.	<0.006	n r.	<0.006
Muscle	n.r.	<0.006	n r.	<0.006
Fat	n.r.	<0.006	n r.	<0.006
Kidney	n.r.	<0.006	n r.	0.025
Liver	n.r.	<0.006	n r.	0.009
Urine (day 1)	6.3	n r.	5.0	n r.
Urine (day 2)	7.8	n r.	6.1	n r.
Urine (day 3)	8.2	n r.	7.0	n r.
Urine (day 4)	8.5	n r.	6.9	n r.
Urine (day 5)	**8.5**	n r.	**7.0**	n r.
Feces (day 1)	53.6	n r.	72.2	n r.
Feces (day 2)	68.2	n r.	83.0	n r.
Feces (day 3)	78.7	n r.	84.2	n r.
Feces (day 4)	84.7	n r.	88.1	n r.
Feces (day 5)	**90.4**	n r.	**90.0**	n r.

TRR = total radioactive residue
n r. = not reported

As shown in Table 6.2.3-3, all milk and tissue samples contained less than 0.006 mg/kg of TRR, the detection limit, except the kidney and liver samples from goat treated at the high dose rate. These two samples were used to characterise the nature of the residues.

Kidney: Radioactive residues were extracted from kidney using acetone/water (3:1, v/v) as solvent. The overall recovery in the extractable portion was >100% (by comparison with the TRR obtained by combustion); no radioactivity was observed in the PES. The concentrated extract was analysed by HPLC to determine the nature of the radioactive residue. The parent compound (CL 312622) was the predominant radioactive residue (60.0%). The impurity known to be present in the dosing solution (CL 152795) was also found in the extract at 11.0% of TRR.

Minor polar unknowns (total 10.3% of TRR) and non-polar unknowns (total 11.9% of TRR) were also present in the kidney extract. Since these fractions were equivalent to 0.003 mg/kg and contained multiple components, no further characterisation was attempted.

Liver: Radioactive residues were extracted from liver according to a modification of the kidney extraction procedure. The percent extractable radioactivity was 98.7% and the percent TRR in the PES by combustion/LSC analysis yielded residues less than the validated detection limit.

The concentrated extract was analysed by HPLC after solid phase extraction (SPE) cleanup. CL 312622 in the liver was present at 38.2% of TRR. The impurity known to be present in the dosing solution (CL 152795) was also found in the liver extract at 19.0% of the TRR as determined by HPLC. Polar radioactive components were present in the liver extract at 31.2% of the TRR. The majority of that fraction (22.9%) was unretained by the SPE cartridge, indicating it to be very polar material. Some non-polar metabolites were also present at a total concentration of 11.3% of the TRR. Since these fractions were equivalent to 0.003 mg/kg or lower and contained multiple components, no further characterisation was attempted.

Data on the distribution of the recovered radioactivity from the kidney and the liver extract, as determined by HPLC, are summarized below.

Table 6.2.3-4: Distribution of radioactivity in high dose goat kidney and liver

Compound	Kidney (0.025 mg/kg)		Liver (0.009 mg/kg)	
	% TRR	mg/kg	% TRR	mg/kg
CL 312622	60.0	0.015	38.2	0.003
CL 152795	11.0	0.003	19.0	0.002
Polar unknowns	10.3	0.003	31.2	0.003
Non-polar unknowns	11.9	0.003	11.3	0.001

TRR = total radioactive residue

III. CONCLUSION

The results of this lactating goat study showed that the major route of elimination of CL 312622 was via feces and to a lesser extent via urine. At the end of the 5-day dosing period, 98.9% (8.5% in urine and 90.4% in feces) of the administered cumulative low dose was recovered and 97.0% (7.0% in urine and 90.0% in feces) of the administered cumulative dose was recovered in the high dose treatment group.
The results of this CL 312622 study showed that CL 312622 and CL 152795 (present as an impurity in the dosing solution) were excreted with minimal retention by the kidney and the liver at the exaggerated dose of 33.4 mg/kg in the feed. There was no accumulation in any other edible goat tissue or in the milk. There were no residues found in the tissues or milk of the low dose treated goat.

CA 6.2.4 Pigs

The metabolic fate of imazamox in rodents (rats) and ruminants (goats) did not differ significantly. All studies showed a rapid excretion of mainly unchanged parent compound. Therefore investigations on the metabolism in pigs were not required and consequently, a metabolism study on imazamox in pigs has not been conducted.

CA 6.2.5 Fish

According to Commission regulation 283/2013, metabolism studies in fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications. In principal, seeds of soybean and sunflower as well as their meal and oilseed rape meal may be considered to serve as feed item for fish. However, residues of imazamox in these crop parts are usually not detectable except for sunflower where low residues were seen.
The exact conditions under which such a study should be performed are further described in the Working document of the EU Commission SANCO/11187/2013, rev. 3 on the nature of pesticide residues in fish. The document specifies that the accumulation of compounds with low lipophilicity via the diet is known to be negligible and that fish metabolism studies are therefore only required for active substances with a log P_{OW} equal or greater than 3 (refer to p. 6/ paragraph 4 of the document).
Imazamox is a compound of very low lipophilicity, having a log P_{OW} of 0.7, thus no accumulation in fish tissues is expected. Moreover, the BCF for imazamox (CL 299263) in fish was determined in a study included and evaluated in the previous submission and was found to be <1.
Thus, since the log P_{OW} of imazamox is well below the above mentioned trigger value and since it was demonstrated that imazamox residues do not accumulate, no fish metabolism study is deemed necessary even if it cannot be excluded that fish might be exposed to very low residues of imazamox via sunflower seeds and meal.

Overall Summary Livestock Metabolism

During the initial EU Review of the active substance imazamox the metabolism of imazamox has been studied in goat and hen with 6-pyridine-^{14}C labelled imazamox. These studies with imazamox showed no accumulation of imazamox derived residues in the edible tissues or eggs of poultry or in the edible tissues or milk of goats. Elimination of imazamox as parent compound via the excreta was shown to be rapid and efficient.

In addition to the studies with parent imazamox, this dossier provides studies of the metabolic fate of imazamox metabolites CL 263284 and CL 312622 in laying hens and lactating goats, since it cannot be excluded that livestock animals might be exposed to residues of these metabolites via feed. All studies show the same behaviour as imazamox itself, i.e. rapid and efficient excretion of the mainly unchanged test item and a very low transfer of possible residues of these metabolites from feed into milk, eggs and animal tissues. As further shown in Chapter 6.7, no detectable imazamox-derived residues are expected at the worst case estimated maximum feed intake for all livestock species.

Metabolism of the plant metabolite glucoside CL 189215 was not investigated in a separate study since O-glucosides are known to be easily cleaved under the conditions in the gastrointestinal tract yielding the aglycon CL 263284. As the study with CL 263284 confirms a rapid excretion and virtually no transfer of residues from diet into tissues and milk, an additional cattle study with CL 189215 seems not justified at this point.
Possible residues of CL 189215 in livestock feedstuff have been considered by expressing them in CL 263284 equivalents and considering the sum of CL 263284 and CL 189215, expressed as CL 263284-equivalents in order to derive a worst case feedburden.

CA 6.3 Magnitude of residues trials in plants

Most residue studies were performed using the representative formulation BAS 720 06 H and BAS 831 00 H in combination with the adjuvant DASH (BAS 160 00 S), because in several countries an application with the adjuvant is foreseen. Studies carried out with BAS 720 06 H + DASH or BAS 831 00H + DASH represent the worst-case, also covering application of the formulation without adjuvant. For details on the adjuvant DASH, reference is made to Doc J, CP 1.4.1.

CA 6.3.1 Oilseed rape

Table 6.3.1-1: Critical GAP for the use of BAS 720 H in/on oilseed rape

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
oilseed rape	1 x 0.035 kg BAS 720 H/ha	100 L/ha	n.s.	spray application	BBCH 10-18 BBCH 30-50

PHI = pre-harvest interval

This GAP is currently registered for the product BAS 798 00 H (SC formulation, application rate of 2 L product/ha at BBCH 10-18, with DASH 1L/ha). Registration of this GAP is further sought for the product BAS 831 00 H (SC formulation, application rate of 1 L product/ha at BBCH 10-18 or BBCH 30-50, with DASH 1L/ha.
The following residue trials are submitted in support of the intended use pattern (as shown above) with the formulation BAS 831 00 H.
Please note that the formulation BAS 798 KA H is identical with BAS 798 00 H.

Table 6.3.1-2: GAP information of residue trials conducted in/on oilseed rape between 2007 and 2013 in Northern and Southern Europe

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Northern EU	Germany (2 trials)	BAS 798 KA H, SC (+BAS 160 00 S)[2]	spray application	0.05	0.025	1	0 12-13 100-101
	Germany (1 trial)	BAS 798 00 H, SC (+BAS 160 00 S)[2]	spray application	0.035	0.0175	1	0 94 94
	United Kingdom (1 trial)	BAS 798 00 H, SC (+BAS 160 00 S)[2]	spray application	0.035	0.0175	1	0 99 99
	Germany (1 trial)	BAS 831 00 H, SC + BAS 160 00 S	spray application	0.035	0.0175	1	0 81 81
	United Kingdom (1 trial)	BAS 831 00 H, SC + BAS 160 00 S	spray application	0.035	0.0175	1	0 98 98
Southern EU	France (2 trials)	BAS 798 KA H, SC (+BAS 160 00 S)[2]	spray application	0.05	0.025	1	0 15-19 78-183
	Italy (1 trial)	BAS 798 00 H, SC (+BAS 160 00 S)[2]	spray application	0.035	0.0175	1	0 224 224
	France (1 trial)	BAS 798 00 H, SC (+BAS 160 00 S)[2]	spray application	0.035	0.0175	1	0 111 111
	Spain (1 trial)	BAS 831 00 H, SC + BAS 160 00 S	spray application	0.035	0.0175	1	0 74 74
	Italy (1 trial)	BAS 831 00 H, SC + BAS 160 00 S	spray application	0.035	0.0175	1	0 64 64

0) actual application rates varied by 10% at most
1) days after last application
2) replicate treatment with DASH (BAS 160 00 S) as a tank mix

Report:	CA 6.3.1/1 North L., 2007a Study on the residue behaviour of Imazamox, Metazachlor and Quinmerac in oilseed rape following foliar applications under field conditions in Northern and Southern Europe during 2005-2006 2007/1007939
Guidelines:	<none>
GLP:	Yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 6.3.1/2 North L., 2007b Study on the residue behaviour of Imazamox, Metazachlor and Quinmerac in oilseed rape following foliar applications under field conditions in Northern and Southern Europe during 2005-2006 2007/1007963
Guidelines:	<none>
GLP:	Yes (certified by Staatliches Gewerbeaufsichtsamt, Hildesheim, Germany)

Executive Summary

During the 2005/2006 growing season 4 decline trials in oilseed rape were conducted in different representative growing areas in the Northern EU and Southern EU, to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC). BAS 798 KA H (SC formulation, identical with BAS 798 00 H) containing 25 g/L imazamox, 75 g/L metazachlor and 100 g/L quinmerac was applied once at a rate equivalent to 0.05 kg imazamox/ha in a spray volume of 200 L/ha. One plot of each trial was treated with BAS 798 KA H combined with DASH (adjuvant BAS 160 00 S) in a tank mix. The application was performed 90±3 days before the expected harvest (growth stage BBCH 51-61). Specimens of whole plant without roots were collected immediately and 12-19 days (BBCH 65) after the application. Specimens of seed were collected 78-101 days after the application, at plant maturity (BBCH 89). The specimens were analysed for imazamox and its metabolite CL 263284 with BASF Method No. M3519 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of imazamox and metabolite CL 263284 in mature seed (BBCH 89) were <0.05 mg/kg, resulting in total imazamox residues of <0.10 mg/kg after application with and without adjuvant.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 798 KA H (SC)
Lot/Batch #:	1010 (25 g/L imazamox, nominal), not reported for BAS 160 00S (DASH), for composition see Doc J, CP 1.4.1
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	Seed: 0.05-0.5 mg/kg Whole plant w/o roots: 0.05-5.0 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	PS22-1A-VH
Botanical name:	*Brassica napus*
Crop parts(s) or processed commodity:	Whole plant without roots, seed
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 2005/2006 growing season 4 decline trials in oilseed rape were conducted in different representative growing areas in the Northern EU (Germany) and Southern EU (France) to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC). BAS 798 KA H (SC) containing 25 g/L imazamox, 375 g/L metazachlor and 100 g/L quinmerac was applied once at a rate equivalent to 0.05 kg imazamox/ha in a spray volume of 200 L/ha. One plot of each trial was treated with BAS 798 KA H (SC) combined with adjuvant BAS 160 00 S. The application was performed, 90±3 days before the expected harvest (growth stage BBCH 51-61). Specimens of whole plant without roots were collected immediately and 12-19 days (BBCH 65) after the application. Specimens of seed were collected 78-101 days after the application, at plant maturity (BBCH 89). Samples were stored frozen at or below -18°C for a maximum of 326 days until analysis. In this summary only residues of imazamox are reported.

Table 6.3.1-3: Target application rates and timings for oilseed rape

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2005/2006	4	1	F	BAS 798 KA H (SC) + BAS 160 00 S	BAS 720 H	0.05	200	90 (±3) days before harvest
2005/2006	4	1	F	BAS 798 KA H (SC)	BAS 720 H	0.05	200	90 (±3) days before harvest

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 (Reg. No. 4110773) with BASF Method No. M3519 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues are extracted from oilseed rape matrices using an acidic methanol-water solution, cleaned up using SCX solid phase extraction (SPE) cartridges and measured by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.1-4: Summary of procedural recovery data for imazamox

Matrix	Fortification Level (mg/kg)	Summary Recoveries		
		n	Mean (%)	RSD (%)
BASF Method No. M3519; LOQ = 0.05 mg/kg		**Imazamox (BAS 720 H)**		
Whole plant	0.05, 0.50, 5.0	6	87	7
Seed	0.05-0.50	4	91	8
Overall		10	89	7
BASF Method No. M3519; LOQ = 0.05 mg/kg		**CL 263284 (Reg. No. 4110773)**		
Whole plant	0.05, 0.50, 5.0	6	93	3
Seed	0.05, 0.50	4	90	9
Overall		10	92	6

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-5, detailed residue levels are shown in Table 6.3.1-6 and Table 6.3.1-7.

In the Northern EU residues in oilseed rape treated with BAS 798 KA H and adjuvant DASH (BAS 160 00 S) sampled on the day of the application (BBCH 51) ranged from 0.22-2.22 mg/kg for imazamox. Residues of metabolite CL 263284 were below LOQ (0.05 mg/kg). In the whole-plant commodities sampled 12-13 days after the application (BBCH 65) residues ranged from 0.05-0.19 mg/kg for imazamox and from <0.05-0.05 mg/kg for metabolite CL 263284. No residues at or above the LOQ of the analytical method (0.05 mg/kg) of imazamox or its metabolite CL 263284 were found in seed samples at harvest 78-101 days after the application (BBCH 89). Total imazamox for seeds was <0.10 mg/kg.

In oilseed rape whole plant specimens treated with BAS 798 KAH without an adjuvant, residues at 0 DALA (BBCH 51) ranged from 0.26-0.53 mg/kg for imazamox. Residues of metabolite CL 263284 were below LOQ (0.05 mg/kg). In the whole-plant commodities sampled 100-101 days after the application (BBCH 89) no quantifiable residues of imazamox and CL 263284 were found. No residues at or above the LOQ of the analytical method (0.05 mg/kg) of imazamox or its metabolite were found in seed samples 100-101 days after the application (BBCH 89). Total imazamox for seeds was <0.10 mg/kg.

In the Southern EU residues in oilseed rape treated with BAS 798 KA H and adjuvant DASH (BAS 160 00 S) sampled on the day of the application (BBCH 53-61) ranged from 0.97-1.55 mg/kg for imazamox. Residues of metabolite CL 263284 were below LOQ (0.05 mg/kg). In the whole-plant commodities sampled 12-13 days after the application (BBCH 65) residues ranged from 0.12-0.14 mg/kg for imazamox and from 0.21-0.28 mg/kg for metabolite CL 263284. No residues at or above the LOQ of the analytical method (0.05 mg/kg) of imazamox or its metabolite CL 263284 were found in seed samples at harvest 78-83 days after the application (BBCH 89). Total imazamox for seeds was <0.10 mg/kg.

In oilseed rape whole plant specimens treated with BAS 798 KAH without an adjuvant, residues at 0 DALA (BBCH 53-61) ranged from 0.44-0.68 mg/kg for imazamox. Residues of metabolite CL 263284 were below LOQ (0.05 mg/kg). In the whole-plant commodities sampled 78-83 days after the application (BBCH 89) no quantifiable residues of imazamox and CL 263284 were found. No residues at or above the LOQ of the analytical method (0.05 mg/kg) of imazamox or its metabolite were found in seed samples 78-83 days after the application (BBCH 89). Total imazamox for seeds was <0.10 mg/kg.

Table 6.3.1-5: Summary of residues in oilseed rape treated with BAS 798 KA H

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284 (4110773)	Total imazamox[4]
With Adjuvant DASH							
Northern EU	2005/2006	0	51	Whole plant[3]	0.22-2.20	<0.05	0.27-2.25
		12-13	65	Whole plant[3]	0.05-0.19	<0.05-0.50	0.10-0.71
		100-101	89	Seed	<0.05	<0.05	<0.10
Southern EU	2005/2006	0	53-61	Whole plant[3]	0.97-1.50	<0.05	1.02-1.55
		12-19	65	Whole plant[3]	0.12-0.14	0.21-0.28	0.17-0.19
		78-83	89	Seed	<0.05	<0.05	<0.10
Without Adjuvant DASH							
Northern EU	2005/2006	0	51	Whole plant[3]	0.26-0.53	<0.05	0.31-0.58
		12-13	65	Whole plant[3]	<0.05	<0.05	<0.10
		100-101	89	Seed	<0.05	<0.05	<0.10
Southern EU	2005/2006	0	53-61	Whole plant[3]	0.44-0.68	<0.05	0.49-0.73
		12-19	65	Whole plant[3]	<0.05	<0.05	<0.10
		78-83	89	Seed	<0.05	<0.05	<0.10

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048)

III. CONCLUSION

Residues of imazamox in whole plant at 0 DALA tended to be higher after application with the adjuvant DASH, however already after 12-19 DALA residues of imazamox in whole plant were comparable to the levels after application with ou without adjuvant.

In mature seed, residues of imazamox and metabolite CL 263284 at BBCH 89 were <0.05 mg/kg, resulting in total imazamox residues of <0.10 mg/kg after application with or without adjuvant. The application of adjuvant DASH had no effect on residue levels in edible matrices of oilseed rape.

Table 6.3.1-6: Residues of imazamox in oilseed rape after one application of BAS 798 KA H or a tank mix of BAS 798 KA H and BAS 160 00 S (DASH) in Northern Europe

Trial Details		Crop	Country	Formulation, Application Rate[0)] (kg a.s./ha)	Crop Growth Stage[2)] (BBCH)	DA-LA[1)]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	211921	Oilseed rape	Germany	BAS 798 KA H: 1 x 0.05	51	0	W.plant	0.53	<0.05	0.58
Doc ID:	2007/1007939[3]					13	W.plant	<0.05	<0.05	<0.10
Trial No.:	AF/10114/BA/3					101	Seed	<0.05	<0.05	<0.10
GLP:	yes									
Year:	2005/2006									
Study code:	251536	Oilseed rape	Germany	BAS 798 KA H 1 x 0.05 + 2 L/ha BAS 160 00 S	51	0	W.plant	2.20	<0.05	2.25
Doc ID:	2007/1007963[4]					13	W.plant	0.19	0.50	0.71
Trial No.:	AF/10475/BA/3					101	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2005/2006									
Study code:	211921	Oilseed rape	Germany	BAS 798 KA H: 1 x 0.05	51	0	W.plant	0.26	<0.05	0.31
Doc ID:	2007/1007939[3]					12	W.plant	<0.05	<0.05	<0.10
Trial No.:	AF/10114/BA/4					100	Seed	<0.05	<0.05	<0.10
GLP:	yes									
Year:	2005/2006									
Study code:	251536	Oilseed rape	Germany	BAS 798 KA H 1 x 0.05 + 2 L/ha BAS 160 00 S	51	0	W.plant	0.22	<0.05	0.27
Doc ID:	2007/1007963[4]					12	W.plant	0.05	<0.05	0.10
Trial No.:	AF/10475/BA/4					100	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2005/2006									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) study 2007/1007939 contains 4 rape trials treated with formulation BAS 798 KA H
4) study 2007/1007963 contains 4 rape trials at the same locations as 2007/1007939 treated with formulation BAS 798 KA H plus adjuvant BAS 160 00 S
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.048)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.05) 0.05 mg/kg were used for each analyte.
w.plant whole plant without roots
_ underlined values were used for MRL calculation

Table 6.3.1-7: Residues of imazamox in oilseed rape after one application of BAS 798 KA H or a tank mix of BAS 798 KA H and BAS 160 00 S (DASH) in Southern Europe

Trial Details		Crop	Country	Formulation, Application Rate[0)] (kg a.s./ha)	Crop Growth Stage[2)] (BBCH)	DA-LA[1)]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	211921	Oilseed rape	France	BAS 798 KA H: 1 x 0.05	61	0	W.plant	0.68	<0.05	0.73
Doc ID:	2007/1007939[3]					15	W.plant	<0.05	<0.05	<0.10
Trial No.:	AF/10114/BA/1					78	Seed	<0.05	<0.05	<0.10
GLP:	yes									
Year:	2005/2006									
Study code:	251536	Oilseed rape	France	BAS 798 KA H 1 x 0.05 + 2 L/ha BAS 160 00 S	61	0	W.plant	0.97	<0.05	1.02
Doc ID:	2007/1007963[4]					12	W.plant	0.12	0.21	0.33
Trial No.:	AF/10475/BA/1					78	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2005/2006									
Study code:	211921	Oilseed rape	France	BAS 798 KA H: 1 x 0.05	53	0	W.plant	0.44	<0.05	0.49
Doc ID:	2007/1007939[3]					19	W.plant	<0.05	<0.05	<0.10
Trial No.:	AF/10114/BA/2					83	Seed	<0.05	<0.05	<0.10
GLP:	yes									
Year:	2005/2006									
Study code:	251536	Oilseed rape	France	BAS 798 KA H 1 x 0.05 + 2 L/ha BAS 160 00 S	53	0	W.plant	1.50	<0.05	1.55
Doc ID:	2007/1007963[4]					19	W.plant	0.14	0.28	0.42
Trial No.:	AF/10475/BA/2					83	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2005/2006									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) study 2007/1007939 contains 4 rape trials treated with formulation BAS 798 KA H
4) study 2007/1007963 contains 4 rape trials at the same locations as 2007/1007939 treated with formulation BAS 798 KA H plus adjuvant BAS 160 00 S
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.048)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.05) 0.05 mg/kg were used for each analyte.
w.plant whole plant without roots
_ underlined values were used for MRL calculation

Report:	CA 6.3.1/3 Gabriel E.J., 2013a Study on the residue behaviour of Metazachlor, Quinmerac and Imazamox in oilseed rape after treatment with BAS 798 00 H and BAS 160 00 S under field conditions in Germany and the United Kingdom, 2011 2012/1084182
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

During the 2011 growing season 2 field trials were conducted in the Northern EU to determine the residue level of imazamox and its metabolites CL 263284, CL312622 and CL189215 in or on raw agricultural commodities (RAC) of oilseed rape. BAS 798 00 H, an SC formulation of metazachlor, quinmerac and imazamox, was applied once at a rate equivalent to 0.035 kg a.s./ha of imazamox in a spray volume of 200 L/ha. One plot from every trial was treated with BAS 798 00 H and adjuvant BAS 160 00 S as a tank mixture. The application was performed at growth stage BBCH 18 except for trial L110324, where the BBCH ranged between 16 and 50. Specimens of whole plant without roots were collected immediately and 94-99 days (BBCH 89) after application. Seed samples were collected at plant maturity 94-99 days (BBCH 89) after the application.
The specimens were analysed for imazamox and its metabolites CL 263284, CL312622 and CL189215 with BASF Method No. L0188/01 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues of imazamox and all metabolites in mature seed at BBCH 89 after one application of BAS 798 00 H with and without adjuvant DASH (BAS 160 00 S) were <0.01 mg/kg, total imazamox was <0.02 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 798 00 H (SC)
Lot/Batch #:	5011 (17.5 g/L imazamox, nominal) 1095 (BAS 160 00 S (DASH))
Purity:	Not reported
CAS#:	114311-32-9
Development code:	346280_68
Spiking levels:	Seed: 0.01-1.0 mg/kg Whole plant w/o roots: 0.01-4.0 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	SO 0495 Micro CL (IMI-tolerant variety)
Botanical name:	*Brassica napus*
Crop parts(s) or processed commodity:	Whole plant without roots, seed
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season 2 trials in oilseed rape were conducted in different representative growing areas in the Northern EU (Germany, United Kingdom) to determine the residue level of imazamox and its metabolites CL 263284, CL312622 and CL189215 in or on raw agricultural commodities (RAC). BAS 798 00 H (17.5 g/L imazamox, SC) was applied once at a rate equivalent to 0.035 kg imazamox/ha in a spray volume of 200 L/ha. One replicate of each trial was treated combined with adjuvant BAS 160 00 S. The application was performed at growth stage BBCH 18 (but in one trial early flowering occurred due to drought stress). Specimens of whole plant without roots were collected immediately and 94-99 days after the application at harvest. Specimens of seed were collected 94-99 days after the application, at plant harvest (BBCH 89). Samples were stored frozen at or below -18°C for a maximum of 294 days until analysis.

Table 6.3.1-8: Target application rates and timings for oilseed rape

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2011	2	1	F	BAS 798 00 H (SC) +BAS 160 00 S	BAS 720 H	0.035	200	BBCH 18
2011	2	1	F	BAS 798 00 H (SC)	BAS 720 H	0.035	200	BBCH 18

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL312622 (Reg. No. 4110542) and CL189215 (Reg. No. 4110445) with BASF Method No. L0188/01 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues are extracted from oilseed rape matrices using an acidic methanol-water solution. An aliquot of the sample extract was diluted and residues were detected by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.1-9: Summary of procedural recovery data for imazamox

Matrix	Fortification Level (mg/kg)	Summary Recoveries		
		n	Mean (%)	RSD (%)
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**Imazamox (BAS 720 H)**		
Whole plant	0.01, 4.0	6	104	8
Seed	0.01-0.10	6	96	5
Overall		12	100	8
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL 263284 (Reg. No. 4110773)**		
Whole plant	0.01, 4.0	6	109	4
Seed	0.01-0.10	6	98	5
Overall		12	104	7
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL312622 (Reg. No. 4110542)**		
Whole plant	0.01, 4.0	6	107	13
Seed	0.01-0.10	6	94	8
Overall		12	100	12
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL189215 (Reg. No. 4110445)**		
Whole plant	0.01, 4.0	6	111	8
Seed	0.01-0.10	6	96	7
Overall		12	103	10

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-10; detailed residue levels are shown in Table 6.3.1-11.

In the Northern EU residues in oilseed rape treated with BAS 798 00 H and adjuvant DASH (BAS 160 00 S) and sampled 0 days after the application (BBCH 18) ranged from 1.30-2.60 mg/kg for imazamox and <0.01-0.01 mg/kg for metabolite CL 312622. Residues of metabolite CL 263284 and CL 189215 were below LOQ (0.01 mg/kg). In the rest of plant commodities sampled 94-99 days after the application (BBCH 89) residues ranged from 0.02-0.19 mg/kg for metabolite CL 263284. No quantifiable residues of imazamox and its metabolites CL 312622 and CL 189215 were found. No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or all of its metabolites investigated were found in seed samples at harvest 94-99 days after the application (BBCH 89). Total imazamox for seeds was <0.02 mg/kg.

In oilseed rape whole plant specimens treated with BAS 798 00 H without adjuvant, residues at 0 DALA (BBCH 18) ranged from 0.54-1.10 mg/kg for imazamox, were 0.02-0.03 mg/kg for metabolite CL 263284 and <0.01-0.01 mg/kg for metabolite CL 312622. Residues of metabolite CL 189215 were below LOQ (0.01 mg/kg). In the rest of plant commodities sampled 94-99 days after the application (BBCH 89) residues ranged from 0.01-0.05 mg/kg for metabolite CL 263284, were <0.01-0.03 mg/kg for CL 312622 and <0.01-0.02 mg/kg for CL 189215. No quantifiable residues of imazamox were found. No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or all of its metabolites investigated were found in seed samples at harvest 94-99 days after the application (BBCH 89). Total imazamox for seeds was <0.02 mg/kg.

Table 6.3.1-10: Summary of residues in oilseed rape treated with BAS 798 00 H (with and without DASH)

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	Imaza-mox (BAS 720)	CL 263284[5] (4110773)	Total imaza-mox[4]	CL 312622 (4110542)	CL 189215 (4110445)
With Adjuvant DASH									
Northern EU	2011	0	18	Whole plant[3]	1.30-2.60	<0.01	0.55-1.11	<0.01-0.01	<0.01
		94-99	89	Rest plant[3]	<0.01	0.02-0.19	0.03-0.2	<0.01	<0.01
		94-99	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
Without Adjuvant DASH									
Northern EU	2011	0	18	Whole plant[3]	0.54-1.10	0.02-0.03	1.32-2.63	<0.01-0.01	<0.01
		94-99	89	Rest plant[3]	<0.01	0.01-0.05	0.02-0.06	<0.01-0.03	<0.01-0.02
		94-99	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte.
5) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.

III. CONCLUSION

Residues of imazamox in whole plant samples taken directly after application (0 DALA) were a little bit higher after application with adjuvant DASH than without adjuvant.
Residues of imazamox and all metabolites in mature seed at BBCH 89 after one application of BAS 798 00 H with and without adjuvant DASH (BAS 160 00 S) were <0.01 mg/kg, total imazamox residues were <0.02 mg/kg. Thus, the application of adjuvant DASH had no impact on residue levels in edible commodities of oilseed rape.

Table 6.3.1-11: Residues of imazamox in oilseed rape after one application of BAS 798 00 H or a tank mix of BAS 798 00 H and BAS 160 00 S (DASH) in Northern Europe

Trial Details		Crop Variety	Country	Formulation, Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2]	DA LA[1] (days)	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	407228	Oilseed	Germany	BAS 798 00 H:	16-50	0	W.plant	0.54	<0.01	0.55	<0.01	<0.01
Doc ID:	2012/1084182	Rape		1 x 0.035		94	R.plant	<0.01	0.01	0.02	<0.01	<0.01
Trial No.:	L110324	Micro				94	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes	CL										
Year:	2011											
Study code:	407228	Oilseed	Germany	BAS 798 00 H:	16-50	0	W.plant	1.3	0.02	1.32	<0.01	<0.01
Doc ID:	2012/1084182	Rape		1 x 0.035		94	R.plant	<0.01	0.02	0.03	<0.01	<0.01
Trial No.:	L110324[3]	Micro		+ 2 L/ha		94	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	<0.01
GLP:	yes	CL		BAS 160 00 S								
Year:	2011											
Study code:	407228	Oilseed	United	BAS 798 00 H:	18	0	W.plant	1.1	<0.01	1.11	0.01	<0.01
Doc ID:	2012/1084182	Rape	Kingdom	1 x 0.035		99	R.plant	<0.01	0.05	0.06	<0.01	<0.01
Trial No.:	L110325	Micro				99	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes	CL										
Year:	2011											
Study code:	407228	Oilseed	United	BAS 798 00 H:	18	0	W.plant	2.6	0.03	2.63	0.01	<0.01
Doc ID:	2012/1084182	Rape	Kingdom	1 x 0.035		99	R.plant	<0.01	0.19	0.20	0.03	0.02
Trial No.:	L110325[3]	Micro		+ 2 L/ha		99	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	<0.01
GLP:	yes	CL		BAS 160 00 S								
Year:	2011											

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) study 2012/1084182 contains 2 rape trials. At each location one plot was treated with formulation BAS 798 00 H plus adjuvant BAS 160 00 S
I: Imazamox
II: Metabolite CL 263284 (4110773) expressed as parent equivalents (conversion factor 1.04815)
III: Total imazamox (residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte
IV: Metabolite CL312622 (Reg. No. 4110542)
V: Metabolite CL189215 (Reg. No. 4110445)
w.plant whole plant without roots
r.plant rest of plant without roots
_ underlined values were used for MRL calculation

Report:	CA 6.3.1/4 Gabriel E.J., 2013b Study on the residue behaviour of Metazachlor, Quinmerac and Imazamox in oilseed rape after treatment with BAS 798 00 H and BAS 160 00 S under field conditions in Italy and Southern France, 2011/2012 2013/1089639
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

During the 2012 growing season 2 field trials were conducted in the Southern EU to determine the residue level of imazamox and its metabolites CL 263284, CL312622 and CL189215 in or on raw agricultural commodities (RAC) of oilseed rape. BAS 798 00 H, an SC formulation of metazachlor, quinmerac and imazamox, was applied once at a rate equivalent to 0.035 kg a.s./ha of imazamox in a spray volume of 200 L/ha. A plot from every trial was treated with BAS 798 00 H and adjuvant BAS 160 00 S as a tank mixture. Specimens of whole plant without roots and seed were collected immediately and 111-224 days after the application at harvest (BBCH 89).
The specimens were analysed for imazamox and its metabolites CL 263284, CL312622 and CL189215 with BASF Method No. L0188/01 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or all of its metabolites investigated were found in seed samples at harvest (BBCH 89) 111-224 days after one application of BAS 789 00 H with and without adjuvant DASH (BAS 160 00S). Total imazamox for seeds was <0.02 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 798 00 H (SC)
Lot/Batch #:	5011 (17.5 g/L imazamox, nominal) 1095 (BAS 160 00 S (DASH))
Purity:	Not reported
CAS#:	114311-32-9
Development code:	346280_68
Spiking levels:	Seed: 0.01-1.0 mg/kg Whole plant w/o roots: 0.01-4.0 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	SO 0495 Micro CL (IMI-tolerant variety)
Botanical name:	*Brassica napus*
Crop parts(s) or processed commodity:	Whole plant without roots, seed
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 2012 growing period 2 trials in oilseed rape were conducted in different representative growing areas in the Southern EU (France, Italy) to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on raw agricultural commodities (RAC). BAS 798 00 H (17.5 g/L imazamox, SC) was applied once at a rate equivalent to 0.035 kg imazamox/ha in a spray volume of 200 L/ha. One plot of each trial was treated with a tank mix of BAS 798 00 H and adjuvant BAS 160 00 S. The application was performed at growth stage BBCH 18. Specimens of whole plant without roots and seed were collected immediately and 111-224 days after the application (BBCH 89). Samples were stored frozen at or below -18°C for a maximum of 462 days until analysis.

Table 6.3.1-12: Target application rates and timings for oilseed rape

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2012	2	1	F	BAS 798 00 H (SC) (+BAS 160 00 S)	BAS 720 H	0.035	200	BBCH 18
2012	2	1	F	BAS 798 00 H (SC)	BAS 720 H	0.035	200	BBCH 18

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with BASF Method No. L0188/01 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues are extracted from oilseed rape matrices using an acidic methanol-water solution. An aliquot of the sample extract is diluted and analysed by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.1-13: Summary of procedural recovery data for imazamox

Matrix	Fortification Level (mg/kg)	Summary Recoveries		
		n	Mean (%)	RSD (%)
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**Imazamox (BAS 720 H)**		
Whole plant	0.01, 4.0	6	96	10
Seed	0.01-0.05	8	100	4
Overall		14	98	7
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL 263284 (Reg. No. 4110773)**		
Whole plant	0.01, 4.0	6	97	6
Seed	0.01-0.05	8	97	4
Overall		14	97	5
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL312622 (Reg. No. 4110542)**		
Whole plant	0.01, 4.0	6	92	4
Seed	0.01-0.05	8	97	4
Overall		14	95	5
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL189215 (Reg. No. 4110445)**		
Whole plant	0.01, 4.0	6	94	12
Seed	0.01-0.05	8	101	7
Overall		14	98	10

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-14, detailed residue levels are shown in Table 6.3.1-15.

In the Southern EU residues in oilseed rape treated with a tank mix of BAS 798 00 H and adjuvant DASH (BAS 160 00 S), sampled 0 days after the application (BBCH 18), ranged from 0.18-1.80 mg/kg for imazamox, from <0.01-0.01 mg/kg for metabolite CL 263284 and from <0.01-0.01 mg/kg for metabolite CL 312622. Residues of metabolite CL 189215 were below LOQ (0.01 mg/kg). In the rest of plant commodities sampled 111-224 days after the application (BBCH 89) residues ranged from <0.01-0.03 mg/kg for metabolite CL 263284. No quantifiable residues of imazamox and its metabolites CL 312622 and CL 189215 were found. No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or all of its metabolites investigated were found in seed samples at harvest 111-224 days after the application (BBCH 89). Total imazamox in seeds was <0.02 mg/kg.

In oilseed rape whole plant specimens treated with BAS 798 00 H (without adjuvant), residues at 0 DALA (BBCH 18) ranged from 0.10-2.00 mg/kg for imazamox, were <0.01-0.03 mg/kg for metabolite CL 263284 and <0.01-0.02 mg/kg for metabolite CL 312622. Residues of metabolite CL 189215 were below LOQ (0.01 mg/kg). In the rest of plant commodities sampled 111-224 days after the application (BBCH 89) residues ranged from <0.01-0.09 mg/kg for metabolite CL 263284, but no quantifiable residues of imazamox and its metabolites CL 312622 and CL 189215 were found. No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or all of its metabolites investigated were found in seed samples at harvest 111-224 days after the application (BBCH 89). Total imazamox in seeds was <0.02 mg/kg.

No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or any of its metabolites investigated were found in seed samples at harvest (BBCH 89), 111-224 days after one application of BAS 798 00H, with or without adjuvant DASH (BAS 160 00 S). Total imazamox residues in seeds were <0.02 mg/kg.

No residues were found in any of the control samples.

Table 6.3.1-14: Summary of residues in oilseed rape treated with BAS 798 00 H (with and without DASH)

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	Imazamox (BAS 720)	CL 263284[5] (4110773)	Total imaza-mox[4]	CL 312622 (4110542)	CL 189215 (4110445)
With Adjuvant DASH									
Southern EU	2012	0	18	Whole plant[3]	0.18-1.80	<0.01-0.01	0.19-1.81	<0.01-0.01	<0.01
		111-224	89	Rest plant[3]	<0.01	<0.01-0.03	<0.02-0.4	<0.01	<0.01
		111-224	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
Without Adjuvant DASH									
Southern EU	2012	0	18	Whole plant[3]	0.1-2.0	<0.01-0.03	0.11-2.03	<0.01-0.02	<0.01
		111-224	89	Rest plant[3]	<0.01	<0.01-0.09	<0.02-0.10	<0.01	<0.01
		111-224	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte.
5) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.

III. CONCLUSION

No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or any of its metabolites investigated were found in seed samples at harvest (BBCH 89) 111-224 DALA of BAS 798 00H with or without adjuvant DASH (BAS 160 00 S). Total imazamox residues in seeds were <0.02 mg/kg. The application of adjuvant DASH had no significant impact on residue levels in whole plant or edible commodities of oilseed rape.

Table 6.3.1-15: Residues of imazamox in oilseed rape after one application of BAS 798 00 H or a tank mix of BAS 798 00 H and BAS 160 00 S (DASH) in Southern Europe

Trial Details		Crop Variety	Country	Formulation, Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2]	DA LA[1] (days)	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	407228_1	Oilseed	Italy	BAS 798 00 H:	18	0	W.plant	1.80	0.01	1.81	0.01	<0.01
Doc ID:	2013/1089639	Rape		1 x 0.035		224	R.plant	<0.01	0.03	0.04	<0.01	<0.01
Trial No.:	L110461	Micro				224	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes	CL										
Year:	2012											
Study code:	407228_1	Oilseed	Italy	BAS 798 00 H:	18	0	W.plant	2.00	0.03	2.03	0.02	<0.01
Doc ID:	2013/1089639[3]	Rape		1 x 0.035		224	R.plant	<0.01	0.09	0.10	<0.01	<0.01
Trial No.:	L110461	Micro		+ 2 L/ha		224	Seed	<0.01	<0.01	<u><0.02</u>	<0.01	<0.01
GLP:	yes	CL		BAS 160 00 S								
Year:	2012											
Study code:	407228_1	Oilseed	France	BAS 798 00 H:	18	0	W.plant	0.18	<0.01	0.19	<0.01	<0.01
Doc ID:	2013/1089639	Rape		1 x 0.035		111	R.plant	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No.:	L110462	Micro				111	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes	CL										
Year:	2012											
Study code:	407228_1	Oilseed	France	BAS 798 00 H:	18	0	W.plant	0.10	<0.01	0.11	<0.01	<0.01
Doc ID:	2013/1089639[3]	Rape		1 x 0. 035		111	R.plant	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No.:	L110462	Micro		+ 2 L/ha		111	Seed	<0.01	<0.01	<u><0.02</u>	<0.01	<0.01
GLP:	yes	CL		BAS 160 00 S								
Year:	2012											

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) study 2013/1089639 contains 2 rape trials. At each location one plot was treated with formulation BAS 798 00 H plus adjuvant BAS 160 00 S

I: imazamox
II: metabolite CL 263284 (4110773) expressed as parent equivalents (conversion factor 1.04815)
III: total imazamox (residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte
IV: metabolite CL312622 (Reg. No. 4110542)
V: metabolite CL189215 (Reg. No. 4110445)
w.plant = whole plant without roots
r.plant = rest of plant without roots
_underlined values were used for MRL calculation

Report:	CA 6.3.1/5 Martin T., 2013a Study on the residue behavior of Quinmerac (BAS 518 H) and Imazamox (BAS 720 H) on oilseed rape after the application of BAS 831 00 H under field conditions in Germany, United Kingdom, Italy and Spain, 2012 2013/1044540
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	Yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

During the 2012 growing season 4 field trials were conducted in the EU, 2 in the Northern and 2 in the Southern region, to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on raw agricultural commodities (RAC) of oilseed rape. BAS 831 00 H, an SC formulation of quinmerac and imazamox, was applied once at a rate equivalent to 0.035 kg a.s./ha of imazamox in a tank mix with adjuvant DASH (BAS 160 00 S). The spray volume was 200 L/ha. The application was performed at growth stage BBCH 50. Specimens of whole plant without roots were collected immediately and 64-98 days (BBCH 89) after application. Seed samples were collected at plant maturity 64-98 days (BBCH 89) after the application.
The specimens were analysed for imazamox and its metabolites CL 263284, CL 312622 and CL 189215 with BASF Method No. L0188/01 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues of imazamox and all metabolites in mature seed at BBCH 89 were <0.01 mg/kg in the Northern and Southern EU after one application of BAS 831 00 H with adjuvant DASH (BAS 160 00 S) Total imazamox in seed was <0.02 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 831 00 H (SC)
Lot/Batch #:	450008 (35 g/L imazamox, nominal), 70161875L0 (BAS 160 00 S (DASH))
Purity:	Not reported
CAS#:	114311-32-9
Development code:	346280_68
Spiking levels:	Seed: 0.01-0.1 mg/kg Whole plant w/o roots: 0.01-0.1 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	SO 0495 Salsa CL (IMI-tolerant variety)
Botanical name:	*Brassica napus*
Crop parts(s) or processed commodity:	Whole plant without roots, seed
Sample size:	1.0 kg seeds, 0.5 kg rest of plants (12 plants each)

B. STUDY DESIGN

1. Test procedure

During the 2012 growing season 4 trials in oilseed rape were conducted in different representative growing areas in the Northern EU (Germany, United Kingdom) and Southern EU (Spain, Italy) to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on raw agricultural commodities (RAC). BAS 831 00 H (35 g/L imazamox, SC) was applied once in a tank mix with DASH (BAS 160 00 S) at a rate equivalent to 0.035 kg imazamox/ha, in a spray volume of 200 L/ha. The application was performed at growth stage BBCH 50. Specimens of whole plant without roots were collected immediately and 64-98 days after the application (BBCH 89). Specimens of seed were collected 64-98 days after the application, at plant harvest (BBCH 89). Samples were stored frozen at or below -18°C for a maximum of 236 days until analysis.

Table 6.3.1-16: Target application rates and timings for oilseed rape

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2012	4	1	F	BAS 831 00 H (SC) + BAS 160 00 S	BAS 720 H	0.035	200	BBCH 50

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with BASF Method No. L0188/01 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues are extracted from oilseed rape matrices using an acidic methanol-water solution. An aliquot of the sample extract is diluted and analysed by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.1-17: Summary of procedural recovery data for imazamox

Matrix	Fortification Level (mg/kg)	Summary Recoveries		
		n	Mean (%)	RSD (%)
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**Imazamox (BAS 720 H)**		
Whole plant	0.01, 0.1, 2.0	3	93	15
Rest of plant	0.01, 0.1	2	99	N/A
Seed	0.01, 0.1	2	95	N/A
Overall		7	95	13
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL 263284 (Reg. No. 4110773)**		
Whole plant	0.01, 0.1	2	102	N/A
Rest of plant	0.01, 0.1, 2.0	3	100	9
Seed	0.01, 0.1	2	78	N/A
Overall		7	94	13
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL 312622 (Reg. No. 4110542)**		
Whole plant	0.01, 0.1	2	83	N/A
Rest of plant	0.01, 0.1	2	98	N/A
Seed	0.01, 0.1	2	87	N/A
Overall		6	89	11
BASF Method No. L0188/01; LOQ = 0.01 mg/kg		**CL 189215 (Reg. No. 4110445)**		
Whole plant	0.01, 0.1	2	106	N/A
Rest of plant	0.01, 0.1	2	105	N/A
Seed	0.01, 0.1	2	93	N/A
Overall		6	101	12

N/A: not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-18; detailed residue levels are shown in Table 6.3.1-19 for Northern Europe and in Table 6.3.1-20 for Southern Europe.

In the Northern EU, residues in oilseed rape whole plant specimens sampled 0 days after the application (BBCH 50) ranged from 1.80-2.00 mg/kg for imazamox, were 0.03-0.04 mg/kg for metabolite CL 263284 and <0.01-0.01 mg/kg for CL 312622. Residues of metabolite CL 189215 were below LOQ (0.01 mg/kg). In the rest of plant commodities sampled 81-98 days after the application (BBCH 89) residues ranged from 0.01-0.08 mg/kg for metabolite CL 263284. No quantifiable residues of imazamox or its metabolites CL 312622 and CL 189215 were found. No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or any of its metabolites investigated were found in seed samples at harvest 81-98 days after the application (BBCH 89). Total imazamox in seeds was <0.02 mg/kg.

In the Southern EU, residues in oilseed rape whole plant specimens sampled 0 days after the application (BBCH 50) ranged from 0.77-1.20 mg/kg for imazamox and were 0.01-0.02 mg/kg for metabolite CL 263284. Residues of metabolites CL 312622 and CL 189215 were below LOQ (0.01 mg/kg). In the rest of plant commodities sampled 64-74 days after the application (BBCH 89) residues ranged from 0.14-0.27 mg/kg for metabolite CL 263284 and from <0.01-0.02 mg/kg for CL 312622. No quantifiable residues of imazamox orits metabolite CL 189215 were found. No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or any of its metabolites were found in seed samples at harvest 64-74 days after the application (BBCH 89). Total imazamox in seeds was <0.02 mg/kg.

No residues were found in any of the control samples.

Table 6.3.1-18: Summary of residues in oilseed rape treated with BAS 831 98 H and BAS 160 00 S (DASH)

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	Imazamox (BAS 720H)	CL 263284[5] (4110773)	Total imaza-mox[4]	CL 312622 (4110542)	CL 189215 (4110445)
Northern EU	2012	0	50	Whole plant[3]	1.80-2.0	0.03-0.04	1.84-2.03	0.01	<0.01
		81-98	89	Rest plant[3]	<0.01	0.01-0.08	0.02-0.09	<0.01	<0.01
		81-98	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
Southern EU	2012	0	50	Whole plant[3]	0.77-1.2	0.01-0.02	0.78-1.22	<0.01	<0.01
		64-74	89	Rest plant[3]	<0.01	0.14-0.27	0.15-0.28	<0.01-0.02	<0.01
		64-74	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte.
5) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.

III. CONCLUSION

Residues of imazamox and all metabolites in mature seed at BBCH 89 were <0.01 mg/kg in the Northern and Southern EU after one application of BAS 831 00 H with adjuvant DASH (BAS 160 00 S). Total imazamox residues were <0.02 mg/kg.

Table 6.3.1-19: Residues of imazamox in oilseed rape after one application of a tank mix of BAS 831 00 H and BAS 160 00 S (DASH) in Northern Europe

Trial Details		Crop Variety	Country	Formulation, Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2]	DA LA[1] (days)	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	421874	Oilseed	Germany	BAS 831 00 H:	50	0	W.plant	2.0	0.03	2.03	0.01	<0.01
Doc ID:	2013/1044540	Rape		1 x 0.035		81	R.plant	<0.01	0.08	0.09	<0.01	<0.01
Trial No.:	L120254	Salsa CL		+ 1 L/ha		81	Seed	<0.01	<0.01	<u>≤0.02</u>	<0.01	<0.01
GLP:	yes			BAS 160 00 S								
Year:	2012											
Study code:	421874	Oilseed	United	BAS 831 00 H:	50	0	W.plant	1.8	0.04	1.84	0.01	<0.01
Doc ID:	2013/1044540	Rape	Kingdom	1 x 0.035		98	R.plant	<0.01	0.01	0.02	<0.01	<0.01
Trial No.:	L120255	Salsa CL		+ 1 L/ha		98	Seed	<0.01	<0.01	<u>≤0.02</u>	<0.01	<0.01
GLP:	yes			BAS 160 00 S								
Year:	2012											

0) actual application rates varied by 10% at
1) days after the last application
2) at last application
I: imazamox
II: metabolite CL 263284 (4110773) expressed as parent equivalents (conversion factor 1.04815)
III: total imazamox (residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte
IV: metabolite CL312622 (Reg. No. 4110542)
V: metabolite CL189215 (Reg. No. 4110445)
w.plant = whole plant without roots
r.plant = rest of plant without roots
_underlined values were used for MRL calculation

Table 6.3.1-20: Residues of imazamox in oilseed rape after one application of a tank mix of BAS 831 00 H and BAS 160 00 S (DASH) in Southern Europe

Trial Details		Crop Variety	Country	Formulation, Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2]	DA LA[1] (days)	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	421874	Oilseed	Spain	BAS 831 00 H:	50	0	W.plant	0.77	0.01	0.78	<0.01	<0.01
Doc ID:	2013/1044540	Rape		1 x 0.035		74	R.plant	<0.01	0.14	0.15	<0.01	<0.01
Trial No.:	L120256	Salsa CL		+ 1 L/ha		74	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	<0.01
GLP:	yes			BAS 160 00 S								
Year:	2012											
Study code:	421874	Oilseed	Italy	BAS 831 00 H:	50	0	W.plant	1.2	0.02	1.22	<0.01	<0.01
Doc ID:	2013/1044540	Rape		1 x 0.035		64	R.plant	<0.01	0.27	0.28	0.02	<0.01
Trial No.:	L120257	Salsa CL		+ 1 L/ha		64	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	<0.01
GLP:	yes			BAS 160 00 S								
Year:	2012											

0) actual application rates varied by 10% at
1) days after the last application
2) at last application
I: Imazamox
II: Metabolite CL 263284 (4110773) expressed as parent equivalents (conversion factor 1.04815)
III: Total imazamox (residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte
IV: Metabolite CL312622 (4110542)
V: Metabolite CL189215 (4110445)
w.plant = whole plant without root
r.plant = rest of plant without roots
_underlined values were used for MRL calculation

CA 6.3.2 Sunflower

Table 6.3.2-1: Critical GAP for the use of BAS 720 H in/on sunflower

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Sunflower	1 x 0.05 kg BAS 720 H/ha	300 L/ha	F	spray application	BBCH 12-18

PHI = pre-harvest interval

The following residue trials are submitted in support of the intended use pattern (as shown above) with the formulation BAS 720 06 H.
The formulation BAS 720 02 H is the predecessor of BAS 720 06 H. Both formulations are virtually identical and differ only in the included bactericide.
The formulation BAS 720 AM H is identical with BAS 720 10 H and is a newly developed formulation with an in-built adjuvant (Klearfac AA-270). This formulation was tested in bridging trials against the representative formulation BAS 720 06 H (in combination with adjuvant DASH). The formulation BAS 720 BD H is identical with BAS 720 AM H/BAS 720 10 H, with the same ai content (25g/L) and the same type and content of in-built adjuvant (Klearfac AA-270).

Table 6.3.2-2: GAP information of residue trials conducted in sunflower

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Northern EU	France (2 trials)	BAS 720 02 H, SL	spray application	0.05	0.017	1	8-16 37-38 98-99
	France (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 104
	The Netherlands (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 93
	Belgium (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 90
	Germany (1 trial)	BAS 720 06 H, SL (BAS 160 00 S, EC)[2]	spray application	0.05	0.025	1	0 112
	Germany (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 124
	United Kingdom (1 trial)	BAS 720 06 H, SL (BAS 160 00 S, EC)[2]	spray application	0.05	0.025	1	0 117

Table 6.3.2-2: GAP information of residue trials conducted in sunflower

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
	Germany (1 trial)	BAS 720 06 H, SL +BAS 160 00 S, EC BAS 720 AM H	spray application	0.05	0.025	1	0 118
	France-N (1 trial)	BAS 720 06 H, SL +BAS 160 00 S, EC BAS 720 AM H	spray application	0.05	0.025	1	0 121
Southern EU	Spain (4 trials)	BAS 720 02 H, SL	spray application	0.04	0.013	1	14-20 36-46 83-110
	France-S (1 trial)	BAS 720 06 H, SL (BAS 160 00 S, EC)[2]	spray application	0.05	0.025	1	0 111
	France (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 104
	Italy (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 118
	Italy (1 trial)	BAS 720 06 H, SL +BAS 160 00 S, EC	spray application	0.05	0.025	1	0 117
	Spain (1 trial)	BAS 720 06 H, SL BAS 160 00 S, EC)[2] BAS 720 AM H	spray application	0.05	0.025	1	0 107
	Greece (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 86
	Spain (1 trial)	BAS 720 BD H, SL	spray application	0.05	0.025	1	0 104
	Italy (1 trial)	BAS 720 06 H, SL +BAS 160 00 S,EC BAS 720 AM H	spray application	0.05	0.025	1	0 114

0) actual application rates varied by 10% at most
1) days after last application
2) one plot treated with DASH (BAS 160 00 S) in a tank mix
The trials highlighted in yellow are submitted in addition to the original submission

Report:	CA 6.3.2/1 Perny A., 2004a Study on the residue behaviour of Imazamox in Imazamox resistant sunflowers after application of BAS 720 02 H under field conditions in France, in 2003 2004/1000754
Guidelines:	EEC 91/414, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	Yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

During the 2003 growing season 2 trials were conducted in the EU North to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC) of sunflower. BAS 720 02 H was applied once at a rate equivalent to 0.05 kg a.s./ha of imazamox in a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-18. Specimens of whole plant without roots were collected 8-16 days (BBCH 30-32) and 37-38 days (BBCH 61) after the application. Specimens of seed were collected 98-99 days after the application, at plant maturity (BBCH 89).
The specimens were analysed for imazamox and its metabolite CL 263284 with BASF Method No. M3515 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of imazamox and metabolite CL 263284 in mature seeds at BBCH 89 were <0.05 mg/kg, total imazamox residues were <0.10 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 720 02 H (SL)
Lot/Batch #:	2001, 40 g/L imazamox, nominal
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	0.05, 0.5 mg/kg

2. Test Commodity:

Crop:	Sunflower
Type:	Oilseeds
Variety:	Imazamox-resistant varieties DK 3900 CL, CMS425xRHA426SF02
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed commodity:	Whole plant without roots, seed
Sample size:	0.5-1.0 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2003 growing season 2 trials in sunflower were conducted in different representative growing areas in the EU North (France) to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC). BAS 720 02 H (4% imazamox, SL) was applied once at a rate equivalent to 0.05 kg imazamox/ha in a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-18. Specimens of whole plant without roots were collected 8-16 days (BBCH 30-32) and 37-38 days (BBCH 61) after the application. Specimens of seed were collected 98-99 days after the application, at plant maturity (BBCH 89). Samples were stored frozen at or below -18°C for a maximum of 210 days until analysis.

Table 6.3.2-3: Target application rates and timings for sunflower

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2003	2	1	F	BAS 720 02 H (SL)	BAS 720 H	0.05	300	BBCH 14-18

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 with BASF Method No. M3515 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues were extracted from sunflower matrices using an acidic methanol-water solution, cleaned up using SCX solid phase extraction (SPE) cartridges and measured by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.2-4: Summary of recoveries of imazamox and metabolite CL 263284 in sunflower

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No. M3515		BAS 720 H (imazamox)			Metabolite CL 263284		
Whole plant without roots	0.05/0.5	2	95	N/A	2	101	N/A
Seed	0.05/0.5	2	99	N/A	2	113	N/A
Overall	0.05/0.5	4	97	2.4	4	107	10.7

N/A not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-5, detailed residue levels are shown in Table 6.3.2-6.
Residues of parent imazamox (BAS 720 H) and its metabolite CL 263284 were 0.09-0.16 mg/kg and <0.05-0.07 mg/kg in whole plants without roots samples taken 8-16 DALA, respectively; total imazamox residues amounted to 0.16-0.21 mg/kg.
No residues at or above the LOQ of the analytical method (0.05 mg/kg) of imazamox or its metabolite CL 263284 were found in sunflower whole plant specimens taken 37-38 DALA (BBCH 61) or in seed samples taken 83-110 DALA (BBCH 89). Total imazamox in these samples was <0.10 mg/kg.

No residues of imazamox were found in control plants.

Table 6.3.2-5: Summary of residues in sunflower

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284	Total imazamox[4]
EU North	2002	8-16	30-32	Whole plant[3]	0.09-0.16	<0.05-0.07	0.16-0.21
		37-38	61	Whole plant[3]	<0.05	<0.05	<0.10
		98-99	89	Seed	<0.05	<0.05	<0.10

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.05 mg/kg was used for analytes with residues below the LOQ

III. CONCLUSION

Residues of imazamox and metabolite CL 263284 in mature seed at BBCH 89 were <0.05 mg/kg, total imazamox was <0.10 mg/kg.

Table 6.3.2-6: Residues of imazamox in sunflower after one application of BAS 720 02 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)			
							Matrix	Imaza-mox	CL 263284	Total Imaza-mox[4]
Study code:	166093	Sun-flower	France	BAS 720 02 H: 1 x 0.05	14	16	Whole plant[3]	0.16	<0.05	0.21
Doc ID:	2004/1000754					38	Whole plant[3]	<0.05	<0.05	<0.10
Trial No:	FAN/28/03					99	Seed	<0.05	<0.05	<u><0.10</u>
GLP:	yes									
Year:	2003									
Study code:	166093	Sun-flower	France	BAS 720 02 H: 1 x 0.05	18	8	Whole plant[3]	0.09	0.07	0.16
Doc ID:	2004/1000754					37	Whole plant[3]	<0.05	<0.05	<0.10
Trial No:	FBM/18/03					98	Seed	<0.05	<0.05	<u><0.10</u>
GLP:	yes									
Year:	2003									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.05 mg/kg was used for analytes with residues below the LOQ

_ underlined values were used for MRL calculation

Report:	CA 6.3.2/2 Beck J. et al., 2003a Study on the residue behaviour of Imazamox in sunflower after application of BAS 720 02 H under field conditions in Spain, 2002 2002/1012087
Guidelines:	EEC 91/414 (1607/VI/97), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	Yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

During the 2002 growing season 4 trials were conducted in the EU South to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC) of sunflower. BAS 720 02 H was applied once at a rate equivalent to 0.04 kg a.s./ha of imazamox in a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-16. Specimens of whole plant without roots were collected 14-20 days (BBCH 30) and 36-46 days (BBCH 61) after application. Seed samples were collected at plant maturity, 83-110 days (BBCH 89) after the application.
The specimens were analysed for imazamox and its metabolite CL 263284 with BASF Method No. M3515 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of imazamox and metabolite CL 263284 in mature seed at BBCH 89 were <0.05 mg/kg, total imazamox was <0.10 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 720 02 H (SL)
Lot/Batch #:	278117/276912, 40 g/L imazamox, nominal
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	Seed: 0.05-0.10mg/kg Whole plant w/o roots: 0.05-0.10mg/kg

2. Test Commodity:

Crop:	Sunflower
Type:	Oilseeds
Variety:	Mycogene X 81359 (IMI tolerant variety)
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed commodity:	Whole plant without roots, seed
Sample size:	0.5-3.0 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2002 growing season 4 trials in sunflower were conducted in different representative growing areas in the EU South (Spain) to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC). BAS 720 02 H (40 g/L imazamox, SL) was applied once at a rate equivalent to 0.04 kg imazamox/ha in a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-16. Specimens of whole plant without roots were collected 14-20 days (BBCH 30) and 36 to 46 days (BBCH 61) after the application. Specimens of seed were collected 83-110 days after the application, at plant maturity (BBCH 89). Samples were stored frozen at or below -18°C for a maximum of 6 months until analysis.

Table 6.3.2-7: Target application rates and timings for sunflower

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2002	4	1	F	BAS 720 02 H (SL)	BAS 720 H	0.04	300	BBCH 14-16

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 with BASF Method No. M3515 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues are extracted from sunflower matrices using an acidic methanol-water solution, cleaned up using SCX solid phase extraction (SPE) cartridges and measured by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.2-8: Summary of recoveries of imazamox and metabolite CL 263284 in sunflower

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No. M3515		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant without roots	0.05/0.2/0.5	4	78	7.7	4	78	12.4
Seed	0.05/0.1	2	77	N/A	2	79	N/A

N/A not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-9, detailed residue levels are shown in Table 6.3.2-10.
No residues at or above the LOQ of the analytical method (0.05 mg/kg) of imazamox or its metabolite CL 263284 were found in sunflower whole plant specimens taken 14-20 days (BBCH 30) and 36-46 days (BBCH 61) after the application or in seed samples taken 83-110 days (BBCH 89) after application. Total imazamox was <0.10 mg/kg.

No residues of imazamox were found in control plants.

Table 6.3.2-9: Summary of residues in sunflower

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284	Total imazamox[4]
EU South	2002	14-20	30	Whole plant[3]	<0.05	<0.05	<0.10
		36-46	61	Whole plant[3]	<0.05	<0.05	<0.10
		83-110	89	Seed	<0.05	<0.05	<0.10

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.05 mg/kg was used for analytes with residues below the LOQ

III. CONCLUSION

Residues of imazamox and metabolite CL 263284 in mature seed at BBCH 89 were <0.05 mg/kg, total imazamox residues were <0.10 mg/kg.

Table 6.3.2-10: Residues of imazamox in sunflower after one application of BAS 720 02 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)			
							Matrix	Imaza-mox	CL 263284	Total Imaza-mox[4]
Study code:	142855	Sun-flower	Spain	BAS 720 02 H 1 x 0.04	14	17	Whole plant[3]	<0.05	<0.05	<0.10
Doc ID:	2002/1012087					46	Whole plant[3]	<0.05	<0.05	<0.10
Trial No:	ALO/22/02					103	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2002									
Study code:	142855	Sun-flower	Spain	BAS 720 02 H 1 x 0.04	16	17	Whole plant[3]	<0.05	<0.05	<0.10
Doc ID:	2002/1012087					46	Whole plant[3]	<0.05	<0.05	<0.10
Trial No:	ALO/23/02					110	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2002									
Study code:	142855	Sun-flower	Spain	BAS 720 02 H 1 x 0.04	15	14	Whole plant[3]	<0.05	<0.05	<0.10
Doc ID:	2002/1012087					43	Whole plant[3]	<0.05	<0.05	<0.10
Trial No:	AYE/16/02					91	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2002									
Study code:	142855	Sun-flower	Spain	BAS 720 02 H 1 x 0.04	16	20	Whole plant[3]	<0.05	<0.05	<0.10
Doc ID:	2002/1012087					36	Whole plant[3]	<0.05	<0.05	<0.10
Trial No:	AYE/17/02					83	Seed	<0.05	<0.05	<u>0.10</u>
GLP:	yes									
Year:	2002									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.05 mg/kg was used for analytes with residues below the LOQ

_ underlined values were used for MRL calculation

Report:	CA 6.3.2/3 Gabriel E.J., 2013c Study on the residue behaviour of Imazamox in sunflower after treatment with BAS 720 06 H and BAS 160 00 S under field conditions in Germany, the United Kingdom, southern France and Italy, 2011. 2012/1084183
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 6.3.2/4 Meyer M., 2014a Addendum 1 - Study on the residue behaviour of Imazamox in sunflower after treatment with BAS 720 06 H and BAS 160 00 S under field conditions in Germany, the United Kingdom, southern France and Italy, 2011 2014/1162729
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

During the 2011 growing season 4 trials in sunflower were conducted in the EU, 2 in the EU North and 2 in the EU South, to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on raw agricultural commodities (RAC) after the application of BAS 720 06 H alone (40 g/L imazamox, SL) or after the application of BAS 720 06 H in a tank mix with BAS 160 00 S (Adjuvant DASH) on an additional plot. The treatments were applied once at a rate equivalent to 0.05 kg imazamox/ha in spray volumes of 200 L/ha. The application was performed at growth stage BBCH 18. Specimens of whole plant without roots were collected at the day of the application. Seed and rest of plant without roots specimen were collected at plant maturity (BBCH 89), 97-117 DALA. The specimens were analysed for imazamox and its metabolites with BASF Method No. L0188/01 which quantifies the relevant analytes with a limit of quantitation (LOQ) of 0.01 mg/kg. Total imazamox residues in mature sunflower seeds ranged from 0.02-0.03 mg/kg and were below the LOQ of the analytical method of <0.02 mg/kg in the EU North and South, respectively. Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU North were <0.01 mg/kg, 0.01-0.02 mg/kg, <0.01 mg/kg and 0.02-0.03 mg/kg, respectively. Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU South were all below the LOQ of <0.01 mg/kg for each analyte.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 720 06 H (SL), BAS 160 00 S (EC)
Lot/Batch #:	0004147163, 40 g/L imazamox, nominal 1095, adjuvant DASH
Purity:	Not reported
CAS#:	114311-32-9, imazamox
Development code:	Not reported
Spiking levels:	0.01, 0.1, 10 mg/kg

2. Test Commodity:

Crop:	Sunflower
Type:	Oilseeds
Variety:	LN 11180CLplus (Imazamox-tolerant variety)
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed commodity:	Whole plant without roots, rest of plant without roots, seed
Sample size:	0.1-1.0 kg (min. 12 units)

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season 4 trials in sunflower were conducted in the EU, 2 in the EU North (Germany, United Kingdom) and 2 in the EU South (Italy, France) to determine the residue level of imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL312622 (Reg. No. 4110542) and CL189215 (Reg. No. 4110445) in or on raw agricultural commodities (RAC) after the application of BAS 720 06 H alone (40 g/L imazamox, SL) or after the application of BAS 720 06 H in a tank mix with BAS 160 00 S (Adjuvant DASH) on an additional plot. The treatments were applied once at a rate equivalent to 0.05 kg imazamox/ha in spray volumes of 200 L/ha. The application was performed at growth stage BBCH 18. Specimens of whole plant without roots were collected at the day of the application. Seed and rest of plant without roots specimen were collected at plant maturity (BBCH 89), 97-117 DALA. Samples were stored frozen at or below -18°C for a maximum of 350 days until analysis. Data of all trials are reported here, however, for later MRL derivation only the highest residue of each set of comparative trials is relevant.

Table 6.3.2-11: Target application rates and timings for sunflower

Year	No. of Trials (Plots)	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2011	4	1	F	BAS 720 06 H (SL)	BAS 720 H	0.05	200	BBCH 18
2011	4	1	F	BAS 720 06 H (SL) +BAS 160 00 S (EC)	BAS 720 H Adjuvant DASH	0.05	200	BBCH 18

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with BASF Method No. L0188/01 quantifying each analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues were extracted from sunflower matrices using an acidic methanol-water solution. A portion of the extract was centrifuged, filtered through a folded filter and an aliquot of the filtrate was diluted for determination by HPLC-MS/MS.

Table 6.3.2-12: Summary of recoveries of imazamox and its metabolites in sunflower

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No. L0188/01; LOQ=0.01 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant without roots	0.01,0.1,10	2	100	N/A	2	97	N/A
Rest plant	0.01,0.1,10	2	102	N/A	2	78	N/A
Seed	0.01,0.1,10	2	97	N/A	2	96	N/A
Overall	0.01,0.1,10	6	100	4.3	6	90	15.2
BASF Method No. L0188/01; LOQ=0.01 mg/kg		**Metabolite CL 312622**			**Metabolite CL 189215**		
Whole plant without roots	0.01,0.1,10	2	98	N/A	2	94	N/A
Rest plant	0.01,0.1,10	2	89	N/A	2	82	N/A
Seed	0.01,0.1,10	2	92	N/A	2	86	N/A
Overall	0.01,0.1,10	6	93	6.6	6	87	13.1

N/A not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-13Table 6.3.1-14; detailed residue levels are shown in Table 6.3.2-14 and Table 6.3.2-15.

After treatment with BAS 720 06 H in a tank mix with BAS 160 00 S (adjuvant DASH) or without DASH, in the EU North no residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolite CL 312622 were found in sunflower seed specimens at harvest (BBCH 89) while residues of 0.02 mg/kg and 0.02-0.03 mg/kg of metabolites CL 263284 and CL 189215 were measured, respectively. Total imazamox residues were 0.03 mg/kg. Whole plant specimen sampled at the day of the application contained 1.60-4.34 mg/kg, <0.01-0.01 mg/kg, <0.01-0.01 mg/kg and <0.01 mg/kg of imazamox, CL 263284, CL 312622 and CL 189215, respectively. Total imazamox residues were 1.61-4.35 mg/kg. Rest of plant specimen sampled at plant maturity (112-117 DALA) contained no residues at or above the LOQ (0.01 mg/kg). Total imazamox residues were below the LOQ of the analytical method of <0.02 mg/kg.

After treatment with BAS 720 06 H in a tank mix with BAS 160 00 S (adjuvant DASH) or without DASH in the EU South, no residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolites CL 263284, CL 312622 and CL 189215, were found in sunflower seed specimen at harvest (BBCH 89). Total imazamox residues were <0.02 mg/kg. Whole plant specimen sampled at the day of the application contained 2.47-3.20 mg/kg, <0.01-0.01 mg/kg, <0.01-0.02 mg/kg and <0.01 mg/kg of imazamox, CL 263284, CL 312622 and CL 189215, respectively. Total imazamox residues were 2.48-3.21 mg/kg. Rest of plant specimen sampled at plant maturity (97-111 DALA) contained no residues at or above the LOQ (0.01 mg/kg) for imazamox or its metabolites CL 263284, CL 312622 and CL 189215. Total imazamox residues were below the LOQ of the analytical method of <0.02 mg/kg.

No residues of imazamox and its metabolites were found in any of the control samples.

Table 6.3.2-13: Summary of residues in sunflower treated with BAS 720 06 H (with and without DASH)

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	Imazamox (BAS 720)	CL 263284[5]	Total imaza-mox[4]	CL 312622	CL 189215
Without Adjuvant DASH									
Northern EU	2011	0	18	Whole plant[3]	1.74-4.32	<0.01-0.01	1.75-4.33	<0.01-0.01	<0.01
		112-117	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		112-117	89	Seed	<0.01	0.01-0.02	0.02-0.03	<0.01	0.02-0.03
Southern EU	2011	0	18	Whole plant[3]	2.78-2.96	<0.01	2.79-2.97	<0.01	<0.01
		97-111	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		97-111	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
With Adjuvant DASH									
Northern EU	2011	0	18	Whole plant[3]	1.60-4.34	<0.01-0.01	1.61-4.35	<0.01-0.01	<0.01
		112-117	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		112-117	89	Seed	<0.01	0.02	0.03	<0.01	0.02-0.03
Southern EU	2011	0	18	Whole plant[3]	2.47-3.20	<0.01-0.01	2.48-3.21	<0.01-0.02	<0.01
		97-111	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		97-111	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte.
5) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.

III. CONCLUSION

Total imazamox residues in mature sunflower seeds ranged from 0.02-0.03 mg/kg and were below the LOQ of the analytical method (<0.02 mg/kg) in the EU North and EU South, respectively.

Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU North were <0.01 mg/kg, 0.01-0.02 mg/kg, <0.01 mg/kg and 0.02-0.03 mg/kg, respectively. Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU South were all below the LOQ of <0.01 mg/kg for each analyte.

The application of adjuvant DASH had no significant impact on residue levels in whole plant or edible commodities of sunflower.

Table 6.3.2-14: Residues of imazamox and its metabolites in sunflower after one application of BAS 720 06 H or a tank mix of BAS 720 06 H and BAS 160 00 S (DASH) in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	407229	Sun-flower	Germany	BAS 720 06 H: 1 x 0.05	18	0	W. plant[3]	1.74	<0.01	1.75	<0.01	<0.01
Doc ID:	2012/1084183					112	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L110316					112	Seed	<0.01	0.02	0.03	<0.01	0.03
GLP:	yes											
Year:	2011											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	1.60	<0.01	1.61	<0.01	<0.01
						112	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						112	Seed	<0.01	0.02	<u>0.03</u>	<0.01	0.03
Study code:	407229	Sun-flower	United Kingdom	BAS 720 06 H: 1 x 0.05	18	0	W. plant[3]	4.32	0.01	4.33	0.01	<0.01
Doc ID:	2012/1084183					117	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L110317					117	Seed	<0.01	0.01	0.02	<0.01	0.02
GLP:	yes											
Year:	2011											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	4.34	0.01	4.35	0.01	<0.01
						117	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						117	Seed	<0.01	0.02	<u>0.03</u>	<0.01	0.02

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) whole plant without roots
4) rest of plant without roots
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
_ underlined values were used for MRL calculation

Table 6.3.2-15: Residues of imazamox and its metabolites in sunflower after one application of BAS 720 06 H or a tank mix of BAS 720 06 H and BAS 160 00 S (DASH) in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	407229	Sun-flower	France	BAS 720 06 H: 1 x 0.05	18	0	W. plant[3]	2.78	<0.01	2.79	<0.01	<0.01
Doc ID:	2012/1084183					111	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L110318					111	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes											
Year:	2011											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	2.47	<0.01	2.48	<0.01	<0.01
						111	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						111	Seed	<0.01	<0.01	<u><0.02</u>	<0.01	<0.01
Study code:	407229	Sun-flower	Italy	BAS 720 06 H: 1 x 0.05	18	0	W. plant[3]	2.96	<0.01	2.97	<0.01	<0.01
Doc ID:	2012/1084183					117	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L110319					117	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes											
Year:	2011											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	3.20	0.01	3.21	0.02	<0.01
						117	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						117	Seed	<0.01	<0.01	<u><0.02</u>	<0.01	<0.01

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) whole plant without roots
4) rest of plant without roots
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL312622 (Reg. No. 4110542)
V) metabolite CL189215 (Reg. No. 4110445)
_ underlined values were used for MRL calculation

Report:	CA 6.3.2/5 Erdmann H.-P., 2013a Study on the residue behaviour of BAS 720 H (Imazamox) in sunflower after application of either BAS 720 AM H or BAS 720 06 H under field condition in Northern France, Italy, Spain and Germany, 2012 2013/1003728
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	Yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Report:	CA 6.3.2/6 Erdmann H.-P., 2014b Amendment No. 1: Study on the residue behaviour of BAS 720 H (Imazamox) in sunflower after application of either BAS 720 AM H or BAS 720 06 H under field condition in Northern France, Italy, Spain and Germany, 2012 2014/1158174
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Executive Summary

During the 2012 growing season 4 trials in sunflower were conducted in the EU, 2 in the EU North and 2 in the EU South, to determine the residue level of imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) in or on raw agricultural commodities (RAC) after the application of formulation BAS 720 AM H (25 g/L imazamox, SL) or after the application of BAS 720 06 H (40 g/L imazamox, SL) in a tank mix with BAS 160 00 S (Adjuvant DASH) on an additional plot. The treatments were applied once at a rate equivalent to 0.05 kg imazamox/ha, in spray volumes of 200 L/ha. The application was performed at growth stage BBCH 18. Specimens of whole plant without roots were collected at the day of the application. Seed and rest of plant without roots specimen were collected at plant maturity (BBCH 89), 107-121 DALA. The specimens were analysed for imazamox and its metabolites with BASF Method No L0188/01 which quantifies the relevant analytes with a limit of quantitation (LOQ) of 0.01 mg/kg. Total imazamox residues in mature sunflower seeds ranged from 0.02-0.08 mg/kg and from <0.02-0.03 mg/kg in the EU North and EU South, respectively. Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU North were <0.01 mg/kg, 0.01-0.07 mg/kg, <0.01 mg/kg and 0.05-0.13 mg/kg, respectively. Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU South were <0.01 mg/kg, <0.01-0.02 mg/kg, <0.01mg/kg and <0.01-0.05 mg/kg, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 720 06 H (SL), BAS 160 00 S (EC) BAS 720 AM H (SL)
Lot/Batch #:	0005014720, 40 g/L imazamox, nominal, 70161875L0, BAS 16000S (adjuvant DASH) 403050, 25 g/L imazamox, nominal
Purity:	Not reported
CAS#:	114311-32-9, imazamox
Development code:	Not reported
Spiking levels:	Seed: 0.01, 0.1, 1.0 mg/kg Whole plant w/o roots: 0.01, 0.1, 1.0, 10 mg/kg Rest of plant w/o roots: 0.01, 0.1, 1.0 mg/kg

2. Test Commodity:

Crop:	Sunflower
Type:	Oilseeds
Variety:	Paraiso 1001 (IMI tolerant variety)
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed commodity:	Whole plant without roots, seed
Sample size:	0.1-1.0 kg (min. 12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2012 growing season 4 trials in sunflower were conducted in the EU, 2 in the EU North (Germany, France) and 2 in the EU South (Italy, France), to determine the residue level of imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) in or on raw agricultural commodities (RAC) after the application of formulation BAS 720 AM H (25 g/L imazamox, SL) or after the application of BAS 720 06 H (40 g/L imazamox, SL) in a tank mix with BAS 160 00 S (Adjuvant DASH) on an additional plot. The formulation BAS 720 AM H is identical with BAS 720 10 H and is a newly developed formulation with an in-built adjuvant (Klearfac AA-270). The treatments were applied once at a rate equivalent to 0.05 kg imazamox/ha in spray volumes of 200 L/ha. The application was performed at growth stage BBCH 18. Specimens of whole plant without roots were collected at the day of the application. Seed and rest of plant without roots specimen were collected at plant maturity (BBCH 89) 107-121 DALA. Samples were stored frozen at or below -18°C for a maximum of 242 days until analysis. Data of all trials are reported here, however, for later MRL derivation only the highest residue of each set of comparative trials is relevant.

Table 6.3.2-16: Target application rates and timings for sunflower

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2012	4	1	F	BAS 720 AM H (SL)	BAS 720 H	0.05	200	BBCH 18
2012	4	1	F	BAS 720 06 H (SL) +BAS 160 00 S (EC)	BAS 720 H Adjuvant DASH	0.05	200	BBCH 18

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with BASF Method No. L0188/01 quantifying each analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues are extracted from sunflower matrices using an acidic methanol-water solution. A portion of the extract was centrifuged, filtered through a folded filter and an aliquot of the filtrate was diluted tor determination by HPLC-MS/MS.

Table 6.3.2-17: Summary of recoveries of imazamox and its metabolites in sunflower

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No. L0188/01; LOQ=0.01 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant without roots	0.01/0.1/1/10*	6	99	5.8	3	94	7.4
Rest plant without roots	0.01/0.1/1	3	106	7.2	3	101	0.9
Seed	0.01/0.1/1	3	114	4.3	3	108	6
Overall	0.01/0.1/1/10	12	104	7.6	9	101	7.7
BASF Method No. L0188/01; LOQ=0.01 mg/kg		**Metabolite CL 312622**			**Metabolite CL 189215**		
Whole plant without roots	0.01,0.1,10	3	97	4.0	3	103	5.4
Rest plant without roots	0.01,0.1,10	3	101	6.4	3	93	7.4
Seed	0.01,0.1,10	3	102	6.7	3	107	1.9
Overall	0.01,0.1,10	9	100	5.6	9	101	7.8

* imazamox only

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-18; detailed residue levels are shown in Table 6.3.2-19 and Table 6.3.2-20.

After treatment with BAS 720 AM H or with BAS 720 06 H in a tank mix with BAS 160 00 S (adjuvant DASH) in the EU North no residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolite CL 312622 were found in sunflower seed specimens at harvest (BBCH 89). Residues ranged from <0.01-0.07 mg/kg and from 0.05-0.13 mg/kg for metabolites CL 263284 and CL 189215, respectively. Total imazamox residues were 0.02-0.08 mg/kg. Whole plant specimen sampled at the day of the application contained 2.4 - 3.3 mg/kg, <0.01-0.01 mg/kg, <0.01-0.02 mg/kg and <0.01 mg/kg of imazamox, CL 263284, CL 312622 and CL 189215, respectively. Total imazamox residues were 2.41-3.31 mg/kg. Rest of plant specimen sampled at plant maturity (118-121 DALA) contained no residues at or above the LOQ of 0.01 mg/kg. Total imazamox residues were below the LOQ of the analytical method of <0.02 mg/kg.

After treatment with BAS 720 AM H or with BAS 720 06 H in a tank mix with BAS 160 00 S (adjuvant DASH) in the EU South no residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolites were found in sunflower seed specimens at harvest (BBCH 89). For its metabolites CL 263284 and CL 189215 residues of <0.01-0.02 mg/kg, and <0.01-0.05 mg/kg were found. Total imazamox residues ranged between <0.02 and 0.03 mg/kg. Whole plant specimens sampled at the day of the application contained 2.70-6.60 mg/kg, <0.01-0.01 mg/kg, <0.01-0.02 mg/kg and <0.01 mg/kg of imazamox, CL 263284, CL 312622 and CL 189215, respectively. Total imazamox residues were 2.71-6.61 mg/kg. Rest of plant specimen sampled at plant maturity (107-114 DALA) contained no residues at or above the LOQ of 0.01 mg/kg for imazamox or its metabolites CL 263284, CL 312622 and CL 189215. Total imazamox residues were below the LOQ of the analytical method of <0.02 mg/kg.

No residues of imazamox and its metabolites were found in any of the control samples.

Table 6.3.2-18: Summary of residues in sunflower treated with BAS 720 AM H or BAS 720 06 H and BAS 160 00 S (Adjuvant DASH) in a tank mix

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	Imazamox (BAS 720 H)	CL 263284[5]	Total imazamox[4]	CL 312622	CL 189215
Plot with BAS 720 AM H (in-built adjuvant)									
Northern EU	2012	0	18	Whole plant[3]	2.40-3.30	<0.01-0.01	2.41-3.31	<0.01-0.01	<0.01
		118-121	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		118-121	89	Seed	<0.01	0.02-0.07	0.03-0.08	<0.01	0.07-0.12
Southern EU	2012	0	18	Whole plant[3]	4.80-6.60	<0.01-0.01	4.81-6.61	0.01-0.02	<0.01
		107-114	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		107-114	89	Seed	<0.01	<0.01-0.02	<0.02-0.03	<0.01	<0.01-0.05
Plot with BAS 720 06 H + DASH									
Northern EU	2012	0	18	Whole plant[3]	2.40-2.60	<0.01	2.41-2.61	<0.01-0.02	<0.01
		118-121	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		118-121	89	Seed	<0.01	0.01-0.07	0.02-0.08	<0.01	0.05-0.13
Southern EU	2012	0	18	Whole plant[3]	2.70-2.90	<0.01	2.71-2.91	<0.01	<0.01
		107-114	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		107-114	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01-0.02

1) days after last application
2) at harvest
3) without roots
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte.
5) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.

III. CONCLUSION

Total imazamox residues in mature sunflower seeds ranged from 0.02-0.08 mg/kg and from <0.02-0.03 mg/kg in the EU North and South, respectively.

Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU North were <0.01 mg/kg, 0.01-0.07 mg/kg, <0.01 mg/kg and 0.05-0.13 mg/kg, respectively. Residues of imazamox (BAS 720 H) and its metabolites CL 263284, CL 312622 and CL 189215 in the EU South were <0.01 mg/kg, <0.01-0.02 mg/kg, <0.01 mg/kg and <0.01-0.05 mg/kg, respectively. Comparing both formulations, residue levels of imazamox in whole plant at 0 DALA had a slight trend to be higher for the formulation BAS 720 AM H, but in edible matrices (seed) residue levels of imazamox and metabolites were similar for both formulations. Thus, the type of adjuvant had no influence on the residue levels in edible commodities of sunflower. .

Table 6.3.2-19: Residues of imazamox and its metabolites in sunflower after one application of BAS 720 AM H or a tank mix of BAS 720 06 H and BAS 160 00 S (DASH) in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BB CH)	DA-LA[1]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	417820	Sun-flower	Germany	BAS 720 AM H: 1 x 0.05	18	0	W. plant[3]	2.40	<0.01	2.41	<0.01	<0.01
Doc ID:	2013/1003728					118	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L120330					118	Seed	<0.01	0.07	0.08	<0.01	0.12
GLP:	yes											
Year:	2012											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	2.60	<0.01	2.61	<0.01	<0.01
						118	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						118	Seed	<0.01	0.07	0.08	<0.01	0.13
Study code:	417820	Sun-flower	France	BAS 720 AM H: 1 x 0.05	18	0	W. plant[3]	3.30	0.01	3.31	0.01	<0.01
Doc ID:	2013/1003728					121	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L120331					121	Seed	<0.01	0.02	0.03	<0.01	0.07
GLP:	yes											
Year:	2012											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	2.40	<0.01	2.41	0.02	<0.01
						121	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						121	Seed	<0.01	0.01	0.02	<0.01	0.05

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) whole plant without roots
4) rest of plant without roots
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
_ underlined values were used for MRL calculation

Table 6.3.2-20: Residues of imazamox and its metabolites in sunflower after one application of BAS 720 AM H or a tank mix of BAS 720 06 H and BAS 160 00 S (DASH) in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BB CH)	DA-LA[1]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	417820	Sun-flower	Spain	BAS 720 AM H: 1 x 0.05	18	0	W. plant[3]	6.60	<0.01	6.61	0.01	<0.01
Doc ID:	2013/1003728					107	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L120332					107	Seed	<0.01	0.02	0.03	<0.01	0.05
GLP:	yes											
Year:	2012											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	2.70	<0.01	2.71	<0.01	<0.01
						107	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						107	Seed	<0.01	<0.01	<0.02	<0.01	0.02
Study code:	417820	Sun-flower	Italy	BAS 720 AM H: 1 x 0.05	18	0	W. plant[3]	4.80	0.01	4.81	0.02	<0.01
Doc ID:	2013/1003728					114	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L120333					114	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes											
Year:	2012											
				BAS 720 06 H: 1 x 0.05 BAS 160 00 S: in tank mix	18	0	W. plant[3]	2.90	<0.01	2.91	<0.01	<0.01
						114	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
						114	Seed	<0.01	<0.01	<0.02	<0.01	<0.01

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) whole plant without roots
4) rest of plant without roots
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
_ underlined values were used for MRL calculation

Report:	CA 6.3.2/7 Erdmann H.P., 2014a Study on the residue behaviour of BAS 720 H (Imazamox) in sunflower after application of BAS 720 BD H under field condition in Germany, The Netherlands, Northern and Southern France, Belgium, Greece, Italy and Spain, 2013 2013/1405201
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Report:	CA 6.3.2/8 Erdmann H.-P., 2014a Study on the residue behaviour of BAS 720 H (Imazamox) in sunflower after application of BAS 720 BD H under field condition in Germany, The Netherlands, Northern and Southern France, Belgium, Greece, Italy and Spain, 2013 2014/1162727
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	BAS 720 BD H (SL)
Lot/Batch #:	403173, 25 g/L imazamox, nominal,
Purity:	Not reported
CAS#:	114311-32-9, imazamox
Development code:	Not reported
Spiking levels:	0.01-10 mg/kg

2. **Test Commodity:**

Crop:	Sunflower
Type:	Oilseeds
Variety:	Paraiso 1000, Lot 958 (IMI tolerant variety)
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed commodity:	Whole plant without roots, rest of plant without roots, seed
Sample size:	0.1-1.0 kg (min. 12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2013 growing season a total of 8 trials in sunflower were conducted in the EU, 4 in the EU North (Germany, The Netherlands, France and Belgium) and 2 in the EU South (France, Greece, Italy and Spain), to determine the residue level of imazamox and its metabolites CL 263284 (Reg. No 4110773), CL 312622 (Reg. No 4110542) and CL 189215 (Reg. No 4110445) in or on raw agricultural commodities (RAC) after the application of formulation BAS 720 BD H (25 g/L imazamox, SL). The treatments were applied once at a rate equivalent to 0.05 kg imazamox/ha in spray volumes of 200 L/ha. The application was performed at growth stage BBCH 18. Specimens of whole plant without roots were collected at the day of application. Seed and rest of plant without roots specimens were collected at plant maturity (BBCH 89). The maximum storage interval from harvest until analysis was 208 days.

Table 6.3/1 Target application rates and timings for sunflower

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2013	8	1	F	BAS 720 BD H (SL)	BAS 720 H	0.05	200	BBCH 18

2. Description of analytical procedures

The specimens were analyzed for imazamox and its metabolites CL 263284 (Reg. No 4110773), CL 312622 (Reg. No 4110542) and CL 189215 (Reg. No 4110445) with BASF method No L0188/01 quantifying each analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues were extracted from sunflower matrices using an acidic methanol-water solution. A portion of the extract was centrifuged, filtered through a disposable syringe filter and an aliquot of the filtrate was diluted for determination by HPLC-MS/MS.

Table 6.3/2 Summary of recoveries of imazamox and its metabolites in sunflower

Matrix	Fortification level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0188/01; LOQ=0.01 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant without roots	0.01/0.1/1/10	4	96.0	5.6	4	88.1	18
Seed	0.01/0.1/1/10	4	93.5	6.2	4	95.8	5.6
Rest plant without roots	0.01/0.1/1/10	4	107	5.4	4	101	3.2
Overall	0.01/0.1/1/10	12	98.7	7.9	12	95.1	11
BASF method No L0188/01; LOQ=0.01 mg/kg		**Metabolite CL 312622**			**Metabolite CL 189215**		
Whole plant without roots	0.01/0.1/1/10	4	90.9	7.5	4	99.3	12
Seed	0.01/0.1/1/10	4	90.5	4.8	4	99.6	4.6
Rest plant without roots	0.01/0.1/1/10	4	102	6.0	4	97.7	7.6
Overall	0.01/0.1/1/10	12	94.5	8.2	12	98.8	7.7

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/3; detailed residue levels are shown in Table 6.3/4 and Table 6.3/5.

After treatment with BAS 720 BD H in the EU North no residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolites CL 312622 were found in sunflower seed specimens at harvest (BBCH 89). Residues ranged from 0.012-0.038 mg/kg and from 0.011-0.046 mg/kg for metabolites CL 263284 and CL 189215, respectively. Total imazamox residues were 0.022-0.048 mg/kg. Whole plant specimens sampled at the day of the application contained 2.4 -3.5 mg/kg, <0.01-0.01 mg/kg, <0.01 mg/kg and <0.01 mg/kg of imazamox, CL 263284, CL 312622 and CL 189215, respectively. Total imazamox residues were 2.4-3.5 mg/kg. Rest of plant specimens sampled at plant maturity (90-124 DALA) contained no residues at or above the LOQ of 0.01 mg/kg. Total imazamox residues were below the LOQ of the analytical method of <0.02 mg/kg.

After treatment with BAS 720 BD H in the EU South no residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolites were found in sunflower seed specimens at harvest (BBCH 89). For its metabolite CL 189215 residues of <0.01-0.019 mg/kg were found. Total imazamox residues were below the LOQ (<0.02 mg/kg). Whole plant specimens sampled at the day of the application contained 1.7-3.7 mg/kg, <0.01-0.012 mg/kg, <0.01 mg/kg and <0.01 mg/kg of imazamox, CL 263284, CL 312622 and CL 189215, respectively. Total imazamox residues were 1.7-3.7 mg/kg. Rest of plant specimens sampled at plant maturity (86-118 DALA) contained no residues at or above the LOQ of 0.01 mg/kg for imazamox or its metabolites. Total imazamox residues were below the LOQ of the analytical method of <0.02 mg/kg.

No residues of imazamox and its metabolites were found in any of the control samples.

Table 6.3/3 Summary of residues in sunflower treated with BAS 720 BD H

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)					
				Matrix	Imazamox (BAS 720 H)	CL 263284[5]	Total imazamox[4]	CL 312622[6]	CL 189215[7]
Northern EU	2013	0	18	Whole plant[3]	2.4-3.5	<0.01-0.01	2.4-3.5	<0.01	<0.01
		90-124	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		90-124	89	Seed	<0.01	0.012-0.038	0.022-0.048	<0.01	0.011-0.046
Southern EU		0	18	Whole plant[3]	1.7-3.7	<0.01-0.012	1.7-3.7	<0.01-0.011	<0.01
		86-118	89	Rest plant[3]	<0.01	<0.01	<0.02	<0.01	<0.01
		86-118	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01-0.019

1 Days after last application
2 At harvest
3 Without roots
4 Total imazamox = residues parent (BAS 720 H) + (residues CL 263284 expressed as parent equivalents). When calculating total imazamox residues, for values below the LOQ (<0.01) 0.01 mg/kg were used for each analyte.
5 Expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.048.
6 Expressed as parent equivalents. The conversion factor from CL 312622 to BAS 720 H is 1.00011.
7 Expressed as parent equivalents. The conversion factor from CL 189215 to BAS 720 H is 0.673359.

III. CONCLUSION

Total imazamox residues in mature sunflower seeds ranged from 0.022-0.048 mg/kg and were below the LOQ (<0.02 mg/kg) in the EU North and South, respectively.

Table 6.3/4 Residues of imazamox and its metabolites in sunflower after one application of BAS 720 BD H in Northern Europe

Trial details		Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	417821	Sunflower	Germany	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	3.0	<0.01	3.0	<0.01	<0.01
DocID:	2013/1405201					124	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130478					124	Seed	<0.01	0.029	0.038	<0.01	0.034
GLP:	Yes											
Year:	2013											
Study code:	417821	Sunflower	The Netherlands	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	2.4	0.010	2.4	<0.01	<0.01
DocID:	2013/1405201					93	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130479					93	Seed	<0.01	0.038	0.048	<0.01	0.046
GLP:	Yes											
Year:	2013											
Study code:	417821	Sunflower	France	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	2.4	<0.01	2.4	<0.01	<0.01
DocID:	2013/1405201					104	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130480					104	Seed	<0.01	0.012	0.022	<0.01	0.024
GLP:	Yes											
Year:	2013											
Study code:	417821	Sunflower	Belgium	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	3.5	<0.01	3.5	<0.01	<0.01
DocID:	2013/1405201					90	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130481					90	Seed	<0.01	0.022	0.032	<0.01	0.011
GLP:	Yes											
Year:	2013											

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Whole plant without roots
4 Rest of plant without roots
I Imazamox
II Metabolite CL 263284, expressed as parent equivalent (conversion factor 1.048)
III Total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
IV Metabolite CL 312622 (Reg. No. 4110542), expressed as parent equivalent (conversion factor 1.00011)
V Metabolite CL 189215 (Reg. No. 4110445), expressed as parent equivalent (conversion factor 0.673359)
_ Underlined values were used for MRL calculation

Table 6.3/5 Residues of imazamox and its metabolites in sunflower after one application of BAS 720 BD H in Southern Europe

Trial details		Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	417821	Sunflower	France	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	3.7	<0.01	3.7	<0.01	<0.01
DocID:	2013/1405201					104	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130482					104	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	Yes											
Year:	2013											
Study code:	417821	Sunflower	Greece	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	3.4	0.012	3.4	<0.01	<0.01
DocID:	2013/1405201					86	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130483					86	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	Yes											
Year:	2013											
Study code:	417821	Sunflower	Italy	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	3.6	<0.01	3.6	<0.01	<0.01
DocID:	2013/1405201					118	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130484					118	Seed	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	Yes											
Year:	2013											
Study code:	417821	Sunflower	Spain	BAS 720 BD H: 1 x 0.05	18	0	W. plant[3]	1.7	<0.01	1.7	0.011	<0.01
DocID:	2013/1405201					104	R. plant[4]	<0.01	<0.01	<0.02	<0.01	<0.01
Trial No:	L130485					104	Seed	<0.01	<0.01	<0.02	<0.01	0.019
GLP:	Yes											
Year:	2013											

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Whole plant without roots
4 Rest of plant without roots
I Imazamox
II Metabolite CL 263284, expressed as parent equivalent (conversion factor 1.048)
III Total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
IV Metabolite CL 312622 (Reg. No. 4110542), expressed as parent equivalent (conversion factor 1.00011)
V Metabolite CL 189215 (Reg. No. 4110445), expressed as parent equivalent (conversion factor 0.673359)
_ Underlined values were used for MRL calculation

CA 6.3.3 Soya bean

Table 6.3.3-1: Critical GAP for the use of BAS 720 H in/on soya bean

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Soya bean	1 x 0.05 kg BAS 720 H/ha	100 L/ha	n.s.	spray application	BBCH 12-14

PHI = pre-harvest interval

This use pattern is registered with the formulation Pulsar (BAS 720 02/06 H, 40 g/L SL formulation) and residue data have been generated with a 120 g/L or 40g/L solo formulation. However, those trials were analyzed only for parent imazamox, which is not in line with the proposed residue definition, thus they were not included in this submission. Those trials show a no residue situation for imazamox in soya bean seeds with an LOQ of 0.05 mg/kg. In support of the proposed residue definition the following residue trials are included which were conducted with a mix formulation of imazamox and bentazone (Corum, BAS 762 01 H, SL formulation). The formulation type is the same as with Pulsar and the application rate of 42 g/ha is covering the intended Pulsar application rate of 50 g/ha. Since the application in these trials was carried out a bit later (up to BBCH 25), they represent a worst case.

Table 6.3.3-2: GAP information of residue trials conducted in soya bean between 2007 and 2013

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Northern EU	France (1 trial)	BAS 762 01 H (+BAS 9047 0S)[2]	spray application	0.042	0.021	1	90
	Germany (2 trials)	BAS 762 01 H	spray application	0.042	0.021	1	0 27 78-98
	France (2 trials)	BAS 762 01 H	spray application	0.042	0.021	1	0 28 69-104
Southern EU	France (2 trials)	BAS 762 01 H (+BAS 9047 0S)[2]	spray application	0.042	0.021	1	90-104
	Italy (2 trials)	BAS 762 01 H (+BAS 9047 0S)[2]	spray application	0.042	0.021	1	85-88

0) actual application rates varied by 10% at most
1) days after last application
2) replicate treatment with DASH (BAS 9047 0S) as a tank mix

Report:	CA 6.3.3/1 Kreke N., 2009a Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with a tankmix of BAS 762 01 H (22.4/480 g/L) and BAS 9047 0S (DASH HC) from one open field trial in Northern France in 2008 2008/1034457
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.3/2 Kreke N., 2010c Amendment: BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with the tankmix from one open field trial in Northern France, 2008 2010/1155811
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.3/3 Kreke N., 2009b BAS 762 01 H: Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L Imazamox/Bentazone) from one open field trial in Northern France in 2008 2008/1034456
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.3/4 Kreke N., 2010d First amendment: Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L Imazamox/Bentazone) from one open field in Northern France, 2008 2010/1155810
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2008 growing season 1 field trial was conducted in the Northern EU to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC) of soya bean. BAS 762 01 H, an SL formulation of bentazone and imazamox, was applied once at a rate equivalent to 0.042 kg a.s./ha of imazamox in a spray volume of 205 L/ha. Data on bentazone are not reported. A replicate trial was treated with BAS 762 01 H and adjuvant DASH HC (BAS 9047 0S) in a tank mixture. The application was performed at growth stage BBCH 13. Seeds were collected at harvest 90 days (BBCH 89) after application.
The specimens were analysed for imazamox and its metabolite CL 263284 with Method M 3178 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues of imazamox and metabolite CL 263284 in mature seed at growth stage BBCH 89 were <0.01 mg/kg with or without the use of adjuvant DASH, total imazamox residues were <0.02 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SC) + DASH (BAS 9047 0S)
Lot/Batch #:	402003 (22.4 g/L imazamox, nominal) 1071 (BAS 9047 0S (DASH HC))
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	Seed: 0.01-0.1 mg/kg

2. Test Commodity:

Crop:	Soya bean
Type:	Oilseeds
Variety:	Esor
Botanical name:	*Glycine max*
Crop parts(s) or processed Commodity:	Seed
Sample size:	>1 kg

B. STUDY DESIGN

1. Test procedure

During the 2008 growing season 1 trial in soya bean was conducted in Northern France, to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC). BAS 762 01 H (22.4 g/L imazamox, SL) was applied once at a rate equivalent to 0.042 kg imazamox/ha in a spray volume of 200 L/ha. One replicate trial was treated combined with adjuvant BAS 9047 0S. The application was performed at growth stage BBCH 13. Seeds were collected at harvest 90 days (BBCH 89) after application. Samples were stored frozen at or below -18°C for a maximum of two months until analysis.

Table 6.3.3-3: Target application rates and timings for soya bean

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2008	1	1	F	BAS 762 01 H (SL)	BAS 720 H	0.042	100-400	BBCH 12-25
2008	1	1	F	BAS 762 01 H (SL) (+BAS 9047 0S)	BAS 720 H Adjuvant DASH	0.042	100-400	BBCH 12-25

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues are extracted from soya bean matrices using an acidified methanol-water solution. An aliquot of the sample extract is centrifuged and the supernatant was transferred and evaporated. The residue was re-dissolved in methanol-water for analysis. Measurement of the residues is accomplished by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.3-4: Summary of procedural recovery data for imazamox

Matrix	Fortification Level (mg/kg)	Summary Recoveries		
		n	Mean (%)	RSD (%)
Method: M 3178; LOQ = 0.01 mg/kg		**Imazamox (BAS 720 H)**		
Seed	0.01-0.10	4	98	2
Method: M 3178; LOQ = 0.01 mg/kg		**CL 263284 (Reg. No. 4110773)**		
Seed	0.01-0.10	4	97	7

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-5, detailed residue levels are shown in Table 6.3.3-6.
No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolite CL 263284 were found in seed samples at harvest (BBCH 89), 90 days after the application with or without the use of adjuvant DASH. Total imazamox for seeds was <0.02 mg/kg. No residues have been found in any control sample.

Table 6.3.3-5: Summary of residues in soya bean treated with BAS 762 01 H (with and without DASH)

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284[3]	Total imazamox[4]
With Adjuvant DASH							
Northern EU	2008	90	89	Seed	<0.01	<0.01	<0.02
Without Adjuvant DASH							
Northern EU	2008	90	89	Seed	<0.01	<0.01	<0.02

1) days after last application
2) at harvest
3) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048)

III. CONCLUSION

Residues of imazamox and metabolite CL 263284 in mature seed at BBCH 89 were <0.01 mg/kg with or without the use of adjuvant DASH, total imazamox residues were <0.02 mg/kg. The application of adjuvant DASH had no impact on residue levels in edible commodities of soybean.

Table 6.3.3-6: Residues of imazamox in soya bean after one application of BAS 762 01 H or a tank mix of BAS 762 01 H and BAS 9047 0S (DASH HC) in Northern Europe

Trial Details		Crop	Country	Formulation, Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	B89774	Soya bean	France	BAS 762 01 H: 1 x 0.042	13	90	Seed	<0.01	<0.01	<0.02
Doc ID:	2008/1034456									
Trial No.:	A/NF/H/08/48									
GLP:	yes									
Year:	2008									
Study code:	B89785	Soya bean	France	BAS 762 01 H: 1 x 0.042 + 0.5 % BAS 9047 0S	13	90	Seed	<0.01	<0.01	<u>≤0.02</u>
Doc ID:	2008/1034457[3]									
Trial No.:	A/NF/H/08/49									
GLP:	yes									
Year:	2008									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) though different study and trial number, the trial site is identical with the trial site above and is therefore regarded as replicate
I imazamox
II metabolite CL 263284 (Reg. No. 4110773), expressed as parent equivalent (conversion factor 1.048)
III total imazamox =residues parent (BAS 720 H) + residues CL 263284 expressed as parent equivalents. When calculating total imazamox residues, residue values below the LOQ (<0.01) were set at LOQ (0.01 mg/kg)
_underlined values were used for MRL calculation

Report:	CA 6.3.3/5 Kreke N., 2008a BAS 762 01 H - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L Imazamox/Bentazone) from four open field trials in Italy and Southern France, 2007 2007/1023134
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.3/6 Kreke N., 2010e First amendment: BAS 762 01 H - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L) from four open field trials in Italy and Southern France in 2007 2010/1155806
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.3/7 Kreke N., 2008b BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with this tankmix from four open field trials, Italy and Southern France, 2007 2007/1028359
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.3/8 Kreke N., 2010f First amendment: BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with this tankmix from four open field trials, Italy and Southern France, 2007 2010/1155807
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2007 growing season 4 field trials were conducted in the Southern EU (France, Italy) to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC) of soya bean. BAS 762 01 H, an SL formulation of bentazone and imazamox, was applied once at a rate equivalent to 0.042 kg a.s./ha of imazamox in a spray volume of 200 L/ha in France and 400 L/ha in Italy. Data on bentazone are not reported. A replicate from every trial was treated with BAS 762 01 H and adjuvant DASH HC (BAS 9047 0S) in a tank mixture. The application was performed at growth stage BBCH 13-24. Seeds were collected at harvest 85-104 days (BBCH 89) after application.
The specimens were analysed for imazamox and its metabolite CL 263284 with Method M 3178 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues of imazamox and metabolite CL 263284 in mature seed at growth stage BBCH 89 were <0.01 mg/kg with or without the use of adjuvant DASH, total imazamox was <0.02 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL) + DASH (BAS 9047 0S)
Lot/Batch #:	402009 (22.4 g/L imazamox, nominal) 1070 (BAS 9047 0S (DASH HC))
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	Seed: 0.01-0.1 mg/kg

2. Test Commodity:

Crop:	Soya bean
Type:	Oilseeds
Variety:	Quito, Deka big, M10 (Pioneer), B92 (Pioneer)
Botanical name:	*Glycine max*
Crop parts(s) or processed Commodity:	Seed
Sample size:	>1 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season 4 trials in soya bean were conducted in different representative growing areas in the Southern EU (France, Italy) to determine the residue level of imazamox and its metabolite CL 263284 in or on raw agricultural commodities (RAC). BAS 762 01 H (22.4 g/L imazamox, SL) was applied once at a rate equivalent to 0.042 kg imazamox/ha in a spray volume of 200-400 L/ha. One replicate of each trial was treated combined with adjuvant BAS 9047 0S. The application was performed at growth stage BBCH 13-24. Although the intended growth stage for application in soya bean is BBCH 12-14 the trials with application time BBCH 23-25 are considered valid worst case since they showed residues below LOQ. Seeds were collected at harvest 85-104 days (BBCH 89) after application. Samples were stored frozen at or below -18°C for a maximum of 3 months until analysis.

Table 6.3.3-7: Target application rates and timings for soya bean

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	4	1	F	BAS 762 01 H (SL)	BAS 720 H	0.042	100-400	BBCH 12-25
2007	4	1	F	BAS 762 01 H (SL) +BAS 9047 0S	BAS 720 H	0.042	100-400	BBCH 12-25

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues are extracted from soya bean matrices using an acidified methanol-water solution. An aliquot of the sample extract is centrifuged and the supernatant was transferred and evaporated. The residue was re-dissolved in methanol-water for analysis. Measurement of the residues is accomplished by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.3-8: Summary of procedural recovery data for imazamox

Matrix	Fortification Level (mg/kg)	Summary Recoveries		
		n	Mean (%)	RSD (%)
Method: M 3178; LOQ = 0.01 mg/kg		**Imazamox (BAS 720 H)**		
Seed	0.01-0.10	4	98	2
Method: M 3178; LOQ = 0.01 mg/kg		**CL 263284 (Reg. No. 4110773)**		
Seed	0.01-0.10	4	97	7

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-9, detailed residue levels are shown in Table 6.3.3-10.
No residues at or above the LOQ of the analytical method (0.01 mg/kg) of imazamox or its metabolite CL 263284 were found in seed samples at harvest (BBCH 89), 85-104 days after the application with or without adjuvant DASH. Total imazamox residues in seeds were <0.02 mg/kg.

No residues have been found in any of the control samples.

Table 6.3.3-9: Summary of residues in soya bean treated with BAS 762 01 H (with and without DASH HC)

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284[3]	Total imazamox[4]
With Adjuvant DASH							
Southern EU	2007	85-104	89	Seed	<0.01	<0.01	<0.02
Without Adjuvant DASH							
Southern EU	2007	85-104	89	Seed	<0.01	<0.01	<0.02

1) days after last application
2) at harvest
3) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048)

III. CONCLUSION

Residues of imazamox and metabolite CL 263284 in mature seed at growth stage BBCH 89 were <0.01 mg/kg with or without the use of adjuvant DASH, total imazamox residues were <0.02 mg/kg.

The application of adjuvant DASH had no impact on residue levels in edible commodities of soybean.

Table 6.3.3-10: Residues of imazamox in soya bean after one application of BAS 762 01 H and a tank mix of BAS 762 01 H and BAS 9047 0S (DASH HC) in Southern Europe

Trial Details		Crop	Country	Formulation, Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B42941 2007/1023134 A/SF/H/07/142 yes 2007	Soya bean	France	BAS 762 01 H: 1 x 0.042	13	90	Seed	<0.01	<0.01	<0.02
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B44111 2007/1028359 A/SF/H/07/165[3] yes 2007	Soya bean	France	BAS 762 01 H: 1 x 0.042 + 0.5 % BAS 9047 0S	13	90	Seed	<0.01	<0.01	<u><0.02</u>
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B42941 2007/1023134 A/SF/H/07/143 yes 2007	Soya bean	France	BAS 762 01 H: 1 x 0.042	13	104	Seed	<0.01	<0.01	<0.02
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B44111 2007/1028359 A/SF/H/07/166[3] yes 2007	Soya bean	France	BAS 762 01 H: 1 x 0.042 + 0.5 % BAS 9047 0S	13	104	Seed	<0.01	<0.01	<u><0.02</u>
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B42941 2007/1023134 A/SF/H/07/144 yes 2007	Soya bean	Italy	BAS 762 01 H: 1 x 0.042	23-24	88	Seed	<0.01	<0.01	<0.02
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B44111 2007/1028359 A/SF/H/07/167[3] yes 2007	Soya bean	Italy	BAS 762 01 H: 1 x 0.042 + 0.5 % BAS 9047 0S	23-24	88	Seed	<0.01	<0.01	<u><0.02</u>
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B42941 2007/1023134 A/SF/H/07/145 yes 2007	Soya bean	Italy	BAS 762 01 H: 1 x 0.042	24	85	Seed	<0.01	<0.01	<0.02
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	B44111 2007/1028359 A/SF/H/07/168[3] yes 2007	Soya bean	Italy	BAS 762 01 H: 1 x 0.042 + 0.5 % BAS 9047 0S	24	85	Seed	<0.01	<0.01	<u><0.02</u>

0) actual application rates varied by 10% at most
1) days after last application
2) at last treatment
3) though different trial number, the trial site is identical with the trial site above and is therefore regarded as replicate
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.048)
III total imazamox = residues parent (BAS 720 H) + residues CL 263284 expressed as parent equivalents. When calculating total imazamox residues, residue values below the LOQ (<0.01) were set at LOQ (0.01 mg/kg)
_ underlined values were used for MRL calculation

Report:	CA 6.3.3/9 Erdmann H.-P., 2012a Study on the residue behaviour of BAS 720 H (Imazamox) and BAS 351 H (Bentazone) in soybeans after application of BAS 762 01 H under field condition in Northern France and Germany, 2011 2011/1112578
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	Yes (certified by Land Brandenburg Ministerium fuer Laendliche Entwicklung, Umwelt und Verbraucherschutz, Potsdam, Germany)

Executive Summary

During the 2011 growing season 4 field trials were conducted in the Northern EU to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on raw agricultural commodities (RAC) of soya bean. BAS 762 01 H, an SL formulation of bentazone and imazamox, was applied once at a rate equivalent to 0.042 kg a.s./ha of imazamox in a spray volume of 200 L/ha. Data on bentazone are not reported. The application was performed at growth stage BBCH 25, except in trials L110242 and L110244 where the application was performed at BBCH 21-51 and BBCH 69, respectively. Soya bean specimens were collected immediately after application, 27-28 days after the application and at harvest 69-104 days after application (BBCH 89). Seeds were collected at harvest 69-104 days (BBCH 89) after application. The specimens were analysed for imazamox and its metabolite CL 263284 with BASF Method L0188/01, which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues of imazamox and of all metabolites were <0.01 mg/kg in mature seed (BBCH 89), except for residues of CL 189215 which were 0.04 mg/kg in one trial. Total imazamox residues were <0.02 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SC)
Lot/Batch #:	402025 (22.4 g/L imazamox, nominal)
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	Seed: 0.01-0.1 mg/kg Plant: 0.01-4.0 mg/kg

2. Test Commodity:

Crop:	Soya bean
Type:	Oilseeds
Variety:	Merlin, Sigalia, Prolina
Botanical name:	*Glycine max*
Crop parts(s) or processed Commodity:	Seed, whole plant, rest of plant
Sample size:	>1 kg

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season 4 trials in soya bean were conducted in different representative growing areas in Northern Europe (France and Germany), to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on raw agricultural commodities (RAC). BAS 762 01 H (22.4 g/L imazamox, SL) was applied once at a rate equivalent to 0.042 kg imazamox/ha in a spray volume of 200 L/ha. The application was performed at growth stage BBCH 21-69. Although the intended growth stage for application in soya bean is BBCH 12-14 trials with later application times are considered valid worst case since they showed residues below LOQ. Soya bean specimens were collected immediately after application, 27-28 days after the application and at harvest 69-104 days after application (BBCH 89). Seeds were collected at harvest 69-104 days (BBCH 89) after application. Samples were stored frozen at or below -18°C for a maximum of 323 days until analysis.

Table 6.3.3-11: Target application rates and timings for soya bean

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2011	4	1	F	BAS 762 01 H (SL)	BAS 720 H	0.042	200	BBCH 25

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with BASF Method No L0188/01 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues were extracted from soya bean matrices using an methanol/water/1N hydrochloric acid solution (1200/780/20; v/v/v). An aliquot of the sample extract was centrifuged, filtrated and diluted in acetic water followed by liquid chromatography with mass selective detection (LC-MS/MS).

Table 6.3.3-12: Summary of procedural recovery data for imazamox and its metabolites

Matrix	Fortification Level (mg/kg)	n	Mean (%)		RSD (%)	
			306/261	306/193	306/261	306/193
Method: L0188/01; LOQ = 0.01 mg/kg		**Imazamox (BAS 720 H)**				
Whole plant	0.01, 0.10, 4.0	8	104	105	8.4	7.1
Rest of Plant	0.01-0.10	6	104	96	11	12
Seed	0.01-0.10	6	104	105	17	4.4
Method: L0188/01; LOQ = 0.01 mg/kg		**CL 263284 (4110773)**				
Whole plant	0.01-0.10	6	108	108	7.9	1.3
Rest of Plant	0.01-0.10	6	102	108	18	4.2
Seeds	0.01-0.10	6	107	109	7.4	2.1
.Method: L0188/01; LOQ = 0.01 mg/kg		**CL 312622 (4110542)**				
Whole plant	0.01-0.10	6	101	103	8.7	3.6
Rest of Plant	0.01-0.10	6	96	86	15	8.9
Seed	0.01-0.10	6	101	100	10	6.1
Method: L0188/01; LOQ = 0.01 mg/kg		**CL 189215 (4110445)**				
Whole plant	0.01-0.10	6	105	102	5.6	11
Rest of Plant	0.01-0.10	6	105	104	7.1	9.4
Seed	0.01-0.10	6	106	103	4.6	6.0

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-13, detailed residue levels are shown in Table 6.3.3-14.
In whole plant directly after the application, residues of imazamox ranged from 1.5 to 3.8 mg/kg. They declined to <0.01 mg/kg 28 days thereafter. In seeds and rest of plants taken at BBCH 89, residues were <0.01 mg/kg.
Residues of CL 263284 were below the LOQ at 0 DALA in whole plants and in seeds; in whole plants at 27-28 DALA and in rest of plant, they ranged from <0.01-0.01 mg/kg and <0.01-0.05 mg/kg, respectively.
Consequently, total imazamox residues were 1.51-3.81, <0.02-0.02, <0.02-0.06 and <0.02 mg/kg in whole plants at 0 and 27-28 DALA, rest of plants and seeds, respectively.
Residues of CL 189215 were <0.01, 0.04-0.14, <0.01-0.08 and <0.01-0.04 mg/kg in whole plants at 0 and 27-28 DALA, and in rest of plants and seeds, respectively.
Residues of CL 312622 were below the LOQ of 0.01 mg/kg at all sampling times.

No residues were found in any of the control samples.

Table 6.3.3-13: Summary of residues in soya bean treated with BAS 762 01 H

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	Imaza-mox	CL 263284[4]	Total imazamox[5]	CL312622	CL189215
Northern EU	2011	0	21-69	Whole plant[3]	1.5-3.8	<0.01	1.51-3.81	<0.01	<0.01
		27-28	69-73	Whole plant[3]	<0.01	<0.01-0.01	<0.02-0.02	<0.01	0.04-0.14
		69-104	89	Rest of plant[3]	<0.01	<0.01-0.05	<0.02-0.06	<0.01	<0.01-0.08
		69-104	89	Seed	<0.01	<0.01	<0.02	<0.01	<0.01-0.04

1) days after last application
2) at harvest
2) without roots
4) expressed as parent equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.
5) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048)

III. CONCLUSION

Residues of imazamox and all metabolites were <0.01 mg/kg in mature seed (BBCH 89), except for CL 189215 which was detected at 0.04 mg/kg in one trial. Total imazamox residues were <0.02 mg/kg.

Table 6.3.3-14: Residues of imazamox and its metabolites in soya bean after one application of BAS 762 01 H in Northern Europe

Trial Details		Crop	Country	Formulation, Application Rate[0)] (kg a.s./ha)	Crop Growth Stage[2)] (BBCH)	DALA[1)]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	402845	Soya	Germany	BAS 762 01 H:	25	0	W.plant	2.2	<0.01	2.21	<0.01	<0.01
Doc ID:	2011/1112578	bean		1 x 0.042		27	W.plant	<0.01	<0.01	<0.02	<0.01	0.05
Trial No.:	L110241					78	R.plant	<0.01	<0.01	<0.02	<0.01	0.01
GLP:	yes					78	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	<0.01
Year:	2011											
Study code:	402845	Soya	Germany	BAS 762 01 H:	21-51	0	W.plant	3.8	<0.01	3.81	<0.01	<0.01
Doc ID:	2011/1112578	bean		1 x 0.042		27	W.plant	<0.01	<0.01	<0.02	<0.01	0.07
Trial No.:	L110242					98	R.plant	<0.01	0.01	0.02	<0.01	<0.01
GLP:	yes					98	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	<0.01
Year:	2011											
Study code:	402845	Soya	France	BAS 762 01 H:	25-55	0	W.plant	1.5	<0.01	1.51	<0.01	<0.01
Doc ID:	2011/1112578	bean		1 x 0.042		28	W.plant	<0.01	<0.01	<0.02	<0.01	0.04
Trial No.:	L110243					104	R.plant	<0.01	<0.01	<0.02	<0.01	<0.01
GLP:	yes					104	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	<0.01
Year:	2011											
Study code:	402845	Soya	France	BAS 762 01 H:	69	0	W.plant	2.1	<0.01	2.11	<0.01	<0.01
Doc ID:	2011/1112578	bean		1 x 0.042		28	W.plant	<0.01	0.01	0.02	<0.01	0.14
Trial No.:	L110244					69	R.plant	<0.01	0.05	0.06	<0.01	0.08
GLP:	yes					69	Seed	<0.01	<0.01	<u>0.02</u>	<0.01	0.04
Year:	2011											

0) actual application rates varied by 10% at most
1) days after last application
2) at application
I imazamox
II metabolite CL 263284 (Reg. No. 4110773), expressed as parent equivalent (conversion factor 1.048)
III total imazamox = residues parent (BAS 720 H) + residues CL 263284 expressed as parent equivalents. When calculating total imazamox residues, residue values below the LOQ (<0.01) were set at LOQ (0.01 mg/kg)
IV: metabolite CL312622 (4110542)
V: metabolite CL189215 (4110445)
w.plant whole plant without roots
r.plant rest of plant without roots
_ underlined values were used for MRL calculation

CA 6.3.4 Alfalfa

Table 6.3.4-1: Critical GAP for the use of BAS 720 H in/on alfalfa

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Alfalfa	1 x 0.05 kg BAS 720 H/ha	100-600 L/ha	F	spray application	BBCH 12-16

PHI = pre-harvest interval

For imazamox in alfalfa, several use patterns and formulations are registered, the most critical one being the use pattern registered in France with the product Nirvana (EC formulation with imazamox and pendimethalin, application rate of 4 L product/ha = 66.8 g Imazamox/ha). The residue trials performed with this formulation are the most recent ones and are supposed to cover also the intended use pattern with Pulsar in alfalfa (shown in the table above).

Available data with two other formulations (Corum, BAS 762 01 H, and SF09464, identical with BAS 720 02 H, Pulsar) are additionally included to complete the residue picture of imazamox in alfalfa.

Table 6.3.4-2: GAP information of residue trials conducted in/on alfalfa

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Northern EU	France (1 trial)	BAS 762 01 H, SL (BAS 9047 0S, EC)[2]	spray application	0.028	0.007	1	14 23
	France (4 trials)	BAS 721 03 H, EC	spray application	0.067	0.0134	1	30-34 48-49
Southern EU	Spain (1 trial)	BAS 762 01 H, SL (BAS 9047 0S, EC)[2]	spray application	0.028	0.007	1	28 30
	Italy (1 trial)	BAS 762 01 H, SL (BAS 9047 0S, EC)[2]	spray application	0.028	0.007	1	41
	Spain (1 trial)	SF09464, SL (BAS 720 02 H*)	spray application	0.070	0.023	1	35 49 56 63
	Spain (1 trial)	SF09464, SL (BAS 720 02 H*)	spray application	0.0725	0.028	1	7 21 28 35
	Spain (2 trials)	SF09464, SL (BAS 720 02 H*)	spray application	0.070	0.023	1	40
	Greece (1 trial)	SF09464, SL (BAS 720 02 H*)	spray application	0.075	0.016	1	21 29 44
	France (4 trials)	BAS 721 03 H, EC	spray application	0.067	0.0134	1	28-29 43-44

0) actual application rates varied by 10% at most
1) days after last application
2) one plot treated with DASH HC (BAS 9047 0S) in a tank mix
* formulation SF09464 is identical with BAS 720 02 H

Report:	CA 6.3.4/1 Kreke N., 2008c BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter and hay) following one treatment with a tankmix from three open field trials, Northern France, Italy, Spain, 2007 2007/1028360
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.4/2 Kreke N., 2010a Amendment: Residues at harvest of Imazamox, Bentazone in alfalfa (RAC green matter, hay) following one treatment with a tankmix of BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC), three open field trials, Northern France, Italy Spain 2007 2010/1155809
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.4/3 Kreke N., 2008d BAS 762 AA H - Determination of residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter and hay) following one treatment with BAS 762 AA H (22.4 / 480 g/L) from three open field trials in Northern and Southern Europe 2007 2007/1023135
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.4/4 Kreke N., 2008e First amendment: Determination of residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter and hay) following one treatment with BAS 762 AA H (22.4/480 g/L) from three open field trials, Northern and Southern Europe, 2007 2008/1097982
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.3.4/5 Kreke N., 2010b Second amendment: Determination of residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter, hay) following one treatment with BAS 762 01 H (22.4/480 g/L) from three open field trials in Northern and Southern Europe, 2007 2010/1155808
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2007 growing season 3 trials with alfalfa were conducted in different representative growing areas in the EU, 1 in the EU North and 2 in the EU South to determine the residue level of imazamox and its metabolite CL 263284 in or on green matter and hay after the application of formulation BAS 762 01 H alone, an SL formulation containing 22.4 g/L imazamox and 480 g/L bentazone or after the application of BAS 762 01 H in a tank mix with BAS 9047 0S (adjuvant DASH HC, EC) on an additional plot. Data on bentazone are not reported. The treatments were applied once at growth stage BBCH 11-37, at a rate equivalent to 0.028 kg imazamox/ha in a spray volume of 400 L/ha. Specimens of green matter and hay were collected 14-41 days after the application (BBCH 49-73). The specimens were analysed for imazamox and its metabolite CL 263284 with Method M 3178 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues of imazamox, metabolite CL 263284 and total imazamox were higher after application of BAS 762 01 H in combination with adjuvant DASH HC (BAS 9047 0S) compared to the application of BAS 762 01 H alone and are thus the relevant values for MRL derivation. Residues of total imazamox in the EU after application of BAS 762 01 H in combination with adjuvant DASH HC in a tank mix ranged from <0.02-0.09 mg/kg in alfalfa green matter and from <0.02-0.13 mg/kg in alfalfa hay. Residues of total imazamox in the EU after application of BAS 762 01 H alone ranged from <0.02-0.03 mg/kg in alfalfa green matter and from <0.02-0.06 mg/kg in alfalfa hay.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL) BAS 9047 0S (EC)
Lot/Batch #:	402009, 22.4 g/L imazamox, nominal 480 g/L bentazone, nominal 1071, DASH HC
Purity:	Not reported
CAS#:	114311-32-9 (imazamox)
Development code:	Not reported
Spiking levels:	0.01-0.10mg/kg

2. Test Commodity:

Crop:	Alfalfa
Type:	Legumes (fresh)
Variety:	Symphonie, Emiliana, Brago
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Green matter, hay
Sample size:	0.2-2.1 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season 3 trials with alfalfa were conducted in different representative growing areas in the EU, 1 in the EU North (France) and 2 in the EU South (Italy, Spain) to determine the residue level of imazamox and its metabolite CL 263284 in or on green matter and hay after the application of formulation BAS 762 01 H alone, an SL formulation containing 22.4 g/L imazamox and 480 g/L bentazone or after the application of BAS 762 01 H in a tank mix with BAS 9047 0S (adjuvant DASH HC, EC) on an additional plot. The treatments were applied once at growth stage BBCH 11-37, at a rate equivalent to 0.028 kg imazamox/ha in a spray volume of 400 L/ha. Since the application rate in these trials was below the cGAP±25% the results are considered as supplementary data. Specimens of green matter and hay were collected 14-41 days after the application (BBCH 49-73). Samples were stored frozen at or below -18°C for a maximum of 222 days until analysis. Since only residues of imazamox are relevant in the dossier bentazone residues are not reported.

Table 6.3.4-3: Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	3	1	F	BAS 762 01 H (SL) +BAS 9047 0S (EC)	BAS 720 H Adjuvant DASH	0.028	400	BBCH 12-19
2007	3	1	F	BAS 762 01 H (SL)	BAS 720 H	0.028	400	BBCH 12-19

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. For the analysis of imazamox and its metabolite CL 263284 (Reg. No. 4110773), alfalfa samples (RAC green matter and hay) were extracted with an acidified methanol/water mixture, diluted and the residue levels were determined by mass spectrometric detection after liquid chromatographic separation (HPLC-MS/MS).

Table 6.3.4-4: Summary of procedural recoveries for imazamox and metabolite CL 263284

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method: M 3178; LOQ=0.01 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Green matter	0.01/0.1	8	96	4.2	8	93	2.8
Hay	0.01/0.1	8	90	8.5	8	94	3.3

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-5, detailed residue levels are shown in Table 6.3.4-6 and Table 6.3.4-7.

Trials with adjuvant DASH

In the EU North residues of imazamox and its metabolite CL 263284 were 0.01 mg/kg and 0.075 mg/kg (imazamox equivalents) in alfalfa green matter specimens taken 14 days after application of a tank mix with BAS 762 01 H and BAS 9047 0S (Adjuvant DASH HC) at BBCH 49. Total imazamox was 0.09 mg/kg. Residues of imazamox, its metabolite CL 263284 and total imazamox were 0.018 mg/kg, 0.115 mg/kg and 0.13 mg/kg respectively in alfalfa hay taken 23 days after application (BBCH >50).

In the EU South residues of imazamox, its metabolite CL 263284 and total imazamox were all below the LOQ of the analytical method, that is <0.01 mg/kg for the individual analytes and <0.02 mg/kg for total imazamox, in green matter as well as hay samples taken 28-41 DALA (BBCH 65-73).

Trials without adjuvant DASH
In the EU North residues of imazamox and its metabolite CL 263284 were <0.01 mg/kg and 0.023 mg/kg (imazamox equivalents) in alfalfa green matter specimens taken 14 days after application of BAS 762 01 H at BBCH 49. Total imazamox was 0.03 mg/kg. Residues of imazamox, its metabolite CL 263284 and total imazamox were <0.01 mg/kg, 0.047 mg/kg and 0.06 mg/kg respectively in alfalfa hay taken 23 days after application.

In the EU South residues of imazamox, its metabolite CL 263284 and total imazamox were all below the LOQ of the analytical method, that is <0.01 mg/kg for the individual analytes and <0.02 mg/kg for total imazamox, in green matter as well as hay samples taken 28-41 DALA (BBCH 65-73).

No residues at or above LOQ were detected in any control sample.

Table 6.3.4-5: Summary of residues in/on alfalfa after application of BAS 762 01 H

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284[3]	Total imazamox[4]
With Adjuvant DASH							
EU North	2007	14	49	Green matter	0.010	0.075	0.09
		23		Hay	0.018	0.115	0.13
EU South	2007	28-41	65-73	Green matter	<0.01	<0.01	<0.02
		30-41	65-73	Hay	<0.01	<0.01	<0.02
Without Adjuvant DASH							
EU North	2007	14	49	Green matter	<0.01	0.023	0.03
		23		Hay	<0.01	0.047	0.06
EU South	2007	28-41	65-73	Green matter	<0.01	<0.01	<0.02
		30-41	65-73	Hay	<0.01	<0.01	<0.02

1) days after last application
2) at harvest
3) expressed as imazamox equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ

III. CONCLUSION

Residues of imazamox, metabolite CL 263284 and total imazamox were slightly higher after application of BAS 762 01 H in combination with Adjuvant DASH HC (BAS 9047 0S) compared to the application of BAS 762 01 H alone, but only in the Northern trials. Residues of total imazamox after application of BAS 762 01 H in combination with adjuvant DASH HC in a tank mix ranged from <0.02-0.09 mg/kg in alfalfa green matter and from <0.02-0.13 mg/kg in alfalfa hay. Residues of total imazamox after application of BAS 762 01 H alone ranged from <0.02-0.03 mg/kg in alfalfa green matter and from <0.02-0.06 mg/kg in alfalfa hay.

Table 6.3.4-6: Residues of imazamox in/on alfalfa after one application of BAS 762 01 H plus BAS 9047 0S (DASH) compared to one application of BAS 762 AA H alone in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	B44122	Alfalfa	France	BAS 762 01 H: 1 x 0.028 and BAS 9047 0S: Adjuvant DASH	37	14	Green matter	0.010	0.075	0.09
Doc ID:	2007/1028360					23	Hay	0.018	0.115	0.13
Trial No:	A/NF/H/07/169									
GLP:	yes									
Year:	2007									
Study code:	B42952	Alfalfa	France	BAS 762 01 H: 1 x 0.028	37	14	Green matter	<0.010	0.023	0.03
Doc ID:	2007/1023135					23	Hay	<0.010	0.047	0.06
Trial No:	A/NF/H/07/146									
GLP:	yes									
Year:	2007									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ_

Table 6.3.4-7: Residues of imazamox in/on alfalfa after one application of BAS 762 01 H plus BAS 9047 0S (DASH) compared to one application of BAS 762 AA H alone in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	B44122	Alfalfa	Italy	BAS 762 01 H: 1 x 0.028 and BAS 9047 0S: Adjuvant DASH	11-13	41	Green matter	<0.01	<0.01	<0.02
Doc ID:	2007/1028360					41	Hay	<0.01	<0.01	<0.02
Trial No:	A/IT/H/07/170									
GLP:	yes									
Year:	2007									
Study code:	B42952	Alfalfa	Italy	BAS 762 01 H: 1 x 0.028	11-13	41	Green matter	<0.01	<0.01	<0.02
Doc ID:	2007/1023135					41	Hay	<0.01	<0.01	<0.02
Trial No:	A/IT/H/07/147									
GLP:	yes									
Year:	2007									
Study code:	B44122	Alfalfa	Spain	BAS 762 01 H: 1 x 0.028 and BAS 9047 0S: Adjuvant DASH	12-19	28	Green matter	<0.01	<0.01	<0.02
Doc ID:	2007/1028360					30	Hay	<0.01	<0.01	<0.02
Trial No:	A/SP/H/07/171									
GLP:	yes									
Year:	2007									
Study code:	B42952	Alfalfa	Spain	BAS 762 01 H: 1 x 0.028	12-19	28	Green matter	<0.01	<0.01	<0.02
Doc ID:	2007/1023135					30	Hay	<0.01	<0.01	<0.02
Trial No:	A/SP/H/07/148									
GLP:	yes									
Year:	2007									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ

Report: CA 6.3.4/6
Cronin J.A., 1997a
AC 299, 263 40g/L SL (SF 09464): Decline curve residue study on AC 299, 263 and CL263, 284 in alfalfa (Spain, 1996)
ID-731-001
Guidelines: EEC 91/414
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 1996 growing season one trial with alfalfa was conducted in the EU South to determine the residue level of imazamox and its metabolite CL 263284 in/on alfalfa whole plants (green and dried). SF09464, an SL formulation containing 40 g/L imazamox (identical with BAS 720 02 H) was applied once at a rate equivalent to 0.070 kg imazamox/ha in a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-16. Specimens of whole plants (green and dried) were collected 35-63 days and 63 days after the application, respectively. The specimens were analysed for imazamox and its metabolite CL 263284 with Method CEM-236 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of imazamox, metabolite CL 263284 and total imazamox were below the LOQ of the analytical method (<0.05 mg/kg for each analyte and <0.10 mg/kg for total imazamox) in all green and dried alfalfa samples grown in the EU South and harvested 35 to 63 days after application of 0.07 kg a.s./ha.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	SF09464 (SL), identical with BAS 720 02 H
Lot/Batch #:	001, 40 g/L imazamox, nominal
Purity:	Not reported
CAS#:	114311-32-9 (imazamox)
Development code:	Not reported
Spiking levels:	0.05 mg/kg

2. Test Commodity:

Crop:	Alfalfa
Type:	Legumes (fresh)
Variety:	Aragon
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Whole plant green, whole plant dried
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1996 growing season one trial with alfalfa was conducted in the EU South (Spain) to determine the residue level of imazamox and its metabolite CL 263284 in/on alfalfa whole plants (green and dried). SF09464, an SL formulation containing 40 g/L imazamox (identical to BAS 720 02 H) was applied once at a rate equivalent to 0.070 kg imazamox/ha in a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-16. Specimens of whole plants (green) were collected 35-63 days after the application. Whole plant (dried) samples, corresponding to alfalfa hay, were generated by sun-drying whole plant (green) samples for 24 h. All samples were stored frozen at or below -18°C until analysis.

Table 6.3.4-8: Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1996	1	1	F	SF09464 (SL)	BAS 720 H	0.070	300	BBCH 14-16

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 (Reg. No. 4110773) with Method CEM-236/001 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. For the analysis alfalfa samples (green matter and hay) were extracted with an acidified methanol/water mixture. After clean-up of the extract by liquid/liquid partition and solid phase extraction, residues were determined by UV detection after liquid chromatographic separation (HPLC-UV).

Table 6.3.4-9: Summary of recoveries of imazamox and metabolite CL 263284 in/on alfalfa

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method No. CEM-236/001; LOQ=0.05 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant	0.05	1	82	N/A	1	72	N/A

N/A not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the trial detailed residue levels are shown in Table 6.3.1-11 and Table 6.3.4-11.

Residues of imazamox and its metabolite CL 263284 in all alfalfa whole plant (green and dried) samples harvested 35 to 63 days after a single application of SF09464 at a rate of 0.07 kg imazamox/ha were below the LOQ of the analytical method of 0.05 mg/kg. Consequently, total imazamox was <0.10 mg/kg in all alfalfa samples.

No residues at or above LOQ were detected in any control sample.

Table 6.3.4-10: Summary of residues in/on alfalfa

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284[3]	Total imazamox[4]
EU South	1996	35	n.r.	W. plant (green)	<0.05	<0.05	<0.10
		49		W. plant (green)	<0.05	<0.05	<0.10
		56		W. plant (green)	<0.05	<0.05	<0.10
		63		W. plant (green)	<0.05	<0.05	<0.10
		63		W. plant (dried)	<0.05	<0.05	<0.10

1) days after last application
2) at harvest
3) expressed as imazamox equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.05 mg/kg was used for analytes with residues below the LOQ

n.r. not reported

III. CONCLUSION

Residues of imazamox, metabolite CL 263284 and total imazamox were below the LOQ of the analytical method (<0.05 mg/kg for each analyte and <0.10 mg/kg for total imazamox) in all green and dried alfalfa samples grown in the EU South and harvested 35 to 63 days after a single application of 0.07 kg a.s./ha at BBCH 14-16.

Table 6.3.4-11: Residues of imazamox in alfalfa after one application of SF09464 in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	ID-SP-96-613	Alfalfa	Spain	SF09464: 1 x 0.07	14-16	35	Whole plant (green)	<0.05	<0.05	<0.10
Doc ID:	ID-731-001					49	Whole plant (green)	<0.05	<0.05	<0.10
Trial No:	96-613-24					56	Whole plant (green)	<0.05	<0.05	<0.10
GLP:	yes					63	Whole plant (green)	<0.05	<0.05	<u>≤0.10</u>
Year:	1996					63	Whole plant (dried)	<0.05	<0.05	<u>≤0.10</u>

0) actual application rates varied by 10% at most
1) days after last application
2) at application
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.05 mg/kg was used for analytes with residues below the LOQ
_ underlined values were used for pseudo MRL calculation

Report:	CA 6.3.4/7 Cronin J.A., 1998a AC 299263 40 g a.i./L SL (SF 09464): Decline curve residue study on AC 299263 and its metabolites CL 263284, CL 312622 and CL 189215 in alfalfa - Spain, 1997 ID-731-002
Guidelines:	EPA 171-4, EEC 91/414 Annex II 6, EEC 91/414 Annex III 8
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 1997 growing season one trial with alfalfa was conducted in the EU South to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in/on alfalfa whole plants (green and dried). SF09464, a SL formulation containing 40 g/L imazamox (identical with BAS 720 02 H) was applied once at a target rate equivalent to 0.0725 kg imazamox/ha and a spray volume of 300 L/ha. The application was performed at growth stage BBCH 15-17. Specimens of whole plants (green) were collected 7-35 days after the application. Whole plant (dried) samples, corresponding to alfalfa hay, were generated by sun-drying whole plant (green) samples for 24 h. The specimens were analysed for imazamox and its metabolites CL 263284, CL 312622 and CL 189215 with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.10 mg/kg. Only in green plants harvested 7 DALA residues of 0.37 mg/kg, 0.68 mg/kg and 0.28 mg/kg were found for total imazamox and metabolites CL 312622 and CL 189215, respectively. Residues of total imazamox, its metabolites CL 312622 and CL 189215 were below the LOQ of the analytical method (<0.10 mg/kg and <0.20 mg/kg, respectively) in all green and dried alfalfa samples grown in the EU South and harvested 21-35 DALA.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	SF09464 (SL) (identical with BAS 720 02 H)
Lot/Batch #:	001, 40 g/L imazamox, nominal
Purity:	Not reported
CAS#:	114311-32-9 (imazamox)
Development code:	Not reported
Spiking levels:	0.1, 1.0 mg/kg

2. Test Commodity:

Crop:	Alfalfa
Type:	Legumes (fresh)
Variety:	Aragon
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Whole plant green, whole plant dried
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1997 growing season one trial with alfalfa was conducted in the EU South (Spain) to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on alfalfa whole plants (green and dried). SF09464, an SL formulation containing 40 g/L imazamox (identical to BAS 720 02 H) was applied once at a target rate equivalent to 0.0725 kg imazamox/ha and a spray volume of 300 L/ha. The application was performed at growth stage BBCH 15-17. Specimens of whole plants (green) were collected 7-35 days after the application. Whole plant (dried) samples, corresponding to alfalfa hay, were generated by sun-drying whole plant (green) samples for 24 h. All samples were stored frozen at or below -18°C until analysis.

Table 6.3.4-12: Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1997	1	1	F	SF09464 (SL)	BAS 720 H	0.0725	300	BBCH 15-17

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.10 mg/kg. For the analysis of imazamox and its metabolites, alfalfa samples (green and dry matter) were extracted with an acidified methanol/water mixture. For imazamox, CL 263284 and CL 189215, the extracts were cleaned up by precipitation, centrifugation and Solid-Phase Extraction techniques. Measurement of the residues was accomplished by reverse-phase HPLC with mass spectrometric detection (LC-MS) and monitoring product ions (LC-MS/MS). Results were calculated for imazamox, CL 263284, CL 189215 and CL 312622 by direct comparison of the peak response in the samples to those of external standards.

Table 6.3.4-13: Summary of recoveries of imazamox and its metabolites in/on alfalfa

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method M 3178; LOQ=0.01 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant/ forage/hay	0.1/1.0	2	82	N/A	2	80	N/A
Method M 3178; LOQ=0.01 mg/kg		**Metabolite CL 312622**			**Metabolite CL 189215**		
Whole plant/ forage/hay	0.1/1.0	2	88	N/A	2	79	N/A

N/A not applicable

II. RESULTS AND DISCUSSION

The residue ranges and detailed residue levels for the trial are shown in Table 6.3.4-14 and Table 6.3.4-15.

In the EU South residues of imazamox and its metabolites CL 263284, CL 312622, CL 189215 and total imazamox in all alfalfa samples at all harvest times, except for whole plants (green) harvested 7 DALA, were below the LOQ of the analytical method (0.10 mg/kg for single analytes and 0.20 mg/kg for total imazamox) after a single application of SF09464 at a rate of 0.0725 kg imazamox/ha. Residues in whole plants (green) harvested 7 DALA were <0.10 mg/kg, 0.27 mg/kg, 0.37 mg/kg, 0.68 mg/kg and 0.28 mg/kg for imazamox, metabolite CL 263284, total imazamox, metabolite CL 312622 and metabolite CL 189215.

No residues at or above LOQ were detected in any control sample.

Table 6.3.4-14: Summary of residues in/on alfalfa

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	I	II	III	IV	V
EU South	1997	7	n r.	W. plant (green)	<0.10	0.27	0.37	0.68	0.28
		21		W. plant (green)	<0.10	<0.10	<0.20	<0.10	<0.10
		28		W. plant (green)	<0.10	<0.10	<0.20	<0.10	<0.10
		35		W. plant (green)	<0.10	<0.10	<0.20	<0.10	<0.10
		35		W. plant (dried)	<0.10	<0.10	<0.20	<0.10	<0.10

1) days after last application
2) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.10mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)

III. CONCLUSION

Only in green plants harvested 7 DALA residues of 0.37 mg/kg, 0.68 mg/kg and 0.28 mg/kg were found for total imazamox and metabolites CL 312622 and CL 189215, respectively.
Residues of total imazamox and its metabolites CL 312622 and CL 189215 were below the LOQ of the analytical method (<0.20 mg/kg and <0.10 mg/kg, respectively) in all green and dried alfalfa samples grown in the EU South and harvested 21-35 DALA.

Table 6.3.4-15: Residues of imazamox in/on alfalfa after one application of SF09464 in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	ID-SP-97-805	Alfalfa	Spain	SF09464: 1 x 0.0725	15-17	7	W. pl.[3]	<0.10	0.27	0.37	0.68	0.28
Doc ID:	ID-731-002					21	W. pl.[3]	<0.10	<0.10	<0.20	<0.10	<0.10
Trial No:	97-805-04					28	W. pl.[3]	<0.10	<0.10	<0.20	<0.10	<0.10
GLP:	yes					35	W. pl.[3]	<0.10	<0.10	<0.20	<0.10	<0.10
Year:	1997					35	W. pl.[4]	<0.10	<0.10	<0.20	<0.10	<0.10

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) whole plant green
4) whole plant dried
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.10mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
_ underlined values were used for pseudo MRL calculation

Report:	CA 6.3.4/8 Cronin J.A., 1998b AC 299263 40 g a.i./L SL (SF 09464): At harvest residue study on AC 299263 and its metabolites CL 263284, CL 312622 and CL 189215 in alfalfa - Spain, 1996 ID-731-003
Guidelines:	EEC 96/68, EEC 91/414 Annex II 6, EEC 91/414 Annex III 8
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 1996 growing season two trials with alfalfa were conducted in the EU South to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on alfalfa whole plants. SF09464, an SL formulation containing 40 g/L imazamox was applied once at target rates equivalent to 0.070 kg imazamox/ha and a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-17. Specimens of whole plants were collected 40 days after the application. Whole plant (dried) samples, corresponding to alfalfa hay, were generated by sun-drying whole plant (green) samples for 24 h. The specimens were analysed for imazamox and its metabolites CL 263284, CL 312622, and CL 189215 with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.10 mg/kg. In the EU South residues of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 as well as total imazamox in all fresh and dried alfalfa samples harvested 40 DALA were below the LOQ of the analytical method (0.10 mg/kg for single analytes and 0.20 mg/kg for total imazamox).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	SF09464 (SL) identical with BAS 720 02 H
Lot/Batch #:	001, 40 g/L imazamox, nominal
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	0.1-1.0 mg/kg

2. Test Commodity:

Crop:	Alfalfa
Type:	Legumes (fresh)
Variety:	Aragon
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Whole plant green and dried
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1996 growing season two trials with alfalfa were conducted in the EU South (Spain) to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on alfalfa whole plants. SF09464, an SL formulation containing 40 g/L imazamox (identical to BAS 720 02 H) was applied once at a target rate equivalent to 0.070 kg imazamox/ha and a spray volume of 300 L/ha. The application was performed at growth stage BBCH 14-17. Specimens of whole plants were collected 40 days after the application. Whole plant (dried) samples, corresponding to alfalfa hay, were generated by sun-drying whole plant (green) samples for 24 h. All samples were stored frozen at or below -18°C until analysis.

Table 6.3.4-16: Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1996	2	1	F	SF09464 (SL)	BAS 720 H	0.070	300	BBCH 14-17

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.10 mg/kg. For the analysis of imazamox and its metabolites alfalfa samples (green and dry matter) were extracted with an acidified methanol/water mixture. For imazamox, CL 263284 and CL 189215, the extracts were cleaned up with precipitation, centrifugation and Solid-Phase Extraction techniques. Measurement of the residues was accomplished by reverse-phase HPLC with mass spectrometric detection (LC-MS) and monitoring product ions (LC-MS/MS). Results were calculated for imazamox, CL 263284, CL 189215 and CL 312622 by direct comparison of the peak response in the samples to those of external standards.

Table 6.3.4-17: Summary of recoveries of imazamox and its metabolites in/on alfalfa

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method M 3178; LOQ=0.01 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant forage	0.10/1.0	2	75	N/A	2	76	N/A
Method M 3178; LOQ=0.01 mg/kg		**Metabolite CL 312622**			**Metabolite CL 189215**		
Whole plant forage	0.10/1.0	2	92	N/A	2	70	N/A

N/A not applicable

II. RESULTS AND DISCUSSION

The residue ranges and detailed residue levels for the trial are shown in Table 6.3.4-18 and Table 6.3.4-19.

In the EU South, residues of imazamox and its metabolites CL 263284, CL 312622, CL 189215 as well as total imazamox in all fresh and dried alfalfa samples harvested 40 DALA were below the LOQ of the analytical method (0.10 mg/kg for single analytes and 0.2 mg/kg for total imazamox) after a single application of SF09464 at a rate of 0.070 kg imazamox/ha.

No residues at or above LOQ were detected in any control sample.

Table 6.3.4-18: Summary of residues in/on alfalfa

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	I	II	III	IV	V
EU South	1996	40	n.r.	Whole plant (green)	<0.10	<0.10	<0.20	<0.10	<0.10
				Whole plant (dried)	<0.10	<0.10	<0.20	<0.10	<0.10

1) days after last application
2) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.10mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)

III. CONCLUSION

In the EU South, residues of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 as well as total imazamox in all fresh and dried alfalfa samples harvested 40 DALA were below the LOQ of the analytical method (0.10 mg/kg for single analytes and 0.20 mg/kg for total imazamox).

Table 6.3.4-19: Residues of imazamox in/on alfalfa after one application of SF09464 in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	ID-SP-96-612	Alfalfa	Spain	SF09464: 1 x 0.070	14-16	40	W. pl.[3]	<0.10	<0.10	<u><0.20</u>	<0.10	<0.10
Doc ID:	ID-731-003					40	W. pl.[4]	<0.10	<0.10	<u><0.20</u>	<0.10	<0.10
Trial No:	96-612-25											
GLP:	yes											
Year:	1996											
Study code:	ID-SP-96-612	Alfalfa	Spain	SF09464: 1 x 0.070	15-17	40	W. pl.[3]	<0.10	<0.10	<u><0.20</u>	<0.10	<0.10
Doc ID:	ID-731-003					40	W. pl.[4]	<0.10	<0.10	<u><0.20</u>	<0.10	<0.10
Trial No:	96-612-26											
GLP:	yes											
Year:	1996											

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) whole plant green
4) whole plant dried
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.10mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
_ underlined values were used for pseudo MRL calculation

Report:	CA 6.3.4/9 Bozoglou K., 2000a Imazamox (AC 299263) 40 g a.s./L SL (SF 09464) and its metabolites (AC 263, 284, AC 189, 215, AC 312, 622) - At harvest residue study of Imazamox and its metabolites in alfalfa (Hellas 1999) ID-731-023
Guidelines:	EEC 96/68, EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 7029/VI/95 (22 July 1997)
GLP:	yes (certified by Hellenic Republic Ministry of Rural Development and Food, Athens, Greece)

Executive Summary

During the 1999 growing season one trial with alfalfa was conducted in the EU South to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in/on alfalfa whole plants. SF09464, an SL formulation containing 40 g/L imazamox (identical with BAS 720 02 H) was applied once at a target rate equivalent to 0.075 kg imazamox/ha and a spray volume of 480 L/ha. The application was performed at growth stage BBCH 19. Specimens of whole plants were collected 21, 29 and 42 days after the application. The specimens were analysed for imazamox and its metabolites with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.10 mg/kg. In the EU South, residues of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 as well as total imazamox in all fresh alfalfa samples harvested 21, 29 and 42 DALA were below the LOQ of the analytical method (0.10 mg/kg for single analytes and 0.20 mg/kg for total imazamox).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	SF09464 (SL) (=BAS 720 02 H)
Lot/Batch #:	F017898, 40 g/L imazamox, nominal
Purity:	Not reported
CAS#:	114311-32-9
Development code:	Not reported
Spiking levels:	0.5-5.0 mg/kg

2. Test Commodity:

Crop:	Alfalfa
Type:	Legumes (fresh)
Variety:	Ipati
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Whole plant green
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1999 growing season one trial with alfalfa was conducted in the EU South (Greece) to determine the residue level of imazamox and its metabolites CL 263284, CL 312622 and CL 189215 in or on alfalfa whole plants. SF09464, a SL formulation containing 40 g/L imazamox (identical to BAS 720 02 H) was applied once at a target rate equivalent to 0.075 kg imazamox/ha and a spray volume of 480 L/ha. The application was performed at growth stage BBCH 19. Specimens of whole plants were collected 21, 29 and 42 days after the application. All samples were stored frozen at or below -18°C until analysis.

Table 6.3.4-20: Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	1	1	F	SF09464 (SL)	BAS 720 H	0.075	480	BBCH 19

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with Method M 3178 quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.10 mg/kg. For the analysis of imazamox and its metabolites alfalfa samples (green and dry matter) were extracted with an acidified methanol/water mixture. For imazamox, CL 263284 and CL 189215, the extracts were cleaned up with precipitation, centrifugation and Solid-Phase Extraction techniques. Measurement of the residues was accomplished by reverse-phase HPLC with mass spectrometric detection (LC-MS) and monitoring product ions (LC-MS/MS). Results were calculated for imazamox, CL 263284, CL 189215 and CL 312622 by direct comparison of the peak response in the samples to those of external standards.

Table 6.3.4-21: Summary of recoveries of imazamox and its metabolites in/on alfalfa

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method M 3178; LOQ=0.01 mg/kg		**BAS 720 H (imazamox)**			**Metabolite CL 263284**		
Whole plant forage	0.50/5.0	2	83	N/A	2	73	N/A
Method M 3178; LOQ=0.01 mg/kg		**Metabolite CL 312622**			**Metabolite CL 189215**		
Whole plant forage	0.50/5.0	2	88	N/A	2	66	N/A

N/A not applicable

II. RESULTS AND DISCUSSION

The residue ranges and detailed residue levels are shown in Table 6.3.4-22 and Table 6.3.4-23.

In the EU South residues of imazamox and its metabolites CL 263284, CL 312622, CL 189215 as well as total imazamox in all fresh alfalfa samples harvested 21, 29 and 42 DALA were below the LOQ of the analytical method (0.10 mg/kg for single analytes and 0.20 mg/kg for total imazamox) after a single application of SF09464 at a rate of 0.075 kg imazamox/ha.

No residues at or above LOQ were detected in any control sample.

Table 6.3.4-22: Summary of residues in/on alfalfa

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)					
				Matrix	I	II	III	IV	V
EU South	1999	21	61	W. plant (green)	<0.10	<0.10	<0.20	<0.10	<0.10
		29	65	W. plant (green)	<0.10	<0.10	<0.20	<0.10	<0.10
		42	67	W. plant (green)	<0.10	<0.10	<0.20	<0.10	<0.10

1) days after last application
2) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.10mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)

III. CONCLUSION

In the EU South residues of imazamox and its metabolites CL 263284, C 312622 and CL 189215 as well as total imazamox in all fresh alfalfa samples harvested 21, 29 and 42 DALA were below the LOQ of the analytical method (0.10 mg/kg for single analytes and 0.20 mg/kg for total imazamox).

Table 6.3.4-23: Residues of imazamox in/on alfalfa after one application of SF09464 in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)					
							Matrix	I	II	III	IV	V
Study code:	ID-HE-99-12	Alfalfa	Greece	SF09464:	19	21	W. plant[3]	<0.10	<0.10	<0.20	<0.10	<0.10
Doc ID:	ID-731-023			1 x 0.075		29	W. plant[3]	<0.10	<0.10	<0.20	<0.10	<0.10
Trial No:	99-12-01					42	W. plant[3]	<0.10	<0.10	<u>0.20</u>	<0.10	<0.10
GLP:	yes											
Year:	1999											

0) actual application rates varied by 10% at most
1) days after last application
2) at application
3) whole plant green
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.10mg/kg was used for analytes with residues below the LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
_ underlined values were used for pseudo MRL calculation

Report:	CA 6.3.4/10 Malet J.C.,Allard L., 2011a Residues of Imazamox and Pendimethalin, after 1 application of Nirvana S in lucerne in support of the registration 2011/1294331
Guidelines:	OECD-ENV/JM/MONO(2002)/9, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Report:	CA 6.3.4/11 Malet J.C., 2013a Amendment No. 11/60 - Mesure du niveau de residu de l'Imazamox et de la Pendimethalin, apres 1 application de la preparation Nirvana S sur luzerne dans le cadre d'une extension d'usage sur la culture 2013/1211060
Guidelines:	OECD-ENV/JM/MONO(2002)/9, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5
GLP:	Yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

During the 2009 and 2010 growing seasons 8 trials with alfalfa were conducted in different representative growing areas in the EU to determine the residue level of imazamox and its metabolite CL 263284 in or on green matter (RAC) and hay. BAS 721 03 H, an EC formulation containing 16.7 g/L imazamox and 250 g/L pendimethalin was applied once at a target rate equivalent to 0.067 kg imazamox/ha in a spray volume of 500 L/ha. Data on pendimethalin are not reported. The applications were performed at growth stage BBCH 13-40. Specimens of green matter and hay were collected 28-49 and 34-49 days after the application. The specimens were analysed for imazamox and its metabolite CL 263284 with a HPLC-MS/MS-based method quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Total imazamox ranged from <0.02-0.136 mg/kg in alfalfa green matter harvested 28-49 DALA. Total imazamox in alfalfa hay ranged from 0.054-0.136 mg/kg in samples taken 34-49 DALA.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 721 03 H (EC)
Lot/Batch #:	Not reported, 16.7 g/L imazamox, nominal 250 g/L pendimethalin, nominal
Purity:	Not reported
CAS#:	114311-32-9 (imazamox)
Development code:	Not reported
Spiking levels:	0.01-0.10 mg/kg

2. Test Commodity:

Crop:	Alfalfa
Type:	Legumes (fresh)
Variety:	Gea, Symphonie, Salsa, Diane, Sanditi, Provence, Oro
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Green matter, dry matter (hay)
Sample size:	<0.405 kg

B. STUDY DESIGN

1. Test procedure

During the 2009 and 2010 growing seasons 8 trials with alfalfa were conducted in different representative growing areas in the EU (4 France North, 4 France South) to determine the residue level of imazamox and its metabolite CL 263284 in or on green matter (RAC) and hay. BAS 721 03 H, an EC formulation containing 16.7 g/L imazamox and 250 g/L pendimethalin was applied once at a target rate equivalent to 0.067 kg imazamox/ha in a spray volume of 500 L/ha. The applications were performed at growth stage BBCH 13-40. Specimens of green matter and hay were collected 28-49 and 34-49 days after the application. Samples were stored frozen until analysis. Since only residues of imazamox are relevant in the dossier at hand pendimethalin residues are not reported.

Table 6.3.4-24: Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2009/2010	8	1	F	BAS 721 03 H (EC)	Imazamox Pendimethalin	0.067 1.000	500	BBCH 13-40

2. Description of analytical procedures

The specimens were analysed for imazamox and its metabolite CL 263284 with a method quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. For the analysis of imazamox and its metabolite CL 263284 (Reg. No. 4110773), alfalfa samples (green matter and hay) were extracted with a methanol/water/HCl mixture (60/39/1 v/v/v), diluted and the residue levels were determined by mass spectrometric detection after liquid chromatographic separation (HPLC-MS/MS).

Table 6.3.4-25: Summary of recoveries of imazamox and metabolite CL 263284 in alfalfa

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method Name: not reported		BAS 720 H (imazamox)			Metabolite CL 263284		
Green matter	0.01/0.1	20	89	8	20	94	13
Hay	0.01/0.1	10	73	9	10	73	7

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-26, detailed residue levels are shown in Table 6.3.4-27 and Table 6.3.4-28.

In France North, residues of imazamox in alfalfa green matter were <0.01 mg/kg in samples taken at 30-49 DALA and residues of metabolite CL 263284 ranged from 0.052-0.126 mg/kg (imazamox equivalents) and from 0.019-0.056 mg/kg in specimens taken 30-34 DALA and 48-49 DALA, respectively. Total imazamox ranged from 0.062-0.136 mg/kg and from 0.029-0.06 mg/kg in alfalfa green matter taken 30-34 and 48-49 DALA, respectively. Residues of imazamox in alfalfa hay were <0.01 mg/kg in samples taken at 34-49 DALA and residues of metabolite CL 263284 were 0.126 mg/kg (imazamox equivalents) and ranged from 0.044-0.052 mg/kg in specimens taken 34-49 DALA, respectively. Total imazamox was 0.136 mg/kg and ranged from 0.054-0.062 mg/kg in alfalfa hay taken 34 and 48-49 DALA, respectively.

In France South, residues of imazamox in alfalfa green matter were <0.01 mg/kg in samples taken at 28-44 DALA and residues of metabolite CL 263284 ranged from 0.014-0.025 mg/kg (imazamox equivalents) and were <0.01 mg/kg in specimens taken 28-29 DALA and 43-44 DALA, respectively. Total imazamox ranged from 0.024-0.035 mg/kg and was <0.02 mg/kg in alfalfa green matter taken 28-29 and 43-44 DALA, respectively.

No residues at or above LOQ were detected in any control sample.

Table 6.3.4-26: Summary of residues in/on alfalfa

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)			
				Matrix	Imazamox	CL 263284[3]	Total imazamox[4]
EU North	2009/2010	30-34	n r.	Green matter	<0.010	0.052-0.126	0.062-0.136
		48-49		Green matter	<0.010	0.019-0.056	0.029-0.06
		34		Hay	<0.010	0.126	0.136
		48-49		Hay	<0.010	0.044-0.052	0.054-0.062
EU South	2009/2010	28-29	n r.	Green matter	<0.010	0.014-0.025	0.024-0.035
		43-44		Green matter	<0.010	<0.010	<0.020

1) days after last application
2) at harvest
3) expressed as imazamox equivalents. The conversion factor from CL 263284 to BAS 720 H is 1.04815.
4) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ

n.r. not reported

III. CONCLUSION

In these trials, total imazamox in alfalfa green matter and hay ranged from <0.02-0.136 mg/kg in samples harvested 28-49 DALA and from 0.054-0.136 mg/kg in samples taken 34-49 DALA, respectively.

Table 6.3.4-27: Residues of imazamox in alfalfa after one application of BAS 721 03 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	16-17	34	Green matter	<0.010	0.052	0.062
Doc ID:	2013/1211060*					49	Green matter	<0.010	0.056	<u>0.066</u>
Trial No:	RE10011					34	Hay	<0.010	0.126	<u>0.136</u>
GLP:	yes					49	Hay	<0.010	0.052	0.062
Year:	2009/2010									
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	15	30	Green matter	<0.010	0.103	<u>0.113</u>
Doc ID:	2013/1211060*					48	Green matter	<0.010	0.019	0.029
Trial No:	RE10012					48	Hay	<0.010	0.044	<u>0.054</u>
GLP:	yes									
Year:	2009/2010									
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	13	30	Green matter	<0.010	0.126	<u>0.136</u>
Doc ID:	2013/1211060*					48	Green matter	<0.010	0.025	0.035
Trial No:	RE10013					49	Hay	<0.010	0.046	<u>0.056</u>
GLP:	yes									
Year:	2009/2010									
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	15	30	Green matter	<0.010	0.126	<u>0.136</u>
Doc ID:	2013/1211060*									
Trial No:	RE10014									
GLP:	yes									
Year:	2009/2010									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
* amended report only valid in combination with report BASF DocID 2011/1294331
underlined values were used for MRL calculation

Table 6.3.4-28: Residues of imazamox in alfalfa after one application of BAS 721 03 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DA-LA[1]	Residues Found (mg/kg)			
							Matrix	I	II	III
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	15-16	28	Green matter	<0.010	0.025	0.035
Doc ID:	2013/1211060*					43	Green matter	<0.010	<0.010	<0.02
Trial No:	RE10007									
GLP:	yes									
Year:	2009/2010									
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	14-15	29	Green matter	<0.010	0.020	0.030
Doc ID:	2013/1211060*					44	Green matter	<0.010	<0.010	<0.02
Trial No:	RE10008									
GLP:	yes									
Year:	2009/2010									
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	40	29	Green matter	<0.010	0.020	0.030
Doc ID:	2013/1211060*					44	Green matter	<0.010	<0.010	<0.02
Trial No:	RE10009									
GLP:	yes									
Year:	2009/2010									
Study code:	RXLU00410	Alfalfa	France	BAS 721 03 H: 1 x 0.067	40	29	Green matter	<0.010	0.014	0.024
Doc ID:	2013/1211060*					44	Green matter	<0.010	<0.010	<0.02
Trial No:	RE10010									
GLP:	yes									
Year:	2009/2010									

0) actual application rates varied by 10% at most
1) days after last application
2) at application
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), 0.01 mg/kg was used for analytes with residues below the LOQ
* amended report only valid in combination with report BASF DocID 2011/1294331
underlined values were used for MRL calculation

CA 6.4 Feeding studies

According to Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market as well as the Appendix G (Lundehn document 7031/VI/95 rev.4, July 1996) and OECD guidelines feeding studies are required

(1) if significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to livestock,

and

(2) if metabolism studies indicate that significant residues (above 0.01 mg/kg for each analyte) may occur in any edible animal tissue, taking into account the residue levels in potential feeding stuff obtained at the 1x dose rate.

(3) However, feeding studies shall not be required where intake is below 0.004 mg/kg bw/d, except in cases where the residue, that is to say the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate.

Intended uses with the two representative formulations BAS 720 06 H and BAS 831 00 H covered in this submission include the crops oilseed rape, sunflower, soya bean and alfalfa. All these crops are relevant feed items (rape seed, sunflower seed, soya bean seed and their meals as well as rape forage and alfalfa green forage and hay).

The relevant residue for monitoring imazamox residues in products of animal origin is proposed as sum of parent imazamox + CL 263284, expressed as imazamox equivalents. Additionally, since metabolism studies and residue trials showed the occurrence of the glucoside metabolite CL 189215 in feed item matrices, residues of those three analytes were considered for calculation of dietary feed burden.

Table 6.4.1-1 and Table 6.4.1-2 are showing the anticipated maximum dietary burden for poultry, pigs and ruminants (dairy cattle and beef cattle) calculated with the EFSA feedburden calculator, as provided in the PROFile (based on Appendix G of the Lundehn document, see above). Details on the calculation and the selected input values can be found in chapter 6.7 where then also the proposed EU MRLs for animal products are derived.

CA 6.4.1 Poultry

A feeding study in poultry is not required as the dietary burden of parent imazamox and the sum of CL 263284 + CL189215 (expressed as CL263284), for poultry are below 0.1 mg/kg DM and <0.004 mg/kg bw/day (see Table 6.4.1-1 and Table 6.4.1-2). Also, based on the results of available poultry metabolism studies with Imazamox or CL 263284, no detectable residues (<0.01 mg/kg) are expected in poultry products. Possible residues of CL 189215 in feedstuff have been considered by expressing them in CL 263284 equivalents and considering the sum of CL 263284 and CL 189215, expressed as CL 263284-equivalents in order to derive a worst case feedburden. For details of the feedburden calculation please refer to chapter 6.7.2.2.

Table 6.4.1-1: Estimated maximum dietary burden of imazamox[1] residues for poultry and pigs

Crop	Dry Matter Content (%)	Residue Level[2] (HR/STMR mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Rape forage	15	0.19	-	-	15	0.00054
VI Oil seed						
Sunflower seed meal	86	0.11	10	0.000081	20	0.000051
Dietary burden:		mg/kg bw/day		0.00081		0.0092
		mg/animal/day		0.002		0.69
		mg/kg total feed (DM)		0.013		0.23

* feed intake 0.120 kg DM, body weight (bw) 1.9kg
** feed intake 3 kg DM, body weight (bw) 75 kg
1 parent imazamox
2 for derivation of residue values see **Fehler! Verweisquelle konnte nicht gefunden werden.**

Table 6.4.1-2: Estimated maximum dietary burden of CL 263284[1] residues for poultry and pigs

Crop	Dry matter content (%)	Residue Level (HR/STMR mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Rape forage	15	0.51	-	-	15	0.022
VI Oil seed						
Sunflower seed meal	86	0.11	10	0.001763	20	0.0022
Dietary burden:	mg/kg bw/day		0.0018		0.024	
	mg/animal/day		0.0034		1.80	
	mg/kg total feed (DM)		0.03		0.60	

** feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg
1 considers sum of CL 263284 + CL 189215, calculated as CL 263284-equivalents
2 for derivation of residue values see **Fehler! Verweisquelle konnte nicht gefunden werden.**

The metabolism studies in poultry with imazamox (part of the previous submission, not included in this dossier) and with CL 263284 showed that at dose levels of 2.11 and 10.2 mg/kg and of 2.14 and 10.9 mg/kg diet for imazamox and CL 263284, respectively, total radioactive residues were all below the LOQ of 0.01 mg/kg in poultry eggs and edible tissues (skin with adhering fat, muscle, liver, kidney and blood). The dose levels used in the metabolism studies represent a considerable overdosing with regard to the maximum anticipated feed burden for poultry of 0.013 mg/kg feed DM for imazamox and 0.03 mg/kg feed DM for CL 263284, calculated (see Table 6.4.1-1 and Table 6.4.1-2).

CA 6.4.2 Ruminants

As Table 6.4.2-1 and Table 6.4.2-2 show, the estimated maximum dietary burden for beef and dairy cattle is above 0.1 mg/kg feed DM or 0.004 mg/kg bw/day and therefore the first trigger for a cattle feeding study is fulfilled. However, if no detectable residues can be anticipated based on the results of the metabolism studies, no such feeding study is required. Possible residues of CL 189215 in feedstuff have been considered by expressing them in CL 263284 equivalents and considering the sum of CL 263284 and CL 189215, expressed as CL 263284-equivalents in order to derive a worst case feedburden. For details of the feedburden calculation please refer to chapter 6.7.2.2.

Table 6.4.2-1: Estimated maximum dietary burden of imazamox[1] residues for cattle

Crop	Dry Matter Content (%)	Residue Level[2] (HR/STMR mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Alfalfa silage	20	0.10	100	0.018	100	0.021
Dietary burden:	mg/kg bw/day		0.018		0.021	
	mg/animal/day		9.9		7.4	
	mg/kg total feed (DM)		0.50		0.50	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg
1 parent imazamox
2 for derivation of residue values see **Fehler! Verweisquelle konnte nicht gefunden werden.**

Table 6.4.2-2: Estimated maximum dietary burden of CL 263284[1] residues for cattle

Crop	Dry Matter Content (%)	Residue Level[2] (HR/STMR mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Alfalfa silage	20	0.44	100	0.080	100	0.00094
Dietary burden:	mg/kg bw/day		0.08		0.094	
	mg/animal/day		44.0		32.9	
	mg/kg total feed (DM)		2.20		2.20	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg
1 considers sum of CL 263284 + CL 189215, calculated as CL 263284-equivalents
2 for derivation of residue values see **Fehler! Verweisquelle konnte nicht gefunden werden.**

Parent imazamox

In a metabolism study in goats, animals were dosed with imazamox at actual dose levels of 2.08 or 11.6 mg/kg diet (see chapter 6.2.3). The lower dose level used in the metabolism study is four times greater than the feedburden for imazamox parent of 0.50 mg/kg feed DM (0.021 mg/kg bw/d) calculated for beef cattle (see Table 6.4.2-1). The highest dose level was about 23 times the anticipated feedburden. No residues of imazamox at or above the LOQ of 0.01 mg/kg were detected in goat milk, blood, liver, muscle and fat analyzed during the metabolism study. Only in kidney, imazamox residues of 0.02 and 0.06 mg/kg were found for the low and high dose, respectively. Adjusting the residues in kidney by the respective factors (x0.25 for lower dose level and x0.04 for higher dose level) results in anticipated residues of <0.01 mg/kg for the two dosing levels. Thus, taking into account all worst case conditions (highest residues in the RAC and maximum dietary burden for cattle) the anticipated residues of imazamox in all ruminant matrices is expected to be below the LOQ of <0.01 mg/kg in all tissues, milk and blood.

Metabolite CL 263284

In a separate metabolism study goats were dosed with the hydroxyl metabolite CL 263284 at actual dose levels of 2.33 or 14.5 mg/kg feed. No residues of the metabolite at or above the LOQ (0.01 mg/kg) were found in milk and tissues, except for CL 263284 in liver (0.03 mg/kg) at the high dose level (see chapter 6.2.3).

The feedburden of 2.20 mg CL 263284/kg feed DM (0.094 mg/kg bw/d) calculated for beef cattle (considering the sum of CL 263284 and CL 189215 residues, expressed as CL 263284; see Table 6.4.2-2) approximately matches the low dosing level used in the metabolism study and no residues of CL 263284 at or above the LOQ (0.01 mg/kg) were detected in any goat matrix (milk, blood, liver, kidney, muscle and fat) analyzed during the metabolism study.

No residues above the LOQ (<0.01 mg/kg) are thus expected for CL 263284.

CA 6.4.3 Pigs

As Table 6.4.1-1 and Table 6.4.1-2 show, the estimated maximum dietary burden for pig is above 0.1 mg/kg feed DM or 0.004 mg/kg bw/day and therefore the first trigger for a feeding study is fulfilled. However, a feeding study in pigs is not required if residues in pig products are predicted to be below 0.01 mg/kg. Since the metabolism of imazamox in rat and ruminants is similar, no separate metabolism study for pigs has been conducted and extrapolation is based on the metabolism studies with ruminants. Possible residues of CL 189215 in feedstuff have been considered by expressing them in CL 263284 equivalents and considering the sum of CL 263284 and CL 189215, expressed as CL 263284-equivalents in order to derive a worst case feedburden.

Parent imazamox
In a metabolism study in goats, animals were dosed with imazamox at 2.08 or 11.6 mg/kg diet (see chapter 6.2.3). The dose levels used in the goat metabolism study represent a considerable overdosing (9 to 50 times) with regard to the maximum anticipated feed burden for pigs of 0.23 mg/kg feed DM for imazamox (see Table 6.4.1-1).
No residues of imazamox at or above the LOQ of 0.01 mg/kg were detected in goat milk, blood, liver, muscle and fat analyzed during the metabolism study. Only in kidney, imazamox residues of 0.02 and 0.06 mg/kg were found for the low and high dose, respectively. Adjusting the residues in kidney by the respective factors (x0.11 for lower dose level and x0.02 for higher dose level) results in anticipated residues of <0.01 mg/kg for both dose levels. Thus, taking into account all worst case conditions (highest residues in the RAC and maximum dietary burden for pigs) the anticipated imazamox residue in all pig matrices is expected to be below the LOQ of <0.01 mg/kg.

Metabolite CL 263284
In a separate metabolism study animals were dosed with the hydroxyl metabolite CL 263284 (2.33 or 14.5 mg/kg feed). No residues of the metabolite at or above the LOQ (0.01 mg/kg) were found in milk and tissues, except for CL 263284 in liver (0.03 mg/kg) at the high dose level (see chapter 6.2.3). The dose levels used in the goat metabolism study represent a considerable overdosing (4 to 24 times) with regard to the maximum anticipated feed burden of 0.6 mg/kg feed DM for CL 263284, calculated for pigs (see Table 6.4.1-2).
Adjusting the CL 263284 in liver by the respective factor (x0.04 for high dose level) results in anticipated residues below the LOQ (<0.01 mg/kg) for CL 263284.

CA 6.4.4 Fish

A fish feeding study may be required where residues at levels above 0.01 mg/kg may be reasonably expected in edible tissues, based on the findings of the fish metabolism study and the estimated maximum residues which might occur in fish feed. Particular attention should be laid on lipophilic substances with an intrinsic tendency for accumulation (Commission Regulation (EU) No 283/2013 from March 2013).

Since the log P_{ow} for imazamox is <<3 (log P_{ow} 0.73; please refer to Draft Assessment Report compiled by RMS France), imazamox is not anticipated to accumulate in tissues of fish. Thus a fish metabolism is not required (see Reg (EU) No 283/2013 from March 2013). Additionally, the bio-concentration factor (BCF) for imazamox (CL 299263) in fish, determined in a separate study (evaluated during the EU review of the active substance imazamox), was found to be <1.

Among the crops covered by this submission, seeds of soya bean and sunflower as well as their meal and oilseed rape meal may be considered to serve as feed items for fish. However, in light of the fact that residues of imazamox in these crop matrices are usually not detectable (except for low residues in sunflower seeds) and that due to the very low lipophilicity of imazamox residues do not accumulate in fish, no imazamox derived residues above 0.01 mg/kg can be reasonably expected in edible tissues of fish. Thus, a fish feeding study is not considered necessary.

CA 6.5 Effects of Processing

Studies are provided investigating the nature of residues after processing as well as the magnitude of residues after processing of sunflowers. None of the studies were reviewed in the previous active substance evaluation.

CA 6.5.1 Nature of the residue

Since no processing studies were triggered by the intended uses in the previous evaluation, no study investigating the nature of residues in processing had been submitted so far. According to the data requirements as laid down in Commission regulation (EC) 283/2013, such a study shall be provided where residues in products of plant or animal origin subject to processing may occur at a level of or higher than 0.01 mg/kg (based on the residue definition for risk assessment for the raw commodity). Since this is the case for the intended use in sunflowers, such a study is now provided. Since all plant and animal metabolism studies showed that the molecule is not cleaved between the two ring systems, performance of the study with only one radiolabeled position (in the pyridine ring) was deemed sufficient.

Report:	CA 6.5.1/1 Hassink J., 2012a Imazamox: Hydrolysis at 90°C, 100°C and 120°C 2011/1286214
Guidelines:	EEC 7035/VI/95 rev. 5, OECD Test Guideline 507 - Nature of the residues in processed commodities - High temperature hydrolysis
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

To estimate the degradation behaviour of ^{14}C-labeled imazamox during industrial processing or household preparation, different processes (pasteurisation, baking, brewing, boiling, and sterilisation) were simulated.
Imazamox was not significantly degraded during the simulation of pasteurisation (pH 4, 90°C, 20 min), during the simulation of baking, boiling, brewing (pH 5, 100°C, 60 min) and during sterilisation (pH 6, 120°C, 20 min). It was demonstrated that no degradation products at amounts of 5% TAR or more occurred.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test item

Internal code: BAS 720 H
Reg.No.: 4096483
Chem. name: (RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Molar mass: 305.33 g/mol (unlabelled)
Label: Pyridine-3-^{14}C, imidazolone-3-^{15}N
Batch-No.: 1004-1001
Specific act.: 6.65 MBq/mg
Radiochem. Purity: 99.7%

2. Test system

The test item was suspended in aqueous buffer solutions of different pH-values, to give a final concentration of ca. 0.92 µg/mL.

B. STUDY DESIGN

pH 4 and 90°C - pasteurisation
The test solutions were pasteurised in in a round-bottom flask under rotation in a water bath for 20 minutes at 90°C. The pH-value remained constant with 4.14 at the beginning and 3.97 at the end of the test.

pH 5 and 100°C - baking, brewing, boiling
The test solutions were treated in an Erlenmeyer flask under reflux at 100°C for 60 minutes. To avoid an influence of light, the glassware was wrapped. The pH-value remained constant with 5.27 at the beginning and 5.36 at the end of the test.

pH 6 and 120°C - sterilisation
Sterilisation of the samples was performed at about 120°C in an autoclave for 20 minutes. The measurement was conducted three times. The pH-value remained constant with 6.34 at the beginning and 6.35-6.45 at the end of the test.

For each set of conditions, the total radioactivity present was determined at the end of the incubation period after cooling and compared to the theoretical radioactivity before incubation. The total radioactivity in each sample was determined by LSC.

Radioactivity in the samples was analysed by LSC immediately upon collection or after freezer storage if necessary. Characterisation of the radioactivity in the buffered samples after incubation was performed by HPLC.

II. RESULTS & DISCUSSION

The recovery of applied radioactivity was 98.3-103.5% (see Table 6.5.1-1) after incubation compared to the theoretical radioactivity before incubation. At the end of the incubation periods, no breakdown of the parent into any degradation products was observed. In the first sterilisation test (pH 6 120°C) an unknown peak with 5.8% TAR occurred at a retention time of about 15 min. However, when repeating the test twice, the unknown peak was detected below 5% TAR with a mean value of 4.7%. Furthermore, by fractionation of the peak and further analysis using HPLC, the peak was split into at least two peaks with a ratio about 1:1. Hence, it could be demonstrated that no unknown peaks with more than 5% TAR occurred in the test system. Imazamox was found to be stable during incubation under pasteurisation, baking/brewing/boiling and sterilisation conditions, respectively.

Table 6.5.1-1: Recovery after processing simulation tests with ^{14}C-BAS 720 H

Process represented	Test conditions	Recovery %		
		Total	Imazamox	Unknown
Pasteurisation	pH 4, 90°C, 20 minutes	102.2	102.2	-
Baking/brewing/boiling	pH 5, 100°C, 60 minutes	103.5	103.5	-
Sterilisation*	pH 6, 120°C, 20 minutes	99.9*	95.2*	4.7*

* Means of three tests (Unknown Test 1: 5.8% TAR, Test 2: 3.8% TAR, Test 3: 4.6% TAR)

III. CONCLUSION

Imazamox was demonstrated to be stable under conditions representing pasteurisation, baking/brewing/boiling and sterilisation. No degradates/hydrolysis products >5% TAR were detected.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

Not relevant for the intended uses in oilseed rape, sunflower, soybean and alfalfa.

CA 6.5.3 Magnitude of residues in processed commodities

In determining whether a processing study is needed or not, the following points shall be taken into consideration (according to Commission regulation 283/2013):

(a) the dietary burden of a processed product in the human (such as apples) or animal diet (such as apple pomace);

(b) the level of residue in the plant or plant product to be processed (normally ≥ 0.1 mg/kg)

(c) the physical and chemical properties of the active substance and its relevant metabolites (such as fat-solubility in case of oil seed processing); and

(d) the possibility that breakdown products of toxicological significance may occur after processing of the plant or plant product.

If the level of residues is less than 0.1 mg/kg, processing studies shall be carried out if the contribution of the commodity under consideration to the theoretical maximum daily intake (TMDI) is ≥ 10 % of the ADI or if the estimated daily intake is ≥ 10 % of the ARfD for any European consumer group diet.

In the present dossier detectable residues (>0.01 mg/kg) for imazamox or CL 263284 have only arisen in sunflower seeds (0.08 mg/kg total imazamox). Thus, the total imazamox residues are still below the trigger of 0.1 mg/kg. Taking into account the very low lipophilicity of imazamox, an accumulation of imazamox derived residues in products for human consumption (such as oil, margarine) is not expected.

However, since there are some sunflower processing studies available, they are shown in the following. The studies confirm the assumption that residues only accumulate in sunflower processed products used as feedstuffs (sunflower meal/press cake) but not in oil. These sunflower processing studies may also stand for processing steps used for other oilseeds such as oilseed rape and soya bean for which uses are being described within the present dossier.

Report: CA 6.5.3/1
Johnston R., 2003a
The magnitude of Imazamox (BAS 720 H) and Imazapyr (BAS 693H) residues in sunflower and sunflower processed fractions
2002/5004111
Guidelines: EPA 860.1500, EPA 860.1520
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

Sunflower processing studies were conducted to determine the potential for concentration of residues of imazamox (BAS 720 H) in the processed fractions of sunflower. During the 2000 growing season, six field trials were carried out in the United States. Sunflowers were treated with Raptor® 1AS (SC formulation, 11.78% imazamox) and a formulation containing imazapyr. At all trial sites, one trial plot was untreated to provide control samples, and one trial plot received one foliar tank-mix application at a rate of 0.056 kg imazamox/ha. At a single test site, imazamox and imazapyr were also applied once as a foliar spray to a separate treated plot targeting an exaggerated rate of imazamox of 0.112 kg a.s./ha (Treatment 3, 2x). Another treated plot at this site received a single foliar application of imazamox alone at 0.14 kg a.s./ha (Treatment 4, 2.5x). The applications were made broadcast post-emergence targeting when the plants were 30 to 46 cm tall (6 to 8 leaf growth stage). Replicate sunflower seed RAC samples were harvested at normal crop maturity, 72-100 days after treatment (DAT) from each plot from all sites. One additional bulk whole seed sample was collected at 88 DAT from each of the plots at one trial and later processed according to simulated commercial practices into meal and refined oil.
Sunflower samples were analysed for residues of imazamox (BAS 720 H) and its hydroxy and di-acid metabolites CL 263284 and CL 312622 using LC-MSD. The validated limit of quantitation was 0.05 mg/kg. It was shown that imazamox and metabolite CL 263284 slightly concentrated in meal. For all other analyte-commodity-combinations, the processing factors showed a continuance at the same level.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Raptor® 1AS, SC/L
Lot/Batch #:	AC 11404-25U; 11.78% w/w imazamox
CAS#:	114311-32-9

2. Test Commodity:

Crop:	Sunflower
Type:	Oilseeds
Variety:	Not reported (IMI sunflower hybrid), CMS HA425 / RHA426
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed Commodity:	Seed, meal, refined oil

B. STUDY DESIGN

1. Test procedure

Sunflower processing studies were conducted to determine the potential for concentration of residues of imazamox (BAS 720 H) in the processed fractions of sunflower. During the 2000 growing season, six field trials were carried out in the United States, two in Nebraska, three in North Dakota and one in Colorado. Sunflowers were treated with Raptor® 1AS (SC formulation, 11.78% imazamox) and a formulation containing imazapyr (Arsenal 2AS). At all trial sites, one trial plot was untreated to provide control samples, and one trial plot received one foliar tank-mix application at a rate of 0.05 lb imazamox/A (0.056 kg a.s./ha). At a single test site in North Dakota (07219.00-ND03), imazamox and imazapyr were also applied once as a foliar spray to a separate treated plot targeting an exaggerated rate of imazamox of 0.10 lb a.s./A (0.112 kg a.s./ha, Treatment 3, 2x). Another treated plot at this site received a single foliar application of imazamox alone at 0.125 lb a.s./A (0.14 kg a.s./ha, Treatment 4, 2.5x). The applications were made broadcast post-emergence targeting when the plants were 12 to 18 inches (30 to 46 cm) tall (6 to 8 leaf growth stage). All sprays were applied in combination with methylated seed oil (1.0% v/v) as a spray adjuvant. The spray volume was 10-21 GPA (94-199 L/ha).

Replicate sunflower seed RAC samples were harvested at normal crop maturity, 72-100 days after treatment (DAT) from each plot from all sites. One additional bulk whole seed sample was collected at 88 DAT from each of the plots at trial 07219.00-ND03 and later processed according to simulated commercial practices into meal and refined oil. All sunflower samples were held frozen after harvest or fraction collection for a maximum of 7 months prior to analysis.

Only the trial used for processing is further reported.

2. Description of analytical procedures

Sunflower samples were analysed for residues of imazamox (BAS 720 H) and its hydroxy and dicarboxylic acid metabolites CL 263284 and CL 312622 using a method developed by ABC Laboratories (HPLC-MS method). Imazapyr residues are not reported in the following since it is not relevant in this context.

Residues were extracted from sunflower seed and meal samples with an acidified methanol/water solution, filtered and concentrated. The residues were purified on a C_{18} solid phase extraction (SPE) column eluted with 25% MeOH in 0.05 M aqueous ammonium acetate and brought to final volume with water. Residues in refined oil samples were diluted in hexane and extracted by shaking with acidified acetonitrile/water solution. Following phase separation, the lower aqueous ACN layer was collected and adjusted to final volume with water. The final chromatography analysis was performed using LC-MSD. The validated limit of quantitation was 0.05 mg/kg.

Procedural recoveries ranged between 77 and 113% at spiking levels of 0.05-0.5 mg/kg.

II. RESULTS AND DISCUSSION

In the processing portion of the study, residues of imazamox were <0.05 mg/kg in all seed samples (n=3) collected 88 days after treatment with imazamox at 0.056, 0.112, and 0.14 kg a.s./ha. Residues of imazamox concentrated slightly in meal, by a factor of 1.4, but residues did not concentrate in refined oil. Residues in sunflower seed RAC and processed fractions are given in Table 6.5.3-1.

III. CONCLUSION

It was shown that imazamox and metabolite CL 263284 slightly concentrated in meal. For all other analyte-commodity-combinations, the processing factors showed a continuance at the same level.

Table 6.5.3-1: Residues of imazamox in sunflower processed fractions

Trial Location / Trial Number	Processed Commodity	Application Rate (kg a.s./ha)	DALA[1]	Residues Found (mg/kg)				Processing Factor[3] for Parent Imazamox	Processing Factor[3] for Total Residue[2]
				Imazamox	CL 263284 (M715H001)	Total[2]	CL 312622		
Minot, ND, USA (07219.00-ND03) 2000	**seed (RAC)**	1 x 0.056*	88	**<0.05**	<0.05	**<0.10**	<0.05	-	-
	meal			<0.05	<0.05	<0.10	<0.05	1	1
	refined oil			<0.05	<0.05	<0.10	<0.05	1	1
	seed (RAC)	1 x 0.112*	88	**<0.05**	<0.05	**<0.10**	<0.05	-	-
	meal			0.07	0.07	0.14	<0.05	1.4	***1.4***
	refined oil			<0.05	<0.05	<0.10	<0.05	1	1
	seed (RAC)	1 x 0.14	88	**<0.05**	<0.05	**<0.10**	<0.05	-	-
	meal			0.05	0.06	0.11	<0.05	1	1.1
	refined oil			<0.05	<0.05	<0.10	<0.05	1	1

1) days after last application
2) sum of imazamox and CL 263284, expressed as imazamox; the conversion factor for CL 263284 to parent equivalent is 1.048
3) the processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
* tank mix with imazapyr
Value in *italics* indicates worst case

Report:	CA 6.5.3/2 Norris F.A., 2009a The magnitude of Imazamox and Imazapyr residues in Clearfield sunflower and Clearfield sunflower processed fractions following application of BAS 723 00 H 2008/7019225
Guidelines:	EPA 860.1500, EPA 860.1520, PMRA 98-02 Section 9, PMRA 98-02 Section 10
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

Sunflower processing studies were conducted to determine the potential for concentration of residues of imazamox (BAS 720 H) in the processed fractions of imidazolinone-tolerant sunflower. During the 2008 growing season, five residue field trials were carried out in Canada. Sunflowers were treated with a single foliar application of BAS 723 00 H (SL formulation containing imazamox) targeting 0.02 kg a.s./ha. On an additional plot of one trial, an exaggerated rate targeting 0.1 kg a.s./ha was applied for processing. The applications were made broadcast post-emergence targeting stem elongation (BBCH 33-39) to late flowering growth stages (BBCH 69), at 69±1 days prior to the harvest of mature (dry) seed. Replicate sunflower seed RAC samples were harvested at normal crop maturity, 68-70 days after treatment (DAT) from each plot from all sites. At trial 080101 one control and two treated bulks were harvested for processing into meal and refined oil according to simulated commercial procedures.

The sunflower RAC and processed commodity samples were analysed for residues of imazamox and its hydroxyl metabolite CL 263284 using BASF Method M3519, modified. The limit of quantitation was 0.05 mg/kg. It was shown that the sum of imazamox and metabolite CL 263284 concentrated in meal yielding a mean processing factor of 3. In refined oil, residues declined below the LOQ of 0.05 mg/kg for both imazamox and CL 263284. Information on imazapyr is not reported since it is not relevant for this dossier.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 723 00 H (SL; imazamox 33%, imazapyr 15% nominal)
Lot/Batch #:	FRE-000467
CAS#:	114311-32-9 (imazamox)

2. Test Commodity:

Crop:	Sunflower
Type:	Oilseeds
Variety:	Clearfield® Sunflower: Viper
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed Commodity:	Seed, meal, refined oil

B. STUDY DESIGN

1. Test procedure

Sunflower processing studies were conducted to determine the potential for concentration of residues of imazamox (BAS 720 H) in the processed fractions of imidazolinone-tolerant sunflower. During the 2008 growing season, five residue field trials were carried out in Canada, four in Manitoba and one in Saskatchewan. Sunflowers were treated with a single foliar application of BAS 723 00 H (SL formulation containing 33% imazamox and 15% imazapyr) targeting 0.02 kg a.s./ha). On an additional plot of one trial, an exaggerated rate targeting 0.1 kg a.s./ha was applied for processing. The applications were made broadcast post-emergence targeting stem elongation (BBCH 33-39) to late flowering growth stages (BBCH 69), at 69±1 days prior to the harvest of mature (dry) seed. All sprays were applied in combination with a spray adjuvant. The spray volume was 10-11 GPA (95-101 L/ha).

Replicate sunflower seed RAC samples were harvested at normal crop maturity, 68-70 days after treatment (DAT) from each plot from all sites. At trial R080100 one control and two treated bulks were harvested for processing into meal and refined oil according to simulated commercial procedures. Samples were stored frozen after harvest or fraction collection for a maximum of 157 days prior to analysis.

Only the trial used for processing is further reported.

2. Description of analytical procedures

The sunflower RAC and processed commodity samples were analysed for residues of imazamox and its hydroxyl metabolite CL 263284 using BASF Method M 3519, modified. The method modifications include, among other minor items, the elimination of the solid phase extraction clean-up, which is not needed after the introduction of a centrifugation step.

Residues and metabolites were extracted using an acidic methanol-water solution. An aliquot of the sample extract was diluted with water, methanol and formic acid solution prior analysis of the residues by LC-MS/MS.

The validated limit of quantitation was 0.05 mg/kg. Procedural recoveries of imazamox ranged between 70 and 110% at spiking levels of 0.05-0.5 mg/kg.

II. RESULTS AND DISCUSSION

In the processing portion of the study, mean residues of total imazamox were 0.54 mg/kg in seeds collected 70 days after treatment with imazamox at 0.1 kg a.s./ha. Residues of total imazamox concentrated in meal, by a factor of 3, but residues did not concentrate in refined oil. Residues in sunflower seed RAC and processed fractions are given in the table below.

III. CONCLUSION

It was shown that imazamox and metabolite CL 263284 concentrated in meal yielding a worst case processing factor of 3. In refined oil, residues declined below the LOQ of 0.05 mg/kg for both imazamox and CL 263284.

Table 6.5.3-2: Residues of imazamox in sunflower processed fractions

Trial Location / Trial Number	Processed Commodity	Application Rate (kg a.s./ha)	DALA[1]	Replicate	Residues Found (mg/kg)			Processing Factor[3] for Parent Imazamox	Processing Factor[3] for Total Residue[2]
					Imazamox	CL 263284 (M715H001)	Total[2]		
Dunder, SK, Canada (RCN R080100) 2008	Seed (RAC)	0.1*	70	A	0.20	0.24	0.45	-	-
				B	0.26 (mean **0.23**)	0.35 (mean 0.30)	0.63 (mean **0.54**)	-	-
	Meal	0.1*	70	A	0.53	1.02	1.60	2.3	***3.0***
				B	0.51	0.96	1.52	2.2	2.8
	Refined oil	0.1*	70	A	<0.05	<0.05	<0.10	0.2	0.2
				B	<0.05	<0.05	<0.10	0.2	0.2

1) days after last application
2) sum of imazamox and CL 263284, expressed as imazamox; the conversion factor for CL 263284 to parent equivalent is 1.048
3) the processing factor is calculated by dividing the residue in the processed fraction by the mean residue in the RAC sample; residues <LOQ were set LOQ
* and 0.045 kg a.s./ha of imazapyr (mix formulation)
Value in *italics* indicates worst case

Report:	CA 6.5.3/3 Johnston R.L., 2009a Magnitude of Imazamox and Imazethapyr residues in sunflower RAC and processed fractions following applications of BAS 724 00 H 2008/7008101
Guidelines:	EPA 860.1500, EPA 860.1520, PMRA 98-02 Section 10, PMRA 98-02 Section 9
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

Sunflower processing studies were conducted to determine the potential for concentration of residues of imazamox (BAS 720 H) in the processed fractions of imidazolinone-tolerant sunflower. During the 2007 growing season, five residue field trials were carried out in the United Stated and Canada. Sunflowers were treated with a single foliar application of Odyssey® (70% WDG) containing imazamox and imazethapyr (35% WG each) targeting on 15-17 g a.s./ha. On one site, 34 g a.s./ha were accidently applied. One exaggerated rate targeting on 75 g a.s./ha was applied for processing studies in one site. The applications were made broadcast post-emergence targeting when the sunflowers were at flower buds visible to late flowering growth stages, targeting a 60±1 day pre-harvest interval (PHI). At all trial sites, one control and duplicate treated sunflower seed RAC were harvested. At one trial one control and two treated bulk seed sample weighing approximately 30 kg each were harvested by combine, but have been processed separately (replicate A & B) into sunflower processed commodities.
Sunflower samples were analysed for residues of imazamox and its hydroxyl metabolite CL 263284 using GENCS method SOP-PA.0288. The limit of quantitation was 0.05 mg/kg It was shown that metabolite CL 263284 concentrated in meal. For all other analyte-commodity-combinations, the processing factors showed a residue decline or a continuance at the same level. The worst case processing factor found for total imazamox was 2.1.
Information on imazethapyr is not reported any further since they are not relevant for this dossier.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 724 00 H (WG)
Lot/Batch #:	4062M01MP; imazamox and imazethapyr 35% each (nominal)
CAS#:	114311-32-9 (imazamox)

2. Test Commodity:

Crop:	Sunflower
Type:	Oilseeds
Variety:	Clearfield® Sunflower: DKF38-80CL
Botanical name:	*Helianthus annuus*
Crop parts(s) or processed Commodity:	Seed, meal, refined oil

B. STUDY DESIGN

1. Test procedure

Sunflower processing studies were conducted to determine the potential for concentration of residues of imazamox (BAS 720 H) in the processed fractions of imidazolinone-tolerant sunflower. During the 2007 growing season, five residue field trials were carried out in the United States (two in North Dakota and one in Wisconsin) and Canada (one in Manitoba and one in Saskatchewan). Sunflowers were treated with a single foliar application of BAS 724 00 H (WG, imazamox and imazethapyr 35% each) at 0.015-0.017 kg a.s./ha. On one site, 34 g a.s./ha were accidently applied. On an additional plot of one trial, an exaggerated rate of 0.075 kg a.s./ha was applied for processing. The applications were made broadcast post-emergence when the sunflowers were at flower buds visible to late flowering growth stages, targeting 60±1 days prior to the harvest of mature (dry) seed. All sprays were applied in combination with an adjuvant. The spray volume was 10-15 GPA (94-142 L/ha).

Replicate sunflower seed RAC samples were harvested at normal crop maturity, 58-60 days after treatment (DAT) from each plot from all sites. At one trial (R070271), one control and two treated bulks were harvested for processing into meal and refined oil according to simulated commercial procedures. Samples were stored frozen after harvest or fraction collection for a maximum of 239 days prior to analysis.

Only the trial used for processing is further reported.

2. Description of analytical procedures

Sunflower samples were analysed for residues of imazamox (BAS 720 H) and its hydroxyl metabolite CL 263284 using GENCS method SOP-PA.0288. Residues were extracted from sunflower seed and meal samples with an acidified methanol/water solution, centrifuged and the organic phase evaporated. Thereafter the pH was adjusted for liquid-liquid partition with dichloromethane. An aliquot of organic phase was taken to evaporate until dryness. The eluates were diluted with water, methanol and formic acid solution prior to analysis via LC-MS/MS. The validated limit of quantitation was 0.05 mg/kg. Procedural recoveries ranged between 70 and 110% at spiking levels of 0.05-5 mg/kg.

II. RESULTS AND DISCUSSION

In the processing portion of the study, mean residues of total imazamox were 0.20 mg/kg in seed samples collected 59 days after treatment with imazamox at 0.075 kg a.s./ha. Residues of parent imazamox were below LOQ (<0.05 mg/kg) in all processed fractions while CL 263284 concentrated in meal, but not in refined oil. Residues in sunflower seed RAC and processed fractions are given in the table below.

III. CONCLUSION

It was shown that metabolite CL 263284 concentrated in meal. For all other analyte-commodity-combinations, the processing factors showed a residue decline or a continuance at the same level. The worst case processing factor found for total imazamox was 2.1.

Table 6.5.3-3: Residues of imazamox in sunflower processed fractions

Trial Location / Trial Number	Processed Commodity	Application Rate (kg a.s./ha)	DALA[1]	Replicate	Residues Found (mg/kg)			Processing Factor[3] for Parent Imazamox	Processing Factor[3] for Total Residue[2]
					Imazamox	CL 263284 (M715H001)	Total[2]		
McHenry, ND, USA (RCN R070271) 2007	Seed (RAC)	1 x 0.075*	59	A	<0.05	0.16	0.22	-	-
				B	<0.05	0.11	0.17	-	-
					(mean <**0.05**)	(mean **0.14**)	(mean **0.20**)		
	Meal	1 x 0.075*	59	A	<0.05	0.24	0.30	1	1.5
				B	<0.05	0.34	0.41	1	***2.1***
	Refined oil	1 x 0.075*	59	A	<0.05	<0.05	<0.10	1	1
				B	<0.05	<0.05	<0.10	1	1

1) days after last application
2) sum of imazamox and CL 263284, expressed as imazamox; the conversion factor for CL 263284 to parent equivalent is 1.048
3) the processing factor is calculated by dividing the residue in the processed fraction by the mean residue in the RAC sample; residues <LOQ were set LOQ
* and 0.075 kg a.s./ha of imazethapyr (mix formulation)
Value in *italics* indicates worst case

As a conclusion of all three processing studies, the following mean processing factors can be derived for sunflower meal and refined oil.

Table 6.5.3-4: Overall mean processing factors for total BAS 720 H residues of sunflower processed fractions

Trial Location / Trial Number	Meal	Refined Oil
Minot, ND, USA 07219.00-ND03	1.4	1.0
Dunder, SK, Canada RCN R080100	3.0	0.2
McHenry, ND, USA RCN R070271	2.1	1.0
Mean	**2.2 (n=3)**	**0.7 (n=3)**

The following study provides information on the effect of processing on the level of residues for metabolite CL 189215 in soya bean seed. The data are considered suitable to describe the concentration of residues in sunflower seeds since both crops belong to the group of oilseeds.

Report:	CA 6.5.3/4 Jones B., 2011a Study of residues of Imazapyr and Imazapic in soybean cultivance (grains) and processed fractions (meal, oil, laminated soybean and hulls), after treatment with BAS 714 01 H under field conditions in Brazil 2012/1044747
Guidelines:	Resolucao RDC No. 216 - ANVISA, FAO manual on submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed - Roma 2002 1st edition
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 714 01 H, WG
Lot/Batch #:	4044-3B; 525 g/kg imazapyr, 175 g/kg imazapic
CAS#:	Imazapyr: 81334-34-1; imazapic: 81334-60-3; CL 189215: none

2. Test Commodity:

Crop:	Soya bean
Type:	Oilseeds
Variety:	Cultivance / CV 127
Botanical name:	*Glycine max*
Crop parts(s) or processed Commodity:	Defatted meal, toasted defatted meal, laminated soya bean, oil

B. STUDY DESIGN

1. Test procedure

Soya bean processing studies were conducted to determine the potential for concentration of residues of imazapyr and imazapic, the latter sharing shares metabolites CL 263284 and CL 189215 with imazamox, in the processed fractions of soya bean. In the following, only CL 189215 data are further reported as those were requested by ANSES. During the 2008/2009 growing season, two field trials were carried out in Brazil. Soya beans were treated with BAS 714 01 H (WG formulation, 525 g/kg imazapyr + 175 g/kg imazapic). At both trial sites, one trial plot was untreated to provide control samples, and one trial plot received one foliar application at a rate of either 0.210 kg a.s./ha (0.1575 kg imazapyr/ha + 0.0525 kg imazapic/ha) or 0.140 kg a.s./ha (0.105 kg imazapyr/ha + 0.035 kg imazapic/ha). The applications were made at BBCH 67 or 69 in a spray volume of 200 L/ha. All sprays were applied in combination with adjuvant Dash (0.25%; v/v). Soya bean seed RAC samples were harvested at crop maturity, 60 days after treatment (DAT) from each trial and processed according to simulated commercial practices into defatted meal, toasted defatted meal, oil and laminated soya bean. All soya bean samples were held frozen after harvest for a maximum of 16 months prior to analysis.

2. Description of analytical procedures

Soya bean samples were analyzed for residues of CL 189215 using method SOP-PA.0288 (HPLC-MS/MS).

Soya bean samples were extracted with methanol/water/HCl (60:39:1, v/v/v), and following centrifugation, an aliquot of the extract was taken to be analyzed by LC-MS/MS. The validated limit of quantitation was 0.01 mg/kg. The transition used for quantitation was m/z 454 → 292 for CL 189215.

Procedural recoveries ranged between 71 and 103% at spiking levels of 0.01-1.0 mg/kg.

II. RESULTS AND DISCUSSION

In the processing portion of the study, residues of CL 189215 were 0.02 mg/kg in seed after treatment with 0.0525 kg imazapic/ha and <0.01 mg/kg after treatment with 0.035 kg imazapic/ha. Thus, processing factors were only determined for samples of the higher treatment rate. Residues of CL 189215 concentrated slightly in defatted meal, by a factor of 1.5, but residues did not concentrate in toasted defatted meal, oil or laminated soya bean. Residues in soya bean seed RAC and processed fractions are given in Table 6.5.3-1.

III. CONCLUSION

It was shown that imazamox metabolite CL 189215 slightly concentrated in defatted soya bean meal. For all other commodities, the processing factors showed a decrease or continuance at the same level.

Table 6.5.3-1: Residues of CL 189215 in soya bean processed fractions

Trial location / Trial number	Processed commodity	Application rate Imazapic (kg a i./ha)	DALA[1]	Residues found (mg/kg)	Processing factor[2]
				CL 189215	
Santo Antonio de Posse, SP, Brazil (G090003) 2008/2009	**Seeds (RAC)**	1 x 0.0525*	60	**0.02**	-
	Defatted meal			0.03	1.5
	Toasted defatted meal			0.02	1.0
	Oil			<0.01	0.5
	Laminated soya bean			<0.01	1.0
Londrina, PR, Brazil (G090004) 2008/2009	**Seeds (RAC)**	1 x 0.035**	60	**<0.01**	-
	Defatted meal			n r.	-
	Toasted defatted meal			n r.	-
	Oil			n r.	-
	Laminated soya bean			<0.01	-

1) Days after last application
2) The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
* Mix formulation of imazapic (0.0525 kg/ha) and imazapyr (0.1575 kg/ha)
** Mix formulation of imazapic (0.035 kg/ha) and imazapyr (0.105 kg/ha)
n r. Samples not analyzed since residues of CL 189215 in seed (RAC) were <LOQ

CA 6.6 Residues in Rotational Crops

A confined rotational crop study has been evaluated during the initial EU Review of the active substance imazamox. Results from this rotational crop study conducted with ^{14}C-labelled imazamox (pyridine label) at a dose rate equivalent to 0.07 kg/ha showed no accumulation of residues in succeeding crops.

In order to address the fate of the imidazolinone moiety in the molecule, a new confined rotational crop study was conducted with ^{14}C, ^{15}N-labelled imazamox (imidazolinone label). The pyridine ring label was investigated in parallel within the new study in order to allow for direct comparison of both radiolabelled forms.

This new study largely confirmed the results of the previous study with the pyridine label. Typically, total radioactive residues were <0.01 mg/kg in all follow crops. Only in cereal matrices this trigger was exceeded, but identification of residues showed that residues of imazamox and CL 263284 (the two components relevant for the residue definition) are always <0.01 mg/kg. Thus, it is not anticipated that imazamox derived residues will be found in succeeding crops.

CA 6.6.1 Metabolism in rotational crops

Report:	CA 6.6.1/1 Funk D.,Possienke M., 2013a Nachbaustudie mit 14C-BAS 720 H - Confined rotational crop study with 14C-BAS 720 H 2013/1085609
Guidelines:	EPA 860.1850: EPA Residue Chemistry Test Guidelines, EPA 860.1850: Confined Accumulation in Rotational Crops, EPA 860.1000: Background - PMRA Section 97.13 (Canada): Residue Chemistry Guidelines Confined Accumulation in Rotational Crops (June 1997), EPA 860.1000: EPA Residue Chemistry Test Guidelines, BBA IV 3-10, EEC 7524/VI/95 rev. 2 (July 22 1997), OECD 502 Metabolism in Rotational Crops (January 2007)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 6.6.1/2 Funk D.,Dempe J., 2014a Amendment No. 1 - Nachbaustudie mit 14C-BAS 720 H - Confined rotational crop study with 14C-BAS 720 H 2014/1162701
Guidelines:	EPA 860.1850: EPA Residue Chemistry Test Guidelines, EPA 860.1850: Confined Accumulation in Rotational Crops, EPA 860.1000: Background - PMRA Section 97.13 (Canada): Residue Chemistry Guidelines Confined Accumulation in Rotational Crops (June 1997), EPA 860.1000: EPA Residue Chemistry Test Guidelines, BBA IV 3-10, EEC 7524/VI/95 rev. 2 (July 22 1997), OECD 502 Metabolism in Rotational Crops (January 2007)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The objective of this study was to investigate the amount of imazamox (BAS 720 H) residues and the nature of the degradation products in rotational crops. For this purpose two different radiolabels (imidazolinone and pyridine) of imazamox were applied to bare soil at the maximum annual application rate of 75 g a.s./ha.
The nature and the level of radioactive residues were investigated in spinach (immature and mature), white radish (root and top) and spring wheat (forage, hay, straw and grain). For the imidazolinone label, samples from the plant back intervals 31, 119 and 364 days were investigated, while for the pyridine label plant back intervals of 29, 123 and 365 days were used. Plant samples were harvested at maturity and additional immature spinach samples as well as spring wheat forage samples (in part dried to hay) were taken 23 to 33 days and 50 to 75 days after sowing, respectively. Soil samples were taken after ploughing and after harvest of the mature crops for each plant back interval. The radioactive residues in these samples and in the soil samples were determined by combustion analysis.
Low levels of radioactive residues were observed in all rotational crop matrices at all analysed plant back intervals in this study. The calculated total radioactive residues (TRR) in spinach did not exceed 0.008 mg/kg for all plant back intervals. Likewise, the TRR in white radish did not exceed 0.007 mg/kg for all plant back intervals. In spring wheat after a plant back interval of 364 DAT, the levels of radioactive residues was equal to 0.003 mg/kg or below. For all other matrices the TRR calculated was determined whereby the highest residue levels were measured in straw (0.025 mg/kg to 0.111 mg/kg). In hay the levels ranged from 0.020 mg/kg to 0.074 mg/kg. The total radioactive residues in grain accounted for 0.005 mg/kg to 0.051 mg/kg. Lower levels were found in spring wheat forage ranging from 0.004 mg/kg to 0.012 mg/kg. The residue concentration in the top soil layer after aging and ploughing slightly decreased to the plant back intervals of 31/29 DAT and 119/123 DAT and remained then more or less stable.
HPLC analysis of the partition phases of the methanol extracts, water extracts and samples obtained thereof resulted for all spring wheat matrices and plant back intervals in a metabolite pattern with only a few peaks. The parent imazamox was identified in both labels in levels equal or below 0.007 mg/kg. In addition, the metabolite M715H001 (CL 263284) was identified by co-chromatography analyses in selected rotational crop matrices of the pyridine label in a minor extent (up to 0.002 mg/kg). The metabolite M715H001 is characterised by a hydroxyl group, derived from the cleavage of the methyl ether group (demethylation) of the parent. Furthermore, degradation products in minor concentrations were characterised by their chromatographic properties. These components probably were intermediates in the degradation of imazamox to C1 or C2 units which finally entered the biosynthetic pathway of carbohydrates. The solubilization and characterization of considerable parts of these non-extractable residues by enzymatic cleavage of natural macromolecules indicates a subsequent incorporation of ^{14}C-labelled C1 or C2 units into plant polysaccharides. Additionally, the precipitation of the water extract in spring wheat grain with acetone and subsequent protease treatment indicated that a substantial part of the ^{14}C-labelled C1 or C2 units was incorporated into proteins. Extracts of spinach and radish matrices were not analysed by HPLC due to low levels of radioactive residues.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	$^{14}C/^{15}N$-imidazolinone-labelled (5/3 position) imazamox ^{14}C-pyridine (3 position)/^{15}N imidazolinone-labelled (3 position) imazamox Unlabelled imazamox
Lot/Batch #:	1003-1001 (imidazolinone label) 1004-1001 (pyridine label) AC 12820-7 (unlabelled)
Purity:	Radiochemical purity: 98.7% (imidazolinone label) 99.7% (pyridine label) Chemical purity: 95.3% (imidazolinone label) 97.2% (pyridine label) 99.5% (unlabelled) Specific activity: 7.0 MBq/mg (imidazolinone label) 6.65 MBq/mg (pyridine label)
CAS#:	114311-32-9

2. Test Commodity:

Crop:	Spinach	White radish	Spring wheat
Type:	Leafy vegetables	Root & tuber vegetables	Cereals
Variety:	Corvette F1	April Cross	Thassos
Botanical name:	*Spinacia oleracea*	*Raphanus sativus*	*Triticum aestivum*

Crop part / processed commodity: Immature and mature spinach; white radish tops and roots; spring wheat forage, hay, straw and grain

Sample size: Min. 25 g plant material

3. Soil: A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.6.1-1).

Table 6.6.1-1: Soil Physicochemical Properties

Soil Series	Soil Type*	pH**	OM %	Sand %	Silt %	Clay %	Maximal water holding capacity g/100 g dry soil	CEC[1)] $cmol^+/kg$
Bruch West (imidazolinone label)	Sandy loam	7.1	1.46***	67.1*	22.1*	10.8*	28.8	12.6
Bruch West (pyridine label)	Sandy loam	7.2	1.48***	66.4*	21.7*	11.9*	30.2	11.8

1) Cation Exchange Capacity
* USDA scheme
** $CaCl_2$
*** total organic carbon

B. STUDY DESIGN

The study was conducted during the period October, 2010 to September, 2013 by BASF SE in Limburgerhof, Germany.

1. Test procedure

A confined rotational crop study was conducted with $^{14}C/^{15}N$-imazamox. The active substance was applied to bare sandy loam soil (USDA scheme) in plastic containers at a nominal application rate of 75 g a.s./ha (approximately 0.067 Ib/A) using an automatic spray track system. In two separate setups differently labelled test items were applied ($^{14}C/^{15}N$-labelled in the imidazolinone ring or ^{14}C-labelled in the pyridine ring and ^{15}N-labelled in the imidazolinone moiety). The intended ratio of labelled to unlabelled test item was for both labels 2:1. The nature and the level of radioactive residues were investigated in spinach (immature and mature), white radish (root and top) and spring wheat (forage, hay, straw and grain). For the imidazolinone label samples from the plant back intervals 31, 119 and 364 days were investigated, while for the pyridine label plant back intervals of 29, 123 and 365 days were used.

2. Sampling

Plant samples were harvested at maturity and additional immature spinach samples as well as spring wheat forage samples (in part dried to hay) were taken 23 to 33 days and 50 to 75 days after sowing, respectively. Mature and immature spinach leaves were sampled and the roots remained in the soil. Ripe white radishes were pulled from the soil and separated into the edible parts (root) and the remaining green parts (top). Immature green plants of spring wheat were sampled (wheat forage) and partly dried to wheat hay. In addition, at harvest, mature wheat ears and straw were cut off with scissors. Straw was cut into pieces, and the ears were separated into grain and chaff using a thresher. The chaff was mixed to the straw.
Soil samples were taken after the individual plant back intervals and after harvest of the mature crops.

All samples were stored in a freezer at -18°C or below immediately after they were taken and until they were transferred to the metabolism laboratory. The storage conditions stayed the same until analysis started and during the whole period of the study. The extracts were stored in a refrigerator or, for longer periods, in a freezer.

3. Description of analytical procedures

Prior to extraction and determination of the TRR, sample material was homogenised. Plant matrices with a sufficient level of radioactivity were extracted with solvents, while for samples with low concentrations of radiolabelled compounds the amount of the total radioactive residues (TRR) was obtained only by combustion analysis. Soil samples were directly subjected to combustion.
Extraction: Aliquots of homogenised plant material were extracted three times with methanol. The combined methanol extracts were measured by LSC. The residue was further extracted in the same way with water (twice). The combined water extracts were also radioassayed by LSC. The combined results of methanol extractions and water extractions are referred to as extractable radioactive residues (ERR). The residue after solvent extraction of each sample was dried. Subsequently, the residues were homogenised. Aliquots were combusted for the determination of the residual radioactive residue (RRR). The total radioactive residues (TRR) were obtained by calculating the sum of ERR and RRR values and additionally by combustion of sample aliquots. The TRR included, in some spring wheat hay and straw samples, radioactive residue of pendimethalin (BAS 455 H), which was identified as a contamination in this study. Throughout this summary, pendimethalin is reported in the tables like a metabolite would be but it is a contamination and no product of metabolisation of imazamox.
The obtained methanol extracts of most spring wheat matrices were partitioned three times with ethyl acetate, cyclohexane and/or n-hexane.
The residual radioactive residues (RRR) after extraction with methanol and water with a sufficient level of radioactivity were subsequently extracted twice with ammonia. After ammonia extraction, the residues were dried and subsequently solubilized with different enzymes (macerozyme and cellulase; β-glucosidase and hesperidinase; α-amylase, β-amylase and amyloglucosidase; or tyrosinase and laccase).
Combustion: Appropriate aliquots of soil and solid plant samples were combusted by means of a sample oxidizer. ^{14}C-standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly (recovery values between 94 and 100%). In order to determine the background radioactivity, aliquots of untreated wheat straw samples (forage, straw and grain) were combusted under the same conditions.
LSC: For the quantitation of radioactivity in liquid samples, a liquid scintillation counter was used. Aliquots of liquid samples were mixed with an appropriate volume of a suitable scintillator prior to LSC measurement. All data were corrected using appropriate quench curves and are expressed in decays per minute (dpm).
HPLC analysis was performed of the methanol and water extracts for spring wheat matrices; extracts of spinach and radish matrices were not analysed by HPLC due to low levels of radioactive residues.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

During the course of the present study, the total radioactive residues (TRR) were determined by direct combustion analysis of small aliquots of homogenised sample material as well as by calculating the sum of the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) after solvent extraction. For spring wheat hay (123 DAT, pyridine label) the partition phases of the methanol extract and the water extract were summarized as ERR. The calculated TRR of all extracted rotational crop matrices showed no major differences to the TRR values obtained by combustion. For all further calculations the TRR calculated was used as 100% TRR.
At all plant back intervals, the highest TRR levels were found in spring wheat hay, straw and grain. The concentration of residues in grain decreased significantly after a plant back interval of approximately 365 days. For the other plant matrices much lower TRR values were found.

Table 6.6.1-2: Total radioactive residues in crops after treatment with ^{14}C-imazamox

Matrix (Days After Sowing /Planting, DAP)	TRR Combusted [mg/kg]		TRR Calculated [mg/kg]	
	Imidazolinone Label	Pyridine Label	Imidazolinone Label	Pyridine Label
Plant back interval: 30 DAT	**31 DAT**	**29 DAT**	**31 DAT**	**29 DAT**
Spinach (immature) (25/26 DAP)	0.006	0.009	0.005	0.008
Spinach (mature) (43/41 DAP)	0.003	0.005	0.003	0.004
White radish top (64/67 DAP)	0.005	0.006	0.006	0.005
White radish root (64/67 DAP)	0.003	0.003	0.003	0.003
Spring wheat forage (52/52 DAP)	0.008	0.014	0.008	0.012
Spring wheat hay (52/52 DAP)	0.046	0.078	0.042	0.074** (0.072)
Spring wheat straw (incl. chaff) (115/108 DAP)	0.044	0.077	0.040** (0.035)	0.080** (0.072)
Spring wheat grain (115/108 DAP)	0.032	0.053	0.030	0.051
Plant back interval: 119 DAT	**119 DAT**	**123 DAT**	**119 DAT**	**123 DAT**
Spinach (immature) (26/23 DAP)	0.005	0.002	0.004	0.002
Spinach (mature) (42/39 DAP)	0.005	0.002	0.004	0.002
White radish top (60/65 DAP)	0.009	0.003	0.007	0.002
White radish root (60/65 DAP)	0.002	0.002	0.001	0.001
Spring wheat forage (53/50 DAP)	0.012	0.004	0.011	0.004
Spring wheat hay (53/50 DAP)	0.050	0.023	0.043	0.020
Spring wheat straw (incl. chaff) (97/108 DAP)	0.132	0.026	0.111** (0.076)	0.025
Spring wheat grain (97/108)	0.035	0.019	0.036	0.019
Plant back interval: 364 DAT	**364 DAT**	**365 DAT**	**364 DAT***	**365 DAT**
Spinach (immature) (33/33 DAP)	<0.001	0.004	-	0.004
Spinach (mature) (46/48 DAP)	<0.001	0.005	-	0.004
White radish top (60/76 DAP)	<0.001	0.006	-	0.006
White radish root (60/76 DAP)	<0.001	0.001	-	0.001
Spring wheat forage (55/75 DAP)	<0.001	0.008	-	0.008
Spring wheat hay (55/75 DAP)	0.002	0.052	-	0.051
Spring wheat straw (incl. chaff) (137/129 DAP)	0.003	0.034	-	0.033
Spring wheat grain (137/129 DAP)	0.002	0.004	-	0.005

DAT: Days after treatment
* TRR calculated was not determined because of low amount of radioactivity.
** Including a contamination of pendimethalin; values in brackets represent the corrected values (TRR-detected amount of pendimethalin

For all soil samples, the residue levels (TRR) were determined by direct combustion analysis of subsamples. Measurements were carried out before as well as after soil aging and ploughing. Additionally, soil was sampled after harvest of the individual mature crops for each plant back interval.

For the imidazolinone label, the residue concentration in the top soil layer decreased after aging and ploughing. After harvest of the mature crops, the residue levels in soil remained more or less stable for the plant back intervals of 31, 119 and 364 DAT.

For the pyridine label, the residue concentration in the top soil layer decreased after aging and ploughing until 123 DAT. Then, the level remained stable up to a year after application (365 DAT). After harvest of the mature crops, the residue levels in soil remained more or less stable for the plant back intervals of 29, 123 and 365 DAT.

Table 6.6.1-3: Total radioactive residues in soil samples following treatment with ^{14}C-imazamox

Soil Samples (Days After Treatment, DAT)	TRR Determined by Direct Combustion [mg/kg]	
	Imidazolinone Label	Pyridine Label
(0 DAT)	0.670	0.694
Plant back interval:	**31 DAT**	**29 DAT**
After ploughing		
(31 DAT)	0.019	0.050
After harvest of mature crops		
Spinach (74 DAT)	0.011	0.011
White radish (95 DAT)	0.013	0.016
Spring wheat (146 DAT)	0.012	0.015
Plant back interval:	**119 DAT**	**123 DAT**
After ploughing		
(119 DAT)	0.014	0.018
After harvest of mature crops		
Spinach (161 DAT)	0.014	0.019
White radish (179 DAT)	0.011	0.016
Spring wheat (216 DAT)	0.013	0.017
Plant back interval:	**364 DAT**	**365 DAT**
After ploughing		
(364 DAT)	0.013	0.018
After harvest of mature crops		
Spinach (410 DAT)	0.010	0.014
White radish (424 DAT)	0.011	0.015
Spring wheat (501 DAT)	0.009	0.014

B. EXTRACTION AND CHARACTERIZATION OF RESIDUES

1. Extraction and characterization of residues in rotational crops

The extractability of the radioactive residues with methanol and water ranged for rotational crop matrices from 17.1% to 77.1% TRR in the imidazolinone label and from 17.8% to 77.9% TRR for the pyridine label. For spring wheat grain, a tendency for low extractability with both solvents was found (less than 30% TRR). The major portions of the radioactive residues were generally extracted with methanol, except for spring wheat grain (all plant back intervals, both labels), hay (123 DAT, pyridine label) and straw (365 DAT, pyridine label) where similar portions were extracted with methanol and water.

Table 6.6.1-4: Extractability of radioactive residues in rotational crop samples (imidazolinone label)

Days After Treatment DAT	TRR Calculated* [mg/kg]	Methanol Extract [mg/kg]	Methanol Extract [% TRR]	Water Extract [mg/kg]	Water Extract [% TRR]	ERR** [mg/kg]	ERR** [% TRR]	RRR [mg/kg]	RRR [% TRR]
Immature spinach									
31	0.005	0.003	50.1	<0.001	6.0	0.003	56.1	0.002	43.9
119	0.004	0.003	74.7	<0.001	2.4	0.003	77.1	0.001	22.9
Mature spinach									
31	0.003	0.002	52.7	<0.001	8.2	0.002	60.9	0.001	39.1
119	0.004	0.002	64.7	<0.001	3.4	0.002	68.1	0.001	31.9
White radish top									
31	0.006	0.003	53.8	0.001	11.1	0.004	64.9	0.002	35.1
119	0.007	0.004	63.0	0.001	9.7	0.005	72.7	0.002	27.3
White radish root									
31	0.003	0.002	60.3	<0.001	4.3	0.002	64.6	0.001	35.4
119	0.001	0.001	56.0	<0.001	0.0	0.001	56.0	0.001	44.0
Spring wheat forage									
31	0.008	0.003	35.5	<0.001	5.2	0.003	40.7	0.005	59.3
119	0.011	0.005	48.7	0.001	6.3	0.006	55.0	0.005	45.0
Spring wheat hay									
31	0.042	0.017	39.4	0.001	2.9	0.018	42.3	0.024	57.7
119	0.043	0.018	42.0	0.006	14.0	0.024	56.0	0.019	44.0
Spring wheat straw									
31	0.040	0.016	41.2	0.003	7.9	0.019	49.2	0.020	50.8
119	0.111	0.056	50.6	0.018	16.1	0.074	66.7	0.037	33.3
Spring wheat grain									
31	0.030	0.004	12.6	0.004	13.0	0.008	25.5	0.022	74.5
119	0.036	0.004	10.7	0.002	6.4	0.006	17.1	0.030	82.9

* TRR was calculated as the sum of ERR + RRR and set 100%

** ERR calculated as sum of methanol and water extract

Table 6.6.1-5: Extractability of radioactive residues in rotational crop samples (pyridine label)

Days After Treatment DAT	TRR Calculated* [mg/kg]	Methanol Extract [mg/kg]	Methanol Extract [% TRR]	Water Extract [mg/kg]	Water Extract [% TRR]	ERR** [mg/kg]	ERR** [% TRR]	RRR [mg/kg]	RRR [% TRR]
Immature spinach									
29	0.008	0.004	50.7	0.001	6.2	0.005	56.8	0.004	43.2
123	0.002	0.001	54.0	<0.001	9.9	0.001	63.9	0.001	36.1
365	0.004	0.003	72.2	<0.001	5.7	0.003	77.9	0.001	22.1
Mature spinach									
29	0.004	0.002	42.4	<0.001	8.2	0.002	50.6	0.002	49.4
123	0.002	0.001	45.1	<0.001	9.8	0.001	54.9	0.001	45.1
365	0.004	0.002	59.6	<0.001	7.1	0.002	66.7	0.001	33.3
White radish top									
29	0.005	0.003	49.4	0.001	10.0	0.003	59.4	0.002	40.6
123	0.002	0.001	42.5	<0.001	12.4	0.001	54.9	0.001	45.1
365	0.006	0.004	62.1	0.001	11.4	0.004	73.5	0.002	26.5
White radish root									
29	0.003	0.002	69.6	<0.001	2.9	0.002	72.5	0.001	27.5
123	0.001	0.001	67.4	<0.001	2.6	0.001	70.0	<0.001	30.0
365	0.001	0.001	66.8	<0.001	4.0	0.001	70.8	<0.001	29.2
Spring wheat forage									
29	0.012	0.005	41.2	<0.001	3.8	0.005	45.0	0.006	55.0
123	0.004	0.001	24.0	<0.001	4.8	0.001	28.8	0.003	71.2
365	0.008	0.004	44.4	<0.001	6.0	0.004	50.4	0.004	49.6
Spring wheat hay									
29	0.074	0.025	33.7	0.007	9.8	0.032	43.4	0.042	56.6
123	0.020	0.002	9.1	0.002	8.7	0.004	17.8	0.014	67.8
365	0.051	0.020	39.1	0.011	21.2	0.030	60.3	0.020	39.7
Spring wheat straw									
29	0.080	0.039	48.6	0.010	12.6	0.049	61.3	0.031	38.7
123	0.025	0.006	23.6	0.003	11.4	0.009	35.0	0.016	65.0
365	0.033	0.007	21.5	0.007	20.5	0.014	42.0	0.019	58.0
Spring wheat grain									
29	0.051	0.008	15.9	0.007	13.4	0.015	29.3	0.036	70.7
123	0.019	0.002	8.7	0.002	9.3	0.003	18.0	0.015	82.0
365	0.005	0.001	11.8	0.001	16.0	0.001	27.8	0.003	72.2

* TRR was calculated as the sum of ERR + RRR and set 100%
** ERR calculated as sum of methanol and water extract

In order to characterize the methanol extractable radioactive residues as organosoluble or water soluble fractions, liquid / liquid partitions of the different wheat matrices were carried out using ethyl acetate and cyclohexane (only spring wheat grain) as organic solvents. In most cases, higher portions of the radioactive residues extracted with methanol were water soluble, and lower portions were found in the organic fractions. In some cases, comparable portions were found in the organic phases and in the water phases (spring wheat grain 119 DAT, imidazolinone label and spring wheat straw and grain, both 123 DAT, pyridine label). Higher portions in the organic phase were only found for spring wheat straw at the plant back intervals of approximately 30 DAT (both labels) and 119 DAT (imidazolinone label).

2. Identification, characterization and quantification of radioactive residues in rotational crops

HPLC analysis of the partition phases of the methanol extracts, water extracts and samples obtained thereof resulted for all spring wheat matrices and plant back intervals in a metabolite pattern with only a few peaks. The parent imazamox was identified in both labels in levels equal or below 0.007 mg/kg. In addition, the metabolite M715H001 (CL 263284) was identified in selected rotational crop matrices of the pyridine label (wheat, up to 0.002 mg/kg). The metabolite M715H001 is characterised by a hydroxyl group, derived from the cleavage of the methyl ether group (demethylation) of the parent. Identification of parent and metabolite was performed by retention time comparison with reference items in two HPLC systems and by co-chromatography experiments. Furthermore, degradation products in minor concentrations were characterised by their chromatographic properties in two chromatographic systems. These components probably were intermediates in the degradation of imazamox to C1 or C2 units which finally entered the biosynthetic pathway of carbohydrates. The solubilization and characterization of considerable parts of these non-extractable residues by enzymatic cleavage of natural macromolecules indicates a subsequent incorporation of ^{14}C-labelled C1 or C2 units into plant polysaccharides.
Additionally, the precipitation of components in the water extract in spring wheat grain with acetone and subsequent protease treatment indicated that a substantial part of the ^{14}C-labelled C1 or C2 units was incorporated into proteins. In several spring wheat matrices pendimethalin was identified by co-chromatography analyses and retention time comparison as contamination.
Extracts of spinach and radish matrices were not analysed by HPLC and the residues after solvent extraction not further investigated due to low levels of radioactive residues.

Table 6.6.1-6: Total identified residues in spring wheat samples following treatment with ^{14}C-imazamox

Crop Matrix	Imazamox (BAS 720 H)		M715H001 (CL 263284)	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
31 DAT (Imidazolinone Label)				
Spring wheat forage	n.d.	n.d.	n.d.	n.d.
Spring wheat hay	n.d.	n.d.	n.d.	n.d.
Spring wheat straw	n.d.	n.d.	n.d.	n.d.
Spring wheat grain	0.002	6.2	n.d.	n.d.
119 DAT (Imidazolinone Label)				
Spring wheat forage	n.d.	n.d.	n.d.	n.d.
Spring wheat hay	n.d.	n.d.	n.d.	n.d.
Spring wheat straw	n.d.	n.d.	n.d.	n.d.
Spring wheat grain	n.d.	n.d.	n.d.	n.d.
29 DAT (Pyridine Label)				
Spring wheat forage	n.d.	n.d.	n.d.	n.d.
Spring wheat hay	0.007	9.1	0.002	2.3
Spring wheat straw	0.002	2.1	n.d.	n.d.
Spring wheat grain	0.005	10.1	0.001	1.4
123 DAT (Pyridine Label)				
Spring wheat forage	n.d.	n.d.	n.d.	n.d.
Spring wheat hay	0.001	4.0	n.d.	n.d.
Spring wheat straw	n.d.	n.d.	0.001	5.5
Spring wheat grain	0.001	2.7	n.d.	n.d.
365 DAT (Pyridine Label)				
Spring wheat hay	n.d.	n.d.	n.d.	n.d.
Spring wheat straw	n.d.	n.d.	n.d.	n.d.

n.d. not detected

In most of the spring wheat matrices analysed, considerable amounts of the radioactive residues were not extractable with methanol and water. The residual radioactive residues after solvent extraction of particular spring wheat matrices were further characterised using an individual combination of sequential solubilization steps applying ammonia treatment, solubilization with macerozyme / cellulase, glucosidase / hesperidinase, tyrosinase / laccase and amylase / amyloglucosidase. The solubilized residues had possibly been associated with or embedded / incorporated in insoluble plant material (e. g. proteins, cell wall polymers and starch). The most effective solubilization steps were the treatment with macerozyme (solubilization of up to 55% TRR) and with aqueous ammonia (up to 14.4% TRR). In the case of spring wheat grain (pyridine label), incubation with amylase also released substantial amounts of radioactivity (about 20% TRR). Fermentation procedures with the supernatants after macerozyme incubation and subsequent distillation yielded ethanol. This indicated an incorporation of ^{14}C-labelled C1 or C2 units derived from imazamox degradation products into plant carbohydrates.

3. Proposed metabolic pathway

The proposed metabolic pathway of imazamox in rotational crops is shown in Figure 6.6.1-1. The key step of the metabolism of imazamox in the matrices of the rotational crop spring wheat is cleavage of the methyl ether group (demethylation) resulting in metabolite M715H001, characterised by a hydroxyl group.

4. Storage stability

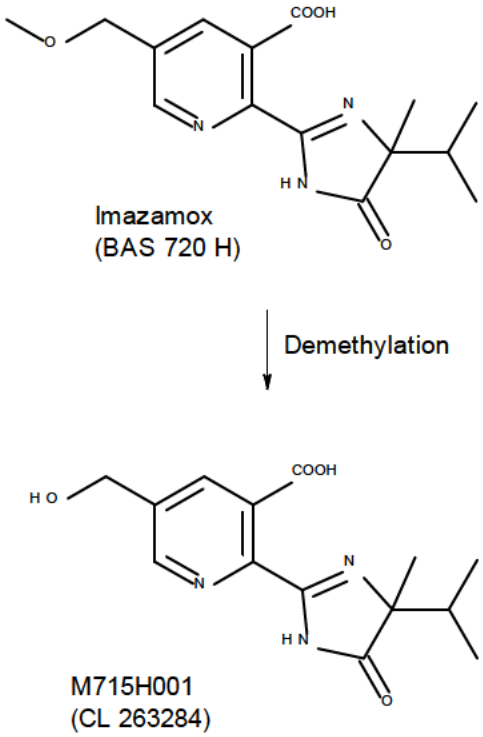
All samples were stored in a freezer at approximately -18°C or below during the course of the study. A comparison of the extractabilities and of the metabolite patterns obtained at the beginning and at the end of the investigation period showed that there was no relevant change in the nature of the radioactive residues of imazamox during storage of the plant samples over the period of investigation. The stability in stored extracts was demonstrated over periods of up to 14 months.

III. CONCLUSION

The metabolism of $^{14}C/^{15}N$-imazamox in confined rotational crops was investigated after spray application of the test item to bare soil at a nominal rate of 75 g a.s./ha. Two differently labelled test items were used in this study. After application, the soil was aged for approximately 30 days (simulating an emergency plant back), 120 days (simulating a fall plant back) and 365 days (one year after treatment). Afterwards, the crops spinach, white radish and spring wheat were sowed.
Low levels of radioactive residues were observed in all rotation crop matrices at all analysed plant back intervals in this study. The total radioactive residues (TRR) in spinach (immature and mature samples) did not exceed 0.008 mg/kg for all plant back intervals. Likewise, the TRR in white radish top and root did not exceed 0.007 mg/kg for all plant back intervals.
Total radioactive residues in spring wheat matrices were generally highest at the shortest plant back interval of 29-31 days. In wheat forage residues were 0.012 mg/kg after 30 d plant back interval and declined to 0.008 mg/kg at the 365 d plant back interval. Residues in hay and straw were mostly at a similar level, declining from 0.072 mg/kg to 0.051 mg/kg (hay) and from 0.072 mg/kg to 0.033 mg/kg (straw) at the 365 d plant back interval, for the pyridine label. For the imidazolinone label, residues in wheat hay ranged from 0.042 mg/kg to 0.002 mg/kg and in straw residues were declining from 0.040 mg/kg to 0.003 mg/kg with a peak value of 0.076 mg/kg after the 120 d plant back interval. Residues in wheat grain constantly declined over time from initially 0.051 mg/kg (at the 30 d plant back interval) down to 0.005 mg/kg (at the 365 d plant back interval).
The residue concentration in the top soil layer after aging and ploughing slightly decreased to the plant back intervals of 31/29 DAT and 119/123 DAT and remained then more or less stable.
HPLC analysis of the partition phases of the methanol extract, water extracts and samples obtained thereof resulted for all spring wheat matrices and plant back intervals in a metabolite pattern with only a few peaks. The parent imazamox was identified in both labels in levels equal or below 0.007 mg/kg.

In addition, the metabolite M715H001 (CL 263284) was identified by co-chromatography analyses in selected rotational crop matrices of the pyridine label in a minor extent (up to 0.002 mg/kg). The metabolite M715H001 is characterised by a hydroxyl group, derived from the cleavage of the methyl ether group (demethylation) of the parent. Furthermore, degradation products in minor concentrations were characterised by their chromatographic properties. These components probably were intermediates in the degradation of imazamox to C1 or C2 units which finally entered the biosynthetic pathway of carbohydrates. The solubilization and characterization of considerable parts of the non-extractable residues by enzymatic cleavage of natural macromolecules indicates a subsequent incorporation of ^{14}C-labelled C1 or C2 units into plant polysaccharides. Additionally, the precipitation of the water extract in spring wheat grain with acetone and subsequent protease treatment indicated that a substantial part of the ^{14}C-labelled C1 or C2 units was incorporated into proteins.
Extracts of spinach and radish matrices were not analysed by HPLC and the residues after solvent extraction not further investigated due to low levels of radioactive residues.

Figure 6.6.1-1: Proposed Metabolic Pathway of Imazamox in Rotational Crops



CA 6.6.2 Magnitude of residues in rotational crops

According to the data requirement as laid down in Commission Regulation (EC) 283/2013, the magnitude of residues in rotational crops has to be investigated if the metabolism studies indicate that residues of the active substance or of relevant metabolites or breakdown products either from plant or soil metabolism may occur (> 0.01 mg/kg).

Since the new confined rotational crop study demonstrated that residues of imazamox and the metabolite CL 263284, the two components constituting the residue definition, are below the LOQ of 0.01 mg/kg in all investigated succeeding crops and also no other single component occurs in amounts above 0.01 mg/kg, the conduct of a field rotational crop study is not considered necessary at this point in time.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

Plant matrices

As the outcome of the previous review of imazamox, in the Monograph compiled by the RMS France the residue definition for edible commodities of plant products was defined as parent imazamox plus the hydroxymethyl metabolite CL 263284 (expressed as imazamox), based on metabolism studies in soybean, peas, oilseed rape and maize.

The three new metabolism studies (in oilseed rape, wheat and paddy rice), partly conducted with radiolabel in the imidazolinone ring, provide the same picture of the metabolic fate of imazamox in plants and are thus congruent with the previous understanding of imazamox metabolism in crops. Metabolism of imazamox has been confirmed to proceed via O-demethylation of the methoxymethyl group to form the hydroxymethyl metabolite (CL 263284) which undergoes further metabolism via oxidation and/or glucose conjugation to yield minor amounts of the dicarboxylic acid metabolite (CL 312622), and the glucose conjugate (CL 189215).
The new confined rotational crop study also supports this metabolic pathway of imazamox in plants with imazamox and metabolite CL 263284 being the only components identified in measurable amounts. Further degradation products in minor concentrations were characterized as possible intermediates of the degradation of imazamox to C1 or C2 units, which finally enter the biosynthetic pathway of carbohydrates/polysaccharides and proteins.
In summary, the most predominant residue besides parent imazamox through all edible plant matrices is the hydroxymethyl metabolite CL 263284, while residue levels of the metabolites CL 189215 and CL 312622 were subordinate and mostly occurred in matrices used as feedstuff.
New residue trials that were analysed for all above mentioned metabolites support this picture.

The following residue definition is therefore proposed for MRL setting and dietary risk assessment:

Sum of parent imazamox + hydroxymethyl metabolite CL 263284 (Reg. No. 4110773), expressed as imazamox equivalents.

The same residue definition for plant matrices was proposed in the 2013 JMPR submission for imazamox.

Animal matrices

Livestock metabolism studies with imazamox as submitted in the previous peer review showed no accumulation of imazamox residues in the edible tissues or eggs of poultry or in the edible tissues or milk of goats. Elimination of imazamox as unchanged parent compound via the excreta is rapid and efficient. Metabolism studies in the rat showed a similar rapid excretion of mainly unchanged parent imazamox.

Since livestock animals may be exposed to residues of the hydroxymethyl metabolite CL 263284, available livestock metabolism studies with this metabolite as well as with CL 312622 were included in this submission. All studies show a similar behaviour as with the parent compound, i.e. rapid and efficient excretion of the mostly unchanged test substance. No separate study is available for the metabolite CL 189215 that might be present in feedstuff as well since this is the O-glucoside of CL 263284. O-glucosides are generally known to be readily cleavable in the gastrointestinal tract of livestock animals yielding then CL 263284 as aglycon in this case. With that in mind, and having seen the rapid excretion and minimal transfer of residues of CL 263284 from feed into animal tissues, it was not deemed necessary to investigate the behaviour of CL 189215 residues in livestock animals separately, but to regard it as covered by the data for CL 263284.

Thus, as livestock animals will be primarily exposed to residues of imazamox, CL 263284 and its glucoside CL 189215 via feedstuff, the following residue definition is proposed for MRL setting and risk assessment purposes:

Sum of parent imazamox + hydroxymethyl metabolite CL 263284 (Reg. No. 4110773), expressed as imazamox equivalents.

The same residue definition for animal matrices was proposed in the 2013 JMPR submission for imazamox.

The exposure of livestock animals to possible residues of CL 189215 via feed is covered in the feedburden calculation and subsequent derivation of MRLs (see chapter 6.7.2.2).

Metabolites

In order to address the relevance of the main plant metabolites CL 263284, CL 189215 and CL 312622, the approach as laid down in the Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, (EFSA Journal 2012;10(07): 2799) was mainly followed. Since the exposure assessment (see MCA, chapter 6.9) showed for all three metabolites to be above the TTC threshold for genotoxicity of 0.0025 µg/kg bw /d, genotoxicity testing is provided for these metabolites in MCA, chapter 5.8. Additionally, since metabolite CL 263284 is the most predominant metabolite in plants and is proposed to be included into the residue definition, a 28-day study in rats was performed with this metabolite as well (see also MCA, chapter 5.8.). The exposure assessment for the three metabolites is found in MCA, chapter 6.9.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The following table shows the existing EU MRLs, the tentative MRLs that have recently been published by EFSA according to Article 12(2) of Regulation (EC) No 396/2005 as well as the MRLs proposed in this dossier.

Code Number	Commodity	Existing EU MRL (mg/kg)	MRL [Art. 12][#] (mg/kg)	MRL Proposed by BASF (mg/kg)
Existing enforcement residue definition: imazamox/ **Proposed residue definition:** sum imazamox parent + CL263284 (in parent equivalents)				
Code Number	**Commodity**	**Existing EU MRL (mg/kg)**	**MRL [Art. 12][#] (mg/kg)**	**MRL Proposed by BASF (mg/kg)**
260010	Beans (with pods)	0.05*	0.05*	-
260040	Peas (without pods)	0.05*	0.05*	-
300010	Beans (dry)	0.05*	0.05*	-
300020	Lentils (dry)	0.05*	0.05*	-
300030	Peas (dry)	0.05*	0.05*	-
401050	Sunflower seed	0.05*	0.05	~~0.3~~[1] 0.2[1]
401060	Rape seed	0.05*	0.05	0.1
401070	Soya bean	0.05*	0.05	0.02
500030	Maize	0.05*	0.05*	-
500060	Rice	0.05*	0.05*	-
1010000	Animal tissue	-	-	0.02
1020000	Milk	-	-	0.02
1030000	Bird eggs	-	-	0.02

according to 'Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for imazamox according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(6):3282,34 pp. doi:10.2903/j.efsa.2013.3282'

1 with the addition of 8 additional sunflower trials (please refer to report 6.3.2/6) a lower MRL proposal compared to the first submission (0.3 mg/kg – January2014) was derived

* indicates the lower limit of analytical determination

MRLs in this dossier are based on residue trials performed after the EU Review of imazamox with application rates according to the current cGAPs. In these more recent magnitude of residue studies not only parent imazamox but also metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) were analysed. MRLs are proposed based on the rounded MRLs of total imazamox (sum of parent imazamox + metabolite CL 263284, expressed as imazamox equivalents) derived with the OECD calculator (OECD calculator spreadsheet: http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html).

The residue data for imazamox in the present dossier was analysed mainly using two different analytical methods (BASF Method No M3519 and L0188/01). M3519 analyses the parent compound imazamox and the hydroxymethyl metabolite CL 263284 with an LOQ of 0.05 mg/kg each while BASF Method No L0188/01 analyses parent imazamox, and metabolites CL 263284 (Reg. No. 4110773), CL 312622 (Reg. No. 4110542) and CL 189215 (Reg. No. 4110445) with an LOQ of 0.01 mg/kg for each analyte, respectively. Thus, according to the residue definition, the LOQ for the sum of imazamox and its metabolite CL 263284 is 0.10 mg/kg and 0.02 mg/kg for the two different methods, respectively. Therefore an overall LOQ of 0.10 mg/kg is considered suitable for all plant commodities. For animal matrices the LOQ of 0.02 mg/kg should apply since the analytical method measures each of the analytes with an LOQ of 0.01 mg/kg.

Therefore it is proposed that:

1. MRL calculations for plants should be done using LOQs of 0.10 mg/kg and 0.02 mg/kg or a combination of both, whichever is available, according to the proposed residue definition for plant and the LOQ of the analytical method.

2. A default MRL of 0.02 mg/kg should apply for all ruminant, swine and poultry products according to the proposed residue definition, LOQ of the analytical method, and no anticipation of residues in animal products above the LOQ.

Rape seed

For rape seed 12 trials were performed in the EU, 6 in the EU North and 6 in the EU South in the period from 2005 to 2012. 8 trials were conducted according to the cGAP (0.035 kg a.s./ha), 4 in the North and 4 in the South. In the other 4 trials (2 in EU North and 2 in EU South), an application rate of 0.05 kg a.s./ha (0.035 kg a.s./ha+43%) was used. However, these worst case trials are considered suitable for MRL derivation, since they contain residues below the respective LOQ of the analytical method. In the trials the following total residues of the parent imazamox and metabolite CL 263284 (hydroxymethyl metabolite) were found at harvest:

<0.02 (4x), <0.10 (2x)	Northern Europe
<0.02 (4x), <0.10 (2x)	Southern Europe

All residues were below the LOQ of the analytical methods applied (0.1 mg/kg and 0.02 mg/kg) and thus the number of trials for each EU region is sufficient for MRL derivation. As all residues were below LOQ, 0.1 mg/kg and 0.02 mg/kg were used for calculation purposes, respectively [see Table 6.7.2-1].

Table 6.7.2-1: MRL calculation for rape seed

OECD Calculator	Total Imazamox [mg/kg]	
	N-EU	S-EU
Highest residue	0.100	0.100
Mean + 4 SD	0.212	0.212
CF x 3 Mean	0.047	0.047
Rounded MRL	**0.1**	**0.1**
STMR	0.02	0.02

The tentative EU MRL was set to 0.05 mg/kg according to the residue definition of parent imazamox only and the residue values used for MRL derivation being all below the LOQ of the respective analytical method (<0.05 mg/kg).

An

EU MRL of **0.1 mg/kg** for **rape seed**

is proposed according to the proposed residue definition (sum of imazamox and metabolite CL 263284) and the present residue data and based on the highest LOQ for total imazamox residues.

Sunflower

For sunflower seed 14 trials were performed in the EU, 6 in the EU North and 8 in the EU South in the period from 2002 to 2012. An additional 8 trials were conducted in 2013. All trials were performed according to the cGAP (0.05 kg a.s./ha±25%). The following total residues of the parent imazamox plus metabolite CL 263284 (hydroxymethyl metabolite) were found at harvest:

0.022, 0.03 (3x), 0.032, 0.038, 0.048 0.08, <0.10 (2x)	Northern Europe
<0.02 (37x), 0.03, <0.10 (4x)	Southern Europe

MRLs were calculated as shown in Table 6.7.2-2. For residues below the respective LOQ, (<0.1 mg/kg or <0.02 mg/kg) 0.1 mg/kg or 0.02 mg/kg were used for calculation purposes, respectively.

Table 6.7.2-2: MRL calculation for sunflower seed

OECD Calculator	Total Imazamox [mg/kg]	
	N-EU	S-EU
Highest residue	0.100	0.100
Mean + 4 SD	0.173	0.203
CF x 3 Mean	0.133	0.055
Rounded MRL	**0.2**	**0.2**
STMR	0.035	0.020

The tentative EU MRL was set to 0.05 mg/kg according to the residue definition of parent imazamox only and according to the LOQ of the respective analytical method (<0.05 mg/kg).

An EU MRL of 0.2 mg/kg was derived for sunflower seed according to the proposed residue definition (sum of imazamox and metabolite CL 263284) and based on the present residue data in support of the critical GAP.

With the data at hand a

EU MRL of **0.2 mg/kg** for sunflower seed is proposed.

Soya bean

For soya bean 9 trials were performed in the EU, 5 in the EU North and 4 in the EU South in the period from 2007 to 2011. All trials were conducted according to the cGAP (0.05 kg a.s./ha ±25%). In the trials the following total residues of the parent imazamox and metabolite CL 263284 (hydroxymethyl metabolite) were found at harvest:

<0.02 (5x)	Northern Europe
<0.02 (4x)	Southern Europe

All residues were below the LOQ of the analytical methods applied (<0.02 mg/kg) and thus the number of trials for each EU region is sufficient for MRL derivation. MRLs were calculated as shown in Table 6.7.2-3. For all residues 0.02 mg/kg was used for calculation purposes, since all values were below the LOQ.

Table 6.7.2-3: MRL calculation for soya bean seed

OECD Calculator	Total Imazamox [mg/kg]	
	N-EU	S-EU
Highest residue	0.020	0.020
Mean + 4 SD	0.020	0.020
CF x 3 Mean	0.020	0.020
Rounded MRL	**0.020**	0.020
STMR	0.020	0.020

The temporary EU MRL was set to 0.05 mg/kg according to the residue definition of parent imazamox only and according to the LOQ of the respective analytical method (<0.05 mg/kg).

An

EU MRL of **0.02 mg/kg** for **soya bean seed**

is proposed according to the proposed residue definition (sum of imazamox and metabolite CL 263284) and based on the available trials and the LOQ for total imazamox.

Alfalfa

For alfalfa 13 trials were performed in the EU in the period from 1996 to 2010 according to the EU cGAP (0.0668 kg a.s./ha±25%, registered with the product Nirvana), which is more critical than the registered GAP for the representative formulation BAS 720 06H (0.050 kg a.s./ha). 8 of these 13 trials also support the 50g/ha use rate for BAS 720 06 H (within the 25% corridor). Since EU MRL derivation should be based on the cGAP, the residue values of all 13 trials were used for MRL calculation. The following total residues of the parent imazamox and metabolite CL 263284 (hydroxymethyl metabolite) were found at harvest:

0.066, 0.113, 0.136 (2x)	Green matter	Northern Europe
0.054, 0.056, 0.136	Hay	
0.024, 0.03 (2x), 0.035, <0.10, <0.20 (3x), 0.37	Green matter	Southern Europe
<0.10, <0.20 (3x)	Hay	

MRL calculation was performed considering residue values for both green matter and hay (see Table 6.7.2-4).

Table 6.7.2-4: MRL calculation for alfalfa green matter and hay

OECD Calculator	Total Imazamox [mg/kg]	
	Green Matter	**Hay**
Highest residue	0.370	0.200
Mean + 4 SD	0.522	0.402
CF x 3 Mean	0.320	0.251
Rounded MRL	**0.6**	**0.4**
STMR	0.113	0.136

EU MRLs for feedstuffs have not been set in the past but will be needed in the future. Thus pseudo-MRLs are proposed here for alfalfa green matter and hay. Due to the comparability of the data set and in order to improve the statistical evaluation, data sets for the EU North and South were combined and evaluated together.

It is proposed to establish

a pseudo EU MRL of **0.6 mg/kg** for **alfalfa green matter**

and

a pseudo EU MRL of **0.4 mg/kg for alfalfa hay**

based proposed residue definition (sum of imazamox and metabolite CL 263284).

Animal matrices

Estimation of residues in livestock feed

A worst case diet was derived for different livestock species with the EFSA dietary burden calculator (based on Appendix G of the Lundehn guidance document 7031/VI/95 rev.4, July 1996). From each group of crops/commodities the item with the highest potential residue contribution on a dry matter basis is chosen. Then, the total diet is composed beginning with the group representing the highest contribution and filling the rest with feed from the other groups in descending order.
The evaluation is based on the following formula:

$$\text{Uptake [mg/kg bw/day]} = \frac{\text{Total intake of dry matter [kg/ animal/day]} \times \text{\% of diet} \times \text{Residue in feed item [mg/kg]}}{\text{Dry matter content of feed item [\%]} \times \text{Bodyweight [kg]}}$$

The following tables show the calculations of the maximum dietary burden for each relevant livestock species, which are based on the highest or median residue levels of imazamox residues, depending on the commodity. These maximum dietary burdens are then used to derive suitable MRLs for products of animal origin.

Since livestock animals may be exposed to residues of imazamox and the plant metabolites CL 263284 and its glucoside CL 189215, and since separate livestock metabolism studies were conducted for both parent and CL 263284, separate feedburden calculations were carried out for parent imazamox on the one hand and for the sum of CL 263284 plus CL 189215. Measured residues of CL 189215 were converted into CL 263284-equivalents and then the sum of both metabolites was considered. In the cases where the metabolite CL 189215 has not been analysed, a crop specific factor (derived from metabolism studies) is applied in order to ensure the derivation of a worst case feedburden.
The two estimated maximum feedburdens (one for parent and one for the sum of CL 263284 plus CL 189215) were then compared to the dose levels of the respective metabolism studies with imazamox and CL 263284 in goats and hens and expected residues in animal tissues, milk and eggs were extrapolated for deriving suitable MRL proposals.

The tables below summarise the residue values used for the dietary burden calculations for parent imazamox as well as CL 263284 and their origin. Following the usual practice, highest residues (HR) were used as input values for forages and median residues (STMR / $STMR_P$) for bulk or processed commodities.

Table 6.7.2-5: Residue values used for calculation of the feedburden for parent imazamox

Crop	STMR/ $STMR_P$ [mg/kg]	HR / HR_P [mg/kg]	Origin
Alfalfa (fresh)	-	0.10	HR from all alfalfa trials (cGAP) in EU (=LOQ of older trials)
Alfalfa silage	-	0.10	
Alfalfa hay	-	0.10	
Rape forage	-	0.19	Value rest of plant at 12d PHI (coming from trial AF/10475/BA/3, DocID 2007/1007963)*
Rape seed	0.05#	-	Based on all oilseed rape trials in EU (=LOQ of older trials)
Rape seed meal	0.10	-	STMR seed x $PF_{default}$ 2
Sunflower seed	0.05#Δ	-	Based on all sunflower trials in EU (=LOQ of older trials)
Sunflower seed meal	0.11Δ	-	STMR x $PF_{measured}$ 2.2 **
Soya bean seed	0.01#	-	Based on all soya bean trials in EU (=LOQ)
Soya bean meal	0.013	-	STMR x $PF_{default}$ 1.3

LOQ
*No data from PHI 0days was considered
** Mean processing factor as derived from three studies see, chapter 6.5
ΔThe additional trials from study report 6.3.2/6 lower the STMR value calculated for the EU North and South to 0.035 and 0.02 total imazamox in mg/kg. Thus the following feedburden calculation covers also the additional sunflower residue values.

Table 6.7.2-6: Residue values used for calculation of the feedburden for CL 263284 (sum of CL 263284 and CL 189215) [1)]

Crop	STMR/ STMR$_P$ [mg/kg]	HR / HR$_P$ [mg/kg]	Origin
Alfalfa (fresh)	-	0.44	Value for whole plant PHI 7 days, from trial 97-805-04 (Doc ID-731-002), sum of CL263284+CL189215 in CL263284 equivalents
Alfalfa silage	-	0.44	
Alfalfa hay	-	0.36	Sum of CL 263284-value for hay PHI 34 d from trial RE10011 (DocID 2013/1211060) + CL 263284-value x Factor 2 [2)]
Rape forage	-	0.51	Sum of CL 263284-value for rest of plant PHI 13d from trial AF/10475/BA/3 (DocID 2007/107963) + CL 263284-value x Factor 0.07 [3)]
Rape seed	0.02	-	Based on all oilseed rape trials; where CL 189215 was not measured a factor of 1 was applied[4)] to the CL 263284 values to derive the sum; residues of CL 263284 were all <LOQ
Rape seed meal	0.04	-	STMR x PF $_{default}$ 2
Sunflower seed	0.11[Δ]	-	Based on all sunflower trials; where CL 189215 was not measured a factor of 2.25 was applied[5)] to the CL 263284 values to derive the sum and then the median value was determined
Sunflower seed meal	0.24[Δ]	-	STMR x PF$_{measured}$ 2.2*
Soya bean seed	0.04	-	Based on all soya bean trials; where CL 189215 was not measured a factor of 3 was applied[6)] to the CL 263284 values (which were <0.01 mg/kg) to derive the sum and then the median value was determined
Soya bean meal	0.05	-	STMR x PF $_{default}$ 1.3

1) Conversion factor for CL189215 to CL263284 is 0.642425
2) since CL189215 was not measured a factor of 2 (from metabolism study) was applied to CL263284
3) since CL189215 was not measured in the trial with the highest residue of CL 263284, a factor of 0.07 (from metabolism studies and recent residue trials) was applied to CL263284
4) the factor for oilseed rape seed of 1 was derived from the recent residue trials that were analysed for both CL 263284 and CL 189215; in all trials residues of both analytes were always <0.01 mg/kg
5) the factor for sunflower seed of 2.25 was derived from recent residue trials that were analysed for both CL 263284 and CL 189215;
6) the factor for soybean seed of 3 was derived from recent residue trials that were analysed for both CL 263284 and CL 189215;
* Mean processing factor as derived from three studies see, chapter 6.5
ΔThe additional trials from study report 6.3.2/6 lower the STMR value calculated for the EU North and South to 0.035 and 0.02 total imazamox in mg/kg. Thus the following feedburden calculation covers also the additional sunflower residue values.

For cattle, poultry and pigs calculations were done with the relevant residues (parent imazamox or sum CL 263284 + CL189215 expressed as CL263284 equivalents, see Table 6.7.2-5 and Table 6.7.2-6) and the results are shown in Table 6.7.2-7 to Table 6.7.2-10.

Table 6.7.2-7: Estimated maximum dietary burden of imazamox[1] residues for cattle

Crop	Dry Matter Content (%)	Residue Level[2] (HR/STMR mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Alfalfa silage	20	0.10	100	0.018	100	0.021
Dietary burden:	mg/kg bw/day		0.018		0.021	
	mg/animal/day		9.9		7.4	
	mg/kg total feed (DM)		0.50		0.50	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg
1 parent imazamox
2 for derivation of residue values see Table 6.7.2-5

Table 6.7.2-8: Estimated maximum dietary burden of imazamox[1] residues for poultry and pigs

Crop	Dry Matter Content (%)	Residue Level[2] (HR/STMR mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Rape forage	15	0.19	-	-	15	0.00054
VI Oil seed						
Sunflower seed meal	86	0.11[Δ]	10	0.000081	20	0.000051
Dietary burden:	mg/kg bw/day		0.00081		0.0092	
	mg/animal/day		0.002		0.69	
	mg/kg total feed (DM)		0.013		0.23	

* feed intake 0.120 kg DM, body weight (bw) 1.9kg
** feed intake 3 kg DM, body weight (bw) 75 kg
1 parent imazamox
2 for derivation of residue values see Table 6.7.2-5
ΔThe additional trials from study report 6.3.2/6 lower the STMR value calculated for the EU North and South to 0.035 and 0.02 total imazamox in mg/kg. Thus the feedburden calculation covers also the additional sunflower residue values.

Table 6.7.2-9: Estimated maximum dietary burden of CL 263284[1] residues for cattle

Crop	Dry Matter Content (%)	Residue Level[2] (HR/STMR mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Alfalfa silage	20	0.44	100	0.080	100	0.00094
Dietary burden:	mg/kg bw/day		0.08		0.094	
	mg/animal/day		44.0		32.9	
	mg/kg total feed (DM)		2.20		2.20	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg
1 considers sum of CL 263284 + CL 189215, calculated as CL 263284-equivalents
2 for derivation of residue values see Table 6.7.2-6

Table 6.7.2-10: Estimated maximum dietary burden of CL 263284[1] residues for poultry and pigs

Crop	Dry matter content (%)	Residue Level (HR/STMR mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Rape forage	15	0.51	-	-	15	0.022
VI Oil seed						
Sunflower seed meal	86	0.11[Δ]	10	0.001763	20	0.0022
Dietary burden:	mg/kg bw/day		0.0018		0.024	
	mg/animal/day		0.0034		1.80	
	mg/kg total feed (DM)		0.03		0.60	

** feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg
1 considers sum of CL 263284 + CL 189215, calculated as CL 263284-equivalents
2 for derivation of residue values see Table 6.7.2-6
ΔThe additional trials from study report 6.3.2/6 lower the STMR value calculated for the EU North and South to 0.035 and 0.02 total imazamox in mg/kg. Thus the feedburden calculation covers also the additional sunflower residue values.

Thus, the doses to be used when estimating the maximum residues in products of animal origin are:

for parent imazamox residues

dairy cattle	0.018 mg/kg bw/d (0.50 mg/kg DM)
beef cattle	0.021 mg/kg bw/d (0.50 mg/kg DM)
poultry	0.00081 mg/kg bw/d (0.013 mg/kg DM)
pigs	0.0092 mg/kg bw/d (0.23 mg/kg DM)

for CL 263284-derived residues

dairy cattle	0.080 mg/kg bw/d (2.20 mg/kg DM)
beef cattle	0.094 mg/kg bw/d (2.20 mg/kg DM)
poultry	0.0018 mg/kg bw/d (0.03 mg/kg DM)
pigs	0.024 mg/kg bw/d (0.60 mg/kg DM)

It should be noted that the doses assume that the diet completely consists of plant material which had been treated with imazamox considering worst case assumptions such as intake of the highest residue (HR).

Cattle products

Parent imazamox

No feeding study with Imazamox in ruminants is available and none is required as residues of ruminant products are predicted to be below the LOQ (0.01 mg/kg) at the estimated maximum feedburden based on the residue levels observed in the respective goat metabolism study with imazamox (for justification see chapter 6.4).

In a metabolism study in goats, animals were dosed with imazamox at actual dose levels of 2.08 or 11.6 mg/kg diet (see chapter 6.2.3). The lower dose level used in the metabolism study is four times greater than the feedburden for imazamox parent of 0.50 mg/kg feed DM (0.021 mg/kg bw/d) calculated for beef cattle (see Table 6.7.2-7). The highest dose level was about 23 times the anticipated feedburden. No residues of imazamox at or above the LOQ of 0.01 mg/kg were detected in goat milk, blood, liver, muscle and fat analyzed during the metabolism study. Only in kidney, imazamox residues of 0.02 and 0.06 mg/kg were found for the low and high dose, respectively. Adjusting the residues in kidney by the respective factors (x0.25 for lower dose level and x0.04 for higher dose level) results in anticipated residues of <0.01 mg/kg for the two dosing levels. Thus, taking into account all worst case conditions (highest residues in the RAC and maximum dietary burden for cattle) the anticipated residues of imazamox in all ruminant matrices is expected to be below the LOQ of <0.01 mg/kg in all tissues, milk and blood.

CL 263284

In a separate metabolism study goats were dosed with the hydroxyl metabolite CL 263284 at actual dose levels of 2.33 or 14.5 mg/kg feed. No residues of the metabolite at or above the LOQ (0.01 mg/kg) were found in milk and tissues, except for CL 263284 in liver (0.03 mg/kg) at the high dose level (see chapter 6.2.3).

The feedburden of 2.20 mg CL 263284/kg feed DM (0.094 mg/kg bw/d) calculated for beef cattle (considering the sum of CL 263284 and CL 189215 residues, expressed as CL 263284; see Table 6.7.2-9) approximately matches the low dosing level used in the metabolism study and no residues of CL 263284 at or above the LOQ (0.01 mg/kg) were detected in any goat matrix (milk, blood, liver, kidney, muscle and fat) analyzed during the metabolism study.

No residues above the LOQ (<0.01 mg/kg) are thus expected for CL 263284.

Thus, it can be stated that for the sum of imazamox and metabolite CL 263284 (=residue definition for MRL setting in animal matrices), no residues above the LOQ (<0.02 mg/kg) are expected for cattle products and milk.

Poultry products
A feeding study in poultry is not required as the estimated maximum feedburden (seeTable 6.7.2-8 and Table 6.7.2-10) for poultry is below the current trigger of <0.004 mg/kg bw/day and based on the metabolism studies in laying hens, residues in poultry products are predicted to be below 0.01 mg/kg for both imazamox and metabolite CL 263284.

Parent imazamox
In a metabolism study in poultry, animals were dosed with imazamox at 2.11 or 10.2 mg/kg diet (part of the previous submission, not included in this dossier). The dose levels used in the metabolism study represent a considerable overdosing with regard to the highest feedburden of 0.013 mg/kg feed DM calculated for poultry (see Table 6.7.2-8). No residues of imazamox at or above the LOQ of 0.01 mg/kg were detected in poultry eggs and edible tissues (skin with adhering fat, muscle, liver, kidney and blood) analyzed during the metabolism study.

CL 263284
In a separate metabolism study animals were dosed with hydroxyl metabolite CL 263284 (2.14 or 10.9 mg/kg feed). Again, the estimated maximum feedburden in poultry for the sum of CL 263284 and CL 189215, expressed as CL 263284 (0.03 mg/kg feed), is much lower than the low dose used in the metabolism study, and no residues of the metabolites at or above the LOQ (0.01 mg/kg) were found in eggs and tissues.

Thus, it can be stated that for the sum of imazamox and metabolite CL 263284 (=residue definition for MRL setting in animal matrices), no residues above the LOQ (<0.02 mg/kg) are expected for poultry products and eggs.

Pig products
A feeding study in pigs is only required, if the metabolic pathways differ significantly in rats as compared to ruminants. Since the metabolism of imazamox in rat and ruminants is similar, no separate metabolism study for pigs has been conducted and extrapolation is based on the metabolism studies with ruminants.

Parent imazamox
In a metabolism study in goats, animals were dosed with imazamox at 2.08 or 11.6 mg/kg diet (see chapter 6.2.3). The dose levels used in the goat metabolism study represent a considerable overdosing (9 to 50 times) with regard to the maximum anticipated feed burden for pigs of 0.23 mg/kg feed DM for imazamox (see Table 6.7.2-8).
No residues of imazamox at or above the LOQ of 0.01 mg/kg were detected in goat milk, blood, liver, muscle and fat analyzed during the metabolism study. Only in kidney, imazamox residues of 0.02 and 0.06 mg/kg were found for the low and high dose, respectively. Adjusting the residues in kidney by the respective factors (x0.11 for lower dose level and x0.02 for higher dose level) results in anticipated residues of <0.01 mg/kg for both dose levels. Thus, taking into account all worst case conditions (highest residues in the RAC and maximum dietary burden for pigs) the anticipated imazamox residue in all pig matrices is expected to be below the LOQ of <0.01 mg/kg.

Metabolite CL 263284

In a separate metabolism study animals were dosed with the hydroxyl metabolite CL 263284 (2.33 or 14.5 mg/kg feed). No residues of the metabolite at or above the LOQ (0.01 mg/kg) were found in milk and tissues, except in liver (0.03 mg/kg) at the high dose level (see chapter 6.2.3). The dose levels used in the goat metabolism study represent a considerable overdosing (4 to 24 times) with regard to the maximum anticipated feed burden of 0.6 mg/kg feed DM for CL 263284, calculated for pigs (see Table 6.7.2-10).
Adjusting the CL 263284 in liver by the respective factor (x0.04 for high dose level) results in anticipated residues below the LOQ (<0.01 mg/kg) for CL 263284.

Thus, it can be stated that for the sum of imazamox and metabolite CL 263284 (=residue definition for MRL setting in animal matrices), no residues above the LOQ (<0.02 mg/kg) are expected for pig products.

The additionally presented 8 sunflower trials (please refer to report 6.3.2/6) are covered by the already presented feed burden calculations and do thus not alter the MRL proposals for animal matrices. The following MRLs in animal products are proposed:

0.02 mg/kg for meat, fat , liver, kidney, edible offals and other products for swine, sheep, cattle, goats, horses, poultry and other farm animals

0.02 mg/kg for milk and cream of all species

0.02 mg/kg for bird’s eggs

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

Plant Products

According to the proposed residue definition for MRL setting of

Sum parent imazamox + CL263284 expressed as parent equivalents;

and referring to MRL derivations in chapter CA 6.7.2.1 it is proposed to establish EU MRLs of:

0.10 mg/kg for rape seed
0.02 mg/kg for soya bean seed
0.20 mg/kg for sunflower seed (updated MRL proposal including 8 additional sunflower trials)
0.60 mg/kg for alfalfa forage (pseudo MRL)
0.40 mg/kg for alfalfa hay (pseudo MRL)

Animal products

According to the proposed residue definition for MRL setting of

Sum parent imazamox + CL263284 expressed as parent equivalents;

and referring to MRL derivations in chapter CA 6.7.2.2 it is proposed to establish EU MRLs of:

0.02 mg/kg for all animal tissues, bird's eggs and milk/cream.

The proposed MRL is at the LOQ of the analytical method for the sum of imazamox and metabolite CL 263284, expressed as imazamox.

CA 6.8 Proposed safety intervals

Imazamox is being recommended for post emergence use of the crop (BBCH 10-18 for sunflower, soya bean and alfalfa and BBCH 30-50 for oilseed rape) the timing of use is governed by the growth stage of the crop and weeds and is not in relation to the time of harvest. Residue trials have been conducted with applications made at the latest recommended crop growth stage with harvest taking place at the time of crop maturity following good agricultural practice.

Pre-harvest interval
In sunflower, soya bean and alfalfa one post-emergence application at the growth stage BBCH 10-18 and in oilseed rape at BBCH 30-50 is intended, with the pre-harvest interval being fixed by the conditions of use.

Re-entry period for livestock to areas to be grazed
Because imazamox is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

Re-entry period for man to treated crops
Imazamox will be applied pre-emergence or early post-emergence to oilseed rape, sunflower, soya bean and alfalfa. Because of the plant size at the growth stages for the intended use patterns, most of the active substance will be applied to soil and interception by crop foliage is minimal. Also, the result of the risk assessments indicate that re-entry of treated field crops is possible after the spray solution has dried. The respective assessments are detailed under MCP Chapter 7.2.3 in the supplemental Dossiers for the representative formulations BAS 720 06 H and BAS 831 00 H.

Witholding period for animal feed stuffs
Treated alfalfa, oilseed rape, sunflower and soya bean may be used as fodder for livestock. Imazamox derived residues in those feed items are assessed in this dossier by providing updated calculations of livestock dietary burdens and deriving suitable MRL for animal products covering the intended uses. There is no additional withholding period needed for animal feeds with regard to imazamox derived residues.

Waiting period between application and crop sowing or planting the crop to be protected
No waiting period is necessary since imazamox containing products are intended for both pre-emergence and post emergence uses.

Waiting period between application and handling treated produce
This is not relevant here since a post-harvest treatment is not intended.

Waiting period between last application and sowing or planting succeeding crops
No waiting period is necessary. Due to the fact that no relevant accumulation of Imazamox or its degradation products were observed in the confined rotational crop study (see document MCA, Chapter 6.6), no limitation concerning the succeeding crops is necessary.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

CA 6.9.1 Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI calculations

No new TMDI calculations are necessary through the addition of 8 sunflower trials (please refer to report 6.3.2/6) to this update as the residues as well as the resulting MRLs are covered by the dietary assessments already submitted in the original dossier.

The MRL values used in the dietary risk assessments in this chapter are listed in Table 6.9.1-1 below.

The chronic consumer risk assessment for imazamox was performed using the EU MRLs proposed in the 'Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for imazamox according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(6):3282,34 pp. doi:10.2903/j.efsa.2013.3282'. In addition, new MRLs for oilseed rape, sunflower and soya bean seed as well as for animal products proposed in this dossier are used in the risk assessment (see Table 6.9.1-1). For all crops listed in the residues section of this dossier MRLs have been derived on the basis of the conducted residue trials. MRL values for animal products are based on extrapolation from the residue levels as observed in metabolism studies on goats and laying hens at the theoretical worst case feed burden calculations (see chapters 6.7.2.2). For the chronic risk assessment, the ADI of Imazamox of 9 mg/kg bw/d (as derived in the last peer review process and laid down in the European Commission Review Report for the active substance Imazamox SANCO/4325/2000 –Final, 29 November 2002) was applied.

Additionally, an exposure assessment was performed for the three main plant metabolites of imazamox, CL 263284, CL 189215 and CL 312622 and compared to the Cramer Class III threshold value of 1.5 µg/kg bw/d according the TTC concept.
The residue values used for the individual exposure assessments are shown in Table 6.9.1-2, Table 6.9.1-3 and Table 6.9.1-4.

Table 6.9.1-1: Existing and proposed maximum residue levels for imazamox in the EU

Code Number	Commodity	Existing EU MRL (mg/kg)	MRL [Art. 12][#] (mg/kg)	MRL Proposed by BASF (mg/kg)
Existing enforcement residue definition: imazamox/ **Proposed residue definition:** sum imazamox parent + CL263284 (in parent equivalents)				
Code Number	**Commodity**	**Existing EU MRL (mg/kg)**	**MRL [Art. 12][#] (mg/kg)**	**MRL Proposed by BASF (mg/kg)**
260010	Beans (with pods)	0.05*	0.05*	-
260040	Peas (without pods)	0.05*	0.05*	-
300010	Beans (dry)	0.05*	0.05*	-
300020	Lentils (dry)	0.05*	0.05*	-
300030	Peas (dry)	0.05*	0.05*	-
401050	Sunflower seed	0.05*	0.05	~~0.3~~[1] 0.2[1]
401060	Rape seed	0.05*	0.05	0.1
401070	Soya bean	0.05*	0.05	0.02
500030	Maize	0.05*	0.05*	-
500060	Rice	0.05*	0.05*	-
1010000	Animal tissue	-	-	0.02
1020000	Milk	-	-	0.02
1030000	Bird eggs	-	-	0.02

according to 'Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for imazamox according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(6):3282,34 pp. doi:10.2903/j.efsa.2013.3282'

1 with the addition of 8 additional sunflower trials (please refer to report 6.3.2/6) a lower MRL proposal compared to the first submission (0.3 mg/kg) was derived

* indicates the lower limit of analytical determination

For the exposure assessment of metabolites according to the TTC concept, all relevant supported uses were considered and with the selected residue levels a worst case approach was followed.

Table 6.9.1-2: Residue values used for the exposure assessment of CL 263284 according to the TTC concept

Crop(group)	CL 263284 input values	Remarks
Legumes, fresh	0.05	LOQ of old trials, new results do not exceed this
Pulses	0.05	LOQ of old trials, new results do not exceed this
Sunflower	0.063Δ	HR of new trials (N EU with 720 AM H)
Rapeseed	0.01	STMR of all available trials
Soybean	0.01	STMR of all available trials
Rice	0.05	HR of new trials, single application (STMR 0.03)
Animal products	0.03 (for kidney + edible offal, except poultry)	HR from goat metabolism study with CL 263284 at high dose level (14.15 mg/kg feed) as a worst case; realistic maximum feedburden much lower
	0.01 (for all other animal products)	Values from goat and hen metabolism studies with CL 263284; all values <0.01 mg/kg

Max: 36.8% of 0.0015 mg/kg bw/d = 0.552 µg/kg bw/d
Δ Value not altered by the additionally submitted sunflower trials in the present version of the dossier (please refer to report 6.3.2/6)

Table 6.9.1-3: Residue values used for the exposure assessment of CL 189215 according to the TTC concept

Crop(group)	CL 189215 input values	Remarks
Legumes, fresh	0.03	HR of all available new trials (n=22) 0.03; STMR 0.01
Pulses	0.01	HR of all available new trials (n=17) 0.01, STMR 0.01
Sunflower	0.13Δ	HR of new trials (STMR 0.025)
Rapeseed	0.01	HR/STMR of new trials (n=8)
Soybean	0.04	HR of new trials, and parent values of old trials (<0.01) x factor of 4 (derived from new trials parent:CL189215)
Rice	0.03	HR of new trials, single application (STMR 0.02)
Animal products	0.03 (for kidney + edible offal, except poultry) 0.01 (for all other animal products)	Values from metabolism studies with CL 263284, cleavage of glucoside in the GIT assumed

Max: 34.2% of 0.0015 mg/kg bw/d = 0.513 µg/kg bw/d
Δ Value not altered by the additionally submitted sunflower trials in the present version of the dossier (please refer to report 6.3.2/6)

Table 6.9.1-4: Residue values used for the exposure assessment of CL 312622 according to the TTC concept

Crop(group)	CL 312622 input values	Remarks
Legumes, fresh	0.01	STMR of all available new trials (n=22);
Pulses	0.01	STMR of all available new trials (n=17)
Sunflower	0.01Δ	HR/STMR of new trials
Rapeseed	0.01	HR/STMR of new trials (n=8)
Soybean	0.01	HR/STMR of new trials and parent values of old trials (<0.01) x factor of 1 (derived from new trials, parent:CL 312622)
Rice	0.01	STMR of new trials, single application
Animal products	0.025 (for kidney + edible offal, except poultry)	HR from goat metabolism study with CL 312622 at high dose level (30 mg/kg feed) as a worst case; realistic maximum feedburden much lower
	0.01 (for all other animal products)	Values from goat and hen metabolism studies with CL 312622; all values <0.01 mg/kg

Max: 19.0% of 0.0015 mg/kg bw/d = 0.285 µg/kg bw/d

Δ Value not altered by the additionally submitted sunflower trials in the present version of the dossier (please refer to report 6.3.2/6)

Table 6.9.1-5: TMDI calculation for total imazamox with PRIMo Model (rev 2.0) using MRLs according to Art (12) evaluation from 2013 and MRLs proposed by BASF

Imazamox
(sum imazamox + CL 263284 in parent equivalents)

Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.02	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**9**	ARfD (mg/kg bw):	**n.n.**
Source of ADI:	**EFSA**	Source of ARfD:	
Year of evaluation:	**2013**	Year of evaluation:	

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum	
	0.000	0.000
No of diets exceeding ADI	---	0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
0.011	FR toddler	0.009	Milk and cream,	0.001	Beans (with pods)	0.001	Meat, preparations of meat,	0.010
0.011	UK Infant	0.009	Milk and cream,	0.001	Maize	0.000	Meat, preparations of meat,	0.009
0.008	NL child	0.007	Milk and cream,	0.001	Meat, preparations of meat, offals,	0.000	Beans (with pods)	0.007
0.007	FR infant	0.006	Milk and cream,	0.000	Beans (with pods)	0.000	Meat, preparations of meat,	0.006
0.006	WHO Cluster diet B	0.002	Sunflower seed	0.001	Maize	0.001	Meat, preparations of meat,	0.002
0.006	UK Toddler	0.005	Milk and cream,	0.000	Meat, preparations of meat, offals,	0.000	Beans	0.005
0.005	ES child	0.003	Milk and cream,	0.001	Meat, preparations of meat, offals,	0.000	Sunflower seed	0.004
0.004	DE child	0.003	Milk and cream,	0.000	Meat, preparations of meat, offals,	0.000	Sunflower seed	0.004
0.004	WHO cluster diet E	0.001	Sunflower seed	0.001	Milk and cream	0.001	Rape seed	0.002
0.004	SE general population 90th percentile	0.003	Milk and cream,	0.001	Meat, preparations of meat, offals,	0.000	Rice	0.004
0.004	WHO cluster diet D	0.002	Sunflower seed	0.001	Milk and cream,	0.000	Meat, preparations of meat,	0.002
0.004	IE adult	0.001	Maize	0.001	Sunflower seed	0.001	Meat, preparations of meat,	0.001
0.004	DK child	0.003	Milk and cream,	0.001	Meat, preparations of meat, offals,	0.000	Birds' eggs	0.004
0.003	WHO regional European diet	0.001	Milk and cream,	0.001	Meat, preparations of meat, offals,	0.000	Sunflower seed	0.002
0.003	WHO Cluster diet F	0.001	Milk and cream,	0.001	Meat, preparations of meat, offals,	0.000	Rape seed	0.002
0.003	ES adult	0.001	Milk and cream,	0.001	Meat, preparations of meat, offals,	0.000	Sunflower seed	0.002
0.002	NL general	0.001	Milk and cream,	0.000	Meat, preparations of meat, offals,	0.000	Beans (with pods)	0.002
0.002	FR all population	0.001	Sunflower seed	0.001	Milk and cream,	0.000	Meat, preparations of meat,	0.001
0.002	PT General population	0.001	Sunflower seed	0.000	Rice	0.000	Maize	0.000
0.002	DK adult	0.001	Milk and cream,	0.000	Meat, preparations of meat, offals,	0.000	Birds' eggs	0.002
0.002	FI adult	0.001	Milk and cream,	0.000	Meat, preparations of meat, offals,	0.000	Rice	0.002
0.002	LT adult	0.001	Milk and cream,	0.000	Meat, preparations of meat, offals,	0.000	Sunflower seed	0.001
0.001	UK Adult	0.001	Milk and cream,	0.000	Meat, preparations of meat, offals,	0.000	Rice	0.001
0.001	UK vegetarian	0.001	Milk and cream,	0.000	Rice	0.000	Beans	0.001
0.000	IT kids/toddler	0.000	Rice	0.000	Peas (without pods)	0.000	Sunflower seed	0.000
0.000	IT adult	0.000	Rice	0.000	Beans (with pods)	0.000	Peas (without pods)	0.000
0.000	PL general population	0.000	Beans (without pods)	0.000	Sunflower seed	0.000	Beans	0 0

Table 6.9.1-6: Exposure assessment for metabolite CL 263284 with PRIMo Model (rev 2.0) against the Cramer Class III threshold of 0.0015 mg/kg according to the TTC concept

CL 263284			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.0015**	ARfD (mg/kg bw):	
Source of ADI:	**TTC**	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).

The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum	
	0	37
No of diets exceeding ADI	---	0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
36.8	FR toddler	26.4	Milk and cream,	4.8	Legume vegetables (fresh)	1 2	Rice	0 0
32.9	UK Infant	25.8	Milk and cream,	2.1	Rice	2.1	Legume vegetables (fresh)	0 0
27.3	NL child	19.5	Milk and cream,	3.2	Legume vegetables (fresh)	1 2	Rice	0 0
23.3	FR infant	17.2	Milk and cream,	3.7	Legume vegetables (fresh)	0 9	PULSES, DRY	0 0
20.5	UK Toddler	13.8	Milk and cream	2.7	PULSES DRY	1 9	Rice	0 0
16.5	ES child	8.3	Milk and cream,	1.6	Rice	1 6	PULSES, DRY	0 0
13.5	WHO Cluster diet B	3.1	Sunflower seed	2.2	Legume vegetables (fresh)	2.1	Milk and cream,	0 0
13.4	DE child	9.5	Milk and cream,	0.9	Legume vegetables (fresh)	0 9	Rice	0 0
11.3	SE general population 90th percentile	8.3	Milk and cream,	1.3	Rice	1.1	Legume vegetables (fresh)	0 0
10.6	WHO cluster diet E	2.8	Legume vegetables (fresh)	2.0	Milk and cream,	1 5	Sunflower seed	0 0
10.5	WHO regional European diet	3.2	Milk and cream,	2.1	Legume vegetables (fresh)	0 8	Swine: Meat	0 0
10.0	WHO cluster diet D	3.4	Milk and cream,	2.1	Sunflower seed	1 8	Rice	0 0
9.8	DK child	8.4	Milk and cream,	0.6	Birds' eggs	0.4	Poultry -chicken, geese, duck,	0 0
9.8	IE adult	2.2	Legume vegetables (fresh)	1.9	Milk and cream,	1 8	PULSES, DRY	0 0
8.5	NL general	4.4	Milk and cream,	1.6	Legume vegetables (fresh)	0 6	Swine: Meat	0 0
8.3	ES adult	3.3	Milk and cream,	1.1	Legume vegetables (fresh)	0 8	Rice	0 0
7.5	WHO Cluster diet F	2.6	Milk and cream,	0.8	Swine: Meat	0.7	Rice	0 0
6.2	PT General population	2.6	Rice	1.5	Legume vegetables (fresh)	1 2	Sunflower seed	0 0
5.8	UK vegetarian	2.2	Milk and cream,	1.4	PULSES, DRY	1 3	Rice	0 0
5.5	FR all population	1.8	Milk and cream,	1.4	Sunflower seed	0 5	Legume vegetables (fresh)	0 0
5.2	UK Adult	2.0	Milk and cream,	1.2	Rice	0 8	PULSES, DRY	0 0
5.1	DK adult	3.6	Milk and cream	0.4	Legume vegetables (fresh)	0.4	Bovine: Meat	0 0
5.1	LT adult	2.6	Milk and cream,	0.7	Rice	0 6	Swine: Meat	0 0
4.8	FI adult	3.8	Milk and cream,	0.4	Rice	0 2	Poultry -chicken, geese, duck,	0 0
1.9	IT kids/toddler	0.7	Legume vegetables (fresh)	0.6	Rice	0 5	PULSES, DRY	0 0
1.8	IT adult	0.8	Legume vegetables (fresh)	0.6	Rice	0 3	PULSES, DRY	0 0
0.4	PL general population	0.2	Legume vegetables (fresh)	0.2	PULSES, DRY	0 0	Sunflower seed	0 0

Table 6.9.1-7: Exposure assessment for metabolite CL 189215 with PRIMo Model (rev 2.0) against the Cramer Class III threshold of 0.0015 mg/kg according to the TTC concept

CL 189215			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.0015**	ARfD (mg/kg bw):	
Source of ADI:	**TTC**	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.
The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum	
	0	34
No of diets exceeding ADI	---	0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
34.2	FR toddler	26.4	Milk and cream,	2.9	Legume vegetables (fresh)	1.4	Sunflower seed	0 0
29.9	UK Infant	25.8	Milk and cream,	1.3	Rice	1 2	Legume vegetables (fresh)	0 0
25.4	NL child	19.5	Milk and cream,	1.9	Legume vegetables (fresh)	1 0	Swine: Meat	0 0
21.1	FR infant	17.2	Milk and cream,	2.2	Legume vegetables (fresh)	0.4	Bovine: Meat	0 0
17.1	UK Toddler	13.8	Milk and cream	1.2	Rice	0.7	Legume vegetables (fresh)	0 0
15.8	WHO Cluster diet B	6.4	Sunflower seed	2.1	Milk and cream,	1 6	Soya bean	0 0
14.7	ES child	8.3	Milk and cream,	1.0	Sunflower seed	1 0	Rice	0 0
13.1	DE child	9.5	Milk and cream,	0.8	Sunflower seed	0.7	Birds' eggs	0 0
11.8	WHO cluster diet E	3.1	Sunflower seed	2.0	Milk and cream,	1.7	Legume vegetables (fresh)	0 0
11.6	WHO cluster diet D	4.3	Sunflower seed	3.4	Milk and cream,	1.1	Rice	0 0
10.3	SE general population 90th percentile	8.3	Milk and cream,	0.8	Rice	0.7	Legume vegetables (fresh)	0 0
9.8	WHO regional European diet	3.2	Milk and cream,	1.2	Legume vegetables (fresh)	1 2	Sunflower seed	0 0
9.7	DK child	8.4	Milk and cream,	0.6	Birds' eggs	0.4	Poultry -chicken, geese, duck,	0 0
8.6	WHO Cluster diet F	2.6	Milk and cream,	1.7	Soya bean	0 8	Sunflower seed	0 0
7.9	IE adult	1.9	Milk and cream,	1.7	Sunflower seed	1 3	Legume vegetables (fresh)	0 0
7.4	ES adult	3.3	Milk and cream,	0.9	Sunflower seed	0 6	Legume vegetables (fresh)	0 0
7.4	NL general	4.4	Milk and cream,	1.0	Legume vegetables (fresh)	0 6	Swine: Meat	0 0
6.5	FR all population	2.9	Sunflower seed	1.8	Milk and cream,	0.4	Poultry -chicken, geese, duck,	0 0
5.9	PT General population	2.5	Sunflower seed	1.6	Rice	0 9	Legume vegetables (fresh)	0 0
4.9	LT adult	2.6	Milk and cream,	0.6	Swine: Meat	0.4	Rice	0 0
4.8	DK adult	3.6	Milk and cream,	0.4	Bovine: Meat	0 2	Birds' eggs	0 0
4.5	FI adult	3.8	Milk and cream	0.2	Rice	0 2	Poultry -chicken geese duck	0 0
3.9	UK vegetarian	2.2	Milk and cream,	0.8	Rice	0.4	Legume vegetables (fresh)	0 0
3.8	UK Adult	2.0	Milk and cream,	0.7	Rice	0.4	Legume vegetables (fresh)	0 0
1.1	IT kids/toddler	0.4	Legume vegetables (fresh)	0.4	Rice	0.1	Sunflower seed	0 0
1.0	IT adult	0.5	Legume vegetables (fresh)	0.4	Rice	0.1	Sunflower seed	0 0
0.2	PL general population	0.1	Legume vegetables (fresh)	0.1	Sunflower seed	0 0	PULSES, DRY	0 0

Table 6.9.1-8: Exposure assessment for metabolite CL 312622 with PRIMo Model (rev 2.0) against the Cramer Class III threshold of 0.0015 mg/kg according to the TTC concept

CL 312622			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.0015**	ARfD (mg/kg bw):	
Source of ADI:	**TTC**	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).

The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
0	19
No of diets exceeding ADI	--- 0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
19.0	FR toddler	15.8	Milk and cream,	1.0	Legume vegetables (fresh)	0 5	Bovine: Meat	0 0
17.4	UK Infant	15.5	Milk and cream,	0.5	Birds' eggs	0.4	Rice	0 0
14.5	NL child	11.7	Milk and cream,	0.6	Legume vegetables (fresh)	0 6	Swine: Meat	0 0
12.0	FR infant	10.3	Milk and cream,	0.7	Legume vegetables (fresh)	0 2	Bovine: Meat	0 0
10.0	UK Toddler	8.3	Milk and cream,	0.5	PULSES, DRY	0.4	Rice	0 0
8.0	ES child	5.0	Milk and cream	0.6	Bovine: Meat	0 5	Poultry -chicken geese duck	0 0
7.2	DE child	5.7	Milk and cream	0.4	Birds' eggs	0 2	Poultry -chicken geese duck	0 0
5.8	SE general population 90th percentile	5.0	Milk and cream,	0.4	Birds' eggs	0 3	Rice	0 0
5.8	DK child	5.1	Milk and cream,	0.3	Birds' eggs	0 2	Poultry -chicken, geese, duck,	0 0
5.2	WHO Cluster diet B	1.3	Milk and cream,	0.5	Sunflower seed	0.4	Legume vegetables (fresh)	0 0
4.9	WHO regional European diet	1.9	Milk and cream,	0.5	Swine: Meat	0.4	Bovine: Meat	0 0
4.7	WHO cluster diet E	1.2	Milk and cream,	0.6	Legume vegetables (fresh)	0.4	Poultry -chicken, geese, duck,	0 0
4.3	WHO cluster diet D	2.0	Milk and cream,	0.4	Rice	0 3	Sunflower seed	0 0
4.1	WHO Cluster diet F	1.6	Milk and cream,	0.5	Swine: Meat	0.4	Soya bean	0 0
4.1	NL general	2.6	Milk and cream,	0.4	Swine: Meat	0 3	Legume vegetables (fresh)	0 0
3.8	ES adult	2.0	Milk and cream,	0.3	Bovine: Meat	0 3	Swine: Meat	0 0
3.6	IE adult	1.1	Milk and cream,	0.4	Legume vegetables (fresh)	0.4	PULSES, DRY	0 0
2.8	DK adult	2.1	Milk and cream,	0.2	Bovine: Meat	0.1	Birds' eggs	0 0
2.6	FI adult	2.3	Milk and cream,	0.1	Poultry -chicken, geese, duck,	0.1	Birds' eggs	0 0
2.6	LT adult	1.6	Milk and cream,	0.4	Swine: Meat	0.1	Rice	0 0
2.3	FR all population	1.1	Milk and cream,	0.2	Poultry -chicken, geese, duck,	0 2	Sunflower seed	0 0
2.1	UK vegetarian	1.3	Milk and cream,	0.3	PULSES, DRY	0 3	Rice	0 0
2.0	UK Adult	1.2	Milk and cream,	0.2	Rice	0 2	Poultry -chicken, geese, duck,	0 0
1.4	PT General population	0.5	Rice	0.3	Legume vegetables (fresh)	0 2	Soya bean	0 0
0.4	IT kids/toddler	0.1	Legume vegetables (fresh)	0.1	Rice	0.1	PULSES DRY	0 0
0.3	IT adult	0.2	Legume vegetables (fresh)	0.1	Rice	0.1	PULSES DRY	0 0
0.1	PL general population	0.0	Legume vegetables (fresh)	0.0	PULSES, DRY	0 0	Sunflower seed	0 0

The chronic consumer risk assessment (see Table 6.9.1-1) based on the MRLs proposed according to the Art (12) evaluation by EFSA (Reasoned Opinion 2013) as well as newly proposed EU MRLs and the ADI of 9 mg/kg for the sum of parent imazamox plus hydroxymetabolite CL 263284, yielded ADI utilisation rates of below 1% (0.000-0.011%) with the EFSA PRIMo. According to the presented TMDI calculations, a long-term intake of imazamox residues (sum of parent imazamox and CL263284) is unlikely to present a public health concern.

Risk assessments for the plant metabolites according to the TTC Concept

According to the TTC (Threshold of Toxicological Concern) concept for metabolites the chronic risk assessments (see Table 6.9.1-6, Table 6.9.1-7 and Table 6.9.1-8) for the three main imazamox plant metabolites were performed. The active substance imazamox is a Cramer Class III substance and thus an ADI of 0.0015 mg/kg bw/d (1.5 µg/kg bw/d or 90 µg/person/d) is allocated to its metabolites (see Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment. EFSA Journal 2012;10(07): 2799. [187 pp.] doi:10.2903/j.efsa.2012.2799). The absence of genotoxic properties has been proven for all three metabolites with suitable in vitro and in vivo tests (see MCA, chapter 5.8). As input values for the risk assessments the residue values shown in Table 6.9.1-2, Table 6.9.1-3 and Table 6.9.1-4 were used for the hydroxy metabolite (CL 263284), its glucoside (CL 189215) and the dicarboxylic acid metabolite (CL 312622), respectively. The risk assessments yielded threshold utilization rates of 37%, 34% and 19%, translating into an exposure of 0.552, 0.513, and 0.285 µg/kg bw/d for metabolites CL 263284, CL 189215, and CL 312622, respectively. In all cases the highest exposure was for the French toddler due to the intake of milk and cream products. According to the presented assessments, exposure of the imazamox metabolites CL 263284, CL 189215 or CL 312622 remain well below the acceptable long-term exposure threshold value of 1.5 µg/kg bw/d for Cramer Class III compounds. Thus, a long-term intake of residues of these metabolites is unlikely to present a public health concern and they can be assumed to be not relevant for consumer safety.

NEDI calculations

For all models included in the EFSA model, the use of STMR or $STMR_P$ values in the estimation of the chronic dietary consumer risk is up to this point in time not necessary since the crude overestimated TMDI of imazamox was 0% of the ADI.

CA 6.9.2 Acute Reference Dose (ARfD) and Dietary Exposure Calculation

NESTI calculations

NESTI calculations are not required at this point in time. No ARfD has been allocated to imazamox as it was not considered necessary.

Other studies

No other/special studies were deemed necessary. The studies and information provided under previous sections are considered adequate and sufficient.

Effect on the residue level in pollen and bee products

The objective of these studies shall be to determine the residue in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

Imazamox is a herbicide with applications intended either pre-emergence or at early post-emergence and residue trials showed a very favourable residue profile, with often a no residue situation. Thus, no significant residues are likely to be expected in the blossoms and thus no residues of imazamox in pollen, honey or other bee products are expected.

The need for such a study was also discussed with the RMS at the presubmission meeting with the outcome that at the moment a residue study for honey and pollen is not deemed necessary.

Also, in the absence of valid test guidelines for such investigations at the time of submission of this dossier, the type and conditions of the studies to be performed (if considered necessary) may be discussed with the national competent authorities upon application for authorization of plant protection products containing the active substance imazamox.

Appendix 1 Tier 1 Summaries of the Supervised Field Residue Trials

Oilseed rape

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	25 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 KA H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
211921 2007/1007939[3] 31311 Uetze Germany AF/10114/BA/3	SO 0495 PS22-1A-VH	1. 01.09.2005 2. Not reported 3. 28.04.-07.08.2006	Foliar spray	0.025	200	0.050	1 28.04.2006	51	W. plant R. plant Seed	0.53 <0.05 <0.05	<0.05 <0.05 <0.05	0.58 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 13 101	BASF Method No. M3519 LOQ 0.05 mg/kg
251536 2007/1007963[4] 31311 Uetze Germany AF/10475/BA/3	SO 0495 PS22-1A-VH	1. 01.09.2005 2. Not reported 3. 28.04.-07.08.2006	Foliar spray	0.025	200	0.050*	1 28.04.2006	51	W. plant R. plant Seed	2.20 0.19 <0.05	<0.05 0.50 <0.05	2.25 0.71 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 13 101	BASF Method No. M3519 LOQ 0.05 mg/kg
211921 2007/1007939[3] Stadorf Germany AF/10114/BA/4	SO 0495 PS22-1A-VH	1. 01.09.2005 2. Not reported 3. 28.04.-07.08.2006	Foliar spray	0.025	200	0.050	1 29.04.2006	51	W. plant R. plant Seed	0.26 <0.05 <0.05	<0.05 <0.05 <0.05	0.31 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 12 100	BASF Method No. M3519 LOQ 0.05 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	25 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 KA H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
251536 2007/1007963[4] Stadorf Germany AF/10475/BA/4	SO 0495 PS22-1A-VH	1. 01.09.2005 2. Not reported 3. 28.04.-07.08.2006	Foliar spray	0.025	200	0.050*	1 29.04.2006	51	W. plant R. plant Seed	0.22 0.05 <0.05	<0.05 <0.05 <0.05	0.27 0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 12 100	BASF Method No. M3519 LOQ 0.05 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) study 2007/1007939 contains 4 rape trials treated with formulation BAS 798 KA H (identical with BAS 798 00H)
4) study 2007/1007963 contains 4 rape trials at the same locations as 2007/1007939 treated with formulation BAS 798 KA H plus adjuvant BAS 160 00 S
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of the sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* imazamox formulation (BAS 798 KA H) in tank mix with Adjuvant DASH (BAS 160 00 S)
w. plant whole plant without roots
r. plant rest of plant without roots
n.a. not analysed

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	17.5 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 00 H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
407228 2012/1084182 67454 Haßloch Germany L110324	SO 0495 Micro CL	1. 30.03.2011 2. 16.05.-27.05.2011 3. 19.08.2011	Foliar spray	0.018	200	0.035	1 17.05.2011	16-50	W. plant R. plant Seed	0.54 <0.01 <0.01	<0.01 0.01 <0.01	0.55 0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 94 94	BASF Method No. L0188/01 LOQ 0.01 mg/kg
407228 2012/1084182 67454 Haßloch Germany L110324	SO 0495 Micro CL	1. 30.03.2011 2. 16.05.-27.05.2011 3. 19.08.2011	Foliar spray	0.018	200	0.035*	1 17.05.2011	16-50	W. plant R. plant Seed	1.3 <0.01 <0.01	0.02 0.02 <0.01	1.32 0.03 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 94 94	BASF Method No. L0188/01 LOQ 0.01 mg/kg
407228 2012/1084182 OX 15 6EP Banbury United Kingdom L110325	SO 0495 Micro CL	1. 08.04.2011 2. 04.07.-05.08.2011 3. 07.09.2011	Foliar spray	0.018	200	0.035	1 31.05.2011	18	W. plant R. plant Seed	1.1 <0.01 <0.01	<0.01 0.05 <0.01	1.11 0.06 <0.02	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 99 99	BASF Method No. L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	17.5 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 00 H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
407228 2012/1084182 OX 15 6EP Banbury United Kingdom L110325	SO 0495 Micro CL	1. 08.04.2011 2. 04.07.-05.08.2011 3. 07.09.2011	Foliar spray	0.018	200	0.035*	1 31.05.2011	18	w. plant r. plant seed	2.6 <0.01 <0.01	0.03 0.19 <0.01	2.63 0.20 <0.02	0.01 0.03 <0.01	<0.01 0.02 <0.01	0 99 99	BASF Method No. L0188/01 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of the sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* imazamox formulation (BAS 798 00 H) in tank mix with Adjuvant DASH (BAS 160 00 S)
w. plant whole plant without roots
r. plant rest of plant without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	35 g/L	(common name and content)	quinmerac
Formulation (e.g. WP)	SC (BAS 831 00 H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s /ha				I	II	III	IV	V		
421874 2013/1044540 67117 Limburgerhof Germany L120254	SO 0495 Salsa CL	1. 19.03.2012 2. 21.05.-30.05.2012 3. 31.07.2012$^{\Delta}$	Foliar spray	0.018	200	0.035*	1 11.05.2012	50	W. plant R. plant Seed	2 <0.01 <0.01	0.03 0.08 <0.01	2.03 0.09 <0.02	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 81 81	BASF Method No. L0188/01 LOQ 0.01 mg/kg
421874 2013/1044540 CO11 2NF Manningtree United Kingdom L120255	SO 0495 Salsa CL	1. 28.03.2012 2. 04.06.-03.07.2012 3. 03.09.2012$^{\Delta}$	Foliar spray	0.018	200	0.035*	1 28.05.2012	50	W. plant R. plant Seed	1.8 <0.01 <0.01	0.04 0.01 <0.01	1.84 0.02 <0.02	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 98 98	BASF Method No. L0188/01 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of the sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* imazamox formulation (BAS 831 00 H) in tank mix with Adjuvant DASH (BAS 160 00 S)
#
Δ date of crop destruction
w. plant whole plant without roots
r. plant rest of plant without roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	25 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 KA H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
211921 2007/1007939[3] 82130 La Francaise France AF/10114/BA/1	SO 0495 PS22-1A-VH	1. 02.09.2005 2. Not reported 3. 29.03.-15.06.06	Foliar spray	0.025	200	0.050	1 29.03.2006	61	W. plant R. plant Seed	0.68 <0.05 <0.05	<0.05 <0.05 <0.05	0.73 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 15 78	BASF Method No. M3519 LOQ 0.05 mg/kg
251536 2007/1007963[4] 82130 La Francaise France AF/10475/BA/1	SO 0495 PS22-1A-VH	1. 02.09.2005 2. Not reported 3. 29.03.-15.06.06	Foliar spray	0.025	200	0.050*	1 29.03.2006	61	W. plant R. plant Seed	0.97 0.12 <0.05	<0.05 0.21 <0.05	1.02 0.33 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 15 78	BASF Method No. M3519 LOQ 0.05 mg/kg
211921 2007/1007939[3] 82000 Montauban France AF/10114/BA/2	SO 0495 PS22-1A-VH	1. 05.09.2005 2. Not reported 3. 29.03.-15.06.2006	Foliar spray	0.025	200	0.050	1 24.03.2006	53	W. plant R. plant Seed	0.44 <0.05 <0.05	<0.05 <0.05 <0.05	0.49 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 19 83	BASF Method No. M3519 LOQ 0.05 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	25 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 KA H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
251536 2007/1007963[4] 82000 Montauban France AF/10475/BA/2	SO 0495 PS22-1A-VH	1. 05.09.2005 2. Not reported 3. 29.03.-15.06.2006	Foliar spray	0.025	200	0.050*	1 24.03.2006	53	W. plant R. plant Seed	1.50 0.14 <0.05	<0.05 0.28 <0.05	1.55 0.42 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	0 19 83	BASF Method No. M3519 LOQ 0.05 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) study 2007/1007939 contains 4 rape trials treated with formulation BAS 798 KA H (identical with BAS 798 00H)
4) study 2007/1007963 contains 4 rape trials at the same locations as 2007/1007939 treated with formulation BAS 798 KA H plus adjuvant BAS 160 00 S
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of the sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* imazamox formulation (BAS 798 KA H) in tank mix with Adjuvant DASH (BAS 160 00 S)
w. plant whole plant without roots
r. plant rest of plant without roots
n.a. not analysed

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	17.5 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 00 H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
407228_1 2013/1089639 40051 Altedo Italy L110461	SO 0495 Micro CL	1. 21.09.2011 2. 06.04.-20.04.2012 3. 11.06.2012	Foliar spray	0.018	200	0.035	1 31.10.2011	18	W. plant R. plant Seed	1.8 <0.01 <0.01	0.01 0.03 <0.01	1.81 0.04 <0.02	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 224 224	BASF Method No. L0188/01 LOQ 0.01 mg/kg
407228_1 2013/1089639[3] 40051 Altedo Italy L110461	SO 0495 Micro CL	1. 21.09.2011 2. 06.04.-20.04.2012 3. 11.06.2012	Foliar spray	0.018	200	0.035*	1 31.10.2011	18	W. plant R. plant Seed	2.0 <0.01 <0.01	0.03 0.09 <0.01	2.03 0.1 <0.02	0.02 <0.01 <0.01	<0.01 <0.01 <0.01	0 224 224	BASF Method No. L0188/01 LOQ 0.01 mg/kg
407228_1 2013/1089639 Sainte Livrade France L110462	SO 0495 Micro CL	1. 24.09.2011 2. 10.04.-20.04.2012 3. 11.07.2012	Foliar spray	0.018	200	0.035	1 22.03.2011	18	W. plant R. plant Seed	0.18 <0.01 <0.01	<0.01 <0.01 <0.01	0.19 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 111 111	BASF Method No. L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	17.5 g/L	(common name and content)	metazachlor , quinmerac
Formulation (e.g. WP)	SC (BAS 798 00 H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
407228_1	SO 0495	1. 24.09.2011	Foliar	0.018	200	0.035*	1	18	W. plant	0.1	<0.01	0.11	<0.01	<0.01	0	BASF Method No.
2013/1089639[3]	Micro CL	2. 10.04.-20.04.2012	spray				22.03.2011		R. plant	<0.01	<0.01	<0.02	<0.01	<0.01	111	L0188/01
Sainte Livrade		3. 11.07.2012							Seed	<0.01	<0.01	<0.02	<0.01	<0.01	111	LOQ 0.01 mg/kg
France																
L110462																

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) study 2013/1089639 contains 2 rape trials. At each location one plot was treated with formulation BAS 798 00 H plus adjuvant BAS 160 00 S
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of the sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* imazamox formulation (BAS 798 00 H) in tank mix with Adjuvant DASH (BAS 160 00 S)
w. plant whole plant without roots
r. plant rest of plant without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Oilseed rape (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	35 g/L	(common name and content)	quinmerac
Formulation (e.g. WP)	SC (BAS 831 00 H) + DASH (BAS 160 00 S)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of : 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment[0]			No of Treatm. and Last Date	Growth Stage (BBCH)[2]	Portion Analysed	Residues (mg/kg)					DA-LA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				I	II	III	IV	V		
421874 2013/1044540 41710 Utrera Spain L120256	SO 0495 Salsa CL	1. 07.03.2012 2. 03.05.-03.06.2012 3. 11.07.2012 Δ	Foliar spray	0.018	200	0.035*	1 27.04.2012	50	W. plant R. plant Seed	0.77 <0.01 <0.01	0.01 0.14 <0.01	0.78 0.15 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 74 74	BASF Method No. L0188/01 LOQ 0.01 mg/kg
421874 2013/1044540 40051 Altedo Italy L120257	SO 0495 Salsa CL	1. 14.03.2012 2. 18.05.-27.05.2012 3. 11.07.2011 Δ	Foliar spray	0.018	200	0.035*	1 08.05.2012	50	W. plant R. plant Seed	1.2 <0.01 <0.01	0.02 0.27 <0.01	1.22 0.28 <0.02	<0.01 0.02 <0.01	<0.01 <0.01 <0.01	0 64 64	BASF Method No. L0188/01 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of the sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* imazamox formulation (BAS 831 00 H) in tank mix with Adjuvant DASH (BAS 160 00 S)
Δ date of crop destruction
w. plant whole plant without roots
r. plant rest of plant without roots

Soya bean

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Soya bean (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	22.4 g/L	(common name and content)	bentazone
Formulation (e.g. WP)	SC (BAS 762 01 H)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
B89774 2008/1034456[3] Heuilley-sur-Saône France A/NF/H/08/48	VP 0541 Essor	1. 08.05.2008 2. 12.07.-25.07.2008 3. 29.09.2008	Foliar spray	0.021	200	0.042	1 01.07.2008	13	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	90	Method M3178 LOQ 0.01 mg/kg
B89785 2008/1034457[3] Heuilley-sur-Saône France A/NF/H/08/49[2)]	VP 0541 Essor	1. 08.05.2008 2. 12.07.-25.07.2008 3. 29.09.2008	Foliar spray	0.021	200	0.042*	1 01.07.2008	13	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	90	Method M3178 LOQ 0.01 mg/kg
402845 2011/1112578 Limburgerhof Germany L110241	VP 0541 Merlin	1. 28.04.2011 2. 25.06.-26.07.2011 3. 08.09.2011	Foliar spray	0.021	200	0.042	1 22.06.2011	25	W. plant W. plant R. plant Seed	2.2 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	2.21 <0.02 <0.02 <0.02	<0.01 <0.01 <0.01 <0.01	<0.01 0.05 0.01 <0.01	0 27 78 78	BASF Method L0188/01 LOQ 0.01 mg/kg
402845 2011/1112578 Lentzke Germany L110242	VP 0541 Merlin	1. 21.04.2011 2. 10.06.-04.07.2011 3. 13.09.2011	Foliar spray	0.021	200	0.042	1 07.06.2011	21-51	W. plant W. plant R. plant Seed	3.8 <0.01 <0.01 <0.01	<0.01 <0.01 0.01 <0.01	3.81 <0.02 0.02 <0.02	<0.01 <0.01 <0.01 <0.01	<0.01 0.07 <0.01 <0.01	0 27 98 98	BASF Method L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Soya bean (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	22.4 g/L	(common name and content)	bentazone
Formulation (e.g. WP)	SC (BAS 762 01 H)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
402845	VP 0541	1. 11.04.2011	Foliar	0.021	200	0.042	1	25-55	W. plant	1.5	<0.01	1.51	<0.01	<0.01	0	BASF Method
2011/1112578	Sigalia	2. 20.06.-01.07.2011	spray				09.06.2011		W. plant	<0.01	<0.01	<0.02	<0.01	0.04	28	L0188/01
Piney		3. 25.09.2011							R. plant	<0.01	<0.01	<0.02	<0.01	<0.01	104	LOQ 0.01 mg/kg
France									Seed	<0.01	<0.01	<0.02	<0.01	<0.01	104	
L110243																
402845	VP 0541	1. 09.05.2011	Foliar	0.021	200	0.042	1	69	W. plant	2.1	<0.01	2.11	<0.01	<0.01	0	BASF Method
2011/1112578	Protina	2. 15.07.-22.07.2011	spray				22.07.2011		W. plant	<0.01	0.01	0.02	<0.01	0.14	28	L0188/01
Cigoné		3. 03.10.2011							R. plant	<0.01	0.05	0.06	<0.01	0.08	69	LOQ 0.01 mg/kg
France									Seed	<0.01	<0.01	<0.02	<0.01	0.04	69	
L110244																

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) Studies 2008/1034456 and 2008/1034457 were performed at the same trial locations, the former without, the latter with adjuvant DASH HC (BAS 9047 0S)
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* with DASH
n.a. not analyzed
w. plant whole plant without roots
r. plant rest of plant without roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Soya bean (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	22.4 g/L	(common name and content)	bentazone
Formulation (e.g. WP)	SC (BAS 762 01 H)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
B42941 2007/1023134[3] Saint-Paul-les-Romans France A/SF/H/07/142	VP 0541 Quito	1. 10.05.2007 2. 27.06.2007 3. 11.09.2007	Foliar spray	0.021	200	0.042	1 13.06.2007	13	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	90	Method M3178 LOQ 0.01 mg/kg
B44111 2007/1028359[4] Saint-Paul-les-Romans France A/SF/H/07/165	VP 0541 Quito	1. 10.05.2007 2. 27.06.-30.07.2007 3. 12.09.2007	Foliar spray	0.021	200	0.042*	1 13.06.2007	13	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	90	Method M3178 LOQ 0.01 mg/kg
B42941 2007/1023134[3] Bevons France A/SF/H/07/143	VP 0541 Deka Big	1. 28.05.2007 2. 17.07.-25.08.2007 3. 22.10.2007	Foliar spray	0.021	200	0.042	1 10.07.2007	13	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	104	Method M3178 LOQ 0.01 mg/kg
B44111 2007/1028359[4] Bevons France A/SF/H/07/166	VP 0541 Deka Big	1. 28.05.2007 2. 17.07.-25.08.2007 3. 25.10.2007	Foliar spray	0.021	200	0.042*	1 10.07.2007	13	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	104	Method M3178 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Soya bean (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	22.4 g/L	(common name and content)	bentazone
Formulation (e.g. WP)	SC (BAS 762 01 H)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
B42941 2007/1023134[3] Gorgonzola Italy A/IT/H/07/144	VP 0541 M 10 (Pioneer)	1. 20.05.2007 2. 08.08.-16.08.2007 3. 08.10.2007	Foliar spray	0.011	400	0.042	1 12.07.2007	23-24	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	88	Method M3178 LOQ 0.01 mg/kg
B44111 2007/1028359[4] Gorgonzola Italy A/IT/H/07/167[5]	VP 0541 M 10 (Pioneer)	1. 20.05.2007 2. 08.08.-16.08.2007 3. 08.10.2007	Foliar spray	0.011	400	0.042*	1 12.07.2007	23-24	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	88	Method M3178 LOQ 0.01 mg/kg
B42941 2007/1023134[3] Liscate Italy A/IT/H/07/145	VP 0541 XB 92 (Pioneer)	1. 22.06.2007 2. 20.08.-30.08.2007 3. 19.10.2007	Foliar spray	0.011	400	0.042	1 26.07.2007	24	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	85	Method M3178 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Soya bean (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	
Content of active substance (g/kg or g/L)	22.4 g/L	(common name and content)	bentazone
Formulation (e.g. WP)	SC (BAS 762 01 H)	Residues calculated as:	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
B44111 2007/1028359[4] Liscate Italy A/IT/H/07/168	VP 0541 XB 92 (Pioneer)	1. 22.06.2007 2. 20.08.-30.08.2007 3. 15.10.2007	Foliar spray	0.011	400	0.042*	1 26.07.2007	24	Seed	<0.01	<0.01	<0.02	n.a.	n.a.	85	Method M3178 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) study 2007/1023134 contains 4 trials treated with formulation BAS 762 01 H
4) study 2007/1028359 contains 4 trials at the same locations as 2007/1023134 treated with formulation BAS 762 01 H plus adjuvant BAS 9047 0S
I imazamox
II metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV: metabolite CL 312622 (Reg. No. 4110542)
V: metabolite CL 189215 (Reg. No. 4110445)
* imazamox (BAS 762 01 H) in tank mix with Adjuvant Dash HC (BAS 9047 0S)
n.a. not analyzed

Alfalfa

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	Adjuvant Dash HC
Content of active substance (g/kg or g/L)	22.4 g/L		
Formulation (e.g. WP)	SL (BAS 762 01 H) EC (BAS 9047 0S)	Residues calculated as:	BAS 720 H (CL 299263) Metabolite CL 263284

1	2	3	4	5			6	7		8	9			10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Growth Stage (BBCH)[3]**	**Portion Analysed**	**Residues (mg/kg)**			**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**		
B44122	AL1021	1. 31.07.2005	Foliar	0.007	400	0.028*	1	37	49	Green matter	0.010	0.075	0.09	14	Method
2007/1028360 51110 Warmeriville Champagne/Ardennes France A/NF/H/07/169	AL1020 Symphonie	2. Not reported 3. 11.10.2007	spray				27.09.2007		49	Hay	0.018	0.115	0.13	23	M3178 LOQ 0.01 mg/kg
B42952	AL1021	1. 31.07.2005	Foliar	0.007	400	0.028	1	37	49	Green matter	<0.010	0.023	0.03	14	Method
2007/1023135 51110 Warmeriville Champagne/Ardennes France A/NF/H/07/146	AL1020 Symphonie	2. Not reported 3. 11.10.2007 + 20.10.2007	spray				27.09.2007		49	Hay	<0.010	0.047	0.06	23	M3178 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
* imazamox (BAS 762 01 H) in tank mix with Adjuvant Dash HC (BAS 9047 0S)

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 455 H
Content of active substance (g/kg or g/L)	16.7 g/L	(common name and content)	Pendimethalin, 250 g/L
Formulation (e.g. WP)	EC (BAS 721 03 H)	Residues calculated as:	BAS 720 H (CL 299263) Metabolite CL 263284

1	2	3	4	5			6	7		8	9			10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Growth Stage (BBCH)[3]**	**Portion Analysed**	**Residues (mg/kg)**			**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**		
RXLU00410	AL1021	1. 18.08.2009	Foliar	0.0134	500	0.067	1	16-17	n.r.	Green matter	<0.010	0.052	0.062	34	Method
2013/1211060*	AL1020	2. Not reported	spray				06.04.2010		n.r.	Green matter	<0.010	0.056	0.066	49	HPLC/MS
35530 Noyal sur Vilaine	Salsa	3. 10.05.-25.05.2010							n.r.	Hay	<0.010	0.126	0.136	34	LOQ
Bretagne France RE10011									n.r.	Hay	<0.010	0.052	0.062	49	0.01 mg/kg
RXLU00410	AL1021	1. 20.08.2009	Foliar	0.0134	500	0.067	1	15	n.r.	Green matter	<0.010	0.103	0.113	30	Method
2013/1211060*	AL1020	2. Not reported	spray				23.03.2010		n.r.	Green matter	<0.010	0.019	0.029	48	HPLC/MS
35680 Moulins	Gea	3. 22.04.-25.05.2010							n.r.	Hay	<0.010	0.044	0.054	48	LOQ
Bretagne France RE10012															0.01 mg/kg
RXLU00410	AL1021	1. 02.09.2009	Foliar	0.0134	500	0.067	1	13	n.r.	Green matter	<0.010	0.126	0.136	30	Method
2013/1211060*	AL1020	2. Not reported	spray				23.03.2010		n.r.	Green matter	<0.010	0.025	0.035	48	HPLC/MS
35150 Janze	Symphonie	3. 10.05.2010							n.r.	Hay	<0.010	0.046	0.056	49	LOQ
Bretagne France RE10013															0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 455 H
Content of active substance (g/kg or g/L)	16.7 g/L	(common name and content)	Pendimethalin, 250 g/L
Formulation (e.g. WP)	EC (BAS 721 03 H)	Residues calculated as:	BAS 720 H (CL 299263) Metabolite CL 263284

1	2	3	4	5			6	7		8	9			10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Growth Stage (BBCH)[3]**	**Portion Analysed**	**Residues (mg/kg)**			**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**		
RXLU00410 2013/1211060* 35150 Amanlis Bretagne France RE100114	AL1021 AL1020 Gea	1. 20.08.2009 2. Not reported 3. 22.04.2010	Foliar spray	0.0134	500	0.067	1 23.03.2010	15	n.r.	Green matter	<0.010	0.126	0.136	30	Method HPLC/MS LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
* amended report only valid in combination with report BASF DocID 2011/1294331
n.r. not reported

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Adjuvant Dash HC
Content of active substance (g/kg or g/L)	22.4 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 762 01 H) EC (BAS 9047 0S)	Residues calculated as:	BAS 720 H (CL 299263) CL 263284, CL 312622, CL 189215

1	2	3	4	5			6	7		8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0)]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2)]**	**Growth Stage (BBCH)[5]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**	**IV**	**V**		
B44122 2007/1028360[4] 94017 Regalbuto Italy A/IT/H/07/170	AL1021 AL1020 Emiliana	1. October 2001 2. End of June 2007 3. 27.07.2007	Foliar spray	0.007	400	0.028*	1 16.06 2007	11-13	65-73 65-73	Green matter Hay	<0.010 <0.010	<0.010 <0.010	<0.02 <0.02	n.a. n.a.	n.a. n.a.	41 41	Method M3178 LOQ 0.01 mg/kg
B42952 2007/1023135[3] 94017 Regalbuto Italy A/IT/H/07/147	AL1021 AL1020 Emiliana	1. October 2001 2. End of June 2007 3. 27.07.2007 October 2001	Foliar spray	0.007	400	0.028	1 16.06 2007	11-13	65-73 65-73	Green matter Hay	<0.010 <0.010	<0.010 <0.010	<0.02 <0.02	n.a. n.a.	n.a. n.a.	41 41	Method M3178 LOQ 0.01 mg/kg
B44122 2007/1028360[4] 25670 Termens Spain A/SP/H/07/171	AL1021 AL1020 Brago	1. February 2005 2. 01.04.2007 3. 16.08.2007	Foliar spray	0.007	400	0.028*	1 19.07 2007	12-19	65 65	Green matter Hay	<0.010 <0.010	<0.010 <0.010	<0.02 <0.02	n.a. n.a.	n.a. n.a.	28 30	Method M3178 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Adjuvant Dash HC
Content of active substance (g/kg or g/L)	22.4 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 762 01 H) EC (BAS 9047 0S)	Residues calculated as:	BAS 720 H (CL 299263) CL 263284, CL 312622, CL 189215

1	2	3	4	5			6	7		8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0)]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2)]**	**Growth Stage (BBCH)[5]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**	**IV**	**V**		
B44122 2007/1023135[3] 25670 Termens Spain A/SP/H/07/148	AL1021 AL1020 Brago	1. February 2005 2. 01.04.2007 3. 16.08.2007 February 2005	Foliar spray	0.007	400	0.028	1 19.07 2007	12-19	65 65	Green matter Hay	<0.010 <0.010	<0.010 <0.010	<0.02 <0.02	n.a. n.a.	n.a. n.a.	28 30	Method M3178 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) study 2007/1023135 contains 4 trials treated with formulation BAS 762 01 H
4) study 2007/1028360 contains 4 trials at the same locations as 2007/1023135 treated with formulation BAS 762 01 H plus adjuvant BAS 9047 0S
5) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
n.a. not analyzed
* imazamox (BAS 762 01 H) in tank mix with Adjuvant Dash HC (BAS 9047 0S)

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (SF09464)	Residues calculated as:	Imazamox (BAS 720 H,CL 299263) CL 312622, CL 189215

1	2	3	4	5			6	7		8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Growth Stage (BBCH)[3]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**	**IV**	**V**		
ID-SP-96-613	AL1021	1. December 1995	Foliar	0.023	300	0.070	1	14-16	n.r.	W. plant#	<0.05	<0.05	<0.1	n.a.	n.a.	35	Method
ID-731-001	AL1020	2. Not reported	spray				04.04.1996		n.r.	W. plant#	<0.05	<0.05	<0.1	n.a.	n.a.	49	CEM-236
25126 Lerida	Aragon	3. May-June 1996							n.r.	W. plant#	<0.05	<0.05	<0.1	n.a.	n.a.	56	LOQ
Spain									Com.h.	W. plant#	<0.05	<0.05	<0.1	n.a.	n.a.	63	0.05 mg/kg
96-613-24									Com.h.	W. plantΔ	<0.05	<0.05	<0.1	n.a.	n.a.	63	
ID-SP-97-805	AL1021	1. April 1997	Foliar	0.028	259	0.0725	1	15-17	17-21	W. plant#	<0 10	0.27	0.37	0.68	0.28	7	Method
ID-731-002	AL1020	2. Not reported	spray				22.05.1997		27-55	W. plant#	<0 10	<0 10	<0.20	<0.10	<0.10	21	M3178
25126 Lerida	Aragon	3. June 1997							61	W. plant#	<0 10	<0 10	<0.20	<0.10	<0.10	28	LOQ
Spain									65	W. plant#	<0 10	<0 10	<0.20	<0.10	<0.10	35	0.1 mg/kg
97-805-04									65	W. plantΔ	<0 10	<0 10	<0.20	<0.10	<0.10	35	
ID-SP-96-612	AL1021	1. April 1996	Foliar	0.023	300	0.070	1	14-16	Com.h.	W. plant#	<0 10	<0 10	<0.20	<0.10	<0.10	40	Method
ID-731-003	AL1020	2. Not reported	spray				17.05.1996		Com.h.	W. plantΔ	<0 10	<0 10	<0.20	<0.10	<0.10	40	M3178
25126 Lerida	Aragon	3. End June 1996															LOQ
Spain																	0.1 mg/kg
96-612-25																	
ID-SP-96-612	AL1021	1. Not reported	Foliar	0.023	300	0.070	1	15-17	Com.h.	W. plant#	<0 10	<0 10	<0.20	<0.10	<0.10	40	Method
ID-731-003	AL1020	2. Not reported	spray				17.05.1996		Com.h.	W. plantΔ	<0 10	<0 10	<0.20	<0.10	<0.10	40	M3178
25126 Lerida	Aragon	3. June 1997															LOQ
Spain																	0.1 mg/kg
96-612-26																	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (SF09464)	Residues calculated as:	Imazamox (BAS 720 H,CL 299263) CL 312622, CL 189215

1	2	3	4	5			6	7		8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Growth Stage (BBCH)[3]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**	**IV**	**V**		
ID-HE-99-12	AL1021	1. 28.03.1999	Foliar	0.016	480	0.075	1	19	61	W. plant#	<0.10	<0.10	<0.20	<0.10	<0.10	21	Method
ID-731-023	AL1020	2. May-August 1999	spray				29.06.1999		65	W. plant#	<0.10	<0.10	<0.20	<0.10	<0.10	29	M3178
Partheni	Ipati	3. 20.07.-12.08.1999							67	W. plant#	<0.10	<0.10	<0.20	<0.10	<0.10	44	LOQ
Thessaloniki Greece 99-12-01																	0.1 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
n.a. not analyzed
Com.h. commercial harvest
green plant without roots
Δ dried plant without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	Nirvana S
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	BAS 455 H Pendimethalin, 250 g/L BAS 720 H (CL 299263) CL 263284
Content of active substance (g/kg or g/L)	16.7 g/L		
Formulation (e.g. WP)	EC (BAS 721 03 H)	Residues calculated as:	

1	2	3	4	5			6	7		8	9			10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Growth Stage (BBCH)[3]**	**Portion Analysed**	**Residues (mg/kg)**			**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**		
RXLU00410 2013/1211060* 35530 Noyal sur Vilaine Bretagne France RE10007	AL1021 AL1020 Oro	1. 2006 2. Not reported 3. 15.-30.04.2010	Foliar spray	0.017	400	0.067	1 18.03.2010	15-16	n.r. n.r.	Green matter Green matter	<0.010 <0.010	0.025 <0.010	0.035 <0.02	28 43	Method HPLC/MS LOQ 0.01 mg/kg
RXLU00410 2013/1211060* 35680 Moulins Bretagne France RE10008	AL1021 AL1020 Provence	1. 2005 2. Not reported 3. 15.-30.04.2010	Foliar spray	0.017	400	0.067	1 17.03.2010	14-15	n.r. n.r.	Green matter Green matter	<0.010 <0.010	0.020 <0.010	0.030 <0.02	29 44	Method HPLC/MS LOQ 0.01 mg/kg
RXLU00410 2013/1211060* 35150 Janze Bretagne France RE10009	AL1021 AL1020 Sanditi	1. 30.03.2009 2. Not reported 3. 15.-30.04.2010	Foliar spray	0.017	400	0.067	1 17.03.2010	40	n.r. n.r.	Green matter Green matter	<0.010 <0.010	0.020 <0.010	0.030 <0.02	29 44	Method HPLC/MS LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	Nirvana S
Crop/crop group:	Alfalfa	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 455 H
Content of active substance (g/kg or g/L)	16.7 g/L	(common name and content)	Pendimethalin, 250 g/L
Formulation (e.g. WP)	EC (BAS 721 03 H)	Residues calculated as:	BAS 720 H (CL 299263) CL 263284

1	2	3	4	5			6	7		8	9			10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Growth Stage (BBCH)[3]**	**Portion Analysed**	**Residues (mg/kg)**			**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**					**I**	**II**	**III**		
RXLU00410	AL1021	1. 27.04.2009	Foliar	0.017	400	0.067	1	40	n.r.	Green matter	<0.010	0.014	0.024	29	Method
2013/1211060*	AL1020	2. Not reported	spray				17.03.2010		n.r.	Green matter	<0.010	<0.010	<0.02	44	HPLC/MS
35150 Amanlis Bretagne France RE10010	Diane	3. 15.-30.04.2010													LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
3) at harvest
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
* amended report only valid in combination with report BASF DocID 2011/1294331
n.r. not reported

Sunflower

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 02 H)	Residues calculated as:	BAS 720 H (CL 299263) CL 263284

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**			**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**		
166093 2004/1000754 67160 Seebach Alsace France FAN/28/03	SO 0702 DK 3900 CL	1. 08.05.2003 2. 01.-31.07.2003 3. 09.09.2003	Foliar spray	0.017	300	0.05	1 02.06.2003	14	Whole plant Whole plant Seed	0.16 <0.05 <0.05	<0.05 <0.05 <0.05	0.21 <0.10 <0.10	16 38 99	Method M3515 LOQ 0.05 mg/kg
166093 2004/1000754 72800 Thoree Pays de la Loire France FBM/18/03	SO 0702 CMS425xRHA4 26 SF02	1. 14.05.2003 2. 18.-26.07.2003 3. 17.09.2003	Foliar spray	0.017	300	0.05	1 11.06.2003	16	Whole plant Whole plant Seed	0.09 <0.05 <0.05	0.07 <0.05 <0.05	0.16 <0.10 <0.10	8 37 98	Method M3515 LOQ 0.05 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	Adjuvant Dash
Content of active substance (g/kg or g/L)	40 g/L	Residues calculated as:	BAS 720 H (CL 299263)
Formulation (e.g. WP)	SL (BAS 720 06 H) SL (BAS 720 AM H)		CL 263284, CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
407229 2012/1084183 49685 Halen Germany L110316	SO0702 LN 11180CL plus	1. 25.05.2011 2. 30.06.-15.07.11 3. 25.10.2011	Foliar spray	0.025	200	0.05*	1 05.07.2011	18	Whole plant Rest plant Seed	1.74 <0.01 <0.01	<0.01 <0.01 0.02	1.75 <0.02 0.03	<0.01 <0.01 <0.01	<0.01 <0.01 0.03	0 112 112	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant Rest plant Seed	1.60 <0.01 <0.01	<0.01 <0.01 0.02	1.61 <0.02 0.03	<0.01 <0.01 <0.01	<0.01 <0.01 0.03	0 112 112	Method L0188/01 LOQ 0.01 mg/kg
407229 2012/1084183 Banbury OX156EP United Kingdom L110317	SO0702 LN 11180CL plus	1. 27.04.2011 2. 12.08.-27.08.11 3. 18.10.2011	Foliar spray	0.025	200	0.05*	1 20.06.2011	18	Whole plant Rest plant Seed	4.32 <0.01 <0.01	0.01 <0.01 0.01	4.33 <0.02 0.02	0.01 <0.01 <0.01	<0.01 <0.01 0.02	0 117 117	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant Rest plant Seed	4.34 <0.01 <0.01	0.01 <0.01 0.02	4.35 <0.02 0.03	0.01 <0.01 <0.01	<0.01 <0.01 0.02	0 117 117	Method L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Adjuvant Dash
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 06 H) SL (BAS 720 AM H)	Residues calculated as:	BAS 720 H (CL 299263) CL 263284, CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417820 2013/1003728 16845 Manker Germany L120330	SO0702 Paraiso 1000	1. 17.04.2012 2. 12.07.-25.07.12 3. 02.10.2012	Foliar spray	0.025	200	0.05***	1 29.05.2012	18	Whole plant Rest plant Seed	2.40 <0.01 <0.01	<0.01 <0.01 0.07	2.41 <0.02 0.08	<0.01 <0.01 <0.01	<0.01 <0.01 0.12	0 118 118	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant Rest plant Seed	2.60 <0.01 <0.01	<0.01 <0.01 0.07	2.61 <0.02 0.08	<0.01 <0.01 <0.01	<0.01 <0.01 0.13	0 118 118	Method L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Adjuvant Dash
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 06 H) SL (BAS 720 AM H)	Residues calculated as:	BAS 720 H (CL 299263) CL 263284, CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417820 2013/1003728 49700 Meigne sous Doue France L120331	SO0702 Paraiso 1000	1. 25.05.2012 2. 15.07.-15.08.12 3. 30.10.2012	Foliar spray	0.025	200	0.05***	1 27.06.2012	18	Whole plant Rest plant Seed	3.30 <0.01 <0.01	0.01 <0.01 0.02	3.31 <0.02 0.03	0.01 <0.01 <0.01	<0.01 <0.01 0.07	0 121 121	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant Rest plant Seed	2.40 <0.01 <0.01	<0.01 <0.01 0.01	2.41 <0.02 0.02	0.02 <0.01 <0.01	<0.01 <0.01 0.05	0 121 121	Method L0188/01 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
* treated with formulation BAS 720 06 H
** treated with formulation BAS 720 06 H in a tank mix with BAS 160 00 S (Adjuvant Dash)
*** treated with formulation BAS 720 AM H (identical with BAS 720 10 H, adjuvant built-in)
Whole plant without roots
Rest plant without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox (BA 720 H)	**Commercial Product (name)**	-
Crop/crop group:	Sunflower (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	
Content of active substance (g/kg or g/L)	25 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (BAS 720 BD H)	**Residues calculated as:**	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**1. Date of sowing/planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of Treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417821 2013/1405201[4] 16845 Manker Germany L130478	SO702 SB Paraiso 958	1. 25.04.2013 2. 16.07.-26.07.2013 3. 08.10.2013	Foliar spray	0.025	200	0.05	1 06.06.2013	18	W.plant[3] Seeds R.plant[3]	3.0 <001 <0.01	<0.01 0.029 <0.01	3.0 0.038 <0.02	<0.01 <0.01 <0.01	<0.01 0.050 <0.01	0 124 124	Method L0188/01 LOQ 0.01 mg/kg
417821 2013/1405201[4] 5811 AW Castenray The Netherlands L130479	SO702 SB Paraiso 958	1. 16.04.2013 2. 19.04.-14.07.2013 3. 09.12.2013	Foliar spray	0.025	200	0.05	1 11.06.2013	18	W.plant[3] Seeds R.plant[3]	2.4 <0.01 <0.01	0.010 0.038 <0.01	2.4 0.048 <0.02	<0.01 <0.01 <0.01	<0.01 0.068 <0.01	0 93 93	Method L0188/01 LOQ 0.01 mg/kg
417821 2013/1405201[4] 72500 S. Pierre da Cheville France (N) L130480	SO702 SB Paraiso 958	1. 26.04.2013 2. 14.07.-10.08.2013 3. 08.10.2013	Foliar spray	0.025	200	0.05	1 25.06.2013	19	W.plant[3] Seeds R.plant[3]	2.4 <0.01 <0.01	<0.01 0.012 <0.01	2.4 0.022 <0.02	<0.01 <0.01 <0.01	<0.01 0.035 <0.01	0 104 104	BASF Method L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox (BA 720 H)	**Commercial Product (name)**	-
Crop/crop group:	Sunflower (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	
Content of active substance (g/kg or g/L)	25 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (BAS 720 BD H)	**Residues calculated as:**	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**1. Date of sowing/planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of Treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417821 2013/1405201[4] 3545 Halen Belgium L130481	SO702 SB Paraiso 958	1. 19.04.2013 2. 20.06.-15.07.2013 3. 11.09.2013	Foliar spray	0.025	200	0.05	1 13.06.2013	18	W.plant[3] Seeds R.plant[3]	3.5 <0.01 <0.01	<0.01 0.022 <0.01	3.5 0.032 <0.02	<0.01 <0.01 <0.01	<0.01 0.016 <0.01	0 90 90	BASF Method L0188/01 LOQ 0.01 mg/kg

0) Actual application rates varied by 10% at most except where noted otherwise
1) Days after last application
2) At treatment
3) Without roots
4) Study only valid in combination with amendment 2014/116727
I Imazamox
II Metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III Total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV: Metabolite CL 312622 (Reg. No. 4110542)
V: Metabolite CL 189215 (Reg. No. 4110445)

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 02 H)	Residues calculated as:	Imazamox BAS 720 H (CL 299263) CL 263284, 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
142855 2002/1012087 41720 Los Palacios Spain ALO/22/02	SO0702 Mycogene X 81359	1. 08.04.2002 2. 18.06.-02.07.02 3. 14.-15.08.2002	Foliar spray	0.013	300	0.04	1 03.05.2002	14	Whole plant# Whole plant# Seed	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	<0.10 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	17 46 103	Method M3515 LOQ 0.05 mg/kg
142855 2002/1012087 41710 Utrera Spain ALO/23/02	SO0702 Mycogene X 81359	1. 08.04.2002 2. 17.06.-05.07.02 3. 21.-22.08.2002	Foliar spray	0.013	300	0.04	1 03.05.2002	16	Whole plant# Whole plant# Seed	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	<0.10 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	17 46 110	Method M3515 LOQ 0.05 mg/kg
142855 2002/1012087 41760 El Coroni Spain AYE/16/02	SO0702 Mycogene X 81359	1. 03.05.2002 2. 15.-27.07.2002 3. 04.-05.09.2002	Foliar spray	0.013	300	0.04	1 05.06.2002	15	Whole plant# Whole plant# Seed	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	<0.10 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	14 43 91	Method M3515 LOQ 0.05 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 02 H)	Residues calculated as:	Imazamox BAS 720 H (CL 299263) CL 263284, 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
142855 2002/1012087 41200 Alcala del Rio Spain AYE/17/02	SO0702 Mycogene X 81359	1. 16.05.2002 2. 15.-25.07.2002 3. 03.-04.09.2002	Foliar spray	0.013	300	0.04	1 12.06.2002	16	Whole plant# Whole plant# Seed	<0.05 <0.05 <0.05	<0.05 <0.05 <0.05	<0.10 <0.10 <0.10	n.a. n.a. n.a.	n.a. n.a. n.a.	20 36 83	Method M3515 LOQ 0.05 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
n.a. not analysed
without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Adjuvant Dash
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 06 H) SL (BAS 720 AM H)	Residues calculated as:	Imazamox BAS 720 H (CL 299263) CL 263284, 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
407229 2012/1084183 47380 Tourtres France L110318	SO0702 LN 11180CL plus	1. 11.05.2011 2. 01.07.-12.07.11 3. 05.10.2011	Foliar spray	0.025	200	0.05*	1 16.06.2011	18	Whole plant# Rest plant# Seed	2.78 <0.01 <0.01	<0.01 <0.01 <0.01	2.79 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 111 111	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant# Rest plant# Seed	2.47 <0.01 <0.01	<0.01 <0.01 <0.01	2.48 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 111 111	Method L0188/01 LOQ 0.01 mg/kg
407229 2012/1084183 40100 Chiesuol Del Fosso Italy L110319	SO0702 LN 11180CL plus	1. 18.05.2011 2. 03.07.-22.07.11 3. 13.09.2011	Foliar spray	0.025	200	0.05*	1 08.06.2011	18	Whole plant# Rest plant# Seed	2 96 <0.01 <0.01	<0.01 <0.01 <0.01	2.97 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 97 97	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant# Rest plant# Seed	3 20 <0.01 <0.01	0.01 <0.01 <0.01	3.21 <0.02 <0.02	0.02 <0.01 <0.01	<0.01 <0.01 <0.01	0 97 97	Method L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Adjuvant Dash
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 06 H) SL (BAS 720 AM H)	Residues calculated as:	Imazamox BAS 720 H (CL 299263) CL 263284, 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417820 2013/1003728 41710 Utrera Spain L120332	SO 0702 Paraiso 1001	1. 30.03.2012 2. 22.06.-04.07.2012 3. 23.08.2012	Foliar spray	0.025	200	0.05***	1 07.05.2012	18	Whole plant[#] Rest plant[#] Seed	6.60 <0.01 <0.01	<0.01 <0.01 0.02	6.61 <0.02 0.03	0.01 <0.01 <0.01	<0.01 <0.01 0.05	0 107 107	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant[#] Rest plant[#] Seed	2.70 <0.01 <0.01	<0.01 <0.01 <0.01	2.71 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 0.02	0 107 107	Method L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Adjuvant Dash
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	
Formulation (e.g. WP)	SL (BAS 720 06 H) SL (BAS 720 AM H)	Residues calculated as:	Imazamox BAS 720 H (CL 299263) CL 263284, 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417820 2013/1003728 40051 Altedo Italy L120333	SO 0702 Paraiso 1001	1. 27.04.2012 2. 10.07.-26.07.12 3. 17.09.2012	Foliar spray	0.025	200	0.05***	1 26.05.2012	18	Whole plant# Rest plant# Seed	4.80 <0.01 <0.01	0.01 <0.01 <0.01	4.81 <0.02 <0.02	0.02 <0.01 <0.01	<0.01 <0.01 <0.01	0 114 114	Method L0188/01 LOQ 0.01 mg/kg
				0.025	200	0.05**			Whole plant# Rest plant# Seed	2 90 <0.01 <0.01	<0.01 <0.01 <0.01	2.91 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 114 114	Method L0188/01 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most except where noted otherwise
1) days after last application
2) at treatment
I) imazamox
II) metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III) total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV) metabolite CL 312622 (Reg. No. 4110542)
V) metabolite CL 189215 (Reg. No. 4110445)
* treated with formulation BAS 720 06 H
** treated with formulation BAS 720 06 H in a tank mix with BAS 160 00 S (Adjuvant Dash)
*** treated with formulation BAS 720 AM H (identical with BAS 720 10H, adjuvant built-in)
# without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox (BA 720 H)	**Commercial Product (name)**	-
Crop/crop group:	Sunflower (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	
Content of active substance (g/kg or g/L)	25 g/L		
Formulation (e.g. WP)	SL (BAS 720 BD H)	**Residues calculated as:**	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**1. Date of sowing/planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of Treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417821 2013/1405201[4] 32220 St Lizier Du Plante France (S) L130482	SO702 SB Paraiso 958	1. 23.04.2013 2. 15.07.-25.07.2013 3. 27.09.2013	Foliar spray	0.025	200	0.05	1 12.06.2013	18	W.plant[3] Seeds R.plant[3]	3.7 <001 <0.01	<0.01 <0.01 <0.01	3.7 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 0.010 <0.01	0 104 104	Method L0188/01 LOQ 0.01 mg/kg
417821 2013/1405201[4] 59032 Platanos Greece L130483	SO702 SB Paraiso 958	1. 27.04.2013 2. 25.06.-15.07.2013 3. 05.08.-15.08.2013	Foliar spray	0.025	200	0.05	1 27.05.2013	18	W.plant[3] Seeds R.plant[3]	3.4 <0.01 <0.01	0.012 <0.01 <0.01	3.4 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 86 86	Method L0188/01 LOQ 0.01 mg/kg
417821 2013/1405201[4] 8124 San Michele di Ravenna Italy L130484	SO702 SB Paraiso 958	1. 09.04.2013 2. 08.06.-24.06.2013 3. 11.09.2013	Foliar spray	0.025	200	0.05	1 16.05.2013	18	W.plant[3] Seeds R.plant[3]	3.6 <0.01 <0.01	<0.01 <0.01 <0.01	3.6 <0.02 <0.02	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0 118 118	BASF Method L0188/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Imazamox (BA 720 H)	**Commercial Product (name)**	-
Crop/crop group:	Sunflower (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	
Content of active substance (g/kg or g/L)	25 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (BAS 720 BD H)	**Residues calculated as:**	imazamox, CL 263284 CL 312622, CL 189215

1	2	3	4	5			6	7	8	9					10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**1. Date of sowing/planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of Treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**					**DA-LA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I**	**II**	**III**	**IV**	**V**		
417821 2013/1405201[4] 41710 Utrera Spain L130485	SO702 SB Paraiso 958	1. 17.04.2013 2. 17.06.-29.06.2013 3. 30.08.2013	Foliar spray	0.025	200	0.05	1 16.05.2013	18	W.plant[3] Seeds R.plant[3]	1.7 <0.01 <0.01	<0.01 <0.01 <0.01	1.7 <0.02 <0.02	0.011 <0.01 <0.01	<0.01 0.028 <0.01	0 104 104	BASF Method L0188/01 LOQ 0.01 mg/kg

0) Actual application rates varied by 10% at most except where noted otherwise
1) Days after last application
2) At treatment
3) Without roots
4) Study only valid in combination with amendment 2014/116727
I Imazamox
II Metabolite CL 263284, expressed as parent equivalent (conversion factor 1.04815)
III Total imazamox = residues parent (BAS 720 H) + (residues CL 263284 x 1.048), for derivation of sum residues below the LOQ were set = LOQ
IV: Metabolite CL 312622 (Reg. No. 4110542)
V: Metabolite CL 189215 (Reg. No. 4110445)

BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Compiled by:

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
17.03.2015	Beigel (2002a) reference added to text in CA 7.1.4.2 (BASF DocID 2002/5004224)	MCP Section 7 Version 1 BASF DocID 2013/1348644

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

CA 7.1.1.1 Aerobic degradation

Report: CA 7.1.1.1/1
Ta C., 2012a
Aerobic soil metabolism of 14C-Imidazolinone BAS 720 H
2011/7002438
Guidelines: EPA 835.4100, OECD 307 (2002)
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The objective of this study was to determine the rate of degradation of BAS 720 H -imazamox, the pattern of formation and decline of significant degradation products, and the identity of any significant degradation products formed.

The aerobic soil degradation of imazamox was conducted using ^{14}C- labelled imazamox, with ^{14}C labelled in the imidazolinone ring. Two soils were used in this study a sandy loam soil (Bruch West) from Germany and a loam soil (New Jersey) from USA. Imazamox was applied at the rate of 0.066 mg a.i. /kg (equivalent to ca. 50 g a.i. /ha assuming a depth of 5 cm and bulk density of 1.5 g/cm^3). The treated soils were incubated at 50% maximum water holding capacity (MWC) at 20 ± 2°C in the dark during 120 days.

Levels of ^{14}C-imazamox were observed to decline rapidly during the study concurrent with the appearance of two major metabolites (> 5% TAR), CL 312622 and CL 354825, several minor metabolites (<5% TAR), and the rapid incorporation of imazamox into bound residues. Among the major metabolites, CL 312622 and CL 354825 were detected at maximum levels of 30.7 and 28% TAR, respectively. Mineralization was observed with levels of CO_2 reaching values of approximately 2.5 to 6.8% TAR after 120 days of incubation.

The DT_{50} values for the degradation of imazamox were calculated to be 38.1 and 23.1 days for sandy loam soil (Bruch West) and loam soil (New Jersey), respectively.

A degradation pathway for imazamox in soil under aerobic conditions was proposed. Imazamox was initially oxidized to CL 312622 (the diacid metabolite), which was further converted to CL 354825 (the hydroxyl acid metabolite). Finally, imazamox and its degradation products are mineralized to CO_2 by soil microorganisms.

Additional investigations on the potential shift of the isomer ratio of imazamox and its metabolites during soil incubation were performed. The results clearly showed that the isomer ratios were almost constant over time. It can be concluded that both isomers are comparably degradable so that combined exposure and risk assessments can be performed without considering the enantiomers separately.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 720 H
CAS Number.:	114311-32-9
Chemical name (IUPAC):	(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2yl)-5-methoxymethylnicotinic acid
Molecular weight:	305.3
Position of radiolabel:	imidazolinon-5-^{14}C, 3-^{15}N
Specific activity:	420,000 dpm/µg
Chemical purity:	95.3%
Radiochemical purity:	98.7%

2. Soils

Soils used in this study were a sandy loam (Bruch West, Germany) and loam (New Jersey, USA). The soils were collected from respective fields (top 8-inch layer) and sieved though a 2 mm sieve before use. The soil characteristics are summarised in Table 7.1.1.1-1.

Table 7.1.1.1-1: Properties of the soils used to investigate the degradation rate of ^{14}C-imazamox under anaerobic conditions

Designation	Bruch West Soil	New Jersey Soil
Origin	Bruch West, Germany	New Jersey, USA
USDA particle size distribution [%] sand > 0.002 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 65.7 23.1 11.2 sandy loam	 28.0 49.0 23.0 loam
Total organic carbon [%]	1.38	1.16
Microbial biomass [µg C/100 g dry soil] at 0 days after treatment	416.0	165.5
Microbial biomass [µg C/100 g dry soil] at 120 days after treatmen	17.3	72.2
CEC [meq/100g]	12.6	7.6
pH (H_2O)	8.1	7.1
Maximum Water Holding Capacity [g H_2O/100 g dry soil]	31.5	37.4

B. STUDY DESIGN

1. Experimental conditions

After weighting portions of 50 g soil (dry weight basis) into individual 250 ml polypropylene wide mouth centrifuge bottles (test vessels), the soil was adjusted to 50% of the maximum water holding capacity and acclimated in a chamber at 20 ± 2° C for 8 days in the dark.

Aliquots of 100 µL of the application solution containing approximately 3.3 µg of test substance were applied into each test vessel, resulting in a concentration of ^{14}C-imazamox of 0.066 ppm in soil (equivalent to 50 g/ha, assuming the applied material distributed evenly into 5 cm depth of soil and bulk density of soil as 1.5 g/cm^3). A total of 40 soil samples were treated (20 for each soil) and additionally, a total of 5 no treated samples and 4 high dose samples for each soil were prepared. Non treated soil was used to determine the microbial activity at the beginning and at the end of the study. High dose samples were used to generate metabolites for identification purposes.

The test vessels were connected to a flow-through system, in which each vessel received a constant air-flow at a rate of approximately 20 mL/min downward over the soil surface and were incubated in the dark at 20 ± 2° C in constant temperature chambers. Before entering the bottles, air was bubbled through a 1N sodium hydroxide (NaOH) scrubber to remove carbon dioxide and to moisturize it. Volatiles and CO_2 were trapped in 30 mL of 1N NaOH absorber to trap CO_2. All the treated soils were kept at 50% of the maximum water holding capacity (MWHC) during the incubation period.

2. Sampling

Duplicate soil samples were taken at 0, 2, 4, 7, 14, 30, 59, 91, and 120 days after treatment (DAT).

The liquid traps were assayed at all sampling times (except time-0) and replaced with fresh 1 N sodium hydroxide solution at each sampling time. All of the soil samples were extracted and processed immediately after they were removed from the flow-through system.

Soil microbial biomass was determined at the beginning of the study (dosing time) and at 4 months of incubation, using the fumigation and extraction method.

3. Description of analytical procedures

The soil samples were extracted once with 100 mL of methanol, followed by two extractions with 50% methanol in water (100 mL each). For each extraction step, the samples were shaken for 30 minutes at 300 strokes per minute at room temperature, and then centrifuged for 15 minutes at 4000 rpm. Aliquots of the extracts were assayed by LSC. The solvent extracts were pooled, concentrated and analysed by HPLC.

Soils were further extracted with 0.5N NaOH solution (100 mL) and the extract was separated into humic and fulvic fractions by acid precipitation. The fulvic fraction was concentrated by solid phase extraction and subjected to HPLC analyses. Identification of parent and its transformation products was performed by retention matching with reference standards using HPLC and confirmation by LC/MS. Radioactivity in the extracts was analysed (LSC). The soils were then air-dried, and the non-extractable residue (NER) was determined by oxidative combustion analysis.

The amounts of CO_2 produced were determined at each sampling time (excluding 0 DAT). The traps were assayed directly by adding aliquots of the trapping solutions into liquid scintillation fluid and counting by liquid scintillation counting (LSC).

4. Calculation of the degradation rate

The calculation of the DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS Kinetics workgroup. Optimization of model parameters, including estimation of parameter standard errors, was performed using the software package KinGUI version 2.2.

Note that the data for this study were independently reanalysed along with other aerobic laboratory data according to FOCUS (2006) kinetics guidance as presented in CA 7.1.2.1.1/1. The DT_{50} and DT_{90} values presented in CA 7.1.2.1.1/1 are considered the definitive endpoints for the aerobic laboratory studies.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The total recoveries of radioactivity from soils treated with ^{14}C-Iabeled imazamox are summarized in Table 7.1.1.1-2 and Table 7.1.1.1-3. The overall average total recovery of the radiolabeled material was 95.5% of the total applied radioactivity (TAR) for the entire samples from 2 soils.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (EER), non-extractable residues (NER, bound residues) and volatiles for each soil is shown in Table 7.1.1.1-2 and Table 7.1.1.1-3.

In Bruch West soil, solvent extractable [^{14}C]-residues of the parent compound (^{14}C-imidazolinone-labeled imazamox) decreased from approximately 97.7-100.1% TAR at 0 DAT to approximately 29.4-38.8% TAR at 120 DAT. There were the two major transformation products (> 5% TAR), CL 312622 and CL 354825, with maximum values of 19.8% and 28% TAR, respectively.

In New Jersey soil, the extractable [^{14}C]-residues of the parent compound decreased from approximately 98.6-99.3% TAR at 0 DAT to approximately 28.4-30.4% TAR at 120 DAT. CL 312622 and CL 354825 were the two major transformation products (> 5% TAR) with maximum values of 30.7% and 21.2% TAR, respectively. Several minor transformation products were also observed, but none of them reached concentration of > 5% TAR.

Table 7.1.1.1-2: Distribution of the radioactivity in Bruch West soil after treatment with ^{14}C-imidazolinone imazamox under aerobic conditions [% TAR]

DAT	ERR				Total Extracts	Volatiles	NER (Humin)	Total Recovery
	Methanol (Extract 1)	Methanol/Water (Extract 2)	Methanol/Water (Extract 3)	NaOH Extract		NaOH Trap		
0	25.5	59.3	11.9	3.2	100.0	NA	1.6	101.6
0	24.5	57.7	12.1	3.4	97.7	NA	1.2	98.9
2	17.4	52.0	13.7	14.2	97.3	0.2	4.0	101.4
2	18.3	51.8	13.6	13.9	97.6	0.2	3.7	101.5
4	14.5	49.5	14.3	17.3	95.6	0.2	5.1	100.9
4	15.4	49.3	13.8	17.1	95.6	0.2	4.4	100.2
7	12.8	45.3	14.7	20.3	93.1	0.3	5.3	98.7
7	13.8	45.6	14.4	20.0	93.8	0.3	5.7	99.8
14	9.7	39.9	16.8	24.4	90.8	0.4	8.6	99.8
14	9.2	40.8	16.3	24.1	90.4	0.4	8.0	98.8
30	6.3	32.1	14.5	32.8	85.7	0.7	10.7	97.1
30	8.2	32.8	13.5	32.3	86.8	0.5	11.2	98.5
59	4.7	23.3	12.0	39.9	79.9	1.8	16.5	98.2
59	7.5	25.9	11.8	38.0	83.2	1.0	14.5	98.7
91	3.5	17.6	9.9	43.0	74.0	3.5	20.9	98.4
91	5.7	19.9	9.4	43.3	78.3	1.7	19.3	99.3
120	2.9	13.9	6.6	49.5	72.9	5.1	22.1	100.1
120	4.4	16.5	7.4	47.5	75.8	2.5	20.2	98.5

DAT = days after treatment
ERR = extractable residues
NER = non-extractable residues (by combustion).
NA = not applicable (no sample analysed). There were no volatile traps for time zero.
All values were rounded to one decimal place.

Table 7.1.1.1-3: Distribution of the radioactivity in New Jersey soil after treatment with ^{14}C-imidazolinone imazamox under aerobic conditions [% TAR]

DAT	ERR				Total Extracts	Volatiles	NER (Humin)	Total Recovery
	Methanol (Extract 1)	Methanol/Water (Extract 2)	Methanol/Water (Extract 3)	NaOH Extract		NaOH Trap		
0	35.5	49.5	11.6	3.3	100.0	NA	1.1	101.1
0	33.6	50.3	12.1	3.3	99.2	NA	0.8	100.1
2	31.4	49.5	12.2	6.4	99.5	0.2	1.8	101.5
2	28.3	48.9	13.6	7.3	98.1	0.2	2.3	100.6
4	22.3	48.8	14.7	9.5	95.3	0.2	3.4	98.9
4	22.8	48.9	15.2	10.3	97.2	0.2	3.5	100.9
7	20.7	45.1	17.6	13.7	97.1	0.3	5.2	102.6
7	19.5	45.2	17.8	13.9	96.4	0.4	5.7	102.5
14	14.7	35.0	20.3	21.1	91.1	0.5	9.3	100.9
14	12.6	34.2	20.3	19.9	87.0	0.6	9.9	97.5
30	12.0	30.4	17.9	27.6	87.8	1.0	12.7	101.5
30	10.3	31.5	18.0	26.7	86.5	1.3	2.8	90.6
59	10.3	25.4	15.5	30.5	81.7	2.2	15.5	99.4
59	8.6	24.5	15.0	31.4	79.5	3.4	15.4	98.3
91	9.2	22.5	14.2	33.5	79.4	3.6	16.8	99.8
91	7.2	21.0	13.5	34.2	75.9	6.1	15.6	97.6
120	8.2	19.5	12.8	36.7	77.2	4.0	18.2	99.4
120	6.2	17.5	11.1	35.8	70.6	6.8	17.7	95.1

DAT = days after treatment
ERR = extractable residues
NER = non-extractable residues (by combustion).
NA = not applicable (no sample analysed). There were no volatile traps for time zero.
All values were rounded to one decimal place

The humic acid fraction reached a maximum level of 2.5 and 2.2% TAR in the Bruch West soil and New Jersey soil at 120 DAT, respectively (Table 7.1.1.1-4 and Table 7.1.1.1-5). The alkali-soluble radioactivity was further fractionated to distinguish between acid-insoluble humic acids and acid-soluble fulvic acids. At the end of incubation period, the fulvic acid fraction had its maximum with 47.0 and 35.7% TAR for the Bruch West soil and New Jersey soils, respectively (Table 7.1.1.1-4 to Table 7.1.1.1-5). In the fulvic acid fraction of the Bruch West soil, imazamox and its major metabolites, CL 312622 and CL 354825, had their maximum values of 18.1, 3.7 and 25.9% TAR, respectively. Considering the New Jersey soil, the parent compound, CL 312622 and CL 354825 had their maximum values of 9.7, 9.0 and 18.3% TAR, respectively.

Table 7.1.1.1-4: Characterization of NaOH extracts in the Bruch West soil [% TAR]

Days after treatment	NaOH Extracts	Fulvic acids	Humic acids[a]	Humins[b]
0	3.2	3.4	0.2	1.6
0	3.4	3.5	0.1	1.2
2	14.2	13.7	0.5	4.0
2	13.9	13.3	0.6	3.7
4	17.3	16.8	0.5	5.1
4	17.1	16.6	0.5	4.4
7	20.3	19.7	0.6	5.3
7	20.0	20.0	0.0	5.7
14	24.4	23.5	0.9	8.6
14	24.1	23.7	0.4	8.0
30	32.8	31.3	1.5	10.7
30	32.3	31.3	1.0	11.2
59	39.9	39.0	0.9	16.5
59	38.0	37.1	0.9	14.5
91	43.0	42.1	0.9	20.9
91	43.3	41.4	1.9	19.3
120	49.5	47.0	2.5	22.1
120	47.5	45.4	2.1	20.2

[a]Humic acids were calculated by subtracting fulvic acids from 0.5N NaOH extract
[b]Humins were post-extraction solids analysed by combustion/LSC

Table 7.1.1.1-5: Characterization of NaOH extracts in the New Jersey soil [% TAR]

DAT	NaOH Extracts	Fulvic acids	Humic acids[a]	Humins[b]
0	3.3	3.3	0.0	1.1
0	3.3	3.1	0.2	0.8
2	6.4	6.2	0.2	1.8
2	7.3	7.2	0.1	2.3
4	9.5	9.5	0.0	3.4
4	10.3	10.2	0.1	3.5
7	13.7	13.5	0.2	5.2
7	13.9	13.5	0.4	5.7
14	21.1	18.8	2.3	9.3
14	19.9	20.0	0.0	9.9
30	27.6	25.9	1.7	12.7
30	26.7	26.0	0.7	2.8
59	30.5	29.4	1.1	15.5
59	31.4	30.4	1.0	15.4
91	33.5	31.2	2.3	16.8
91	34.2	32.5	1.7	15.6
120	36.7	35.7	1.0	18.2
120	35.8	33.6	2.2	17.7

[a]Humic acids were calculated by subtracting fulvic acids from 0.5N NaOH extract
[b]Humins were post-extraction solids analysed by combustion/LSC

C. VOLATILISATION

The mineralisation rate of the organic ^{14}C to $^{14}CO_2$ was observed with levels of $^{14}CO_2$ reaching values of approximately 2.5 to 6.8% TAR after 120 days of incubation (Table 7.1.1.1-2 to Table 7.1.1.1-3).

D. ISOMERIZATION

To investigate the isomerization of imazamox in soil under aerobic conditions, the methanol and methanol/water extracts from both Bruch West and New Jersey soils were subjected to a chiral separation using a chiral method. Results indicated the ratio of R and S isomers of imazamox (1:1 in the dosing solution) was not changed under aerobic conditions, suggesting that there was no conversion from one isomer to another and no preferential isomeric degradation of imazamox in soil. Similarly, the isomer ratio of the second metabolite CL 354825 was almost constant over time. The separation of the two isomers of the first metabolite CL 312622 was not achieved by the present HPLC method. Since parent converted to the diacid, CL 312622 first, then the diacid converted to CL 354825 and the isomers in both parent and the final metabolite CL 354825 were constant over time, the results suggest that the isomer ratio of the intermediate metabolite CL 312622 does not change.

E. TRANSFORMATION OF PARENT COMPOUND

Radio-HPLC analysis of the soil extracts showed degradation of imazamox in two soils. From both soils, the concentration of the parent compound ^{14}C-imidazolinone-labeled imazamox decreased from approximately 97.7-100.1% TAR at 0 DAT to approximately 28.4-38.8% TAR at 120 DAT (Table 7.1.1.1-6). There were two major metabolites (>5% TAR), CL 312622 and CL 354825, which were detected at maximum levels of 19.8-30.7% TAR, and 21.2-28% TAR, respectively. In addition, several minor transformation products were observed, but none of them exceeded 5% TAR.

The identities of the parent compound imazamox (BAS 720 H) and its major metabolites (CL 312622 and CL 354825) were identified by HPLC co-chromatography with reference standards and also confirmed by LC/MS analyses.

In soil and under aerobic conditions, imazamox was initially oxidized to CL 312622 (the diacid metabolite) which was further converted to CL 354825 (the hydroxyl acid metabolite). Ultimately, imazamox and its degradation products are mineralized to CO_2 by soil microorganisms.

Table 7.1.1.1-6: Soil data for the kinetic evaluation

DAT	Bruch West Soil [%TAR]			New Jersey Soil [%TAR]		
	imazamox	CL 312622	CL 354825	imazamox	CL 312622	CL 354825
0	100.1	0.0	0.0	99.3	0.1	0.5
0	97.7	0.0	0.0	98.6	0.0	0.5
2	93.1	3.7	0.0	90.0	8.2	1.0
2	92.6	4.4	0.0	87.8	8.9	1.1
4	89.3	5.9	0.0	77.3	16.1	1.4
4	89.0	5.6	0.3	79.3	15.9	1.5
7	80.6	11.4	0.5	70.2	23.6	2.1
7	83.4	9.4	0.4	71.7	21.3	0.8
14	65.3	16.1	1.7	57.8	25.4	2.9
14	70.1	12.8	0.9	57.1	24.6	2.2
30	48.9	19.8	5.9	48.0	30.2	4.1
30	59.0	14.8	3.7	46.6	30.7	4.8
59	37.9	16.5	14.6	39.4	28.0	9.4
59	50.3	13.1	10.1	34.8	28.3	11.3
91	31.9	11.8	19.8	36.9	22.5	11.0
91	40.6	10.2	16.0	31.7	22.5	13.9
120	29.4	5.5	28.0	30.5	20.0	21.2
120	38.8	4.8	23.6	28.4	18.5	18.1

DAT = days after treatment

Data for the soil kinetic evaluation of imazamox are presented in Table 7.1.1.1-6. The DFOP model provided the best visual and statistical fit to the observed data in both soils. The estimated DT_{50} values of imazamox were calculated to be 23.1 and 38.1 days, and DT_{90} values of imazamox were 347 and 446 days, for the loam soil (New Jersey, USA) and the sandy loam soil (Bruch West, Germany), respectively. Once the best fit was identified, the two major metabolites were added into a pathway model where metabolite rises and decline was described using first-order kinetics.

III. CONCLUSION

Imazamox was rapidly degraded in the soils tested. The DT_{50} values were calculated to be 38.1 days and 23.1 days for the Bruch West and New Jersey soils, respectively. There were two major degradation products formed (> 5% TAR), CL 312622 and CL 354825, which were detected at maximum levels of 30.7 and 28% TAR, respectively. Several minor metabolites were detected at < 5% TAR. Mineralization was observed with levels of CO_2 reaching maximum values of approximately 5.1 to 6.8% TAR after 120 days of incubation.

CA 7.1.1.2 Anaerobic degradation

Report: CA 7.1.1.2/1
Wu S-S. et al., 2013b
Anaerobic soil metabolism of 14C-BAS 720 H
2013/7001808
Guidelines: OECD 307, EPA 835.4200
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The anaerobic metabolism of imazamox (BAS 720 H) in a loam soil from New Jersey (USA) was investigated by flooding the soil with water and using radiolabelled [^{14}C]-imidazolinone labelled imazamox and [^{14}C]-pyridine labelled imazamox.

Soil portions (50 g of soil dry weight) were incubated in darkness at 20 ± 2 °C for five days to reach equilibrium. The soil moisture was adjusted to 50% of maximum water holding capacity. After the equilibration interval and moisture correction, the soil was treated with the dosing solution of [^{14}C]-imidazolinone labelled imazamox or [^{14}C]-pyridine labelled imazamox at a nominal rate of 0.4 mg a.i./kg soil (equivalent to 100 g test item./ha). This application rate was two times higher than the usual field application rate to permit measurement of the disappearance of parent compound and identification of major metabolites formed.

A flow-through system with CO_2-free moist air was used during the aerobic incubation period. Following 30 days of aerobic incubation, the anaerobic phase was initiated by the addition of 100 mL Milli-Q water to each sample container. In the anaerobic phase, the treated systems were connected to a continuous flow-through system with humidified nitrogen and incubated for 120 days. Absorption solutions were set up to collect volatile ^{14}C-residues.

Under aerobic soil conditions, the average total recovery of the radiolabelled [^{14}C]-imidazolinone and [^{14}C]-pyridine material was 98.46% and 97.92% of the total applied radioactivity (TAR), respectively. Under anaerobic soil conditions, the average total recovery of the radiolabelled [^{14}C]-imidazolinone and [^{14}C]-pyridine material was 100.09% and 97.20% TAR, respectively.

This study demonstrated that [^{14}C]-imidazolinone labelled and [^{14}C]-pyridine- labelled imazamox rapidly degraded and mineralized in soil under aerobic conditions and remained constant under anaerobic conditions. Since the [^{14}C]-radiolabelled imazamox showed no decline during the anaerobic phase, the DT_{50} and DT_{90} were not calculated. There was one major metabolite (> 5% TAR) and several minor metabolites (< 5% TAR). Under aerobic conditions, CL 312622 was detected in the [^{14}C]-imidazolinone labelled and [^{14}C]-pyridine-labels treated soils at maximum levels of 29.8 and 38.9% TAR, respectively. Mineralization was observed with levels of $^{14}CO_2$ reaching values of approximately 1.7 to 1.9% TAR after 30 days of aerobic incubation.

Under anaerobic conditions, CL 312622 was detected in the [^{14}C]-imidazolinone labelled and [^{14}C]-pyridine labelled imazamox treated soils at maximum levels of 39.4 and 46.4% TAR, respectively. Mineralization was observed at levels of total $^{14}CO_2$ reaching values of approximately 3% TAR after 120 days of anaerobic incubation.

A degradation pathway for imazamox in soil under anaerobic conditions was proposed: imazamox was initially oxidized to CL 312622 (the diacid metabolite), which was further converted to CL 354825 (the hydroxyl acid metabolite). Finally, imazamox and its degradation products were mineralized to CO_2 by soil microorganisms.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: imazamox

Test Substance: 1

Company reference number:	BAS 720 H
Chemical nomenclature (IUPAC):	(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Site of radiocarbon labelling:	imidazolinon-5-^{14}C, 3-^{15}N
Radiochemical purity:	98.3%
Specific activity:	433,200 dpm/µg
Molecular formula:	$C_{15}H_{19}N_3O_4$
Molecular weight (non-labelled):	305.3

Test Substance: 2

Chemical nomenclature (IUPAC):	(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Site of radiocarbon labelling:	Pyridine-3-^{14}C, imidazolinone-3-^{15}N
Radiochemical purity:	98.6%
Specific activity:	399,000 dpm/µg
Molecular formula:	$C_{15}H_{19}N_3O_4$
Molecular weight (non-labelled):	305.3

2. Soil

The soil used in this study was a loam soil from New Jersey (USA) and was representative of the intended use areas. Soil was collected from the top 6-inch soil layer. Physicochemical characteristics of the test soil and water are summarised in Table 7.1.1.2-1.

After removing the vegetation, larger soil fauna and stones, the soil was sieved through a 2-mm sieve before use. Prior to dosing, the moisture of the soil was adjusted to approximately 50% of its maximum water holding capacity (MWHC) by adding the appropriate amount of Milli-Q water.

Table 7.1.1.2-1: Physicochemical characteristics of the test Soil and water used to investigate the degradation rate of ^{14}C-imazamox under anaerobic conditions

Designation		**New Jersey**
Origin		USA
USDA particle size distribution [%] sand > 0.002 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class		 29 46 25 Loam
Organic matter [%]		2.4
Organic carbon [%]		1.4
Cation exchange capacity [meq/100 g]		9.1
pH (H_2O)		7.0
Max. WHC [%]		37.0
Bulk density g/cc		1.06
Soil biomass [µg/g dry weight basis]	At start of study0 DAT)	52.4
	At end of aerobic phase (30 DAT)	143.6
	At end of anaerobic phase (150 DAT)	971.1
Designation		**Water**
Total organic carbon (TOC) [ppm] (at end of anaerobic phase)		12.5

B. STUDY DESIGN

1. Experimental conditions

Soil portions of 50 g (dry weight basis) were filled into 250 mL wide-mouth polypropylene centrifuge tubes. The moisture content of each sample was adjusted to 50% MWHC by adding water, as necessary. The soil samples from the same ^{14}C label and replicate were arranged in series. The soil in the bottles was allowed to acclimate in a chamber at 20±2°C for 5 days in the dark with moisturized air flow over the soils for the last 4 days.

A total of 44 soil samples were prepared (22 samples for each ^{14}C label) plus 8 extra (4 extra samples for each ^{14}C label). In addition, 13 non-treated soil samples were prepared. Non-treated soil and water was used to measure pH, redox and dissolved oxygen during the anaerobic phase. Non-treated soil was used to determine the microbial activity before study initiation, at the end of aerobic phase and at the end of the study. Total organic carbon was determined in the non-treated water at the end of the study.

The application of the test solutions to the soil samples was performed by applying 100 µL of the ^{14}C-imidazolinone and ^{14}C-pyridine labelled dosing solutions. After dosing, each test vessel was gently shaken by hand to incorporate the test substance into the soil and opened to the atmosphere to evaporate the solvent The nominal final concentration of imazamox was approximately 0.4 ppm, which was equivalent to an application rate of 100 g a.i./ha This application rate was two times higher than the usual field application rate to permit measurement of the disappearance of parent compound and identification of major metabolites formed.

Sample containers with soil treated with each label were reconnected in separate series. A flow of air was passed through 1 N NaOH then over the soil in each container and finally exited through two containers of 1 N NaOH to collect volatile ^{14}C-residues. The soil was maintained aerobically by connecting to an air flow-through system in the dark at 20 ± 2°C for 30 days. Moisture content of the soil samples was kept to 50% MWHC, by adding Milli-Q water as necessary

The treated soil samples were converted to anaerobic conditions after 30 days of aerobic incubation by flooding the soil with 100 mL high-purity bottled water (purged over night with nitrogen). After the addition of the water, the containers were reconnected in series and a stream of moistened ultra-high purity nitrogen gas was directed over the water to facilitate reaching anaerobic conditions.

For anaerobic samples, pH and oxidation-reduction (redox) potential of the water and soil layers and dissolved oxygen (DO) of the water layer were measured.

2. Sampling

Aerobic phase: Following treatment, duplicate incubation vessels of treated soil were taken for analysis at 0 DAT (immediately after application) and after 3, 7, 14 and 30 days following application of test substance (DAT). The solutions in the traps were collected at all sampling times, with exception of 0 DAT. All of the soil samples were extracted and processed immediately after they were removed from the flow-through system.

Anaerobic phase: duplicate water-soil systems were taken 34, 37, 44, 62, 90, and 150 days post-treatment [4, 7, 14, 32, 60, and 120 days post-flooding (DAF), respectively]. All samples were processed immediately after they were removed from the flow-through system by decanting the water layer and extracting the soil samples.

For both aerobic and anaerobic incubation periods, the liquid traps were assayed at all sampling times (except 0 DAT) by directly adding aliquots of the trapping solution into liquid scintillation cocktail and counting by liquid scintillation counting (LSC). The traps were replaced with fresh 1N NaOH solution at each sampling time.

3. Description of analytical procedures

In anaerobic samples, the water and soil layers of anaerobic test systems were separated by centrifugation. The liquid layers were decanted and aliquots were assayed by LSC. The pH of water layer samples was adjusted to 2 with HCl and concentrated by solid phase extraction (SPE) before HPLC analyses.

For both the aerobic and anaerobic phases, the soil samples were first extracted with 100 mL of methanol and followed by two extractions with 50% methanol in water (100 mL each). Aliquots of the extracts were assayed by LSC and then combined. Aliquots of the extracts were assayed using LSC and then combined. Soil was further extracted with 0.5 N solutions, processed and aliquots of the NaOH-extracts were assayed by LSC. The soils were then air-dried, and the non-extractable residue (NER) was determined by oxidative combustion analysis.

The NaOH extracts were fractionated into fulvic acid and humic acid by acid precipitation. Fulvic acid fraction was concentrated by solid phase extraction and analysed by HPLC.

Identification of parent and its transformation products in each sample type was performed by retention matching with reference standards by HPLC and confirmation by LC/MS.

Selective samples from both water and soil were analysed by chiral HPLC to show no conversion between the two isomers of the parent compound and diacid metabolite CL 312622.

4. Calculation of the degradation rate

Kinetic analysis was performed following the recommendations of the FOCUS Kinetics workgroup. The kinetic evaluation was performed for the total flooded data set (total imazamox in soil + water phases) to determine the best-fit kinetic model and corresponding endpoints (DT_{50}, DT_{75} and DT_{90}). The data was evaluated for the best-fit model using the first-order kinetics (SFO). No other kinetic model was tested, as no degradation of imazamox was observed under anaerobic conditions.

II. RESULTS AND DISCUSSION

A.TEST CONDITIONS

The redox potential in water and soil generally decreased throughout the anaerobic phase, while the dissolved oxygen (0.54-0.64 mg/L) and pH (water = 7.0-8.4; soil = 7.2-8.0) remained constant.

B. MASS BALANCE

Under aerobic soil conditions, the average total recovery of the [^{14}C]-imidazolinone and [^{14}C]-pyridine radiolabeled material was 98.46% and 97.92% of the total applied radioactivity (TAR), respectively.

The average total recovery under anaerobic soil conditions of the [^{14}C] imidazolinone and [^{14}C]-pyridine- radiolabeled material was 100.09% and 97.20% TAR, respectively.

C. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (EER), non-extractable residues (NER), CO_2, and other volatiles is shown in Table 7.1.1.2-2 and Table 7.1.1.2-3.

Aerobic conditions: In samples treated with [^{14}C]-imidazolinone imazamox, organic solvent extractable [^{14}C]-residues decreased from 100.30-100.76% TAR at DAT to 51.97–52.37% TAR at 30 DAT. Radioactivity in the NaOH extract increased from 6.70–6.91% TAR at 0 DAT to 30.21–31.17% TAR at 30 DAT. Non-extractable [^{14}C]-residues increased from 1.41-1.44% TAR at 0 DAT to 8.06–8.18% TAR at 30 DAT.

Considering the samples treated with [^{14}C]-pyridine imazamox, organic solvent extractable [^{14}C]-residues decreased from 93.77–95.37% TAR at 0 DAT to 59.09–60.81% TAR at 30 DAT. Radioactivity in the NaOH extract increased from 3.80–3.92% TAR at 0 DAT to 23.00–24.75% TAR at 30 DAT. Non-extractable [^{14}C]-residues increased from 0.60–0.63% TAR at 0 DAT to 7.55–8.95% TAR at 30 DAT.

Anaerobic conditions: From the samples treated with [^{14}C]-imidazolinone labelled imazamox, [^{14}C]-residues in the water layer increased from 47.11–57.08% TAR at 4 DAF to 72.82–8.22% TAR at 120 DAF. Organic solvent extractable [^{14}C]-residues decreased from 19.65-28.64% TAR at 4 DAF to 11.02–13.10% TAR at 120 DAF. The radioactivity detected in the NaOH extract decreased from 17.76–18.61% TAR at 4 DAF to 7.31–7.48% TAR at 120 days. Non-extractable [^{14}C]-residues decreased from approximately 5.51–7.04% TAR at 4 DAF to 3.14–3.21% TAR at 120 DAF.

Considering the samples treated with [^{14}C]-pyridine imazamox, [^{14}C]-residues in the water layer increased from 48.07–53.73% TAR at 4 DAF to 70.59–75.18% TAR at 120 DAF. Organic solvent extractable [^{14}C]-residues decreased from 26.84–28.55% TAR at 4 DAF to 11.60–12.66% TAR at 120 DAF. Radioactivity in the NaOH extract decreased from 10.20–10.93% TAR at 4 DAF to 5.93–6.07% TAR at 120 DAF. [^{14}C]-bound residues decreased slightly from 4.47–5.56% TAR at 4 DAF to 4.05–4.18% TAR at 120 DAF.

Table 7.1.1.2-2: Distribution of radioactivity in a loam soil (New Jersey) after treatment with [^{14}C]-imidazolinone imazamox [% TAR]

DAT	ERR					Total Extracts	NER (Humin)	Volatiles	Total recovery
	Water	Methanol (Extract 1)	Methanol/ Water (Extract 2)	Methanol/ Water (Extract 3)	NaOH Extract			NaOH Trap	
Aerobic									
0	NA	21.32	62.32	16.65	6.91	107.21	1.44	NA	108.65
0	NA	22.47	61.73	16.65	6.70	107.46	1.41	NA	108.87
3	NA	14.80	49.40	15.76	13.83	93.78	2.49	0.10	96.37
3	NA	14.64	49.47	16.90	17.04	98.05	4.02	0.10	102.17
7	NA	11.21	44.25	16.40	19.45	91.32	4.25	0.24	95.80
7	NA	13.61	47.11	17.17	18.61	96.51	4.15	0.23	100.89
14	NA	7.53	38.61	16.60	23.74	86.48	4.67	0.57	91.72
14	NA	8.15	36.98	17.54	25.48	88.15	6.46	0.49	95.09
30	NA	5.25	28.71	18.01	30.21	82.17	8.18	1.75	92.10
30	NA	6.22	29.11	17.03	31.17	83.54	8.06	1.37	92.97
Anaerobic									
4	47.11	5.76	16.18	6.71	18.61	94.36	7.04	1.78	103.18
4	57.08	4.00	10.79	4.87	17.76	94.49	5.51	1.39	101.39
7	54.91	4.30	9.71	3.28	16.31	88.53	7.21	1.82	97.56
7	55.05	4.75	9.17	3.55	17.35	88.86	5.80	1.42	97.08
14	60.14	3.70	8.01	3.44	12.70	87.99	4.65	1.92	94.57
14	66.43	4.21	8.34	3.77	12.84	95.60	4.51	1.49	101.60
32	73.18	3.09	8.88	2.12	9.73	96.99	3.19	2.15	102.33
32	71.63	3.01	8.74	2.08	10.43	95.89	3.92	1.66	101.46
60	67.63	2.84	4.69	2.88	10.08	88.12	4.49	2.50	95.10
60	76.48	3.49	5.28	3.15	10.09	98.50	3.93	1.96	104.39
120	72.82	2.87	5.98	2.17	7.31	91.16	3.21	3.30	97.66
120	78.22	3.99	6.75	2.36	7.48	98.80	3.14	2.80	104.74

NA = not applicable (no sample to be analysed)
NER = non-extractable residues (by combustion)
DAT = days after application
DAF = days after flooding

Table 7.1.1.2-3: Distribution of radioactivity in a loam soil (New Jersey) after treatment with [^{14}C]-pyridine imazamox [% TAR]

DAT	ERR					Total Extracts	NER (Humin)	Volatiles	Total recovery
	Water	Methanol (Extract 1)	Methanol/ Water (Extract 2)	Methanol/ Water (Extract 3)	NaOH Extract			NaOH Trap	
Aerobic									
0	NA	28.67	52.82	12.29	3.92	97.69	0.60	NA	98.29
0	NA	30.19	52.96	12.22	3.80	99.17	0.63	NA	99.80
3	NA	25.73	49.22	13.65	8.18	96.79	2.14	0.08	99.01
3	NA	24.18	48.65	14.63	9.54	96.99	2.91	0.10	100.0
7	NA	18.76	45.14	16.37	14.23	94.50	4.79	0.21	99.49
7	NA	18.87	44.65	16.25	12.64	92.42	4.53	0.25	97.20
14	NA	13.78	40.36	16.71	19.74	90.59	6.59	0.43	97.62
14	NA	13.66	43.00	18.27	17.33	92.27	7.73	0.60	100.59
30	NA	10.25	30.56	18.28	24.75	83.84	7.55	1.10	92.49
30	NA	8.81	31.82	20.18	23.00	83.82	8.95	1.91	94.68
Anaerobic									
4	53.73	8.21	13.31	5.32	10.20	90.77	4.47	1.12	96.36
4	48.07	7.39	15.02	6.13	10.93	87.55	5.56	1.94	95.04
7	54.98	8.66	9.63	4.06	11.79	89.12	5.08	1.14	95.34
7	61.19	7.10	7.84	3.90	10.99	91.03	6.45	1.98	99.45
14	65.82	6.59	7.24	2.97	7.83	90.44	4.20	1.22	95.86
14	67.13	5.60	8.75	3.75	9.14	94.37	5.11	2.09	101.57
32	70.18	3.87	7.95	1.79	6.81	90.59	3.44	1.37	95.40
32	71.03	3.55	8.41	1.87	6.67	91.53	3.08	2.10	96.71
60	73.37	4.55	5.04	2.83	6.72	92.50	3.91	1.59	98.01
60	71.40	3.77	4.81	2.98	7.42	90.38	4.10	2.44	96.91
120	75.18	3.93	6.43	2.30	5.93	93.77	4.18	2.21	100.16
120	70.59	3.35	6.11	2.14	6.07	88.26	4.05	3.23	95.54

N.A. = not applicable (no sample to be analysed
NER = non-extractable residues (by combustion)
DAT = days after application
DAF = days after flooding

C. VOLATILISATION

Under aerobic conditions, the total evolved $^{14}CO_2$ was lower than 2% TAR for both labelled test items. During the anaerobic incubation period, the total evolved $^{14}CO_2$ was below 5% TAR for both [^{14}C]-imidazolinone- and [^{14}C]-pyridine- labelled imazamox.

D. ISOMERIZATION

To investigate the isomerization of imazamox in soil under aerobic and anaerobic conditions, and in water under anaerobic conditions, the water layer and methanol/water extract, as applicable, from both the [^{14}C]-imidazolinone and [^{14}C]-pyridine labels were subjected to a chiral separation. The results indicated the ratio of R and S isomers of imazamox (1:1) was not changed under aerobic and anaerobic conditions, suggesting that there was no conversion from one isomer to another and no preferential isomeric degradation of imazamox in water or soil.

The isomerization of CL 312622 in soil under aerobic and anaerobic conditions and in water under anaerobic conditions was also investigated in representative samples from the water layer and methanol/water extract from the [^{14}C]-imidazolinone label. The results indicated the ratio of R and S isomers of CL 312622 (1:1) was not changed under aerobic and anaerobic conditions, suggesting that there was no conversion from one isomer to another and no preferential isomeric degradation of CL 312622 in water or soil.

E. TRANSFORMATION OF PARENT COMPOUND

Aerobic conditions: The concentration of the parent compound ([^{14}C]-imidazolinone imazamox) decreased from 100.8-100.9% TAR at 0 DAT to 42.7–51.1% TAR at 30 DAT. There was one major transformation product (CL 312622), which was detected at a maximum value of 29.8% TAR. Several minor transformation products were also observed, however none of them reached a concentration of 5% TAR.

Considering the samples treated with [^{14}C]-pyridine imazamox, the concentration of the parent compound decreased from 94.6–95.6% TAR at 0 DAT to 36.8–46.4% TAR at 30 DAT. There was one major transformation product, CL 312622, detected at a maximum value of 38.9% TAR. Several minor transformation products were also observed, however none of them reached concentration of 5% TAR.

Anaerobic conditions: The concentration of [^{14}C]-imidazolinone imazamox remained steady from 49.0–54.0% TAR at 4 DAT to 45.0–57.2% TAR at 120 DAT. The major transformation product was CL 312622 (> 5% TAR), detected at maximum value of 39.4% TAR. Several minor transformation products were also observed, however none of them reached concentration of 5% TAR.

Considering the samples treated with [^{14}C]-pyridine imazamox, the concentration of the parent compound remained steady from 37.7–49.7% TAR at 4 DAT to 37.7–44.7% TAR at 120 DAT. The transformation product CL 312622 was the only one detected at values > 5% TAR, specifically at a maximum value of 46.4% TAR. Several minor transformation products were also observed, however none of them reached concentration of 5% TAR.

Characterisation of non-extractable residues

The non-extractable ^{14}C-residues (NER) in shown in were further characterised by NaOH-extraction and subsequent fractionation into fulvic acids, humic acids and humins. Results are shown in Table 7.1.1.2-4 and Table 7.1.1.2-5.

Table 7.1.1.2-4: Characterisation of bound residues in soil (New Jersey) treated with [^{14}C]-imidazolinone [% TAR]

DAT	NaOH Extracts	Fulvic acids	Humic acids[a]	Humins[b]
Aerobic				
0	6.9	6.2	0.7	1.4
0	6.7	6.1	0.6	1.4
3	13.8	12.9	0.9	2.5
3	17.0	15.6	1.4	4.0
7	19.5	18.0	1.4	4.2
7	18.6	17.3	1.3	4.2
14	23.7	22.0	1.7	4.7
14	25.5	23.5	2.0	6.5
30	30.2	26.7	3.5	8.2
30	31.2	28.0	3.1	8.1
Anaerobic				
DAF	**NaOH Extracts**	**Fulvic acids**	**Humic acids[a]**	**Humins[b]**
4	18.6	16.5	2.1	7.0
4	17.8	15.7	2.0	5.5
7	16.3	14.6	1.7	7.2
7	17.3	15.1	2.3	5.8
14	12.7	11.6	1.1	4.7
14	12.8	11.6	1.3	4.5
32	9.7	8.7	1.0	3.2
32	10.4	9.3	1.1	3.9
60	10.1	9.1	1.0	4.5
60	10.1	9.1	0.9	3.9
120	7.3	6.7	0.6	3.2
120	7.5	6.9	0.6	3.1

[a]Humic acids were calculated by subtracting fulvic acids from 0.5N NaOH extract
[b]Humins were post-extraction solids analysed by combustion/LSC
DAT = days after application
DAF = days after flooding

Table 7.1.1.2-5: Characterisation of bound residues in soil (New Jersey) treated with [^{14}C]-pyridine [% TAR]

DAT	NaOH Extracts	Fulvic acids	Humic acids[a]	Humins[b]
Aerobic				
0	3.9	3.7	0.2	0.6
0	3.8	3.6	0.2	0.6
3	8.2	7.7	0.5	2.1
3	9.5	8.8	0.7	2.9
7	14.2	13.0	1.2	4.8
7	12.6	11.8	0.9	4.5
14	19.7	18.6	1.2	6.6
14	17.3	16.2	1.1	7.7
30	24.7	23.0	1.8	7.6
30	23.0	20.5	2.5	9.0
Anaerobic				
DAF	**NaOH Extracts**	**Fulvic acids**	**Humic acids[a]**	**Humins[b]**
4	10.2	9.2	1.0	4.5
4	10.9	10.0	0.9	5.6
7	11.8	10.2	1.6	5.1
7	11.0	10.8	0.2	6.5
14	7.8	6.8	1.0	4.2
14	9.1	8.1	1.0	5.1
32	6.8	6.0	0.8	3.4
32	6.7	5.6	1.0	3.1
60	6.7	6.0	0.7	3.9
60	7.4	6.7	0.7	4.1
120	5.9	5.4	0.6	4.2
120	6.1	5.2	0.9	4.1

[a]Humic acids were calculated by subtracting fulvic acids from 0.5N NaOH extract
[b]Humins were post-extraction solids analysed by combustion/LSC
DAT = days after application
DAF = days after flooding

As shown in Table 7.1.1.2-6, since imazamox showed no decline during the study period under anaerobic conditions, DT_{50} and DT_{90} values were not calculated.

Table 7.1.1.2-6: Anaerobic soil data used for kinetic evaluation [% TAR]

DAT	DAF	[^{14}C]-imidazolinone			[^{14}C]-pyridine		
		Imazamox	CL 312622	CL 354825	Imazamox	CL 312622	CL 354825
30	0	42.7	29.8	1.4	46.4	30.9	1.6
30	0	51.1	24.5	1.0	36.6	38.9	1.8
34	4	49.0	38.8	1.3	49.7	33.9	0.9
34	4	54.0	32.6	1.0	37.7	42.5	1.6
37	7	47.5	32.2	1.7	48.2	32.9	1.2
37	7	46.2	34.8	1.0	48.9	36.0	1.1
44	14	45.4	36.4	1.2	51.5	31.8	0.8
44	14	55.4	35.0	0.8	41.6	46.4	2.1
62	32	50.9	39.4	1.3	45.8	36.1	1.4
62	32	52.1	36.7	1.5	39.7	43.7	1.8
90	60	44.9	36.8	1.5	49.4	36.9	1.0
90	60	56.8	36.2	1.1	40.0	41.4	2.0
150	120	45.0	37.9	1.0	44.7	41.2	1.2
150	120	57.2	36.2	0.7	37.7	41.0	2.1

DAT = days after application
DAF = days after flooding

III. CONCLUSION

The study demonstrated that [^{14}C]-imidazolinone and [^{14}C]-pyridine labelled imazamox rapidly degraded and mineralised in soil under aerobic conditions, but remained constant under anaerobic conditions. Since imazamox showed no decline during the study period under anaerobic conditions, DT_{50} and DT_{90} values were not calculated.

One major degradation product (> 5% TAR) formed. This degradation product, identified as CL 312622, was detected under aerobic conditions in the [^{14}C]-imidazolinone labeled and [^{14}C]-pyridine labeled imazamox soils at maximum levels of 29.8 and 38.9% TAR, respectively. Mineralization was observed with maximum levels of CO_2 reaching values of 1.7 and 1.9% TAR, respectively, after 30 days of incubation under aerobic conditions.

Metabolite CL 312622 was also detected under anaerobic conditions in the [^{14}C]-imidazolinone and [^{14}C]-pyridine-labelled imazamox treated soils at maximum levels of 39.4 and 46.4% TAR, respectively. Mineralization was observed at maximum levels of CO_2 reaching values of 3.3 to 3.2% TAR, respectively, after 120 days of incubation under anaerobic conditions. CL 312622 appeared essentially stable under anaerobic conditions with levels varying little over the 120 day anaerobic portion of the study.

CA 7.1.1.3 Soil photolysis

Report:	CA 7.1.1.3/1 McCall W.S., Blood A., 2013a BAS 720 H Imazamox: Soil photolysis 2012/7003612
Guidelines:	EPA 835.2410, OECD Draft Guideline Phototransformation of Chemicals on Soil Surfaces (January 2002), EEC 95/36, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

The soil photolysis of BAS 720 H - Imazamox was studied in a loamy soil (Baptistown, New Jersey, USA) for 15 days at 20°C under continuous irradiation. Two labels [imidazolinone and pyridine labels] were used in separate soil samples. ^{14}C-labelled imazamox was applied to the soil at application rate of approximately 0.333 ppm, corresponding to a field application rate of 50 g a.i./ha based on 1.5 g/cm^3 bulk density and 1 cm soil depth.

The soil samples were placed in an Atlas Suntest CPS Plus apparatus and were continuously exposed to a Xenon lamp emitting a light spectrum similar to sunlight (≥ 290 nm), simulating natural sunlight intensity in the spring at 40° N latitude. The dark control samples were prepared similar to the irradiated samples and were stored in the dark in an incubator at 20°C.

The average (of two reps) mass balance for both irradiated and dark control samples for the imidazolinone label ranged from 89.3 to 101.2% TAR The cumulative volatile ^{14}C-residues from the irradiated and dark control samples were 10.7 and 1.2 %TAR, respectively. For the pyridine label, the average (of two reps) mass balance ranged from 98.0% to 102.5% TAR.

A total of 60.5% TAR imazamox in the imidazolinone-labelled samples and 73.0% TAR imazamox in the pyridine-labelled samples was detected after 15 days of irradiation.

The single first order DT_{50} value for imazamox was calculated to be approximately 28.4 days (both labels, n=4) under continuous irradiation and 19.7 days in the dark controls.

One degradation product (<10% TAR) was observed both in irradiated and dark control samples. The compound was identified as the diacid derivative of imazamox (CL 312622). The levels of the diacid metabolite reached a maximum of approximately 8.3% TAR after 3 days and decreased to 8.0% at 15 days of irradiation in the imidazolinone-labelled samples. The diacid was observed at 2.3 %TAR at 0 DAT in the pyridine-labelled irradiated samples and decreased to 1.2% TAR at 3 DAT before steadily increasing to 8.2 %TAR at the end of the study. The degradation patterns observed between the two labels were similar except for the greater amount of radiolabelled CO_2 in the imidazolinone-labelled compound.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material imazamox
Label: imidazolinon-5-^{14}C, 3-^{15}N
Specific activity: 7 MBq/mg
Radiochemical purity: 98.7%
Molecular weight: 305.33 g/mol (unlabelled)
Chemical purity: 95.3%

Label: pyridine-3-C^{14}, imidazolone-3-^{15}N
Specific activity: 6.65 MBq/mg
Radiochemical purity: 99.7%
Molecular weight: 305.33 g/mol (unlabelled)
Chemical purity: 99.2%

2. Soil
The soil used was a loam from the Baptistown, New Jersey, USA. Soil characteristics are summarised in the Table 7.1.1.3-1.

Table 7.1.1.3-1: Characteristics of soil used for soil photolysis of imazamox

Soil designation	**771-82-03**
textural class	Loam (USDA Textural Class)
origin	Baptistown, New Jersey, USA
soil texture [%] sand silt clay	 28 48 24
organic matter [%]	2.3
cation exchange capacity [meq/100 g dry mass]	8.9
pH (1:1 soil:water ratio)	6.9
water holding capacity [mg/100 g dry mass]	49

B. STUDY DESIGN

1. Experimental conditions

For each test (photolysis and dark control), approximately 20 g of soil (dry weight basis) was weighted into small stainless steel trays (9 cm x 4.2 cm). The soil samples were treated with 150 µL of the imidazolinone-labelled imazamox application solution and 300 µL of the pyridine-labelled imazamox application solution. This resulted in a concentration of 0.36 µg/g and 0.02 µg/g relative to soil for the imidazolinone-label and the pyridine-label, respectively. The application solution was added in a manner to cover most of the soil surface to achieve an evenly distribution. Imazamox was applied to get a field application rate of approximately 0.333 ppm, which is equivalent to 50 g a.i./ha (assuming a bulk density of 1.5 g/cm^3 and 1 cm soil depth).

For the irradiated samples, an Atlas Suntest CPS Plus unit was used. The steel trays containing the treated soil samples were placed in the jacketed steel trays and covered with a quartz glass plate, secured with clamps. The soil samples were continuously exposed to a Xenon lamp emitting a light spectrum similar to sunlight (≥ 290 nm). The average light intensity was 528.56 W/m^2, simulating natural sunlight intensity in the spring at 40° N latitude (583 W/m^2).The temperature of the soil was maintained at 20 ± 2°C by circulating coolant through the bottom of the jacketed trays. The jacketed steel tray also contained 3 holes. Two holes were used as inlet and outlet vents for the collection of volatiles and the third hole allowed insertion of a temperature probe to monitor the temperature of the soil during the study. The temperature probe was inserted into a control sample and the soil temperature was recorded each day during the photolysis experiments. Pre-moistened air was supplied to the irradiated samples in jacketed trays by a vacuum pump that drew the air through a 0.5N NaOH scrubber, which was preceded by a 0.2 µm filter. Outgoing air was drawn through a NaOH trap to capture volatile radioactive degradation products.

For the dark control, soil samples were placed in identical jacketed steel trays used for the irradiated samples, sealed but placed in an incubator at 20 ± 2°C in the dark. A steady stream of air was first pulled through a 0.5N NaOH scrubbing solution before passing over the samples. The outgoing air was collected in a 0.5N NaOH trap for $^{14}CO_2$ and analysed for radioactive degradation products afterwards.

2. Sampling

Sampling was performed 0, 1, 3, 7, 11 and 15 days after treatment (DAT) for both the irradiated soil samples and dark controls.

3. Description of analytical procedures

Immediately after sampling, each soil sample was consecutively extracted with 40 mL methanol and two times with methanol: water (1:1, v:v) by shaking for 30 min. at 300 rpm. Each of the extracts were analysed by Liquid Scintillation Counting (LSC) separately to determine the total extractable radioactive residues (ERR) from the soil. The methanol and methanol:water extracts were pooled and subsequently concentrated as appropriate for HPLC analysis.

Bound residue (0.5 N NaOH) extraction/fractionation was performed at each sampling point throughout the study to investigate the partitioning of the radioactive residues into the humic acid, fulvic acid and humins by acid precipitation. With this purpose, bound residue extractions were performed with 40 mL of 0.5N NaOH by shaking at 3000 rpm for 15 minutes. The 0.5N NaOH extracts were adjusted to pH 2 with 6N HCl, mixed and allowed to stand at room temperature for 30 minutes. The extracts were then centrifuged and decanted. Supernatant (fulvic acid) were taken for LSC counts to determine the amount of radioactivity. The fulvic acid fractions were treated with C18 SPE column, and if the fraction contained > 10 %TAR the sample was concentrated under a stream of nitrogen and analysed by HPLC.

4. Kinetic analyses

Kinetic analyses were performed in accordance with the guidance of the FOCUS kinetics workgroup. All analyses were conducted by non-linear regression methods by using the software KinGUII v. 2.2012.202.925. Data for both labels and both replicates for each label were used together in the kinetic analysis giving n=4 data points per sampling.

A. MASS BALANCE

The total recoveries of radioactivity from soil are summarized in Table 7.1.1.3-2 to Table 7.1.1.3-3. The average (of two replicates) material balance ranged from 89.3 to 101.2% TAR for the light treated imidazolinone-labelled samples and from 95.9 to 101.2 %TAR for the dark controls. Considering the pyridine-labelled samples, the average material balance ranged from 98.0 to 101.2 %TAR for the irradiated samples and from 99.0 to 102.5 %TAR for the dark controls.

Table 7.1.1.3-2: Recovery and distribution of radioactivity during soil photolysis of imidazolone-labelled imazamox - mean of two replicates [% TAR]

Irradiation time	Non-extractable residues	NaOH traps (CO_2)	MeOH Extract 1	MeOH/H_2O Extract 2	MeOH/H_2O Extract 3	NaOH Extract	Material balance
irradiated							
0	1.3	n.a.	33.9	48.3	13.5	4.2	101.2
1	4.9	1.0	19.2	49.7	15.0	9.6	99.3
3	6.7	3.2	11.8	46.6	14.5	12.7	95.4
7	8.8	5.8	9.1	41.6	14.5	13.9	93.6
11	10.9	8.9	9.0	33.6	12.2	14.8	89.3
15	9.5	10.7	5.9	35.9	12.7	16.3	90.9
control							
0	1.3	n.a.	33.9	48.3	13.5	4.2	101.2
1	2.5	0.1	30.1	45.4	13.5	5.0	97.5
3	3.1	0.2	27.8	42.8	14.5	7.7	95.9
7	5.6	0.4	20.8	39.7	18.5	12.1	96.7
11	7.4	0.7	16.6	39.7	19.8	14.2	97.9
15	8.8	1.2	10.7	39.2	21.5	18.3	98.9

n.a. = not analysed

Table 7.1.1.3-3: Recovery and distribution of radioactivity during soil photolysis of pyridine- labelled imazamox - mean of two replicates [% TAR]

Irradiation time	Non-extractable residues	NaOH traps (CO_2)	MeOH Extract 1	MeOH/H_2O Extract 2	MeOH/H_2O Extract 3	NaOH Extract	Material balance
irradiated							
0	3.2	n.a.	29.3	30.6	28.9	9.4	101.2
1	4.8	0.1	23.1	49.8	14.7	8.8	101.2
3	7.9	0.2	15.5	48.5	15.3	13.8	101.2
7	9.7	0.4	8.1	48.0	15.6	16.4	98.0
11	14.9	0.6	11.7	39.9	14.2	19.4	100.6
15	12.8	0.9	10.6	36.9	14.3	23.3	98.7
control							
0	3.2	0.0	29.3	30.6	28.9	9.4	101.2
1	3.0	0.0	14.9	58.2	16.8	7.9	100.8
3	4.8	0.1	13.3	55.4	16.9	10.8	101.0
7	7.3	0.1	6.2	52.1	18.2	15.2	99.0
11	9.2	0.1	12.5	44.7	17.9	15.1	99.4
15	10.4	0.1	9.0	42.2	20.0	20.9	102.5

n.a. = not analysed

B. EXTRACTABLE AND BOUND RESIDUES

The extractable radioactive residue (ERR, sum of all extracts) for the irradiated samples for the imidazolinone-label slowly decreased from 99.9% TAR at 0 DAT before levelling off at essentially 70% TAR (69.6 and 70.8% TAR, respectively) for the last two sampling dates (11 & 15 DAT). The dark controls for the imidazolinone-label saw a similar trend of decrease to approximately 90% TAR for the last two sampling events (90.3 and 89.7% TAR for 11 & 15 DAT, respectively). The irradiated samples for the pyridine-labelled material steadily declined from 98.2% TAR at 0 DAT to 85.2% TAR at 15 DAT. The dark controls decreased from 98.2% TAR at 0 DAT to 90.2% TAR at 11 DAT and a slight increase to 92.1% TAR was observed at 15 DAT.

The non-extractable radioactive residues (NER) observed in the irradiated imidazolinone-labelled samples steady increased from 1.3% TAR at 0 DAT to 10.9% at 11 DAT before finally accounting for 9.5 %TAR at the end of the irradiation period (15 DAT). The dark controls for the imidazolinone-label also saw an increase from 1.3 on 0 DAT to 8.8% TAR at 15 DAT. For the irradiated pyridine-labelled samples, NER steady increased from 3.2% TAR at 0 DAT to 14.9% at 11 DAT before finally accounting for 12.8% TAR at the end of the irradiation period (15 DAT). The dark controls for the pyridine-label also increased from 3.2 on 0 DAT to 10.4 %TAR at 15 DAT.

The bound residue fractionation profiles were all similar with no statistically relevant differences observed between the two labels and/or the respective dark controls. The pyridine-label irradiated samples showed the highest levels of each respective fraction. The humic acid fraction increased from 0.2% TAR at 0 DAT to 3.4% TAR at 15 DAT in the irradiated samples. The fulvic acid fraction increased from 13.7% TAR at 0 DAT to 19.9% TAR at 15 DAT in the treated samples. The non-extractable residues (humins) were 4.3% TAR at 0 DAT and reached a high of 12.8% TAR observed in the pyridine-label irradiated samples.

C. VOLATILIZATION

The volatiles collected were significant (up to 10.7% TAR at 15 DAT) for the irradiated imidazolinone-labelled samples. In the dark controls of both labels and the irradiated pyridine-labelled sample, volatiles did not amount to more than 1.2% TAR. All volatiles trapping solutions were treated with $BaCI_2$ to assess the composition of the trapped volatiles. Greater than 99% of the collected volatiles were precipitated as an insoluble precipitate of barium suggesting a carboxylic acid derivative. Therefore, the discrepancy between the two labels can be expected, as the imidazolinone label is placed at the carbonyl carbon of the imadazolinone ring and lends to facile cleavage of volatile radioactive products under experimental conditions.

Carbon dioxide was the only volatile degradation product trapped in the NaOH traps during the soil photolysis in significant amounts.

D. TRANSFORMATION OF PARENT COMPOUND

The imidazolinone-labelled material showed parent decreased from 91.5% TAR at 0 DAT to 60.5% TAR at 15 DAT in the irradiated samples. In the dark controls a similar decreasing trend was observed in going from 91.5% TAR at 0 DAT to 51.7% TAR at 15 DAT of imazamox. A small increase of the diacid metabolite (CL 312622) from 4.1% TAR at 0 DAT to 8.0% TAR at the completion of the study was observed in the irradiated samples. For the dark controls, a more pronounced increase of the metabolite CL 312622 was observed from 4.1% TAR at 0 DAT to 35.7% TAR at 15 DAT.

The amount of pyridine-label imazamox similarly moderately decreased from 93.2% TAR at 0 DAT to 73.0% TAR at 15 DAT of irradiation. In the dark control samples, the parent decreased from 93.2% TAR to 64.2% TAR at 15 DAT. The diacid metabolite increased from 2.3% TAR at 0 DAT to 8.2% TAR at 15 DAT in the irradiated samples. The diacid metabolite increased from 2.3% TAR at 0 DAT to 25.0% TAR at 15 DAT in the dark control samples.

Calculation of the DT_{50} values for imazamox was performed using a single first-order (SFO) model and a first order multi compartment biphasic model (FOMC). The SFO DT_{50} values for imazamox were calculated to be approximately 28.4 days (both labels, n=4) in irradiated samples and 19.7 days in dark control samples.

The result from both SFO and FOMC models are summarized in Table 7.1.1.3-5. Each model resulted in acceptable visual fits and the χ^2 error was low for both models (< 4%). While the fitted FOMC model resulted in lower χ^2 error than with SFO, the FOMC parameters were not estimated with sufficient confidence to justify selection of FOMC. Therefore, the SFO model was selected to represent the data.

Table 7.1.1.3-4: Degradation of imazamox in soil under photolytic conditions and dark control – Distribution of parent and metabolites

Irradiated										
	Imidazolinone Label					**Pyridine Label**				
DAT	**t_R=10 MIN**	**312622 t_R=11.6**	**BAS 720 H t_R=13.55**	**t_R=15 MIN**	**t_R=16 MIN**	**t_R=10 MIN**	**312622 t_R=11.6**	**BAS 720 H t_R=13.55**	**t_R=15 MIN**	**t_R=16 MIN**
0	-	4.1	91.5	-	-	-	2.3	93.2	-	-
1	-	7.5	84.7	0.4	-	0.3	4.3	91.4	-	-
3	-	8.3	76.0	0.5	-	-	1.2	90.3	0.9	-
7	-	8.3	69.3	0.5	-	-	5.6	79.9	1.9	-
11	0.4	8.2	59.4	0.5	-	0.8	5.6	77.7	0.9	-
15	-	8.0	60.5	0.5	-	0.9	8.2	73.0	-	-
Dark Control										
	Imidazolinone Label					**Pyridine Label**				
DAT	**t_R=10 MIN**	**312622 t_R=11.6**	**BAS 720 H t_R=13.55**	**t_R=15 MIN**	**t_R=16 MIN**	**t_R=10 MIN**	**312622 t_R=11.6**	**BAS 720 H t_R=13.55**	**t_R=15 MIN**	**t_R=16 MIN**
0	-	4.1	91.5	-	-	-	2.3	93.2	-	-
1	-	7.4	81.5	-	-	-	4.2	93.2	-	-
3	-	12.7	72.3	-	-	-	7.6	86.8	0.9	-
7	0.1	25.6	63.8	0.7	0.3	-	11.5	80.2	-	-
11	-	41.0	47.7	0.5	0.7	-	19.9	69.7	0.6	-
15	-	35.7	51.7	0.6	0.8	-	25.0	64.2	-	1.0

Table 7.1.1.3-5: Degradation of imazamox in soil under photolytic conditions – Kinetic analysis using SFO and FOMC model

Substance	Method	Sample	DT_{50} [d]	DT_{90} [d]	χ^2 error	p (t-test)	Visual fit
Imazamox	SFO	irradiated	28.4	94.2	2.59	k<<0.01	acceptable
		dark control	19.7	65.3	3.13	k<<0.01	acceptable

Substance	Method	Sample	DT_{50} [d]	DT_{90} [d]	χ^2 error	α, β at 5% level	Visual fit
Imazamox	FOMC	irradiated	100.8	>1000	0.96	not significant	acceptable
		dark control	27.6	>1000	2.31	not significant	acceptable

III. CONCLUSION

A total of 60.5% TAR imazamox in the imidazolinone-labelled samples and 73.0% TAR imazamox in the pyridine-labelled samples remained after 15 days of constant irradiation. One degradation product was observed both in irradiated and dark control samples; it was identified as the diacid metabolite CL 312622. Under irradiation, the levels of CL 312622 steadily increased throughout the study up to 8.0% for imidazolinone-labelled samples and 8.2% TAR for pyridine-labelled samples at the end of the study. In the dark controls, 35.7% TAR for imidazolinone-labelled samples and 25.0% TAR for pyridine-labelled samples was observed as CL 312622 after 15 days.

The degradation patterns observed between the two labels were similar except the greater amount of radiolabelled CO_2 in the imidazolinone-labelled soil samples.

The single first-order DT_{50} of imazamox was calculated to be 28.4 days under continuous irradiation and 19.7 days in the dark controls.

CA 7.1.2 Rate of degradation in soil

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

Report:	CA 7.1.2.1.1/1 Donaldson F.P., 2013a Kinetic evaluation of the aerobic soil degradation of BAS 720 H (imazamox) and its metabolites using FOCUS guidance to derive persistence and modeling endpoints 2013/7001767
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp., SANCO/10058/2005 rev. 2 (FOCUS kinetics report)
GLP:	no

Executive Summary

The purpose of this study was the kinetic evaluation of the aerobic degradation of BAS 720 H - imazamox and the formation of its metabolites CL 312622 and CL 354825 observed under laboratory conditions in seven different soils from four studies.

The appropriate kinetic model to derive non-normalised trigger and normalised modelling endpoints was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics. The best-fit model to derive trigger endpoints was selected based on visual and statistical assessment. The best-fit kinetic model was selected for imazamox while metabolites were modelled with SFO kinetics. Prior to deriving the modelling endpoints, the DT_{50} values obtained under different incubation conditions were normalised to the reference moisture of pF 2 and the reference temperature of 20 °C according to the recommendations of the FOCUS workgroup on degradation kinetics.

Imazamox followed bi-phasic kinetics in most soils. For trigger endpoints, DT_{50} values ranged from 10.8 to 397 days for imazamox, 5.8 to 67.4 days for metabolite CL 312622, and 53.0 to 1000 days for metabolite CL 354825. The geometric mean $DegT_{50}$ values for use as modelling endpoints were calculated to be 164 days for imazamox, 23.6 days for CL 312622, and 327 days for CL 354825. Average metabolite formation fractions were 0.850 for CL 312622 and 0.765 for CL 354825.

I. MATERIAL AND METHODS

A. MATERIALS

The aerobic degradation of imazamox and the formation of two major metabolites, the diacid CL 312622 and the hydroxy acid CL 354825, was analysed in order to derive trigger and modelling endpoints for imazamox and its metabolites, taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp*].

Kinetic modelling strategy

Kinetic evaluation was performed according to FOCUS Kinetics [*FOCUS (2006)*] in order to derive i) degradation parameters that are valid as trigger endpoints as well as ii) appropriate degradation parameters for environmental fate models.

Imazamox was first analysed for a parent-only situation in order to determine the best fit kinetics. If the single first-order (SFO) model was unacceptable, biphasic models including the first-order multi-compartment (FOMC) and double-first-order in parallel (DFOP) were applied. The best-fit model was selected based on visual and statistical assessment and the corresponding DT_{50} and DT_{90} values were reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model. With the appropriate kinetic model selected for imazamox, the metabolites were then fit with SFO kinetics. All parameters except the initial mass of metabolites (fixed to zero) were unconstrained for the final fitting.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

Initial metabolite residues were fixed to zero and any reported initial metabolite residues were added to the parent residue. Non-detections after time zero were omitted up to the last point before the first positive detection, which was set to ½ the lowest detected amount for the analysis. This approach was considered a reasonable approximation of the FOCUS (2006) recommended procedure for handling of LOQ and LOD data as no LOQ/LOD information was reported for these studies. Any non-detections occurring between positive detections were set to ½ the lowest detected amount. The first non-detection after the last positive detection was set to ½ the lowest detected amount, and any subsequent non-detections were omitted.

The software package KinGUII v. 2.2012.202.925 was used for parameter fitting. Model parameters were optimized using the iteratively weighted least squares (IRLS) method until a global minimum was reached.

Normalisation of degradation rates to reference conditions

The DT_{50} values in the laboratory studies were normalised to reference conditions to a temperature of 20 °C and a soil moisture at field capacity of pF 2 [*FOCUS (2002): "Generic guidance for FOCUS groundwater scenarios", v 1.1*].

The temperature normalisation was performed using the temperature correction factor in Equation 7.1.2.1-1 [*FOCUS (2000)*], and a default Q_{10} value of 2.58 was considered [*EFSA (2007): "Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil.", The EFSA Journal 622, 1-32*]. The DT_{50} moisture normalisation was performed using the moisture dependency equations by Walker (Equation 7.1.2.1-2).

Equation 7.1.2.1-1 Temperature correction factor for DT_{50} values

$$f_{temp} = Q_{10}^{\frac{Tact - Tref}{10}}$$

with:	f_{temp}	temperature correction factor	[-]
	T_{act}	incubation temperature	[°C]
	T_{ref}	reference temperature (20 °C)	[°C]
	Q_{10}	factor of increase of degradation rate with an increase in temperature of 10 °C (Q_{10} = 2.58)	[-]

Equation 7.1.2.1-2 Moisture correction factor for DT_{50} values according to Walker

$$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^{0.7} & if \quad \theta_{act} < \theta_{ref} \\ 1 & if \quad \theta_{act} \geq \theta_{ref} \end{cases}$$

¶

with:	f_{moist}	moisture correction factor	[-]
	θ_{ref}	reference soil moisture at field capacity (pF 2, 10 kPa)	[g /g dry soil]
	θ_{act}	actual soil moisture during incubation	[g /g dry soil]

The actual soil moisture was used as specified in the study reports. Unless otherwise noted, the corresponding water content at pF 2 for each soil type was taken from FOCUS [*FOCUS (2000)*].

The DT_{50} values normalised to reference conditions were calculated by multiplying the DT_{50} values at study conditions by the correction factors f_{temp} and f_{moist} as described in Equation 7.1.2.1-3.

Equation 7.1.2.1-3 Calculation of the DT_{50} at reference conditions (20 °C, moisture at pF2)

$$DT_{50\_ref} = DT_{50} \cdot f_{temp} \cdot f_{moist}$$

Experimental data

The kinetic evaluation was based on the findings of four soil degradation studies that have either been reviewed during Annex I inclusion [*Ta C. (1995) - BASF DocID 1995/7000938, Ta C.T. (1997) - BASF DocID 1997/7001038, Ta C.T., Lewis C.J. (1997) - BASF DocID 1997/7000971*] or can be found in section CA 7.1.1.1/1 [*Ta C. (2011) - BASF DocID 2011/7002438*]. An overview of the studies is given Table 7.1.2.1.1-1.

Table 7.1.2.1.1-1: Overview on soil aerobic degradation studies with imazamox

Study	Soil	Soil Texture	Incubation			% TAR at end of study (imazamox, 2 replicates)
			Soil moisture	Temp. [°C]	Time [d]	
Ta (1995)	NJ 1995	Sandy loam[a]	10.6	25	365	13.8 / 11.2
Ta & Lewis (1997)	Limours	Silt loam[b]	26.2	20	122	72.9 / 72.9
	Boissy	Silt loam[b]	26.4	20	157	38.1 / 49.7
	Pontfaverger	Silty clay loam[b]	26.0	20	122	4.0 / 3.9
Ta (1997)	NJ 1997	Sandy loam[a]	10.6	25	365	15.1 / 15.5
Ta (2011)	NJ 2011	Loam[a]	18.7	20	120	30.5 / 28.4
	Bruch West	Sandy loam[a]	15.8			29.4 / 38.8

[a] Soil texture based on USDA classification
[b] Soil texture based on UK classification

II. RESULTS AND DISCUSSION

The kinetic evaluation showed that the bi-phasic kinetics (DFOP, FOMC) were the best-fit models appropriate to derive trigger endpoints for additional work for imazamox in five soils, while the SFO model was appropriate to derive trigger endpoints for imazamox in two soils. Trigger endpoints for metabolites CL 312622 and CL 354825 were derived from the pathway fit using the SFO model. The derived trigger endpoints for imazamox and metabolites CL 312622 and CL 354825 are summarised in Table 7.1.2.1.1-2.

Table 7.1.2.1.1-2: Trigger endpoints for imazamox and its metabolites CL 312622 and CL 354825

Study	Soil	Best-fit model (imazamox)	Imazamox		Kinetic model (metabolites)	CL 312622		CL 354825	
			DT_{50} [d]	DT_{90} [d]		DT_{50} [d]	DT_{90} [d]	DT_{50} [d]	DT_{90} [d]
Ta (1995)	NJ 1995	DFOP	13.4	375	SFO	43.4	144	1000[a]	1000[a]
Ta & Lewis (1997)	Limours	SFO	397	1000[a]	SFO	ND[b]	ND[b]	110	364
	Boissy	SFO	48.6	161	SFO	5.8	19.4	53.0	176
	Pontfaverger	DFOP	10.8	45.2	SFO	18.2	60.5	251	833
Ta (1997)	NJ 1997	FOMC	27.7	609	SFO	18.8	62.6	300	995
Ta (2011)	NJ 2011	FOMC	24.6	1000[a]	SFO	67.4	224	1000[c]	1000[c]
	Bruch West	DFOP	38.1	446	SFO	35.9	119	1000[c]	1000[c]

[a] Reported value > 1000 days. Fixed to 1000 days as a practical conservative approximation
[b] Not determined, as formation rate < degradation rate
[c] Rate constant not robustly estimated. Fixed to 1000 days as a practical conservative approximation

The best-fit model was also used to calculate formation fractions for the metabolites of imazamox. Results are summarised in the table Table 7.1.2.1.1-3.

Table 7.1.2.1.1-3: Summary of formation fractions and associated statistics

Study	Soil	FF imaz-622	$\sigma_{imaz-622}$	df	p	FF 622-825	$\sigma_{622-825}$	df	p
Ta (1995)	NJ 1995	0.945	0.045	72	1.40E-32	0.840	0.063	72	2.25E-21
Ta & Lewis (1997)	Limours	ND	ND	ND	ND	1.0[a]	NA	NA	NA
	Boissy	1.00	0.298	30	1.08E-03	0.960	0.479	30	2.70E-02
	Pontfaverger	0.950	0.031	35	5.37E-27	0.608	0.044	35	5.97E-16
Ta (1997)	NJ 1997	0.947	0.081	65	5.86E-18	0.550	0.056	65	6.62E-15
Ta (2011)	NJ 2011	0.703	0.028	43	2.64E-27	0.594	0.105	43	5.19E-07
	Bruch West	0.556	0.066	41	1.02E-10	0.803	0.190	41	6.37E-05
	Average	**0.850**			**Average**	**0.765**			

FF: formation fraction; σ: standard error
df: degrees of freedom; p: p-value of one-sided t-test
ND: not determined, as complete degradation pathway was not modelled
NA: not applicable, as formation fraction was fixed to 1.0
[a] Fixed to 1.0 as a practical conservative assumption

The kinetic evaluation also showed that the bi-phasic model DFOP was appropriate to derive modelling endpoints for imazamox in five soils and that the SFO model was appropriate to derive modelling endpoints for imazamox in two soils. Modelling endpoints for metabolites CL 312622 and CL 354825 were derived from the pathway fit using SFO model. The DT_{50} values obtained under different incubation conditions were normalised to reference moisture of pF 2 and a temperature of 20 °C. Parameters included in the normalisation procedure for each soil are presented in Table 7.1.2.1.1-4.

Table 7.1.2.1.1-4: Temperature and moisture correction factors used for each study

Study	Soil	T_{act} [°C]	f_{temp} [a]	θ_{act}	θ_{ref} [b]	f_{moist}
Ta (1995)	NJ 1995	25	1.61	10.6	19	0.66
Ta & Lewis (1997)	Limours	20	1.00	26.2	27	0.98
	Boissy	20	1.00	26.4	27	0.98
	Pontfaverger	20	1.00	26.0	30	0.90
Ta (1997)	NJ 1997	25	1.61	10.6	19	0.66
Ta (2011)	NJ 2011	20	1.00	18.7	25	0.82
	Bruch West	20	1.00	15.8	14.6	1.00

T_{act} = study temperature; f_{temp}= temperature correction factor
θ_{act} = study soil moisture; θ_{ref} = reference soil moisture (pF 2); f_{moist} = moisture correction factor
[a] Based on a Q_{10} of 2.58 (EFSA, 2007)
[b] Estimated from FOCUS (2000) based on soil texture except Bruch West, which was measured

Actual and normalised modelling endpoints for imazamox and metabolites CL 312622 and CL 354825 are summarised in Table 7.1.2.1.1-5.

Table 7.1.2.1.1-5: Normalisation of degradation rates to standard conditions

Study	Soil	Kinetic model (imazamox)	Study DT_{50} [d]			Normalised $DegT_{50}$ [d]		
			Imazamox	CL312622	CL354825	Imazamox	CL312622	CL354825
Ta (1995)	NJ 1995	DFOP	277[a]	43.4	1000[b]	295	46.2	1000[b]
Ta & Lewis (1997)	Limours	SFO	397	ND[c]	110	389	ND[c]	107
	Boissy	SFO	48.6	5.8	53.0	47.8	5.7	52.1
	Pontfaverger	DFOP	118[a]	18.2	251	106	16.5	227
Ta (1997)	NJ 1997	DFOP	232[a]	18.8	300	248	20.1	319
Ta (2011)	NJ 2011	DFOP	143[a]	67.4	1000[b]	117	55.0	1000[b]
	Bruch West	DFOP	186[a]	35.9	1000[b]	186	35.9	1000[b]
					Geomean	**164**	**23.6**	**327**

[a] Calculated from $\ln(2)/k_{slow}$
[b] Endpoints conservatively fixed to 1000 days
[c] Not determined, as formation rate < degradation rate

III. CONCLUSION

Trigger and modelling endpoints were derived for imazamox and its metabolites CL 312622 and CL 354825 in four laboratory degradation studies with seven soils. Imazamox followed bi-phasic kinetics in most soils while endpoints for metabolites were derived from SFO kinetics. Trigger endpoints were derived for all soils from non-normalized data. Trigger DT_{50} values ranged from 10.8 to 397 days, 5.8 to 67.4 days and 53.0 to 1000 days for imazamox, metabolite CL 312622, and metabolite CL 354825, respectively. Modelling $DegT_{50}$ values were determined and normalised to reference conditions (20 °C, pF 2). The geometric mean of the modelling endpoints ($DegT_{50}$) was calculated to be 164 days for imazamox, 23.6 days for CL 312622, and 327 days for CL 354825. The average metabolite formation fractions were 0.850 for CL 312622 and 0.765 for CL 354825.

Summary of aerobic soil degradation rates of imazamox from laboratory studies

The non-normalized and normalized degradation rates of imazamox in aerobic soil are summarized below.

Table 7.1.2.1.1-6: Overview of laboratory aerobic soil degradation rates for imazamox

Study (BASF DocID)*	Soil	Soil Texture	Non-normalized trigger endpoint			Normalized modeling endpoint	
			Kinetic model	DT_{50} [d]	DT_{90} [d]	Kinetic model	$DegT_{50}$ [d]
Ta 1995 (1995/7000938)	NJ 1995	Sandy loam[a]	DFOP	13.4	375	DFOP	295
Ta & Lewis 1997 (1997/7000971)	Limours	Silt loam[b]	SFO	397	1000[c]	SFO	389
	Boissy	Silt loam[b]	SFO	48.6	161	SFO	47.8
	Pontfaverger	Silty clay loam[b]	DFOP	10.8	45.2	DFOP	106
Ta 1997 (1997/7001038)	NJ 1997	Sandy loam[a]	FOMC	27.7	609	DFOP	248
Ta 2011 (2011/7002438)	NJ 2011	Loam[a]	FOMC	24.6	1000[c]	DFOP	117
	Bruch West	Sandy loam[a]	DFOP	38.1	446	DFOP	186
						Geomean	**164**

[a] Soil texture based on USDA classification
[b] Soil texture based on UK classification
[c] Reported value > 1000 days. Fixed to 1000 days as a practical conservative approximation
[*] Kinetic evaluation for all soils was performed by Donaldson (BASF DocID 2013/7001767). See CA 7.1.2.1.1/1.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

See CA 7.1.2.1.1/1 for a discussion of the derivation of kinetic endpoints for imazamox degradation products CL 312622 and CL 354825 in aerobic soil laboratory studies. A summary of these values is also given below.

Table 7.1.2.1.2-1: Overview of soil aerobic degradation rates for the imazamox metabolites CL 312622 and CL 354825

Study (BASF DocID)*	Soil	CL 312622			CL 354825		
		Trigger-DT_{50} [d]	Trigger-DT_{90} [d]	Modeling[a]-$DegT_{50}$ [d]	Trigger-DT_{50} [d]	Trigger-DT_{90} [d]	Modeling[a]-$DegT_{50}$ [d]
Ta 1995 (1995/7000938)	NJ 1995	43.4	144	46.2	1000[b]	1000[b]	1000[b]
Ta & Lewis 1997 (1997/7000971)	Limours	ND[c]	ND[c]	ND[c]	110	364	107
	Boissy	5.8	19.4	5.7	53.0	176	52.1
	Pontfaverger	18.2	60.5	16.5	251	833	227
Ta 1997 (1997/7001038)	NJ 1997	18.8	62.6	20.1	300	995	319
Ta 2011 (2011/7002438)	NJ 2011	67.4	224	55.0	1000[d]	1000[d]	1000[d]
	Bruch West	35.9	119	35.9	1000[d]	1000[d]	1000[d]
			Geomean	**23.6**		**Geomean**	**327**

[a] $DegT_{50}$ values have been normalized to standard temperature and moisture conditions (20°C, pF 2)

[b] Reported value > 1000 days. Fixed to 1000 days as a practical conservative approximation

[c] Not determined, as formation rate < degradation rate

[d] Rate constant not robustly estimated. Fixed to 1000 days as a practical conservative approximation

[*] Kinetic evaluation for all soils was performed by Donaldson (BASF DocID 2013/7001767). See CA 7.1.2.1.1/1.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

See CA 7.1.1.2 for discussion of anaerobic degradation of imazamox. Imazamox is stable under anaerobic conditions.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

See CA 7.1.1.2 for discussion of anaerobic degradation of imazamox and degradation products. Imazamox and degradation products appear essentially stable under anaerobic conditions.

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

Report:	CA 7.1.2.2.1/1 Holzer S., 2013a Field soil dissipation study of BAS 720 H (Imazamox) in the formulation BAS 720 06 H on bare soil at 2 different sites in Southern Europe, 2011-2012 2013/1211059
Guidelines:	NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), SETAC Procedures for assessing the environmental fate and behaviour and ecotoxicity of pesticides (March 1995), EFSA Guidance to obtain DegT50 values in soil (2010)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 7.1.2.2.1/2 Holzer S., 2013d 1st addendum to report - Field soil dissipation study of BAS 720 H (Imazamox) in the formulation BAS 720 06 H on bare soil at 2 different sites in Southern Europe, 2011-2012 2013/1347977
Guidelines:	NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), SETAC Procedures for assessing the environmental fate and behaviour and ecotoxicity of pesticides (March 1995), EFSA Guidance to obtain DegT50 values in soil (2010)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The degradation of imazamox (BAS 720 H) and its metabolites CL 312622 (Reg. No. 4110542) and CL 354825 (Reg. No. 4110603) was investigated in soil under field conditions at 2 sites in Europe representative of the southern EU conditions. One trial each was performed in Italy and Spain. Both sites represent typical regions of agricultural practice representative for growing crops including sunflowers that were selected as representative crop for the use of Imazamox.

The product BAS 720 06 H, formulated as a soluble concentrate liquid (SL), was broadcast applied to bare soil in a single application (with two runs) at a nominal rate of 75 g a.s./ha on each subplot and using a target water volume of 200-400 L/ha. Applications were conducted in Spain on 10th May 2011 and in Italy on 12th May 2011 by using a calibrated boom sprayer. In order to protect the applied product from the sun and to exclude any potential impact on the degradation of the test item caused by sunlight, immediately after application and before the subsequent soil sampling, the plots were covered with a layer of sand of approximately 3-5 mm depth. No tillage or fertilization was performed during the course of the study and no crops were grown throughout the trials. The plots were kept free of vegetation via the application of glyphosate.

Soil specimens were taken at intervals up to nominal 360 days after application (DAA) down to a soil depth of up to 90 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at < -18°C within less than 7 hours of being taken and remained frozen until analysis.

Soil specimens were analysed for imazamox and its metabolites CL 312622 and CL 354825 according to the analytical BASF method No. D1102. The analytical method involved extraction of the soil with 0.5 N NaOH, sample clean-up using reverse phase (C18) solid phase extraction followed by a SCX column. The final determination of the analyte was performed by LC-MS/MS. The limit of quantitation (LOQ) was 0.001 mg/kg for each analyte.

No residues above the LOQ of imazamox or its metabolites, CL 312622 and CL 354825, were observed in any of the untreated control specimens proving that there were no interferences of the untreated soil material with the analytical procedures used.

Imazamox degraded quickly under field conditions in soil at the two sites in Spain and Italy. For the trial 11/01899536-01 (Spain), the total amount of imazamox residues observed in the soil profile decreased from 52.2 - 74.7 g/ha on 0 DAA to 2.4 - 6.5 g/ha after 125 DAA with no residues above the LOQ after 245 DAA. For the trial 11/01899536-02 (Italy), the total amount of residues observed in the soil profile decreased from 60.4 – 69.3 g/ha on 0 DAA to 9.1 – 14.4 g/ha after 358 DAA. A kinetic analysis of the data according to FOCUS (2006) kinetics guidance is presented for both sites in a separate modelling report. See CA 7.1.2.2.1/7 for the kinetic analysis.

The main proportion of imazamox residues was found in the upper 10 cm of the soil and only trace amounts close to the LOQ were observed in the 10 - 20 cm layer in the period 14 DAA to 31 DAA (trial 11/01899536-01, Spain) or at later sampling times (11/01899536-02, Italy). No imazamox residues above the LOQ of 0.001 mg/kg were observed in the 20 – 30 cm horizon of both trial sites.

Metabolite CL 354825 was formed in both trials, reaching maximum amounts of 16.2 – 21.2 g/ha in Spain at 125 DAA and 8.2 – 9.6 g/ha in Italy after 273 – 358 DAA. It was exclusively observed in the top 0 - 20 cm soil layer, except for 2 single detects at the LOQ that were observed in the 20 - 30 cm horizon at the trial site in Italy.

Soil concentrations of metabolite CL 312622 were low and mostly close to the LOQ. It was exclusively found in the top soil layer (0-10 cm) and was not longer observed above after 125 DAA and 60 DAA in the trial sites of Spain and Italy, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation):	BAS 720 06 H
Active ingredient:	Imazamox (BAS 720 H, Reg.No. 4096483)
Type of formulation:	soluble concentrate liquid (SL)

2. Test sites

The dissipation of imazamox under field conditions was investigated at two sites in Europe representative of Southern EU conditions. One trial site was located in Spain (11/01899536-01) and the other in Italy (11/01899536-02). The soil characteristics of the soils are presented in Table 7.1.2.2.1-1.

Table 7.1.2.2.1-1: Characteristics of the trial sites used to investigate the field dissipation of BAS 720 H (Imazamox)						
Trial	**11/01899536-01**			**11/01899536-02**		
Location	**Zafarraya, Spain**			**Budrio, Italy**		
Soil properties	0 – 30 cm	30 – 60 cm	60 – 90 cm	0 – 30 cm	30 – 60 cm	60 – 90 cm
Soil texture (DIN 4220)	Silty loamy sand	medium sandy loam	Poor sandy loam	Sandy loamy silt	Silty loamy sand	Poor sitly sand
sand [%]	42.4	38.4	31.9	37.4	40.0	75.3
silt [%]	43.6	39.3	45.2	50.6	49.3	21.2
clay [%]	13.8	22.3	22.8	12.0	10.7	3.6
Soil texture (USDA)	Loam	Loam	Loam	Loam	Loam	Loamy sand
sand [%]	46.1	41.9	36.4	46.3	46.9	80.1 15.6
silt [%]	40.1	38.0	42.8	41.7	42.8	4.4
clay [%]	13.8	20.1	20.8	12.0	10.3	
Organic C (TOC) [%]	1.21	0.61	0.28	0.82	0.65	0.41
Organic matter [%] *	2.09	1.05	0.48	1.41	1.12	0.71
pH [H_2O]	7.55	7.88	7.90	8.60	8.76	8.91
pH [$CaCl_2$]	7.14	7.41	7.40	7.69	7.84	7.97
CEC [mval Ba/100g dry weight]	15.1	15.5	16.5	13.5	10.9	10.2
WHC_{max} [g/100g dry weight]	36.9	35.4	37.8	53.3	42.4	34.6
pF 2.0 [g/100g]	25.6	28.1	30.3	27.0	28.4	20.7
pF 2.5 [g/100g]	21.9	24.2	26.2	21.2	21.8	15.1
Specimen No.	-115**	-116**	-117**	-002	-003	-004
Dry bulk density [g/cm³]	1.68***	n.a.	n.a.	1.56***	n.a.	n.a.
Soil taxonomy	xerochrept			calcaric cambisol		

Summation of particle sizes may differ slightly from 100% due to rounding tolerances
* organic matter = organic carbon x 1.724
** taken at sampling event 295 DAA
*** Specimens taken at 10 - 20 cm depth (mean value of three replicates)
CEC: Cation Exchange Capacity
$WHC_{max:}$ Maximum Water Holding Capacity
pF 2.0 and pF 2.5: Soil moisture according to the Eijkelkamp pressure plate method (determined at two pressures)

The homogeneity of the top 0-20 cm soil layer was verified prior to the start of the trials. The selected fields represented typical regions of agricultural practice for growing crops including sunflowers and they had been under cultivation for many years. The sites were flat without any significant slope. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing, rolled and was then left fallow. No product containing imazamox had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size 24 m^2 in Spain and 60 m^2 in Italy), which was subdivided into 2 subsubplots of equal size, and one treated plot, which consisted of 3 equal sized subplots A, B and C (114 m^2 for each subplot, total treated area was 342 m^2 in Spain and Italy).

The product, formulated as a soluble concentrate liquid (SL, 40 g a.s./L), was broadcast applied to bare soil in a single application (with two runs) at a nominal rate of 75 g a.s./ha and using a target water volume of 200 - 400 L/ha. Applications were conducted in Spain (11/01899536-01) on the 10th of May 2011 and in Italy (11/01899536-02) on the 12th of May 2011 by using a calibrated boom sprayer. A separate spray mixture was prepared (40.0 g. a. s./L) and the test item was applied to each subplot separately in two runs. Spray mixtures were visually checked for homogeneity and 10 mL aliquots of the spray mixture were taken before and after application of each subplot and analysed for imazamox. The average concentration in the spray application mixtures for each subplot was determined to be in a range of 0.125 – 0.179 g/L for the trial 11/01899536-01 (Spain), and in a range ranged between 0.175 – 0.176 g/L for the trial 11/01899536-02 (Italy). Details of application are presented in Table 7.1.2.2.1-2.

The actual application rates determined by quantifying the amount of spray discharged, ranged from 75.0 to 77.6 g a.s./ha (100.0% to 103.5% of the target rate) for trial 11/01899536-01 (Spain) and 73.2 to 77.7 g a.s./ha (97.6% to 103.6% of the target rate) trial 11/01899536-02 (Italy). In addition, the dose was verified by means of sampling Petri dishes filled with fine sieved soil (screened to 2 - 3 mm) from the same site but outside the plot area (approx. 50 g per dish). The Petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (10 in each subplot) before application. On completion of the application, the Petri dishes were sealed with a lid and taped up and frozen with dry ice immediately after sampling on site.

Table 7.1.2.2.1-2: Application parameters of field trial sites treated with BAS 720 06 H

Trial Country	No. of applications*	Application method	Application rate per treatment				No. of treated replicates	Application date
			nominal [g a.s./ha]	Subplot	actual** [g a.s./ha]	dose verification *** [%]		
11/01899536-01 Spain	1	broadcast spray to bare soil	75	A	77.6	107.2	3	10-May-2011
				B	76.6	105.3		
				C	75.0	91.7		
11/01899536-02 Italy	1	broadcast spray to bare soil	75	A	74.6	94.3	3	12-May-2011
				B	73.2	92.3		
				C	77.7	98.8		

* Dose applied: 40.0 g a. s./L was done as two runs.

** determined by calculation of spray liquid applied

*** determined by means of Petri dishes filled with soil [% of nominal rate]

a.s. = imazamox

Immediately after application and before subsequent soil sampling, the control plot and the treated replicates were covered with a thin layer of sand (approx. 5 mm) to protect the applied product from the sun and to exclude any potential impact on the degradation of the test item caused by sunlight. Application of sand was performed by hand for the trial 11/01899536-01 (Spain) or by using a drop spreader for the trial 11/01899536-02 (Italy). Due to a lack of sand in Spain, the control plot was covered 7 days after application. An aliquot of the sand (200 g) was taken after sand application and stored deep frozen. The sand cover was checked and documented for both trials at each sampling event during the whole study. The thickness was between 3 and 5 mm until 14 DAA, which triggered no renewal. This was decided by visual check of the covered area.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout the trials. The plots were kept free of vegetation via the application of glyphosate.

Irrigation was applied to both field trials from the beginning of the study. No irrigation was applied in winter time. The amount applied was documented and verified by means of rain measuring devices, which were placed at each treated replicate and control plots (5 were placed on each treated replicate plot and 2 in the control plot). Irrigation was added at a rate that prevents runoff from the plot surface. Irrigation was applied to the control plot and to the treated plot. If the soil was extremely wet (e.g. by excessive rainfall), then no additional irrigation was applied at the scheduled time intervals. Further details of irrigation are presented in Table 7.1.2.2.1-3.

Climatic conditions were based on records of appropriate weather stations located 300 m of the trial site (Spain) or directly on-site (Italy). Monthly summary results on air temperature, precipitation are presented in Table 7.1.2.2.1-3.

Table 7.1.2.2.1-3: Summary of monthly air temperature, precipitation and irrigation at each field trial site

Trial	11/01899536-01			11/01899536-02		
Location	Zafarraya Spain			Budrio, Italy		
Climatic conditions / Month	T_{mean} Air [°C]	Precipitation [mm]	Irrigation [mm]	T_{mean} Air [°C]	Precipitation [mm]	Irrigation [mm]
Apr 11	13.7	76.2	none	not available	not available	not available
May 11	14.7	67.4	none	18.7	44.6	none
Jun 11	19.3	4.4	43.1	22.0	45.6	28.0
Jul 11	21.9	0.0	58.2	23.3	44.0	28.0
Aug 11	21.7	0.0	84.9	25.2	5.0	34.0
Sep 11	17.3	3.2	63.3	22.0	52.0	40.2
Oct 11	13.5	94.6	27.8	13.1	46.6	58.5
Nov 11	8.7	186.0	none	7.3	34.6	none
Dec 11	6.3	9.4	none	3.3	21.0	none
Jan 12	4.9	38.4	none	0.9	8.4	none
Feb 12	3.4	6.8	none	-0.3	31.8	none
Mar 12	7.9	24.8	none	10.6	2.6	32.0
Apr 12	9.9	112.4	none	12.7	77.4	26.0
May 12	15.9	28.8	none	17.4	78.6	none

2. Sampling

Replicate soil samples (8 per treated subplot, 15 soil specimens at the beginning of the study or 10 soil specimens at the end of the study per control plot) were taken at intervals up to nominally 360 days after application (DAA) down to a maximum soil depth of 90 cm. At 0 DAA, immediately after application, the treated plots were sampled down to 10 cm only. From 1 DAA until 14 DAA the maximum sampling depth was 50 cm, thereafter specimens were taken down to 90 cm. The detailed sampling intervals are presented in Table 7.1.2.2.1-4.

Table 7.1.2.2.1-4: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
11/01899536-01	Spain	-1, 0, 1, 2, 4, 7, 10, 14, 22, 31, 45, 62, 91, 125, 245*, 295* and 367
11/01899536-02	Italy	-2, 0, 1, 2, 4, 7, 10, 14, 20, 29, 43, 60, 89, 120, 180, 273 and 358

* Samplings were postponed to 245 DAA instead of 180 DAA and to 295 DAA instead of 270 DAA, respectively. Due to too wet weather conditions, no sampling was possible during the planned time schedule.

Untreated soil specimens were collected from the control plot between 1 and 2 days before the application down to a depth of 90 cm, and on the final sampling day to a depth of 10 cm. Samples (15) were taken randomly from half of the untreated plot for the first sampling. On the final day of sampling the number of specimens was reduced to 10.

Treated soil samples were taken randomly from 8 points of each of the three treated subplots A to C and designated subsubplots. The specimens of each subplot were handled individually. All soil specimens from 0 - 10 cm depth collected from the treated plots were taken separately using metal tubes of 11.0 cm diameter by pressing a metal tube into the ground and collecting the soil with a spoon or similar device. Specimens taken at 0 DAA were packed in metal boxes. For other sampling dates, 0 – 10 cm specimens were stored in a plastic bag. Soil specimens deeper than 10 cm were collected through the centre of the excavation hole contained by the guard tube, using a common soil corer fitted with plastic liners (coring diameter usually 4.6 cm, in some cases 4.5 cm, length 100 cm). Sampling of these cores was conducted in one run. After sampling, the remaining holes were backfilled with untreated soil from outside the plots.

For soil characterization, a set of 5 cores of 0 – 90 cm depth was taken with the soil corer described above at 1 day before application (DBA) for the trial 11/01899536-01 (Spain) and 2 DBA for trial 11/01899536-02 (Italy). They were cut according to their visually assigned geological horizons and pooled accordingly.

Additionally to the main sampling described above, a second complete sampling (double sampling) was carried out. These reserve specimens were put into the freezers at the field test sites. The double specimens remained at the field test site except for the 0 DAA specimens which were sent to the laboratory for milling and residue analysis.

In order to demonstrate the stability of the residues in soil during storage and any shipments, shipment verification specimens were prepared at nominal sampling events 0, 30, 90, 120 and 180 DAA by fortifying untreated soil from the field site with known amounts of imazamox. These specimens were stored and shipped together with the residue specimens that were taken from the field at the same sampling event.

3. Description of analytical procedure

Field soil specimens (bare soil segments, application verification specimens and shipping verification specimens) were analysed for BAS 720 H (Imazamox) and its metabolites CL 312622 (Reg. No. 4110542) and CL 354825 (Reg. No. 4110603) according to the analytical BASF method No. D1102. The application verification specimens (Petri dishes) and shipping verification speciments were analysed for imazamox only. Segments originating from the treated plots were analysed from the top to the bottom until one segment without residues (residues lower than LOQ) were found. Analysis was conducted down to a maximum soil depth of 40 cm.

The analytical method involved extraction of the soil with 0.5 N NaOH, sample clean-up using reverse phase (C18) solid phase extraction followed by a SCX column. The final determination of the analyte was performed by LC-MS/MS with a limit of quantitation (LOQ) of 0.001 mg/kg for each analyte.

II. RESULTS AND DISCUSSION

A. METHOD VALIDATION

The analytical method has been validated for the determination of imazamox, metabolites CL312622 and CL354825. No residues above the LOQ of imazamox and metabolites CL312622 and CL354825 were observed in any of the control specimens. This proves that there were no interferences of the untreated soil material with the analytical procedures used.

Procedural recovery experiments performed with untreated field soil specimens spiked with the three analytes at concentration levels of 0.001 mg/kg, 0.01 mg/kg, 0.05 mg/kg and 0.1 mg/kg yielded overall mean recovery rates including all fortification levels of 92.0% (imazamox), 87.0% (CL 312622) and 79.0% (CL 354825) for the analysis of the specimens taken from the trial 11/01899536-01 (Spain). Untreated field soil specimens from the trial 11/01899536-02 (Italy) were spiked at same concentration levels and procedural recovery rates were 90.6% for imazamox, 90.6% for CL 312622 and 80% CL 354825. Relative standard deviations of all analytes from each fortification level including both trials were below 12.5%. The results confirm the validity of the analytical method used in this study.

B. VERIFICATION SAMPLES

The average amount of imazamox observed in the application monitors (Petri dishes specimens) from trial 11/01899536-01 (Spain) was 101.4% of the target rate of 75 g a.s./ha, with a relative standard deviation (RSD) of 15.5%. For the trial 11/01899536-02 (Italy), the mean recovery was 95.3% of the nominal rate with a RSD of 12.5%.

Analysis of the shipping verification specimens on imazamox using the analytical BASF method No. D1102 yielded average recovery values (corrected for procedural recovery) of 97 - 103% for the trial 11/01899536-01 (Spain) and 75 - 98% for the trial 11/01899536-02 (Italy) confirming residue stability during all storage and shipment procedures.

C. FINDINGS

All 0-10 cm specimens were analysed in duplicate and the individual numbers averaged to produce a mean for the respective soil specimen. When one of the values was below the LOQ, it was averaged as half of LOQ. For both trials, the double specimens of the 0 - 10 cm soil layer of 0 DAA were also analysed to account for the importance of the day 0 DAA, and the final values were obtained by averaging the mean values of the respective main and double specimens.

In trials 11/01899536-01 (Spain) and 11/01899536-02 (Italy), imazamox in soil showed a rapid decline in all 3 subplots under field conditions. In addition, the main proportion of imazamox residues was found in the top 0 - 10 cm soil layer and only trace amounts close to the LOQ were observed sporadically in the 10 - 20 cm horizon at later sampling times. No imazamox residues above the LOQ of 0.001 mg/kg were observed in the 20 - 30 cm horizon.

Residues of imazamox and its metabolites, CL 312622 and CL 354825, in treated soil samples in the top 0-10 cm soil layer from the trials in Spain and Italy are presented in Table 7.1.2.2.1-5 and Table 7.1.2.2.1-6, respectively. Residue values in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries.

Table 7.1.2.2.1-5: Residues of imazamox and its metabolites CL 312622 and CL 354825 in treated soil samples (0-10 cm) of trial 11/01899536-01 (Spain)

DAA	Imazamox			CL 312622 (Reg. No. 4110542)			CL 354825 (Reg. No. 4110603)		
	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]
0	0.065	0.074	0.049	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1	0.039	0.058	0.058	<0.001	0.001	0.001	<0.001	<0.001	<0.001
2	0.048	0.055	0.043	0.002	0.002	0.002	<0.001	<0.001	<0.001
4	0.047	0.054	0.046	0.001	0.002	0.001	<0.001	<0.001	<0.001
7	0.045	0.046	0.042	0.002	0.002	0.001	<0.001	<0.001	<0.001
10	0.042	0.039	0.030	0.003	0.003	0.003	<0.001	<0.001	<0.001
14	0.020	0.022	0.016	0.002	0.004	0.003	0.002	0.002	0.001
22	0.019	0.017	0.016	0.004	0.003	0.004	0.006	0.006	0.005
31	0.013	0.010	0.009	0.003	0.002	0.002	0.011	0.008	0.009
45	0.007	0.007	0.007	0.001	0.002	0.001	0.012	0.012	0.010
62	0.006	0.003	0.005	<0.001	<0.001	<0.001	0.009	0.011	0.010
91	0.005	0.003	0.004	0.002	<0.001	<0.001	0.012	0.008	0.007
125	<0.001	0.003	0.002	<0.001	<0.001	<0.001	0.016	0.020	0.015
245	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.010	0.011	0.008
295	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	0.011	0.011
367	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.013	0.011	0.008

DAA= days after application

Table 7.1.2.2.1-6: Residues of imazamox and its metabolites CL 312622 and CL 354825 in treated soil samples (0-10 cm) of trial 11/01899536-02 (Italy)

DAA	Imazamox			CL 312622 (Reg. No. 4110542)			CL 354825 (Reg. No. 4110603)		
	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]
0	0.048	0.048	0.053	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
1	0.032	0.034	0.038	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.046	0.045	0.043	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
4	0.040	0.037	0.048	<0.001	<0.001	<0.001	0.002	0.001	<0.001
7	0.041	0.035	0.039	0.001	0.001	0.002	0.002	<0.001	0.001
10	0.032	0.033	0.034	0.001	0.001	0.002	0.002	0.002	<0.001
14	0.034	0.027	0.029	0.001	0.001	<0.001	0.001	0.002	0.001
20	0.029	0.034	0.031	<0.001	0.001	0.001	0.002	0.001	0.001
29	0.026	0.026	0.031	<0.001	0.001	0.002	0.003	0.002	0.002
43	0.017	0.020	0.030	<0.001	0.001	0.002	0.004	0.003	0.003
60	0.025	0.017	0.020	0.001	<0.001	<0.001	0.003	0.004	0.003
89	0.020	0.015	0.016	<0.001	<0.001	<0.001	0.005	0.005	0.004
120	0.012	0.014	0.012	<0.001	<0.001	<0.001	0.005	0.005	0.006
180	0.012	0.011	0.010	<0.001	<0.001	<0.001	0.006	0.006	0.006
273	0.013	0.014	0.012	<0.001	<0.001	<0.001	0.007	0.007	0.007
358	0.007	0.010	0.010	<0.001	<0.001	<0.001	0.006	0.006	0.007

DAA = days after application

Residue levels of the three analytes in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the field specimen, and were summed up for all the analysed depths between 0 and 40 cm.

The total amount of imazamox residues observed in the soil profiles from the trial 11/01899536-01 (Spain) decreased from 52.2 - 74.7 g/ha on 0 DAA to 2.4 - 6.5 g/ha after 125 DAA, with no residues above the LOQ left after 245 days. For the trial 11/01899536-02 (Italy), the total amount of residues observed in the soil profiles decreased from 60.4 – 69.3 g/ha on 0 DAA to 9.1 – 14.4 g/ha after 358 DAA. No imazamox residues above the LOQ of 0.001 mg/kg were observed in the 20 - 30 cm horizon in both trial sites.

In the trial 11/01899536-01 (Spain), residues of metabolite CL 354825 (Reg. No. 4110603) were observed from 14 DAA on, reaching maximum amounts of 16.2 – 21.2 g/ha in the 3 subplots at 125 DAA. Amounts subsequently declined to values between 7.8 and 11.9 g/ha at the end of the study (367DAA). Metabolite CL 354825 was observed only in the top 0-20 cm soil layer and was not observed above the LOQ in the 20-30 cm horizon.

Concentrations of metabolite CL 312622 (Reg. No. 4110542) in the soil were only low and mostly close to the LOQ. Residues were observed in the upper 0 – 10 cm layer from 1 DAA onwards up to a maximum concentration of 5.4 g/ha at 22 DAA. Thereafter, concentrations of the metabolite declined and the analyte was no longer observed after 125 DAA in all 3 subplots. The metabolite CL 312622 was not observed above LOQ in the 10 – 20 cm and 20 – 30 cm horizons in the Spanish trial.

In the field trial 11/01899536-02 (Italy), residues of the metabolite CL 354825 were observed already on the day of application for subplot A and slowly increased during the study to a maximum value of 8.2 - 9.6 g/ha in the 3 subplots after 273 – 358 DAA. Metabolite CL 354825 was only observed in the top 0 - 20 cm soil horizon, except for two single detects at the LOQ that were observed in the 20 - 30 cm horizon. In the 30- 40 cm horizon, CL 354825 was not observed above LOQ in the lower horizons analysed. Considering the metabolite CL 312622, its soil concentrations were very low and always close to the LOQ. CL 312622 residues were only observed between 7 and 60 DAA. This metabolite was exclusively found in the top 0 - 10 cm soil layer and not observed above LOQ in the 10 - 20 cm and 20 - 30 cm horizons.

III. CONCLUSION

Imazamox degraded quickly under field conditions in soil at the two sites in Spain and Italy. The treated plot at both trial sites was covered with 3-5 mm of sand immediately after application to protect the treated soil surface from sunlight. For the trial 11/01899536-01 (Spain), the total amount of imazamox residues observed in the soil profile decreased from 52.2 - 74.7 g/ha on 0 DAA to 2.4 - 6.5 g/ha after 125 DAA with no residues above the LOQ left after 245 DAA. For the trial 11/01899536-02 (Italy), the total amount of residues observed in the soil profile decreased from 60.4 – 69.3 g/ha on 0 DAA to 9.1 – 14.4 g/ha after 358 DAA. A kinetic analysis of the data according to FOCUS (2006) kinetics guidance is presented for both sites in a separate modelling report. See CA 7.1.2.2.1/7 for the kinetic analysis.

The main proportion of imazamox residues was found in the upper 10 cm of the soil and only trace amounts close to the LOQ were observed in the 10 - 20 cm layer in the period 14 DAA to 31 DAA (trial 11/01899536-01, Spain) or at later sampling times (11/01899536-02, Italy). No imazamox residues above the LOQ of 0.001 mg/kg were observed in the 20 – 30 cm horizon of both trial sites.

In both trial sites, the metabolite CL 354825 was formed, reaching maximum amounts of 16.2 – 21.2 g/ha at 125 DAA and 8.2 – 9.6 g/ha after 273 – 358 DAA in the trial sites of Spain and Italy, respectively. This metabolite was exclusively observed in the top 0 - 20 cm soil layer, except for 2 single detects at the LOQ (0.001 mg/kg) that were observed in the 20 - 30 cm horizon at the trial site in Italy.

Soil concentrations of metabolite CL 312622 were low and mainly at or mostly close at the LOQ (0.001 mg/kg). It was exclusively found in the top soil layer (0-10 cm) and was not longer observed above after 125 DAA and 60 DAA in the trial sites of Spain and Italy, respectively.

Report:	CA 7.1.2.2.1/3 Holzer S., 2013c Field soil dissipation study of BAS 720 H (Imazamox) in the formulation BAS 797 00 H on bare soil at 3 different sites in Northern Europe, 2011-2012 2013/1229816
Guidelines:	NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), EFSA Guidance to obtain DegT50 values in soil (2010), SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The dissipation of imazamox (BAS 720 H) and its metabolites CL 312622 (Reg. No. 4110542) and CL 354825 (Reg. No. 4110603) under field conditions in soil was investigated at three sites in Europe representative of northern EU conditions. One trial each was performed in the UK, Northern France and Germany. All sites represent typical regions of agricultural practice representative for growing oilseed rape.

The product BAS 797 00 H, formulated as a suspension concentrate (SC), was broadcast applied to bare soil in a single application (with two runs) at a nominal rate of 75 g a.s./ha and using a target water volume of 200-400 L/ha. Applications were conducted in the UK on 20th June 2011, in France on 05th October 2011 and in Germany on 20th September 2011 by using a calibrated boom sprayer. Immediately after application and before the subsequent soil sampling the plots were covered with a layer of sand of approximately 3 - 5 mm depth to protect the applied product from the sun and to exclude any potential photo-degradation of the test item caused by sunlight.

No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate.

Soil specimens were taken at intervals up to nominal 360 days after application (DAA) down to a soil depth of up to 90 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at ≤ -18°C within less than 9 hours of being taken and remained frozen until analysis.

Soil specimens were analysed for imazamox and its metabolites CL 312622 and CL 354825, according to the analytical BASF method No. D1102. The analytical method involved extraction of the soil with 0.5 N NaOH, sample clean-up using reverse phase (C18) solid phase extraction followed by a SCX column. The final determination of the analyte was performed by LC-MS/MS. The limit of quantitation (LOQ) was 0.001 mg/kg for each analyte. Analysis of soil specimens originating from the treated plots was conducted to a depth until at least one soil segment was free of quantifiable residues (< LOQ of 0.001 mg/kg, maximum depth of analysis: 70 cm).

No residues above the LOQ of imazamox and its metabolites, CL 312622 and CL 354825, were observed in any of the untreated control specimens proving that there were no interferences of the untreated soil material with the analytical procedures used.

Imazamox degraded quickly in soil under field conditions at the three sites in the UK, France and Germany. For 11/01899538-01 (UK), the total amount of residues observed in the soil profiles decreased from 64.0 - 74.4 g/ha on 0 DAA to 3.3 - 4.9 g/ha at 358 DAA. For trial 11/01899538-02 (France), the total amount of residues observed in the soil profiles decreased from 63.7 - 70.5 g/ha on 0 DAA to 7.0 - 20.9 g/ha at 372 DAA. For trial 11/01899538-03 (Germany), the total amount of residues observed in the soil profiles decreased from 67.8 - 70.4 g/ha on 0 DAA to 4.9 - 5.7 g/ha at 274 DAA. At this site, no residues of Imazamox were observed above the LOQ at the last sampling of this study (360 DAA). A kinetic analysis of the data for all sites according to FOCUS (2006) kinetics guidance is presented in a separate modelling report. See CA 7.1.2.2.1/7 for the kinetic analysis.

The main proportion of imazamox residues was found in the upper 0 - 20 cm of the soil and only trace amounts at or close to the LOQ were observed in the 20 - 30 cm soil layers. In the 30 – 60 cm layers residues in the range of the LOQ were only observed at 88 DAA for the site in Germany (trial 11/01899538-03). No imazamox residues above LOQ were observed in the 60 - 70 cm horizon for any of the sites.

The metabolite CL 354825 was formed in all trials reaching maximum amounts of 9 - 12 g/ha in the UK, 17 - 19 g/ha in France and 11 - 15 g/ha in Germany. The main proportion of the residues was observed in the top 0 - 20 cm soil layer and only trace amounts at or close to the LOQ were observed in the 20 - 30 cm soil layer. No metabolite CL 354825 residues above LOQ were observed in the 40 - 70 cm horizon.

Concentrations of the metabolite CL 312622 in the three soils were low and mainly at or close to the LOQ. It was exclusively found in the upper 10 cm of the soils except for one specimen for which residues were found in the range of the LOQ down to 40 cm.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation):	BAS 797 00 H
Active ingredient:	Imazamox (Reg.No. 4096483). The test item also contained Metazachlor but this compound is not of interest for the present study. No residue analysis of metazachlor was performed and therefore following information will only be given for Imazamox
Type of formulation:	Suspension concentrate (SC),

2. Test sites

The dissipation of imazamox under field conditions was investigated at three sites in Europe representative of northern EU conditions. One trial each was performed in the UK (trial 11/01899538-01), Northern France (trial 11/01899538-02) and Germany (trial 11/01899538-03). The soil characteristics of the soils are presented in Table 7.1.2.2.1-7.

The homogeneity of the top 0 - 20 cm soil layer was verified prior to the start of the trials. The selected fields represented typical regions of agricultural practice representative for growing crops including oilseed rape and had been under cultivation for many years. The sites were flat without any significant slope (0 - 1%). Before commencement of the first sampling, the soil at each trial site was prepared as for sowing, rolled and was then left fallow. No product containing the active substance imazamox of the test item had been used on the test plots in the last three years.

Table 7.1.2.2.1-7: Characteristics of the trial sites used to investigate the field dissipation of BAS 720 H (imazamox)

Trial	11/01899538-01			11/01899538-02			11/01899538-03		
Location	Hampton Lucy, UK			Suzanne, France			Emstek, Germany		
Soil properties	0 – 30 [cm]	30 - 60 [cm]	60 – 90 [cm]	0 – 30 [cm]	30 – 60 [cm]	60 – 90 [cm]	0 - 30 [cm]	30 - 60 [cm]	60 - 90 [cm]
Soil texture (DIN 4220)	medium loamy sand	medium loamy sand	medium clay sand	pure silt	pure silt	poor clay silt	medium silty sand	medium silty sand	poor silty sand
sand [%]	77.8	76.7	68.9	7.0	4.8	4.1	67.7	60.8	76.8
silt [%]	13.9	12.7	13.1	88.0	87.6	86.0	30.5	37.1	21.7
clay [%]	8.4	10.8	18.1	4.9	7.7	9.8	1.8	2.0	1.5
Soil texture (USDA)	Loamy sand	Sandy loam	Sandy loam	Silt	Silt	Silt	Loamy sand	Loamy sand	Loamy sand
sand [%]	79.2	77.5	71.5	9.7	7.4	5.8	76.2	78.0	81.4
silt [%]	12.8	12.6	11.9	85.4	84.8	83.6	21.9	20.3	16.9
clay [%]	7.9	9.8	16.5	4.9	7.8	10.5	1.9	1.7	1.8
Organic C (TOC) [%]	0.71	0.33	0.26	1.32	0.68	0.32	2.65	0.87	0.13
Organic matter [%] *	1.22	0.57	0.45	2.28	1.17	0.55	4.57	1.50	0.22
pH [H_2O]	7.28	7.46	7.26	6.82	7.16	7.05	6.02	5.89	6.53
pH [CaC_{l2}]	6.54	6.65	6.57	6.06	6.33	6.10	5.11	5.07	5.64
CEC [mval Ba/100g dry weight]	8.2	6.8	11.1	12.1	12.1	16.9	10.7	3.2	1.7
WHC_{max} [g/100g dry weight]	51.3	49.8	39.1	56.5	56.3	53.9	46.4	40.8	26.8
pF 2.0 [g/100g]	11.5	10.7	16.4	30.2	29.7	31.7	18.3	16.8	11.2
pF 2.5 [g/100g]	10.0	9.1	13.9	27.7	27.3	28.1	15.7	14.2	6.8
Specimen No.	-002	-003	-004	-002	-003	-004	-002	-003	-004
Dry bulk density [g/cm^3]**	1.65	n.a.	n.a.	0.90	n.a.	n.a.	1.38	n.a.	n.a.
Soil taxonomy	Brown soils			Cambisols			Pseudogley-Braunerde		

Summation of particle sizes may differ slightly from 100% due to rounding tolerances
* organic matter = organic carbon x 1.724
** Specimens taken at 10 - 20 cm depth (mean value of three replicates)
CEC: Cation Exchange Capacity
WHCmax: Maximum Water Holding Capacity
pF 2.0 and pF 2.5: Soil moisture according to the Eijkelkamp pressure plate method (determined at two pressures).

B. STUDY DESIGN

1. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size 30 m² in the UK, 60 m² in France and 12 m² in Germany) and one treated plot which consisted of 3 equal sized subplots A, B and C that were assigned for replicates (114 m² for each subplot in the UK and Germany and 120 m² in France).

Imazamox formulated as a suspension concentrate (SC, 17.5 g imazamox/L), was broadcast applied to bare soil in a single application (with two runs at a nominal rate of 75 g a.s./ha and using a target water volume of 200 - 400 L/ha. Applications were conducted in the UK on 20th June 2011, in France on 05th October 2011 and in Germany on 20th September 2011 by using a calibrated boom sprayer. For each subplot replicate, a separate spray mixture was prepared and the test item was applied to each subplot separately in two runs. A separate spray mixture was prepared and the test item (17.5 g imazamox/L SC) was applied to each subplot separately in two runs. Spray mixtures were visually checked for homogeneity and 10 mL aliquots of the spray mixture were taken before and after application of each subplot and analysed for imazamox. The average concentration in the spray application mixtures for each subplot was determined to be in a range of 0.245 - 0.266 g/L for the trial 11/01899538-01 (UK), 0.235 - 0.240 g/L for the trial 11/01899538-02 (France) and 0.191 - 0.192 g/L for trial 11/01899538-03 (Germany).

The actual application rates were determined by quantifying the amount of spray discharged. The actual application rates ranged from 74.4 to 75.7 g a.s./ha (99.2% to 100.9% of the target rate) for trial 11/01899538-01 (UK), 76.8 to 82.3 g a.s./ha (102.4% to 109.7% of the target rate) for trial 11/01899538-02 (France) and 71.6 to 72.6 g a.s./ha (95.5% to 96.8% of the target rate) for trial 11/01899538-03 (Germany). In addition, the dose was verified by means of sampling Petri dishes filled with fine sieved soil (screened to 2 - 3 mm) from the same site but outside the plot area (approx. 50 g per dish). The Petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (10 in each subplot) before application. On completion of the application, the Petri dishes were sealed with a lid and taped up and frozen with dry ice immediately after sampling on site. Details of application are presented in Table 7.1.2.2.1-8.

Table 7.1.2.2.1-8: Application parameters of field trial sites treated with BAS 797 00 H

Trial Country	No. of applications*	Application method	Application rate per treatment				No. of treated replicates	Application date
			nominal [g a.s./ha]	Subplot	actual** [g a.s./ha]	dose verification *** [%]		
11/01899538 -01 (UK)	1	broadcast spray to bare soil	75	A B C	74.4 75.7 74.7	99 94 99	3	20 Jun 2011
11/01899538 -02 (France)	1	broadcast spray to bare soil	75	A B C	76.8 78.6 82.3	90 98 101	3	05 Oct 2011
11/01899538 -03 (Germany)	1	broadcast spray to bare soil	75	A B C	71.6 72.3 72.6	95 97 105	3	20 Sept 2011

* Dose applied: 17.5 g a. s./L SC was done as two runs.
** determined by calculation of spray liquid applied
*** determined by means of Petri dishes filled with soil [% of nominal rate]
a.s. = imazamox

Immediately after application, the control plot and the treated replicates were covered with a layer of sand (approximately 5 mm) to protect the applied product from the sun and to exclude any potential impact on the photo-degradation of the test item caused by sunlight. The sand cover was visually checked and documented for all trials at each sampling event during the whole study. No renewal of the sand cover was performed for any trial.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout the trials. The plots were kept free of vegetation via the application of glyphosate.

Irrigation was applied to all field trials the beginning of the study. No irrigation was applied in winter time. The amount applied was documented and verified by means of rain measuring devices (5 were placed on each treated replicate and 2 in the control plot). Irrigation was added to the control plot and to the treated plot at a rate that prevents runoff from the plot surface. No additional irrigation was applied at the scheduled time intervals if the soil was extremely wet (e.g. by excessive rainfall).

Climatic conditions were based on records of appropriate weather stations located on-site in case of the field trial 11/01899538-01 (UK). The distance between weather station and field trials 11/01899538-02 (France) and 11/01899538-03 (Germany) was 1 km and 0.35 km, respectively. Monthly summary results on air temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-9.

Table 7.1.2.2.1-9: Summary of monthly air temperature, precipitation and irrigation at each field trial site to investigate BAS 797 00 H (SC)

Trial	11/01899538-01			11/01899538-02			11/01899538-03		
Location	Hampton Lucy, UK			Suzanne, France			Emstek, Germany		
Climatic conditions / Month	T_{mean} Air * [°C]	Precipitation [mm]	Irrigation [mm]	T_{mean} Air * [°C]	Precipitation [mm]	Irrigation [mm]	T_{mean} Air * [°C]	Precipitation [mm]	Irrigation [mm]
Jun 11	14.4	28	10	-	-	-	-	-	-
Jul 11	15.8	33	8	-	-	-	-	-	-
Aug 11	15.9	49	40	-	-	-	-	-	-
Sep 11	15.4	24	36	-	-	-	14.9	73	none
Okt 11	12.9	26	none	10.9	46	26	10.0	14	8
Nov 11	9.7	22	none	7.5	8	72	5.5	5.0	72
Dec 11	6.3	47	none	5.3	144	none	5.2	163	none
Jan 12	5.6	33	none	4.0	82	12	3.2	134	none
Feb 12	4.1	12	none	-0.3	26	none	0.1	24	none
Mrz 12	7.7	16	44	8.9	30	43	7.8	16	none
Apr 12	7.2	95	5	8.2	67	none	8.3	31	none
Mai 12	12.1	61	none	14.4	23	63	14.5	79	none
Jun 12	14.3	107	15	15.6	178	none	14.7	87	none
Jul 12	-	-	-	17.3	156	none	17.1	91	none
Aug 12	-	-	-	19.0	20	61	18.5	51	10
Sep 12	-	-	-	13.3	74	17	13.4	40	20
Okt 12	-	-	-	10.6	92	none	9.4	77	none

*Monthly mean of average temperature calculated from two sensors

2. Sampling

Replicate soil specimens were taken at intervals up to one year (372 DAA) down to a maximum soil depth of 90 cm. A total of 8 cores were sampled per treated subplot. At the beginning of the study, 15 cores were sampled per control plot and 10 cores at the end of the study, except for trial 11/01899538-01 (UK) where 12 cores were taken for the double specimen at the last sampling day. At day 0, immediately after application, the treated subplots were sampled down to 10 cm only. From 0 DAA until 14 DAA the max sampling depth was 50 cm, thereafter specimens were taken down to 90 cm. Untreated specimens were collected from the control plot between 7 and 1 days before the application down to a depth of 90 cm, and on the final day of sampling to a depth of 10 cm. Detailed sampling intervals are presented in Table 7.1.2.2.1-10.

Table 7.1.2.2.1-10: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
11/01899538-01	UK	-7, 0, 1, 2, 4, 7, 10, 14, 21, 30, 45, 60, 90, 120, 178, 270 and 358
11/01899538-02	France	-2, 0, 1, 2, 4, 7, 10, 14, 21, 30, 44, 61, 93, 160*, 180, 271 and 372**
11/01899538-03	Germany	-1, 0, 1, 2, 4, 7, 10, 14, 22, 29, 45, 59, 88, 168*, 184, 274 and 360

* Due to ground frost, samplings were postponed to 160 DAA and 168 DAA respectively, instead of 120 DAA.

** sampling was done later as the time schedule planed in the study plan (360 DAA) because of bad weather conditions

Treated soil specimens were taken randomly from 8 points of each of the three treated subplots A to C and designated subsubplots. The specimens from each subplot were handled individually. All soil specimens from 0 - 10 cm depth collected from the treated plots were taken separately using metal tubes of 11.0 cm diameter by pressing a metal tube into the ground and collecting the soil with a spoon or similar device. Specimens taken at 0 DAA were packed in metal boxes. For other sampling dates, 0 - 10 cm specimens were stored in a plastic bag. Soil specimens deeper than 10 cm were collected through the centre of the excavation hole contained by the guard tube, using a common soil corer fitted with plastic liners (length up to 100 cm, coring diameter 4.5 cm for trial 11/01899538-01 (UK) and 4.6 cm for trial 11/01899538-02 (France) and trial 11/01899538-03 (Germany). Sampling of these cores was conducted in one run. After sampling the remaining holes were backfilled with untreated soil from outside the plots.

Additionally to the main sampling described above, a second complete sampling (double sampling) was carried out. These reserve specimens were put into the freezers at the field test sites. The double specimens remained at the field test site except for the 0 DAA specimens which were sent to the laboratory for milling and residue analysis.

In order to demonstrate the stability of the residues in soil during storage and any shipments, shipment verification specimens were prepared at nominal sampling events 0, 30, 90 and 360 DAA (for one trial only) by fortifying untreated soil from the field site with known amounts of imazamox. These specimens were stored and shipped together with the residue specimens that were taken from the field at the same sampling event.

3. Description of analytical procedure

Field soil specimens (bare soil segments, application verification specimens and shipping verification specimens) were analysed for BAS 720 H (Imazamox) and its metabolites CL 312622 (Reg. No. 4110542) and CL 354825 (Reg. No. 4110603) according to the analytical BASF method No. D1102. The application verification specimens (Petri dishes) and shipping verification specimens were analysed for imazamox only. Segments originating from the treated plots were analysed from the top to the bottom until one segment without residues (residues lower than LOQ) were found. Analysis was conducted down to a maximum soil depth of 70 cm.

The analytical method involved extraction of the soil with 0.5 N NaOH, sample clean-up using reverse phase (C18) solid phase extraction followed by a SCX column. The final determination of the analyte was performed by LC-MS/MS with a limit of quantitation (LOQ) of 0.001 mg/kg for each analyte.

II. RESULTS AND DISCUSSION

A. METHOD VALIDATION

The analytical method has been validated for the determination of imazamox, metabolites CL312622 and CL354825. No residues above the LOQ of imazamox and metabolites CL312622 and CL354825 were observed in any of the control specimens. This proves that there were no interferences of the untreated soil material with the analytical procedures used.

Procedural recovery experiments performed with untreated field soil specimens spiked with the three analytes at concentration levels of 0.001, 0.01, 0.03, 0.04, 0.05, 0.06 and 0.1 mg/kg yielded overall mean recovery rates across all fortification levels of Imazamox of 91.9%, 92.4% and 91.4% for trial 11/01899538-01 (UK), trial 11/01899538-02 (France) and trial 11/01899538-03 (Germany), respectively. The obtained mean recoveries of CL 354825 were 76.2%, 79.6% and 83.0% for trial 11/01899538-01 (UK), trial 11/01899538-02 (France) and trial 11/01899538-03 (Germany), respectively. Procedural mean recoveries of CL 312622 were 85.8%, 86.8% and 87.6% for trial 11/01899538-01 (UK), trial 11/01899538-02 (France) and trial 11/01899538-03 (Germany), respectively. Relative standard deviations were below 20% for each analyte. The results confirm the validity of the analytical method used in this study.

B. APPLICATION VERIFICATION

The average amount of imazamox observed in the application monitors (Petri dishes specimens) from trial 11/01899538-01 (UK) was 98% of the target rate of 75 g a.s./ha, with a relative standard deviation (RSD) of 11%. For trial 11/01899538-02 (France) the mean recovery was 96% of the nominal rate with an RSD of 10% and for trial 11/01899538-03 (Germany), the mean recovery was 99% with a RSD of 9%.

Analysis of the shipping verification specimens on imazamox using the analytical BASF method No. D1102 yielded average recovery values of 87 - 96% for trial 11/01899538-01 (UK), 90 - 115% for trial 11/01899538-02 (France) and 90 - 94% for trial 11/01899538-03 (Germany) confirming residue stability during all storage and shipment procedures.

C. FINDINGS

All samples from 0-10 cm soil depth were analysed in duplicate and the individual numbers averaged to produce a mean for the respective soil specimen. Values below the LOQ were averaged as half of LOQ. For all trials, replicates of the 0 - 10 cm soil layer of 0 DAA were also analysed to account for the importance of the day 0 DAA, and the final values were obtained by averaging the mean values of the respective main and double specimens.

For all field trials, residues of the active ingredient imazamox in soil showed a steady decline in all three subplots under field conditions. In addition, the main proportion of imazamox residues was found in the top 0 - 10 cm soil layer in all trial sites. For trial 11/01899538-01 (UK), only trace amounts at the LOQ were observed once in the 10 - 20 cm layer (at 87 DAA in subplot B). Otherwise no residues were observed in the 10 - 20 cm and 20 - 30 cm horizon. For trials 11/01899538-02 (France) and 11/01899538-03 (Germany), was found in the 0 - 20 cm. Trace amounts at or close to the LOQ were observed in the 20 - 30 cm layer. No Imazamox residues above the LOQ were observed at deeper soil horizons, except at one occasion, 88 DAA, down to the 50 - 60 cm layer in the German trial.

Residues of imazamox and its metabolites, CL 312622 and CL 354825, in treated soil samples in the top 0-10 cm soil layer from the trial 11/01899538-01 (UK), 11/01899538-02 (France) and trial 11/01899538-03 (Germany), are presented in Table 7.1.2.2.1-11, Table 7.1.2.2.1-12 And Table 7.1.2.2.1-13, respectively. Residue values in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries.

Table 7.1.2.2.1-11: Residues of imazamox and metabolites CL 312622 and CL 354825 in treated soil samples (0-10 cm) of the trial 11/01899538-01 (UK)

DAT	Imazamox			CL 312622 (Reg. No. 4110542)			CL 354825 (Reg. No. 4110603)		
	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]
0	0.043	0.044	0.050	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1	0.055	0.054	0.045	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.043	0.047	0.041	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4	0.040	0.036	0.042	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
7	0.030	0.040	0.034	<0.001	0.001	0.001	<0.001	<0.001	<0.001
10	0.037	0.050	0.035	0.001	0.002	0.002	<0.001	<0.001	<0.001
14	0.046	0.031	0.033	0.002	0.001	0.001	<0.001	<0.001	<0.001
21	0.030	0.030	0.025	0.002	0.001	0.002	0.002	0.002	0.002
30	0.023	0.024	0.034	0.001	0.002	0.003	0.001	0.003	0.003
45	0.022	0.026	0.020	<0.001	0.001	<0.001	0.002	0.003	0.003
60	0.019	0.018	0.018	<0.001	<0.001	<0.001	0.004	0.004	0.004
87	0.011	0.015	0.009	<0.001	<0.001	<0.001	0.004	0.005	0.004
120	0.008	0.009	0.007	<0.001	<0.001	<0.001	0.005	0.008	0.007
178	0.004	0.009	0.003	<0.001	<0.001	<0.001	0.004	0.004	0.006
270	0.003	0.004	0.004	<0.001	<0.001	<0.001	0.004	0.005	0.005
358	0.003	0.002	0.003	<0.001	<0.001	<0.001	0.005	0.006	0.006

DAT = days after treatment

Table 7.1.2.2.1-12: Residues of imazamox and metabolites CL 312622 and CL 354825 in treated soil samples (0-10 cm) of trial 11/01899538-02 (France)

DAT	Imazamox			CL 312622 (Reg. No. 4110542)			CL 354825 (Reg. No. 4110603)		
	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]
0	0.054	0.060	0.059	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1	0.055	0.052	0.050	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.055	0.040	0.046	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4	0.043	0.047	0.045	<0.001	<0.001	0.030	<0.001	<0.001	<0.001
7	0.051	0.045	0.055	<0.001	0.002	0.050	<0.001	<0.001	<0.001
10	0.045	0.059	0.054	<0.001	0.002	0.007	<0.001	<0.001	<0.001
14	0.040	0.060	0.048	<0.001	0.002	0.003	<0.001	<0.001	<0.001
21	0.054	0.050	0.041	0.002	0.002	0.009	<0.001	<0.001	0.002
30	0.041	0.038	0.036	0.002	0.002	0.009	<0.001	0.001	0.003
44	0.041	0.034	0.038	0.002	0.004	0.005	0.002	0.002	0.003
61	0.038	0.035	0.036	0.001	0.001	0.001	0.003	0.003	0.003
93	0.041	0.038	0.018	<0.001	<0.001	0.005	0.003	0.003	0.008
160	0.035	0.036	0.016	<0.001	<0.001	<0.001	0.005	0.006	0.010
180	0.020	0.029	0.022	<0.001	<0.001	<0.001	0.006	0.008	0.010
271	0.019	0.015	0.009	<0.001	<0.001	<0.001	0.010	0.009	0.013
372	0.014	0.015	0.006	<0.001	<0.001	<0.001	0.011	0.016	0.012

DAT = days after treatment

Table 7.1.2.2.1-13: Residues of imazamox and metabolites CL 312622 and CL 354825 in treated soil samples (0-10 cm) of trial 11/01899538-03 (Germany)

DAT	Imazamox			CL 312622 (Reg. No. 4110542)			CL 354825 (Reg. No. 4110603)		
	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]	Replicate A [mg/kg]	Replicate B [mg/kg]	Replicate C [mg/kg]
0	0.055	0.056	0.056	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1	0.056	0.057	0.057	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.056	0.050	0.060	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4	0.058	0.059	0.060	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
7	0.056	0.054	0.049	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
10	0.057	0.052	0.058	<0.001	<0.001	0.001	<0.001	0.003	<0.001
14	0.046	0.053	0.051	0.001	0.001	0.001	<0.001	<0.001	<0.001
22	0.036	0.040	0.039	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
29	0.033	0.035	0.034	<0.001	<0.001	<0.001	0.002	0.003	<0.001
45	0.033	0.025	0.027	<0.001	<0.001	<0.001	0.005	0.003	0.005
59	0.024	0.025	0.032	<0.001	<0.001	<0.001	0.006	0.006	0.005
88	0.020	0.022	0.022	<0.001	<0.001	<0.001	0.007	0.007	0.005
168	0.009	0.009	0.009	<0.001	<0.001	<0.001	0.007	0.008	0.006
184	0.009	0.006	0.008	<0.001	<0.001	<0.001	0.007	0.006	0.005
274	0.004	0.005	0.005	<0.001	<0.001	<0.001	0.010	0.010	0.009
360	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.008	0.010	0.007

DAT = days after treatment

Residue levels of the three analytes in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the field specimen, and were summed up for all the analysed depths between 0 and 70 cm.

In the trial 11/01899538-01 (UK), the total amount of imazamox residues observed in the soil profiles decreased from 64.0 - 74.4 g/ha on day 0 to 3.3 - 4.9 g/ha at 358 DAA. Residues of metabolite CL 354825 (Reg. No. 4110603) were observed from 21 DAA, with maximum amounts of 9.3 and 11.9 g/ha in the 3 subplots at 358 DAA and 120 DAA, respectively. Residues of the metabolite CL 354825 was typically observed in the top 0 - 10 cm soil layer and only trace amounts at or close to the LOQ were observed in the 10 - 20 cm layer at 120 DAA and 358 DAA. The metabolite CL 354825 was not observed above the LOQ in the 20 - 30 cm horizon. Soil concentrations of metabolite CL 312622 (Reg. No. 4110542) were low and mostly close to the LOQ. Residues were exclusively observed in the upper 0 - 10 cm layer from 7 DAA to 45 DAA up to a maximum concentration of 4.1 g/ha. After this date, the analyte was no longer observed in any of the 3 subplots. The metabolite was not observed above LOQ at deeper soil horizons.

In the trial 11/01899538-02 (France), the total amount of imazamox residues observed in the soil profiles decreased from 63.7 - 70.5 g/ha on day 0 to 7.0 - 20.9 g/ha at 372 DAA. Residues of metabolite CL 354825 (Reg. No. 4110603) were observed from 21 DAA onwards, with maximum amounts of 16.5 - 19.3 g/ha in the 3 subplots after 271 to 372 DAA. This metabolite was mainly observed in the upper 0 - 20 cm of the soil; only traces in the range of the LOQ were observed in the 20 - 30 cm layer in one of the subplots in the period 160 to 372 DAA, but no residues above LOQ were found at deeper horizons. Concentrations of metabolite CL 312622 (Reg. No. 4110542) in the soil were low and typically close to the LOQ, except for subplot C where a maximum of 11.6 g/ha was reached at 30 DAA. Residues were only observed between 4 DAA and 93 DAA. The metabolite CL 312622 was typically not observed above the LOQ in the horizons below 10 cm, except at the 93 DAA sampling event on subplot C, for which the metabolite was found in traces down to 40 cm.

In the trial 11/01899538-03 (Germany), the total amount of imazamox residues observed in the soil profiles decreased from 67.8 - 70.4 g/ha on day 0 to 4.9 - 5.7 g/ha after 274 days. No residues of Imazamox were observed above the LOQ at the last sampling of this study (360 DAA). Residues of metabolite CL 354825 (Reg. No. 4110603) were observed from 10 DAA, with maximum amounts of 11.3 - 14.5 g/ha in the 3 subplots after 274 to 360 DAA. This metabolite was mainly observed in the upper 0 - 20 cm of the soil and only traces close to LOQ were observed in the 20-30 cm layer (168 DAA to 360 DAA). No residues above LOQ were found at deeper horizons, except the 274 DAA sampling event for subplot C, for which CL 354825 residues in the range of the LOQ were also found in the 30 - 40 cm layer. Concentrations of metabolite CL 312622 (Reg. No. 4110542) in the soil were very low and if observed, residues were always at the LOQ. Residues were only observed between 10 and 22 DAA and exclusively in the 0 - 10 cm layer.

III. CONCLUSION

Imazamox degraded steadily under field conditions in soil at three sites in the UK, France and Germany. The treated plot at all trial sites was covered with 3-5 mm of sand immediately after application to protect the treated soil surface from sunlight. At the site in Germany (trial 11/01899538-03), residues above the LOQ of 0.001 mg/kg were no longer detectable following the 274 DAA sampling event. A kinetic analysis of the data according to FOCUS (2006) kinetics guidance is presented for all sites in a separate modelling report. See CA 7.1.2.2.1/7 for the kinetic analysis.

The main proportion of imazamox residues was found in the upper 0 - 20 cm of the soil and only trace amounts at or close to the LOQ (0.001 mg/kg) were observed in the 20 - 30 cm soil layers. In the 30 – 60 cm layers residues in the range of the LOQ were only observed at 88 DAA for the site in Germany (trial 11/01899538-03). No imazamox residues above LOQ were observed in the 60 - 70 cm horizon for any of the sites.

The metabolite CL 354825 was formed in all trials and was mainly observed in the top 0 - 20 cm soil layer and only trace amounts at or close to the LOQ (0.001 mg/kg) were observed in the 20 - 30 cm soil layer. No residues of the metabolite CL 354825 above LOQ were observed in the 40 - 70 cm horizon.

Concentrations of the metabolite CL 312622 in the three soils were only low and mainly at or close to the LOQ (0.001 mg/kg). It was exclusively found in the upper 10 cm of the soils except for one specimen for which residues were found in the range of the LOQ down to 40 cm.

Report:	CA 7.1.2.2.1/4 Holzer S., 2013b Chiral analyses of field soil specimens from existing Imazamox field studies 2013/1235109
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-DOC ENV/MC/CHEM(98)17 Paris 1998
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The scope of this study was to investigate the enantiomeric ratios of imazamox and its metabolites CL 312622 and CL 354825 in existing field soil specimens generated under two previous terrestrial field dissipation studies. The analytical method, which is based on the BASF Analytical Method D1102, was first validated for the chiral analysis. The limit of quantification (LOQ) for all three analytes was 0.001 mg/kg using LC-MS/MS for quantitation.

To confirm the validity of the modified method, five specimens fortified at the LOQ level and five specimens fortified at 10 times the LOQ were analysed. For Imazamox, the fortifications and calibrations were performed with the enantiomers (Reg No. 5835952 S-Enantiomer and Reg.No. 4518133 / CL 334401 R-Enantiomer). For both metabolites, as no single enantiomer was available, the fortification and calibrations were done with the racemic mixture.

Data evaluation was performed by the individual integration of each enantiomer and summation of the received areas. The LOQ of the method was 0.001 mg/kg for all three analytes in soil. As no significant matrix effects were observed, no matrix-matched calibration was performed. The analysis was performed by LC-MS/MS on two parent daughter ion transitions (MRM): one for quantification and one for confirmation. However, for the analyses of the soil specimens only the primary ion transition was evaluated.

Considering that mean recovery values were in the range of 70 - 120 % for each fortification level, with relative standard deviations (RSD) of ≤ 20 %, the validation of the analytical method for the determination of residues with individual integration of each enantiomer of imazamox and its metabolites CL 354825 and CL 312622 was successful. The specificity of the analytical method was acceptable, since no interferences from the specimen matrices were detected at the retention time of interest.

In the analysed field specimens the concentrations of imazamox ranged from 0.003 to 0.185 mg/kg, from < 0.001 mg/kg to 0.012 mg/kg for CL 354825 and from < 0.001 mg/kg to 0.009 mg/kg for CL 312622.

The enantiomeric ratios were determined and ranged from 0.7 to 1.1 for BAS 720 H, from 0.9 to 1.3 for CL 354825 and from 0.7 to 1.1 for CL 312622. The enantiomer ratio of each analyte remained relative stable for all soil specimens and no relation between sampling day and enantiomer ratio was observed.

MATERIAL AND METHODS

I. MATERIALS

Test Materials

Common name: Imazamox
Internal code: BAS 720 H
Registration No.: 4096483
Chemical name (IUPAC): (RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2yl)-5-methoxymethylnicotinic acid
CAS Number.: 114311-32-9
Molecular weight: 305.3 g
Molecular formula: $C_{15}H_{19}N_3O_4$
Chemical purity: 99.5%

Imazamox R-enantiomer CL 3354401
Common name: Imazamox-P
Registration No.: 4518133
Chemical name (IUPAC): 2-((4R)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethyl-nicotinic acid
CAS Number.: 221298-64-2
Molecular weight: 305.3 g
Molecular formula: $C_{15}H_{19}N_3O_4$
Chemical purity: 95.4%

Imazamox S-enantiomer-Reg. No. 5835952
Common name: -
Registration No.: 5835952
Chemical name (IUPAC): 2-((4S)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethyl-nicotinic acid
CAS Number.: 355838-61-8
Molecular weight: 305.3 g
Molecular formula: $C_{15}H_{19}N_3O_4$
Chemical purity: 98.3%

Common name: CL 312622
Registration No.: 4110542
Chemical name (IUPAC): 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)pyridine-3,5-dicarboxylic acid
CAS Number.: 146953-32-4
Molecular weight: 305.3 g
Molecular formula: $C_{14}H_{15}N_3O_5$
Chemical purity: 88.4%

Common name: CL 354825
Registration No.: 4110603
Chemical name (IUPAC): 5-hydroxy-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid
CAS Number.: -
Molecular weight: 277.3 g
Molecular formula: $C_{13}H_{15}N_3O_4$
Chemical purity: 98.2%

Test System

The chiral analyses was performed on existing field soil specimens generated from two field dissipation studies, which can be found in sections CA 7.1.2.2.1/1-3 of this dossier [*Holzer (2013)* – DocID BASF 2013/1229816; *Holzer (2013)* – BASF DocID 2013/1211059]. The trials were located in different regions of Europe (United Kingdom, Germany, Northern France, Spain and Italy), which represent typical regions of agricultural practice. Field soil specimens were collected from the first 10 cm of the top soil layer at different sampling times from a soil depth of 0-10 cm. Those soil specimens having residues above the LOQ of 0.001 mg/kg were preferably selected.

2. DESCRIPTION OF ANALYTICAL PROCEDURE

Soil was processed according to BASF method D1102, which can be found in section CA 4.1.2 of this dossier [*Gooding (2013)* – DocID BASF 2012/7004251]. The soil specimen was extracted by shaking with 0.5 M NaOH. The specimen clean-up was performed by solid phase extraction using a C-18 column followed by a SCX column. The combined eluates were evaporated to dryness by using a nitrogen evaporator with the water bath set to 70°C. Once completely dry, all specimens were reconstituted in 0.5 mL of 0.1 % HCOOH in water/acetonitrile (80/20, v/v) and homogenised by vortex before analyses.

The final determination of the enantiomers of imazamox (Reg No. 5835952 and CL 334401) and the enantiomers of its metabolites CL 312622 and CL 354825 was performed by LC-MS/MS, using a chiral column for separation and monitoring two parent daughter ion transitions. One transition is used for evaluation, the other one for confirmation. The mass transitions for quantification were: m/z 306 → 261 (Imazamox, both enantiomers), m/z 306 → 261 (CL 312622, both enantiomers) and m/z 278 → 233 (CL 354825, both enantiomers).

The detector signals were recorded and each enantiomer was integrated individually. To quantify the concentrations of imazamox or its metabolites CL 312622 and CL 354825, the areas of each enantiomer were summed up. The enantiomeric ratios were determined as the quotient of peak area from the two individually integrated enantiomers.

II. RESULTS AND DISCUSSION

A. METHOD VALIDATION

The described analytical method was successfully validated for the determination of imazamox (CL 334401 and Reg No. 5835952) and its metabolites CL 312622 and CL 354825, with individual integration of each enantiomer in soil. The required limit of quantification for imazamox, Reg. No. 4110542 (CL 312622) and Reg. No. 4110603 (CL 354825), in soil was 0.001 mg/kg. All mean recovery values (mean of 5 replicates per fortification level and analyte) ranged between 70% and 120%, relative standard deviation less than 20%. The detailed results are shown in Table 7.1.2.2.1-14.

Table 7.1.2.2.1-14: Results of method validation phase for the determination of imazamox and its metabolites in soil specimens

Analyte	Ion transition [m/z]	Transition description	No. of fortified specimens	Fortification level [mg/kg]	Mean recovery [%]	SD	RSD [%]
Imazamox	306 → 261	Primary Ion Transition	5	0.001	98	4.8	4.9
				0.01	104	3.3	3.2
	306 → 193	Confirmatory Ion Transition	5	0.001	100	6.2	6.3
				0.01	102	2.1	2.1
CL 312622	306 → 261	Primary Ion Transition	5	0.001	81	3.1	3.7
				0.01	94	1.0	1.1
	306 → 264	Confirmatory Ion Transition	5	0.001	88	2.3	2.6
				0.01	91	3.2	3.5
CL 354825	278 → 233	Primary Ion Transition	5	0.001	79	2.8	3.6
				0.01	83	1.6	1.9
	278 → 165	Confirmatory Ion Transition	5	0.001	90	6.2	6.8
				0.01	81	1.7	2.1

Good linearity ($r \geq 0.999$) was observed in the range of 0.0003 µg/mL to 0.025 µg/mL for both ion transitions of imazamox using standard calibration solutions with increasing concentrations. Similarly, a linear calibration curve ($r \geq 0.999$) was observed in the range from 0.0003 µg/mL to 0.02 µg/mL and from 0.0003 µg/mL to 0.0252 µg/mL for Reg. No. 4110542 (CL 312622) and Reg. No. 4110603 (CL 354825), respectively.

The method was successfully determines residues of the individual enantiomers of imazamox and its metabolites CL 312622 and CL 354825 from the generated soil field specimens by using LC/MS-MS and monitoring two parent daughter ion transitions for each analyte. No interferences were detected at the retention time of interest.

B. RESIDUES AND ENANTIOMER RATIOS

In the analysed soil specimens, the concentrations of imazamox ranged from 0.003 mg/kg to 0.185 mg/kg, from < 0.001 mg/kg to 0.009 mg/kg for CL 312622 and from < 0.001 mg/kg to 0.012 mg/kg for CL 354825.

The enantiomeric ratios were determined as the quotient of the peak area from the two individually integrated enantiomers. It means that for an enantiomer ratio of 1, both enantiomers were present in the same concentration. This value ranged from 0.7 to 1.1 for imazamox, from 0.7 to 1.1 for CL 312622 and from 0.9 to 1.3 for CL 354825, respectively.

Results regarding concentrations in the specimens and the enantiomeric ratios are summarised from Table 7.1.2.2.1-15 to Table 7.1.2.2.1-19.

Table 7.1.2.2.1-15: Summary results for the soil specimens (0-10 cm depth) from the field trial in Spain [*Holzer (2013)* – BASF DocID 2013/1211059]

BASF Field Specimen No.	DAT	Subplot	Imazamox		CL 312622		CL 354825	
			Residue [mg/kg]	ER	Residue [mg/kg]	ER	Residue [mg/kg]	ER
11/01899536-01-016	0	A	0.063	0.9	0.001	1.0	<0.001	-
11/01899536-01-017	0	B	0.083	0.9	0.001	0.9	< 0.001	-
11/01899536-01-018	0	C	0.046	1.0	< 0.001	-	< 0.001	-
11/01899536-01-051	14	B	0.019	0.9	0.004	1.0	0.002	1.1
11/01899536-01-055	22	A	0.016	0.8	0.004	1.1	0.006	1.1
11/01899536-01-059	22	C	0.015	0.8	0.004	0.9	0.006	1.1
11/01899536-01-070	45	B	0.008	0.9	0.002	0.9	0.010	1.0
11/01899536-01-078	62	C	0.006	0.7	< 0.001	-	0.008	1.1
11/01899536-01-087	125	A	0.006	0.8	< 0.001	-	0.010	1.1

ER = Enantiomeric ratio

Table 7.1.2.2.1-16: Summary results for the soil specimens (0-10 cm depth) from the field trial in Italy [*Holzer (2013)* – BASF DocID 2013/1211059]

BASF Field Specimen No.	DAT	Subplot	Imazamox		CL 312622		CL 354825	
			Residue [mg/kg]	ER	Residue [mg/kg]	ER	Residue [mg/kg]	ER
11/01899536-01-016	0	A	0.039	0.9	< 0.001	-	< 0.001	-
11/01899536-01-017	0	B	0.040	1.0	< 0.001	-	< 0.001	-
11/01899536-01-018	0	C	0.049	0.9	< 0.001	-	< 0.001	-
11/01899536-01-072	43	C	0.025	0.9	0.002	1.0	0.003	1.3
11/01899536-01-081	89	A	0.185	0.8	0.001	1.0	0.003	1.1
11/01899536-01-089	120	B	0.014	0.8	< 0.001	-	0.003	1.1
11/01899536-01-106	358	A	0.007	0.8	< 0.001	-	0.006	1.1
11/01899536-01-108	358	B	0.010	0.8	< 0.001	-	0.006	1.1
11/01899536-01-110	358	C	0.008	0.8	< 0.001	-	0.006	1.2

ER = Enantiomeric ratio

Table 7.1.2.2.1-17: Summary results for the soil specimens (0-10 cm depth) from the field trial in United Kingdom [*Holzer (2013)* – DocID BASF 2013/1229816]

BASF Field Specimen No.	DAT	Subplot	Imazamox		CL 312622		CL 354825	
			Residue [mg/kg]	ER	Residue [mg/kg]	ER	Residue [mg/kg]	ER
11/01899538-01-016	0	A	0.049	0.9	< 0.001	-	< 0.001	-
11/01899538-01-017	0	B	0.048	0.9	< 0.001	-	< 0.001	-
11/01899538-01-018	0	C	0.048	1.0	< 0.001	-	< 0.001	-
11/01899538-01-055	21	A	0.032	0.9	0.002	0.8	0.002	1.2
11/01899538-01-066	30	C	0.029	0.9	0.003	0.9	0.003	1.2
11/01899538-01-089	120	B	0.011	0.9	< 0.001	-	0.008	1.0
11/01899538-01-093	178	A	0.005	0.8	< 0.001	-	0.005	1.0
11/01899538-01-101	270	B	0.004	0.9	< 0.001	-	0.005	1.0
11/01899538-01-103	270	C	0.005	0.9	< 0.001	-	0.005	0.9

ER = Enantiomeric ratio

Table 7.1.2.2.1-18: Summary results for the soil specimens (0-10 cm depth) from the field trial in France [*Holzer (2013)* – DocID BASF 2013/1229816]

BASF Field Specimen No.	DAT	Subplot	Imazamox		CL 312622		CL 354825	
			Residue [mg/kg]	ER	Residue [mg/kg]	ER	Residue [mg/kg]	ER
11/01899538-02-016	0	A	0.044	0.9	< 0.001	-	< 0.001	-
11/01899538-02-017	0	B	0.051	0.9	< 0.001	-	< 0.001	-
11/01899538-02-018	0	C	0.007	0.9	< 0.001	-	0.005	1.0
11/01899538-02-066	30	C	0.026	1.0	0.009	1.0	0.002	1.0
11/01899538-02-068	44	A	0.042	0.9	0.002	0.7	0.002	1.1
11/01899538-02-070	44	B	0.024	0.9	0.003	0.9	0.002	1.1
11/01899538-02-106	372	A	0.009	0.9	< 0.001	-	0.007	1.1
11/01899538-02-108	372	B	0.012	0.9	< 0.001	-	0.012	1.1
11/01899538-02-110	372	C	0.005	1.0	< 0.001	-	0.009	1.0

ER = Enantiomeric ratio

Table 7.1.2.2.1-19: Summary results for the soil specimens (0-10 cm depth) from the field trial in Germany [*Holzer (2013)* – DocID BASF 2013/1229816]

BASF Field Specimen No.	DAT	Subplot	Imazamox		CL 312622		CL 354825	
			Residue [mg/kg]	ER	Residue [mg/kg]	ER	Residue [mg/kg]	ER
11/01899538-03-016	0	A	0.080	1.0	< 0.001	-	< 0.001	-
11/01899538-03-017	0	B	0.056	1.0	< 0.001	-	< 0.001	-
11/01899538-03-018	0	C	0.052	1.0	< 0.001	-	< 0.001	-
11/01899538-03-074	59	A	0.013	0.9	< 0.001	-	0.003	1.0
11/01899538-03-078	59	C	0.012	1.0	< 0.001	-	0.003	1.1
11/01899538-03-083	88	B	0.009	1.0	< 0.001	-	0.004	1.0
11/01899538-03-093	184	A	0.006	0.9	< 0.001	-	0.003	1.1
11/01899538-03-097	184	C	0.005	1.0	< 0.001	-	0.003	1.0
11/01899538-03-101	274	B	0.003	1.1	< 0.001	-	0.004	1.0

ER = Enantiomeric ratio

C. STABILITY TESTS OF REFERENCE SOLUTIONS

The results from the stability tests on the reference solutions showed that solutions containing reference item imazamox and its metabolites CL 312622 and CL 354825 were stable during the performance of the laboratory work. The difference between the old solution and the fresh stock solution of imazamox was 5.1%. Considering the stock solutions of CL 312622 and CL 354825, the difference between the old and the freshly prepared solutions containing the test was 3.5% and 2.3%, respectively.

III. CONCLUSION

The analytical method for the chiral determination of residues of imazamox, CL 312622 (Reg. No. 4110542) and CL 354825 (Reg. No. 4110603) in soil was successfully validated.

The imazamox concentrations in the field specimens analysed ranged from 0.003 mg/kg to 0.185 mg/kg. For the test items CL 312622 and CL 354825, the concentration ranged from < 0.001 mg/kg to 0.009 mg/kg and from < 0.001 mg/kg to 0.012 mg/kg, respectively.

The range for the enantiomer ratios of the field soil specimens was determined to be in the same range than in the fortified specimens used for method validation and within the accuracy of the analytical method.

The enantiomer ratio of each analyte remained relative stable for all field soil specimens analysed and no relation between sampling day and enantiomer ratio was observed.

Report:	CA 7.1.2.2.1/5 Knoch E., 2013b Determination of the storage stability of Imazamox (BAS 720 H) and its two metabolites, namely CL 312622 and CL 354825 in soil 2013/1397723
Guidelines:	EEC 7032/VI/95 rev. 5 (22 July 1997), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The aim of this study was to determine the storage stability of imazamox - BAS 720 H- and its two metabolites, namely CL 312622 and CL 354825, in soils from five locations over a time period of 617-620 days (20.6 months) under storage conditions of ≤ -18 °C.

The soils were separately treated with individual standard solutions of imazamox, CL 312622 or CL 354825 to get a concentration of 0.1 mg/kg dry soil for each test item. Thereafter the untreated and treated samples were stored deep-frozen (< -18°C in the dark), with the exception of the soil time 0 samples. At different time intervals, the soil samples were taken from the freezer and analysed according to the BASF method D1102, which with a limit of quantification was set to a 0.001 mg/kg for each analyte.

The working solutions of imazamox, CL 312622 and CL 354825 were found to be stable for at least 4 weeks when stored at 2 - 8 °C. Similarly, imazamox, CL 312622 or CL 354825 in the soil extracts were stable for at least 4 weeks, since the recoveries of the three analytes for stored specimens (2 to 8 °C or < -18 °C) were in the range of 70 to 110 %.

The analytical method D1102 was proved to be specific for the determination of imazamox, CL 312622 and CL 354825 and continuously tested to meet the validity criteria covering a range of 70 - 110 % (exception: 16 months, Germany, BAS 720 H, recovery of 115 %).

The results of the study showed that imazamox, CL 312622 or CL 354825 remained stable in the tested soils when stored deep-frozen (< -18 °C in the dark) over 617-620 days.

I. MATERIAL AND METHODS

A. MATERIALS

1.Test Material

Test item	Imazamox (BAS 720 H)
Reg. No.	4096483
IUPAC-Name:	(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Molecular formula:	$C_{15}H_{19}N_3O_4$
Lot No.:	AC12820-7
Purity:	99.5%
Molecular weight:	305.3 g/mol
Test item	CL312622
Reg. No.	4110542
IUPAC-Name:	2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)pyridine-3,5-dicarboxylic acid
Molecular formula:	$C_{14}H_{15}N_3O_5$
Lot No.:	L82-7
Purity:	88.4%
Molecular weight:	305.3 g/mol

Test item	CL354825
Reg. No.	4110603
IUPAC-Name:	5-hydroxy-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid
Molecular formula:	$C_{13}H_{15}N_3O_4$
Lot No.:	L67-144
Purity:	98.2%
Molecular weight:	277.3 g/mol

2. Soils

The present study was performed on existing field soil specimens taken from two field dissipation studies, which can be found in section CA 7.1.2.2.1/1-3 of this dossier *[Holzer (2013)* – DocID BASF 2013/1229816; *Holzer (2013)* – BASF DocID 2013/1211059]. The trials were located in different regions of Europe (United Kingdom, Germany, Northern France, Spain and Italy), which represent typical regions of agricultural practice. Field soil specimens were collected from the first 20 cm of the top soil layer, sieved through a 2 mm screen and stored under aerobic conditions at 2 – 8 °C prior to use.

B. STUDY DESIGN

1. Experimental Conditions

Stability of Working Solutions and Specimen Extracts
The stability of imazamox, CL 312622 or CL 354825 in the specimen extracts was also tested by re-analysis of specimen extracts (1st assay; soil t_0- specimens at 0.1 mg/kg dry soil) stored at 2 – 8 °C (2nd assay) and ≤ -18 °C (3rd assay) after a period of 4 weeks.

The stability of the working solutions of the mixed reference items (imazamox, CL 312622 or CL 354825) was tested. Working solutions prepared at the beginning of the study were re-analysed and compared with working solutions prepared freshly before analysis.

Storage Stability Testing
From the untreated soil specimens, amounts of 5 g soil were weighed into 50 mL centrifuge tubes. A calculated volume of the individual standard solutions was added to the soil specimens, making the treatment equivalent to 0.1 mg/kg dry soil. The dosing solutions of the test items were performed in pure water. Thereafter, the centrifuge tubes were closed and, with exception of the soil t_0- specimens, deep-frozen at ≤ -18 °C in the dark until analysis. The soil t_0-specimens were extracted immediately after preparation and therefore not deep-frozen.

Together with the spiked samples, an appropriate number of untreated soil aliquots were also deep-frozen, in order to get control and procedural recovery samples for each sampling day. For the procedural recovery samples, some untreated samples (controls) were fortified with 0.2 mL of freshly prepared fortification solutions (~ 2 µg/mL) of the three analytes (0.1 mg/kg) just before working up of the samples. The procedural fortifications indicated the efficiency of the method on the day of analysis.

2. Sampling

All soil samples were stored in a freezer until analysis. At each sampling date, duplicate spiked soil samples and two control samples were removed for analysis. For each analyte, one of the control samples was fortified at a level of 0.1 mg/kg (procedural recoveries), and then analysed along with the control and the stored spiked samples, which contains all analytes individually.

The storage stability test was organized in 8 analytical assays, corresponding to time intervals at 0, 1, 3, 6, 9, 12, 16 months and continued till 620 ± 4 days after the treatment of the specimens to cover the longest period of residue specimens.

3. Description of analytical procedures

All soil samples were analysed according to BASF method D1102, which can be found in section CA 4.1.2 of this dossier [*Gooding (2013)* – DocID BASF 2012/7004251]. The residues from soil specimen were extracted twice by shaking with 0.5 M NaOH. The specimen clean-up was performed with an aliquot of the total extract by solid phase extraction using a C-18 column followed by a SCX column. The combined eluates were evaporated to dryness by using a nitrogen evaporator with the water bath set to 70°C. All specimens were reconstituted in 0.5 mL of 0.1 % HCOOH in water/acetonitrile (80/20, v/v) and homogenised by vortex before analyses.

The final determination of the imazamox, CL 312622 and CL 354825 in the specimen extracts was performed using LC-MS/MS detection. As described in the table below, one transition is used for evaluation, the other one for confirmation.

Test item	Mass transition for quantification	Mass transition for confirmation
BAS 720 H	m/z 306 → 261	m/z 306 → 86
CL 312622	m/z 306 → 261	m/z 306 → 264
CL 354825	m/z 278 → 233	m/z 278 → 165

The limit of quantification (LOQ) was 0.001 mg/kg for each analyte.

II. RESULTS AND DISCUSSION

Stability of Working Solutions and Specimen Extracts

The working solutions of imazamox, CL 312622 or CL 354825 were stable on storage for at least 4 weeks, when stored at 2-8°C, since the quotients for old (stored) solutions were in the range of 90 to 110 % of the freshly prepared solutions.

After 4 weeks and storage at 2 to 8°C or at -18°C (2nd and 3rd assay), the analytes imazamox, CL 312622 and CL 354825 were stable in the specimen extracts, since the recoveries for stored specimens were in the range of 70 to 110 %.

Storage Stability Testing
As shown in Table 7.1.2.2.1-20 to Table 7.1.2.2.1-24, imazamox and its two metabolites, namely CL 312622 and CL 354825, remained stable in the tested soils when stored deep-frozen (< -18 °C in the dark) over 617-620 days.

Results from the procedural recoveries samples showed that the analytical method was proved to be specific for the determination of imazamox, CL 312622 or CL 354825 and continuously tested to meet the validity criteria covering a range of 70 - 110 % (exception: 16 months, Germany, imazamox, recovery of 115 %).

Table 7.1.2.2.1-20: Recovery data of imazamox and its metabolites in frozen soil samples of Location Spain

Storage period [month]	Recovery [%]		
	BAS 720 H	**CL 312622**	**CL 354825**
0	96	94	85
	91	90	82
	87	95	81
	98	97	87
	97	81	81
Mean	**94 %; RSD 5.0 %**	**91 %; RSD 7.2 %**	**83 %; RSD 3.1 %**
1	89	88	76
	84	82	78
Mean	**86**	**85**	**77**
3	96	83	86
	92	84	83
Mean	**94**	**84**	**84**
6	94	87	81
	93	84	86
Mean	**94**	**86**	**84**
9	92	85	84
	94	83	87
Mean	**93**	**84**	**86**
12	90	86	84
	86	87	84
Mean	**88**	**86**	**84**
16	88	88	70
	88	86	74
Mean	**88**	**87**	**72**
618 days	87	76	83
	83	75	88
Mean	**85**	**75**	**85**

Table 7.1.2.2.1-21: Recovery data of imazamox and its metabolites in frozen soil samples of Location Italy

Storage period [month]	Recovery [%]		
	BAS 720 H	**CL 312622**	**CL 354825**
0	92	87	82
	97	91	78
	92	100	73
	95	99	77
	92	87	73
Mean	**94 %; RSD 2.4 %**	**93 %; RSD 7.0 %**	**77 %; RSD 5.1 %**
1	86	92	88
	94	85	79
Mean	**90**	**89**	**83**
3	82	76	78
	97	99	77
Mean	**90**	**88**	**77**
6	99	89	83
	99	88	80
Mean	**99**	**88**	**81**
9	85	85	78
	86	93	77
Mean	**86**	**89**	**78**
12	91	81	82
	91	88	89
Mean	**91**	**85**	**85**
16	91	87	70
	93	83	73
Mean	**92**	**85**	**71**
617 days	93	94	82
	93	86	80
Mean	**93**	**90**	**81**

Table 7.1.2.2.1-22: Recovery data of imazamox and its metabolites in frozen soil samples of Location United Kingdom

Storage period [month]	Recovery [%]		
	BAS 720 H	**CL 312622**	**CL 354825**
0	98	105	81
	107	89	89
	107	92	84
	102	97	85
	93	101	87
Mean	**101 %; RSD 5.8 %**	**97 %; RSD 6.7 %**	**85 %; RSD 3.3 %**
1	90	108	80
	92	103	86
Mean	**91**	**105**	**83**
3	100	88	74
	92	80	78
Mean	**96**	**84**	**76**
6	85	98	81
	97	87	79
Mean	**91**	**93**	**80**
9	88	97	83
	89	91	76
Mean	**88**	**94**	**80**
12	87	84	83
	91	80	83
Mean	**89**	**82**	**83**
16	80	78	74
	85	84	70
Mean	**82**	**81**	**72**
620 days	72	72	98
	78	72	99
Mean	**75**	**72**	**99**

Table 7.1.2.2.1-23: Recovery data of imazamox and its metabolites in frozen soil samples of Location France

Storage period [month]	Recovery [%]		
	BAS 720 H	**CL 312622**	**CL 354825**
0	96	90	77
	103	97	77
	101	93	78
	96	98	83
	96	95	78
Mean	**98 %; RSD 3.3 %**	**95 %; RSD 3.6 %**	**79 %; RSD 3.0 %**
1	89	87	77
	82	106	81
Mean	**85**	**96**	**79**
3	86	100	75
	98	87	74
Mean	**92**	**93**	**74**
6	86	85	82
	90	83	85
Mean	**88**	**84**	**83**
9	90	85	78
	97	86	80
Mean	**94**	**86**	**79**
12	81	77	87
	94	77	89
Mean	**88**	**77**	**88**
16	88	71	99
	101	81	85
Mean	**94**	**76**	**92**
620 days	95	75	101
	105	80	93
Mean	**100**	**78**	**97**

Table 7.1.2.2.1-24: Recovery data of imazamox and its metabolites in frozen soil samples of Location Germany

Storage period [month]	Recovery [%]		
	BAS 720 H	**CL 312622**	**CL 354825**
0	103	81	95
	89	87	93
	91	88	87
	101	99	91
	104	84	86
Mean	**98 %; RSD 6.9 %**	**88 %; RSD 7.8 %**	**90 %; RSD 4.4 %**
1	89	91	88
	99	89	87
Mean	**94**	**90**	**88**
3	107	90	93
	102	99	84
Mean	**104**	**94**	**89**
6	86	78	81
	79	82	88
Mean	**82**	**80**	**85**
9	91	89	89
	94	78	92
Mean	**92**	**83**	**90**
12	90	88	94
	98	88	96
Mean	**94**	**88**	**95**
16	93	81	83
	98	79	75
Mean	**96**	**80**	**79**
621 days	93	78	101
	98	92	90
Mean	**96**	**85**	**96**

III. CONCLUSION

The results of the storage stability study showed that imazamox and its metabolites CL 312622 and CL 354825 were stable in soil over 617-621 days (20.6 months) of storage at temperatures of lower than - 18 °C in the dark.

The working solutions of imazamox, CL 312622 and CL 354825 were stable for at least 4 weeks when stored at 2 - 8 °C. The analytes were proven to be stable in the soil extracts for at least 4 weeks when stored either at 2 to 8 °C or < -18 °C.

Report:	CA 7.1.2.2.1/6 Donaldson F.P., 2013b Kinetic evaluation of fifteen field dissipation trials for BAS 720 H (Imazamox) conducted from 1993-1999 2013/7001766
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp., SANCO/10058/2005 rev. 2 (FOCUS kinetics report) EFSA (2010): Guidance for evaluating laboratory and field dissipation studies to obtain DegT50 values of plant protection products in soil. EFSA Journal 2010; 8(12):1936.
GLP:	no

Executive Summary

A kinetic evaluation of the field dissipation of BAS 720 H – imazamox was conducted on the data of fifteen European terrestrial field dissipation trials, obtained from fourteen studies conducted in Europe between 1993 and 1999.

Each field trial has been kinetically analysed employing guidance from the FOCUS (2006) kinetics workgroup and opinion from EFSA (2010). In particular, attention was given to isolating the slow phase of the dissipation pattern in order to minimise the influence of fast surface processes (photolysis and volatilization).

Based on EFSA (2010) opinion flow charts for field trial assessments, it was concluded that 10 field trials produced acceptable kinetic data for use as modelling endpoints. Data derived from these field trials were normalised to reference conditions (20 °C, pF 2) by time-step normalisation and used in kinetic evaluation in order to derive modelling endpoints for imazamox. The $DegT_{50}$ values ranged between 1.0 and 57.9 days.

Imazamox persistence endpoints were also evaluated for each of the 15 field trials. All but one trial (Limours France, FR2) was modelled with SFO kinetics. The DT_{50} values ranged from 4.3 to 31.1 days, with a geometric mean of 13.9 days.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted on data from fifteen field trials with imazamox obtained from fourteen field dissipation studies conducted between 1993 and 1999 [*Cronin. J.A. (1999): "AC 299263 40 g a.i./L SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil – United Kingdom, 1997-1998"* - DocID 1999/7000802, *Cronin. J.A. (1997): "AC 299263 40g ai/l SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil, Autumn Application – United Kingdom, 1996-1997"* - DocID 1997/7001015, *Trewhitt. J.A. (1999): "Imazamox (AC 299263) 40 g a.s./L SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil, Spring Application – South France, 1999"* - DocID 1999/7000798, *Cronin. J.A. (1997): "AC 299263 40g ai/l SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil, Spring Application – North France 1997"* - DocID 1997/7000978, *Farrell J.A. (1997): "AC 299263 and Related Compound CL 312622 ROD Study in Soil"* - DocID 1997/7000954, *Cronin. J.A. (1997): "AC 299263 120g ai/l SL (RLF 12132): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil, Spring Application – North France, 1995-1996"* - DocID 1997/7001021, *Cronin. J.A. (1997): "AC 299263 40g ai/l SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil, Autumn Application – North France, 1996-1997"* – DocID 1997/7000958, *Cronin. J.A. (1997): "AC 299263 40g ai/l SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil, Autumn Application – North France, 1996-1997"* - DocID 1997/7001018, *Cronin. J.A. (1997): "AC 299263 120g ai/l SL: Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil, Spring Application – North France, 1994-1995"* – DocID 1997/7000959, *Cronin. J.A. (1999): "AC 299263 40 g a.i./L SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil – Germany, 1997-1998"* – DocID 1999/7000805, *Farrell J.A. (1997) "AC 299263 and Related Compound CL 312622 Residue Study in Soil (Soybean)"* – DocID 1997/7000951, *Farrell J.A. (1997): "AC 299263 and Related Compound CL 312622 ROD Study in Soil."* – DocID 1997/7000947, *Cronin. J.A. (1997): "AC 299263 120 g ai/l SL (RLF 12132): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil – Italy, 1995-1996"* - DocID 1997/7001017, *Young H.E. (1998): "AC 299263 40 g a.i./L SL (SF09464): Rate of Dissipation Study on AC 299263, CL 312622 and CL 354825 in Soil – Italy, 1998"* - DocID 1998/7000877].

Imazamox was ground spray broadcast onto bare soil at 12 non-cropped sites, applied pre-emergence to peas at two cropped sites, and applied post-emergence to soybean at one cropped site. Application rates were 75 or 150 g a.i. ha^{-1}. Ten trials were representative of spring/summer applications and five were representative of autumn applications. A summary of each field trial is given in Table 7.1.2.2.1-25.

Table 7.1.2.2.1-25: Field trial summary

Country	Trial code	Location	Target app. rate [g ha^{-1}]	Description	Timing	Study (DocID)
UK	UK1	Stratford-upon-Avon	75	Bare soil	31-Oct-97	1999/7000802
	UK2	Stratford-upon-Avon	75	Bare soil	31-Oct-96	1997/7001015
France	FR1	Cravenceres	75	Bare soil	16-Jun-99	1999/7000798
	FR2	Limours	75	Bare soil	18-Mar-97	1997/7000978
	FR3	Grezille	150	Bare soil	31-Mar-93	1997/7000954
	FR4a	Grezille	75	Pea, pre-emergent	16-Mar-95	1997/7001021
	FR4b	Pontfaverger	75	Pea, pre-emergent	23-Mar-95	1997/7001021
	FR5	Pontfaverger	75	Bare soil	22-Oct-96	1997/7000958
	FR6	Boissy	75	Bare soil	30-Oct-96	1997/7001018
	FR7	Brigne	150	Bare soil	30-Mar-94	1997/7000959
Germany	DE1	Euskirchen	75	Bare soil	21-Oct-97	1999/7000805
Italy	IT1	Fontanelle	75	Soybean, post-emergent	18-May-93	1997/7000951
	IT2	Rubizzano	150	Bare soil	18-Mar-93	1997/7000947
	IT3	Minerbio	150	Bare soil	28-Apr-95	1997/7001017
	IT4	Longara	75	Bare soil	4-Mar-98	1998/7000877

Normalisation procedure

The studies were time-step normalised to a moisture content at pF 2 and temperature of 20 °C according to FOCUS [*FOCUS (2006): "Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration". Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005, v. 2.0, 434 pp.*].

Average daily soil temperature and moisture data were not available from the study reports. Therefore, FOCUS PEARL v. 3.3.3 was used to predict soil temperature and moisture for the duration of each study period.

Soil temperature and moisture contents were derived from the daily minimum and maximum air temperatures, rainfall, and potential evapotranspiration (PET) using FOCUS PEARL v. 3.3.3. Daily measurements of min/max air temperatures and rainfall for the study periods were available in the reports for all the test sites, while PET was used from the MARS grid cell data in which the corresponding trial was located. The weather data was extended beyond the study durations with the appropriate MARS grid cell to facilitate model stabilization and to improve the accuracy of prediction for the study period of interest.

PEARL input files were set up with the van Genuchten parameters being estimated with the HYPRES database (classified or continuous pedotransfer functions determined by available soil moisture data), using the soil characterization data obtained from the study reports. The PEARL model was run using the site-specific soil properties and meteorological data and the estimated daily soil temperature and volumetric moisture content at 0-10 cm depth was used to calculate normalised day lengths. The pF 2 values used in the time-step evaluations for each soil were those derived from the HYPRES database to ensure consistency.

Table 7.1.2.2.1-26 shows the field sampling days for the trial locations and the normalised (20 °C, pF 2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 3.3.3.

Table 7.1.2.2.1-26: Time-step normalised (temperature and moisture) sampling days

Stratford-upon-Avon, UK (UK1)		Stratford-upon-Avon, UK (UK2)		Cravenceres, France (FR1)		Limours, France (FR2)		Grezille, France (FR3)	
DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]
0	0	0	0	0	0	0	0	0	0
1	0.2	1	0.3	1	1.0	1	0.3	7	1.5
3	0.8	3	1.0	3	2.9	3	0.9	15	4.4
5	1.5	5	2.1	5	4.6	5	1.4	29	9.2
7	2.3	7	2.9	7	6.1	7	1.9	58	21.9
10	3.3	10	3.8	10	9.5	10	3.1	90	40.8
14	4.3	14	4.7	14	13.4	15	4.6	120	65.2
19	6.1	18	5.6	19	19.4	17	5.3	185	118.2
24	7.6	24	6.8	26	26.8	23	7.2	363	183.8
31	9.9	30	7.8	28	29.2	30	9.4	--	--
73	20.0	--	--	57	64.2	63	23.7	--	--
90	23.8	--	--	90	103	91	40.9	--	--
118	30.3	--	--	--	--	140	78.2	--	--
Grezille, France (FR4a)		**Pontfaverger, France (FR4b)**		**Pontfaverger, France (FR5)**		**Boissy, France (FR6)**		**Brigne, France (FR7)**	
DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]
0	0	0	0	0	0	0	0	0	0
7	2.6	7	1.8	1	0.3	1	0.3	7	2.6
14	5.8	14	4.2	3	1.1	3	1.0	14	4.8
21	7.5	21	6.5	5	1.9	5	1.9	30	11.3
29	10.7	28	8.6	7	2.7	7	2.7	61	30.3
60	24.8	60	21.9	10	3.7	10	3.9	90	52.8
182	115.3	91	40.2	14	5.5	14	5.0	120	84.8
362	174.0	--	--	18	6.9	18	6.0	--	--
--	--	--	--	24	8.5	24	7.3	--	--
--	--	--	--	30	9.8	30	8.4	--	--
--	--	--	--	--	--	61	13.6	--	--
Euskirchen, Germany (DE1)		**Fontanelle, Italy (IT1)**		**Rubizzano, Italy (IT2)**		**Minerbio, Italy (IT3)**		**Longara, Italy (IT4)**	
DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]
0	0	0	0	0	0	0	0	0	0
1	0.3	34	24.8	8	3.4	14	10.3	1	0.4
3	0.7	106	96.3	14	4.9	28	19.8	3	1.3
6	1.4	197	144.1	33	13.1	60	52.0	5	2.1
8	1.8	372	203.6	63	35.7	90	100.7	7	2.8
10	2.2	--	--	97	76.0	180	196.2	9	3.3
13	2.7	--	--	130	116.2	375	256.2	14	4.9
18	4.3	--	--	189	192.7	--	--	19	6.4
24	6.2	--	--	364	253.1	--	--	24	7.7
30	7.8	--	--	--	--	--	--	30	10.5
57	13.5	--	--	--	--	--	--	61	27.3
90	22.0	--	--	--	--	--	--	89	50.9
--	--	--	--	--	--	--	--	120	90.9

DAT: Days after treatment; D_{norm}: Normalised day length (20 °C, pF 2); --: Not applicable

Kinetic modelling

The software package KinGUII v. 2.2012.202.925 was used for parameter fitting [*Schäfer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007): "KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics". In Del Re, A.A.M. et al (Eds): Proceedings of the XIII Symposium of Pesticide Chemistry, Piacenza, 2007, p. 916-923*]. The iteratively reweighted least squares (IRLS) method was selected to optimise model parameters, estimate parameter standard deviations, determine parameter confidence via the t-test, calculate correlation coefficients (r^2) and χ^2 error percentage, and calculate DT_{50} and DT_{90} dissipation endpoints. The error tolerance and maximum iterations were set to 0.000001 and 100, respectively.

FOCUS guidance for treatment of values below the limit of quantification or detection (LOQ, LOD) was followed [*FOCUS (2006)*]. This guidance was applied to residue data both temporally and spatially. The LOQ for each study was identified as 0.005 mg/kg. The LOD was not provided and therefore was treated as LOQ for the purposes of the kinetic evaluation. The first non-detection prior to a positive identification was set to ½ x LOD. Any non-detections occurring prior to this were set to zero. Non-detections occurring between positive identifications were also set to ½ x LOD. Finally, a non-detection occurring after the last positive identification was set to ½ x LOD, while subsequent non-detections were set to zero.

Soil residues were converted from a mass basis (mg kg^{-1}) to an area basis (g ha^{-1}) using a dry soil bulk density of 1500 kg m^{-3} and the depth of the soil layer.

Metabolite residues at time zero were converted to parent equivalents using molecular weight corrections, added to the parent time zero residues, and then fixed at zero.

Four kinetic models were applied to the data sets: single first-order (SFO), first-order multi-compartment (FOMC), double-first-order in parallel (DFOP), and hockey stick (HS). These models were tested sequentially depending on the desired endpoint (trigger or modelling). Descriptions of each kinetic model as well as the decision trees for model selection/endpoint determination can be found in FOCUS (2006) [*FOCUS (2006)*].

The best fitting kinetic model was determined based on both visual and statistical evaluation. To be considered "best-fit", the model must first result in an acceptable visual fit with residuals randomly scattered about the zero line. Statistically, the χ^2 error and t-tests provide information on the goodness-of-fit and parameter reliability, respectively. An ideal kinetic model should have randomly distributed residuals, χ^2 error < 15%, with estimated model parameters that differ from zero at a significance level of 5% ($p \leq 0.05$). In field studies with higher variation in data, the χ^2 error and t-test significance level can be relaxed to 30% and 10% ($p \leq 0.10$), respectively [*FOCUS (2006)*].

Non-normalized data was kinetically analysed to derive persistence endpoints (DT_{50} and DT_{90}) from the best-fit model.

Time-step normalized data were kinetically analysed to determine modelling endpoints. The current EFSA opinion recommends that rapid loss processes (photolysis and volatilization) should be minimised in order to ensure that any dissipation is the result of degradation in the soil matrix [*EFSA (2010): "EFSA Panel on Plant Protection Products; Guidance for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of plant protection products in soil". EFSA Journal (2010); 8 (12): 1936; 67 pp.*]. The current EFSA opinion was followed with one exception: in some cases, very few data points remained after removing data occurring prior to 10 mm rain, resulting in poor visual and/or statistical fits to the data when fitting the SFO model. In these instances, the DFOP model was applied to the complete data set and the decision tree followed from that step forward. If a biphasic model resulted in an acceptable visual and statistical fit, a conservative $DegT_{50}$ was computed using the slow phase rate constant.

II. RESULTS AND DISCUSSION

The kinetic evaluation of the original datasets (non-normalised) was performed for each of the fifteen field trials to determine trigger endpoints. A summary of the kinetic parameters and the adequate DT_{50} and DT_{90} values to be used as trigger endpoints is given in Table 7.1.2.2.1-27.

Table 7.1.2.2.1-27: Summary of non-normalised trigger endpoints of imazamox derived from European field dissipation trials

Field trial	Best-fit kinetic model	Parameters	p (t-test)	DT_{50} [d]	DT_{90} [d]
UK1	SFO	M_o: 34.2 g ha^{-1} k: 0.022 d^{-1}	k: << 0.01	31.1	103
UK2	SFO	M_o: 28.1 g ha^{-1} k: 0.053 d^{-1}	k: < 0.01	13.0	43.3
FR1	SFO	M_o: 25.2 g ha^{-1} k: 0.042 d^{-1}	k: < 0.01	16.5	54.9
FR2	FOMC	M_o: 46.6 g ha^{-1} α: 1.23 β: 19.4	α: < 0.05 β: < 0.1	14.7	107
FR3	SFO	M_o: 150 g ha^{-1} k: 0.062 d^{-1}	k: < 0.05	11.3	37.4
FR4a	SFO	M_o: 40.2 g ha^{-1} k: 0.160 d^{-1}	k: < 0.1	4.3	14.4
FR4b	SFO	M_o: 44.3 g ha^{-1} k: 0.042 d^{-1}	k: < 0.1	16.4	54.6
FR5	SFO	M_o: 43.8 g ha^{-1} k: 0.073 d^{-1}	k: << 0.01	9.6	31.8
FR6	SFO	M_o: 47.5 g ha^{-1} k: 0.059 d^{-1}	k: << 0.01	11.8	39.0
FR7	SFO	M_o: 104 g ha^{-1} k: 0.082 d^{-1}	k: < 0.1	8.5	28.1
DE1	SFO	M_o: 49.4 g ha^{-1} k: 0.033 d^{-1}	k: << 0.01	20.7	68.9
IT1	SFO	M_o: 67.9 g ha^{-1} k: 0.031 d^{-1}	k: < 0.05	22.4	74.5
IT2	SFO	M_o: 93.0 g ha^{-1} k: 0.065 d^{-1}	k: < 0.05	10.7	35.5
IT3	SFO	M_o: 96.7 g ha^{-1} k: 0.038 d^{-1}	k: << 0.01	18.0	59.9
IT4	SFO	M_o: 77.2 g ha^{-1} k: 0.032 d^{-1}	k: << 0.01	21.7	72.1

The kinetic evaluation of the time-step normalised datasets was performed for all trials to determine modelling ($DegT_{50}$) endpoints. Ten of the fifteen field trials provided acceptable kinetic results in order to derive modelling endpoints. A summary of the kinetic parameters and field half-lives ($DegT_{50}$) adequate to be used in environmental fate modelling are given in Table 7.1.2.2.1-28.

Table 7.1.2.2.1-28: Summary of normalised modelling endpoints of imazamox derived from European field dissipation trials

Field trial	Kinetic model	Parameters		p (t-test)	$DegT_{50}$ [d]
UK1	SFO	M_o: 32.9 g ha^{-1} k: 0.069 d^{-1}		k: 0.016	9.98
UK2	SFO	M_o: 33.2 g ha^{-1} k: 0.196 d^{-1}		k: 0.062	3.53
FR1	HS	M_o: 27.2 g ha^{-1} k_1: 0.133 d^{-1}	k_2: 0.030 d^{-1} t_b: 2.90 d	k_1: 0.014 k_2: 0.093	30.7[a]
FR2	SFO	M_o: 9.66 g ha^{-1} k: 0.012 d^{-1}		k: 0.11	57.9
FR4b	SFO	M_o: 64.3 g ha^{-1} k: 0.223 d^{-1}		k: 0.093	3.11
FR6	SFO	M_o: 149 g ha^{-1} k: 0.382 d^{-1}		k: 0.004	1.82
FR7	SFO	M_o: 445 g ha^{-1} k: 0.666 d^{-1}		k: 0.044	1.04
DE1	HS	M_o: 49.1 g ha^{-1} k_1: 0.140 d^{-1}	k_2: 0.132 d^{-1} t_b: 3.60 d	k_1: 0.004 k_2: 0.010	5.25[a]
IT2	SFO	M_o: 73.7 g ha^{-1} k: 0.125 d^{-1}		k: 0.081	5.56
IT4	SFO	M_o: 92.2 g ha^{-1} k: 0.113 d^{-1}		k: 0.018	6.12
				Geomean	**6.1**

[a] Conservative $DegT_{50}$ value, computed as $ln(2)/k_2$

III. CONCLUSION

A kinetic evaluation of fifteen European terrestrial field dissipation trials with imazamox was conducted in order to derive trigger and modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA opinion on estimating $DegT_{50}$ values in soil for modelling purposes. The field trials were situated in different regions of Europe, considering a range of different soils and climatic conditions.

Trigger endpoints for imazamox were derived for each of the fifteen field trials. SFO kinetics was the appropriate model for all but one of the trials. The non-normalised field half-lives (DT_{50}) ranged from 4.3 to 31.1 days, while DT_{90} values ranged from 14.4 to 107 days.

Kinetic evaluation of the time-step normalised datasets (20 °C, pF 2) resulted in normalised field half-lives ($DegT_{50}$) for imazamox between 1.0 and 57.9 days, with a geometric mean of 6.1 days. Based on Kendall testing, there was not a significant relationship ($P > 0.05$) between $DegT_{50}$ values and soil pH.

Report:	CA 7.1.2.2.1/7 Donaldson F.P., 2013c Kinetic evaluation of five field dissipation trials for BAS 720 H (Imazamox) conducted from 2011-2012 2013/7001768
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp., SANCO/10058/2005 rev. 2 (FOCUS kinetics report)
GLP:	no

Executive Summary

A kinetic evaluation of the field degradation of BAS 720 H – imazamox was conducted on the data of five terrestrial field degradation trials, derived from two studies conducted in Europe between 2011 and 2012. The field trials were situated in different locations of Europe (Northern and Southern regions), considering a range of different soils and climatic conditions. The three northern sites included France (Suzanne), Germany (Emstek), and UK (Hampton Lucy), while the two southern sites included Italy (Budrio) and Spain (Zafarraya). Immediately after application, each of the test plots was covered with 3-5 mm sand in order to minimise surface loss processes (photolysis, volatilisation). This procedure was considered a reasonable approach and consistent with the EFSA (2010) opinion for calculating $DegT_{50}$ values from field dissipation studies. Each field trial was kinetically analysed to derive modelling endpoints taking into account the current guidance of the FOCUS (2006) kinetics workgroup.

Data derived from the field trials were normalised to reference conditions (20 °C, pF 2) by time-step normalisation and used in kinetic evaluation in order to derive degradation parameters that are valid as modelling endpoints. The normalised field half-lives ($DegT_{50}$) for imazamox ranged from 12.4 to 113 days. It was not possible to derive robust modelling endpoints for the soil metabolites of imazamox (CL 312622 and CL 354825) because the kinetic analysis of the dissipation pathway did not produce acceptable visual or statistical results.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for five trials with imazamox from the data of two field dissipation studies conducted from 2011 to 2012. The data can be found in CA 7.1.2.2.1/1-3 [*Holzer S. (2013): "Field soil dissipation study of BAS 720 H (Imazamox) in the formulation BAS 797 00 H on bare soil at 3 different sites in Northern Europe, 2011-2012"* – DocID 2013/1229816, *Holzer S. (2013): "Field soil dissipation study of BAS 720 H (Imazamox) in the formulation BAS 720 06 H on bare soil at 2 different sites in Southern Europe, 2011-2012"* - DocID 2013/1211059]. The trials were situated in northern and southern regions of Europe considering a range of different soils and climatic conditions. The three northern sites included France (Suzanne), Germany (Emstek), and UK (Hampton Lucy), while the two southern sites included Italy (Budrio) and Spain (Zafarraya). Detailed soil characteristics in each trial are reported in the cited studies. Imazamox was applied at a nominal application rate of 75 g a.i. ha^{-1} at all trial sites. Applications were made by spray broadcast onto bare soil and were conducted between May and October 2011. The trial areas were divided into two plots consisting of an untreated control plot and a treated plot. The latter was further divided into three equally-sized subplots assigned for replicates. The treated plots were covered with 3-5 mm layer of sand immediately after application (prior to first sampling) in order to minimize surface loss processes (photolysis, volatilization). This procedure was considered a reasonable approach and consistent with the recommendations of EFSA [*EFSA (2010): "EFSA Panel on Plant Protection Products; Guidance for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of plant protection products in soil". EFSA Journal (2010); 8 (12): 1936; 67 pp.*]. Soil cores were collected to a maximum depth of 90 cm at each site and split into 10 cm segments. The soil samples were analysed for imazamox and its soil metabolites CL 312622 and CL 354825. The limit of quantification (LOQ) for each analyte was 0.001 mg kg^{-1}.

Normalisation procedure

The studies were time-step normalised to a moisture content at pF 2 and temperature of 20 °C according to FOCUS [*FOCUS (2006): "Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration". Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005, v. 2.0, 434 pp.*], following the approach presented by Hardy [*Hardy (2003): "Normalisation of field degradation data for soil temperature and moisture content for use in environmental risk assessments". XII Symposium Pesticide Chemistry, Piacenza, Italy, June (2003), pp. 51-61.*].

Soil temperature and moisture were estimated using FOCUS-PEARL 4.4.4. Weather data was measured on site, typically from the first day of the month in which the initial sample was taken to the last day of the month in which the final sample was taken. The simulation period was set to these dates. Weather data inputs including air temperature (min/max), precipitation, evapotranspiration (ET_o), and global radiation were measured on site. Irrigation amounts were added to the precipitation total on the specified irrigation date.

Soil profiles containing three horizons were created for each site based on the measured soil properties specified for each 30 cm soil depth. The third horizon was continued to 40 cm in order to create a soil profile of one meter depth. Hydraulic characteristics of each horizon were calculated based on the continuous pedotransfer functions available from the HYPRES database.

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20 °C using the Q_{10} approach as described in the report of the FOCUS Groundwater Scenarios Workgroup [see *FOCUS (2000): "FOCUS groundwater scenarios in the EU review of the active substances." Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev. 2, 202 pp*]. The Q_{10} response function was applied for temperatures above 0 °C (see Equation 7.1.2.2-1 b). Below field temperatures of 0 °C it was assumed that no degradation occurs (Equation 7.1.2.2-1 b). For the evaluation, the EFSA opinion on the default Q_{10} value [*EFSA (2007): Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. The EFSA Journal (2007) 622, 1-32.*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture (θ_{ref}) (Equation 7.1.2.2-1 c).

The normalised day lengths were derived according to Equation 7.1.2.2-1 a. For DAT0, no normalisation was considered and application was assumed to occur at the time point zero. Once each day was normalised, each normalised day (after day zero) was cumulatively summed in order to estimate the normalised sampling day.

Equation 7.1.2.2-1: Calculation of normalised day length based on combination of soil moisture and soil temperature correction factors

a) $$D_{norm} = D * f_{temp} * f_{moisture}$$

b) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

c) $$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^{B} & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:	D_{norm} =	normalised day length (temperature and moisture)	
	f_{temp} =	temperature correction factor	[-]
	f_{moist} =	moisture correction factor	[-]
	D =	1 d	[days]
	T_{act} =	actual soil temperature (°C)	[°C]
	T_{ref} =	reference temperature (20 °C)	[°C]
	Q_{10} =	factor of increase of degradation rate with an increase in temperature of 10 °C (Q_{10} = 2.58)	[-]
	θ_{act} =	actual soil moisture (vol. water content)	[m^3 m^{-3}]
	θ_{ref} =	reference soil moisture at pF 2	[m^3 m^{-3}]
	B =	exponent of the moisture response function, B = 0.7	[-]

Table 7.1.2.2.1-29 shows the sampling days for the trial locations and the normalised (20 °C, pF 2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.1.2.2.1-29: Time-step normalised (temperature and moisture) sampling days

Suzanne, France		Emstek, Germany		Hampton Lucy, UK		Budrio, Italy		Zafarraya, Spain	
DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]	DAT [d]	D_{norm} [d]
0	0	0	0	0	0	0	0	0	0
1	0.4	1	0.5	1	0.3	1	0.7	1	0.5
2	0.8	2	1.1	2	0.8	2	1.5	2	1.0
4	1.4	4	2.1	4	1.6	4	2.9	4	2.3
7	2.7	7	3.7	7	3.0	7	4.7	7	4.2
10	3.9	10	5.5	10	4.3	10	6.9	10	5.8
14	5.1	14	8.1	14	5.9	14	10.1	14	8.3
21	6.8	22	12.1	21	8.6	20	14.2	22	13.7
30	10.2	29	14.4	30	12.2	29	22.0	31	18.7
44	14.1	45	19.7	45	18.7	43	35.1	45	29.0
61	18.1	59	23.5	60	24.8	60	50.6	62	41.2
93	25.1	88	30.7	87	39.2	89	73.9	91	62.0
160	34.7	168	45.9	120	55.6	120	102.0	125	84.2
180	40.5	184	50.7	178	71.1	180	139.8	245	127.9
271	78.6	274	91.5	270	93.0	273	155.3	295	137.4
372	135.6	360	156.4	358	127.2	358	181.2	367	158.9

DAT: Days after treatment; D_{norm}: Normalised day length (20 °C, pF 2)

Kinetic modelling

The software package KinGUII v. 2.2012.202.925 was used for parameter fitting [*Schäfer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007): "KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics". In Del Re, A.A.M. et al (Eds): Proceedings of the XIII Symposium of Pesticide Chemistry, Piacenza, 2007, p. 916-923.*]. The iteratively reweighted least squares (IRLS) method was selected to optimize model parameters, estimate parameter standard deviations, determine parameter confidence via the t-test, calculate correlation coefficients (r^2) and χ^2 error percentage, and calculate DT_{50} and DT_{90} endpoints. The error tolerance and maximum iterations were set to 0.000001 and 100, respectively.

FOCUS guidance for treatment of values below the limit of quantification or detection (LOQ, LOD) was followed [*FOCUS (2006)*]. This guidance was applied to residue data both temporally and spatially. The LOQ was reported as 0.001 mg/kg. Values between the LOD and LOQ were not identified; therefore, the LOD was defined as the LOQ for practical purposes. Values below LOD were set to ½ x LOD = 0.0005 mg kg^{-1}.

Soil residues were converted from a concentration basis (mg kg^{-1}) to a mass per area basis (g ha^{-1}) using the measured dry soil bulk density for each sample and the corresponding soil layer depth. Each layer was then summed to obtain a total mass in the soil profile.

Metabolite residues are expected to be zero at the initial (time zero) sampling. FOCUS guidance was taken into consideration in cases where there were observed metabolite residues at time zero [*FOCUS (2006)*]. Metabolite residues at time zero were converted to parent equivalents using molecular weight corrections, added to the parent time zero residues, and then fixed to zero.

Four kinetic models were applied to the data sets: single first-order (SFO), first-order multi-compartment (FOMC), double-first-order in parallel (DFOP), and hockey stick (HS). Descriptions of each kinetic model as well as the decision trees for model selection/endpoint determination can be found in FOCUS [*FOCUS (2006)*].

The best fitting kinetic model was determined based on both visual and statistical evaluation. To be considered "best-fit", the model must first result in an acceptable visual fit with residuals randomly scattered about the zero line. Statistically, the χ^2 error and t-tests provide information on the goodness-of-fit and reliability of parameter estimates, respectively. A kinetic model is selected if the visual evaluation is passed, the χ^2 error is < 15%, and the estimated model parameters differ from zero at a significance level of 5% ($p \leq 0.05$). In field studies with higher variation in the data, the χ^2 error and t-test significance level can be relaxed to 30% and 10% ($p \leq 0.10$), respectively [*FOCUS (2006)*].

The time-step normalised data was kinetically analysed to determine degradation endpoints ($DegT_{50}$) appropriate for use in exposure models according to FOCUS [*FOCUS (2006)*]. The current EFSA opinion recommends that rapid loss processes (photolysis and volatilization) should be minimised in order to ensure that any dissipation is the result of degradation in the soil matrix [*EFSA (2010)*]. Since the plots had been covered with a sand layer, the time-step normalised data was considered acceptable to derive degradation (modelling) endpoints.

II. RESULTS AND DISCUSSION

The kinetic parameters and field half-lives ($DegT_{50}$) adequate to be used in environmental fate modelling are summarised in Table 7.1.2.2.1-30.

Table 7.1.2.2.1-30: Summary of normalised modelling endpoints of imazamox derived from European field dissipation trials

Field trial	Kinetic model	Parameters	p (t-test)	SFO (or surrogate SFO) $DegT_{50}$ [d]
France	SFO	M_o: 65.1 g ha^{-1} k: 0.017 d^{-1}	k: << 0.01	41.3
Germany	SFO	M_o: 75.3 g ha^{-1} k: 0.032 d^{-1}	k: << 0.01	21.9
UK	FOMC	M_o: 70.8 g ha^{-1} α: 1.79 β: 33.9	α: < 0.01 β: < 0.05	26.7[1)]
Italy	DFOP	M_o: 63.4 g ha^{-1} k_{fast}: 0.081 d^{-1} k_{slow}: 0.006 d^{-1} g: 0.429	k_{fast}, k_{slow}, g: < 0.01	113[2)]
Spain	FOMC	M_o: 61.4 g ha^{-1} α: 2.41 β: 25.6	α, β: < 0.1	12.4[1)]

1) Conservatively calculated as $DT_{90}/3.32$
2) Conservatively calculated as $\ln(2)/k_{slow}$

No endpoints were derived for either metabolite, as the kinetic analyses of the dissipation pathway did not produce acceptable visual or statistical results.

III. CONCLUSION

Kinetic evaluation of five field trials with imazamox was conducted in order to derive modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics. The field trials were situated in northern and southern regions of Europe, considering a range of different soils and climatic conditions.

Kinetic evaluation of the time-step normalised datasets (20 °C, pF 2) resulted in normalised field half-lives ($DegT_{50}$) for imazamox between 12.4 and 113 days.

No modelling endpoints for the soil metabolites of imazamox (CL 312622, CL 354825) were derived, since the kinetic analysis of the dissipation pathway did not produce acceptable visual or statistical results.

Summary of field dissipation and field degradation rates of imazamox

The non-normalized and normalized kinetic endpoints from field dissipation studies and field degradation studies (i.e. field plot covered with sand immediately after application to eliminate surface loss processes) are summarized in the table below.

Table 7.1.2.2.1-31: Summary of field dissipation and field degradation kinetic endpoints for imazamox

Country	Trial Code	Location	Soil type (USDA)	Non-normalised trigger-DT_{50} [d]	Non-normalised trigger-DT_{90} [d]	Normalised modeling-$DegT_{50}$ [d]	Study (BASF DocID)
Field dissipation studies 1993-1999*							
UK	UK1	Stratford-upon-Avon	Clay	31.1	103	9.98	Cronin (1999/7000802)
	UK2	Stratford-upon-Avon	Clay	13.0	43.3	3.53	Cronin (1997/7001015)
France	FR1	Cravenceres	Silt loam	16.5	54.9	30.7[a]	Trewhitt (1999/7000798)
	FR2	Limours	Silt loam	14.7	107	57.9	Cronin (1997/7000978)
	FR3	Grezille	Clay	11.3	37.4	-[b]	Farrell (1997/7000954)
	FR4a	Grezille	Sandy clay loam	4.3	14.4	-[b]	Cronin (1997/7001021)
	FR4b	Pontfaverger	Silt loam	16.4	54.6	3.11	
	FR5	Pontfaverger	Silt loam	9.6	31.8	-[c]	Cronin (1997/7000958)
	FR6	Boissy	Silt loam	11.8	39.0	1.82	Cronin (1997/7001018)
	FR7	Brigne	Loam	8.5	28.1	1.04	Cronin (1997/700095)
Germany	DE1	Euskirchen	Loamy sand	20.7	68.9	5.25[a]	Cronin (1999/7000805)
Italy	IT1	Fontanelle	Silt loam	22.4	74.5	-[d]	Farrell (1997/7000951)
	IT2	Rubizzano	Silty clay	10.7	35.5	5.56	Farrell (1997/7000947)
	IT3	Minerbio	Clay	18.0	59.9	-[b]	Cronin (1997/7001017)
	IT4	Longara	Silty clay loam	21.7	72.1	6.12	Young (1998/7000877)

Table 7.1.2.2.1-31: Summary of field dissipation and field degradation kinetic endpoints for imazamox

Country	Trial Code	Location	Soil type (USDA)	Non-normalised trigger-DT_{50} [d]	Non-normalised trigger-DT_{90} [d]	Normalised modeling-$DegT_{50}$ [d]	Study (BASF DocID)
Field degradation studies 2011-2012**							
France		Suzanne	Silt	ND[f]	ND	41.3	Holzer (2013/1229816)
Germany		Emstek	Loamy sand	ND	ND	21.9	
UK		Hampton Lucy	Loamy sand	ND	ND	26.7[e]	
Italy		Budrio	Loam	ND	ND	113[a]	Holzer (2013/1211059)
Spain		Zafarraya	Loam	ND	ND	12.4[e]	
					Geometric mean	**10.5**	

[a] Conservatively calculated as $\ln(2)/k_2$
[b] Trial not used due to lack of appropriate meteorological station (> 20 km) according to EFSA (2010)
[c] Trial not used due to insufficient number of data points after 10 mm rain according to EFSA (2010)
[d] Trial not used due to low statistical confidence in the rate constant
[e] Conservatively calculated as $DT_{90}/3.32$
[f] Not determined - due to the specific design of the studies (i.e. sand covered), the obtained field degradation half-lives were not considered for derivation of trigger endpoints
* Kinetic analysis performed by Donaldson (2013/7001766). See CA 7.1.2.2.1/6.
** Kinetic analysis performed by Donaldson (2013/7001768). See CA 7.1.2.2.1/7.

CA 7.1.2.2.2 Soil accumulation studies

No data were generated or required. The maximum imazamox field dissipation non-normalized DT_{90} was less than one year (107 days).

CA 7.1.3 Absorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

Report:	CA 7.1.3.1.1/1 Vasques A.C., 2012a Adsorption / desorption behavior of 14C-BAS 720 H and metabolites 14C-CL312622 and 14C-CL354825 on different US and European soils 2012/3004061
Guidelines:	OECD 106 (2000), EPA 835.1230
GLP:	Yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The adsorption/desorption behaviour of the radiolabelled imazamox -^{14}C-BAS 720 H – as well as of the radiolabelled metabolites ^{14}C-CL312622 and ^{14}C-CL354825 was investigated on different US and European soils. Specifically, five soils covering a range of pH (in water) from 6.5 to 8.1, a range of organic carbon content from 0.28% to 3.84%, and four different USDA textural classes (Silty Clay Loam, Loam, Loamy Sand and Sandy Loam).

For the determination of the adsorption isotherm, five different concentrations from 0.01 to 1.00 µg/mL of each test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil/test solution for ^{14}C-imazamox and ^{14}C-CL312622 was 1/1, whereas for ^{14}C- CL354825 was 1/5. The test was performed at the adsorption equilibrium time of 24 hours for ^{14}C-imazamox, 48 h for ^{14}C-CL312622 and 4 hours for ^{14}C- CL354825 on the five soils. Desorption was also determined in two steps. The concentrations of the test substance in the soils extracts, adsorption solution as well as in the desorption solutions 1 and 2 and were determined by liquid scintillation counting. The stability of the test substances in the test system was checked by Radio-HPLC analysis.

The following adsorption and desorption parameters were measured and evaluated for ^{14}C-imazamox, ^{14}C-CL312622 and ^{14}C- CL354825 in each soil: distribution coefficients K_d and K_{OC} at five concentration levels, Freundlich adsorption coefficients K_F, the Freundlich exponent 1/n, and the corresponding K_{FOC} values.

The imazamox Freundlich adsorption coefficients K_F ranged from 0.06 to 0.14 mL/g in the five soils, which corresponded to K_{FOC} values from 1.98 to 22.21 mL/g. Freundlich exponents 1/n varied between 0.96 and 1.02. The desorption coefficients K_{Fdes1} and K_{Fdes2} ranged from 0.09 to 0.29 mL/g in the five soils, with K_{FOCdes} values between 3.53 mL/g and 48.94 mL/g.

Freundlich adsorption coefficients K_F for metabolite CL 312622 varied from 0.06 to 0.26 mL/g in the five soils, which corresponded to K_{FOC} values ranging from 4.62 to 20.03 mL/g. Freundlich exponents 1/n ranged from 0.98 to 1.07. The desorption coefficients K_{Fdes1} and K_{Fdes2} ranged from 0.08 to 0.38 mL/g in the five soils, with o K_{FOCdes} values ranging from 6.67 mL/g to 43.52 mL/g.

The metabolite CL354825 Freundlich adsorption coefficients K_F varied from 1.28 to 8.20 mL/g in the tested soils, with K_{FOC} values ranging from 51.32 to 845.16 mL/g. Freundlich exponents 1/n ranged from 0.71 to 0.86. The desorption coefficients K_{Fdes1} and K_{Fdes2} ranged from 3.24 to 19.31 mL/g in the five soils, with corresponded to K_{FOCdes} values of 109.91 to 2255.34 mL/g.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	^{14}C-BAS 720 H
Reg. No.	4096483
Batch No.:	1004-1001
Radiopurity:	99.7%
Molecular weight:	305.3 g/mol

Test item	^{14}C- CL312622
Reg. No.	4110542
Batch No.:	1035-1013
Radiopurity:	97.3%
Molecular weight:	305.3 g/mol

Test item	^{14}C- CL354825
Reg. No.	4110603
Batch No.:	1032-1024
Radiopurity:	97.5%
Molecular weight:	277.3 g/mol

2. Soils

The study was conducted with five different soils from US and Europe. The physico-chemical characterisation of the soils is presented in Table 7.1.3.1.1-1.

Table 7.1.3.1.1-1 Characterisation of soils used to investigate the adsorption and desorption of ^{14}C-labelled imazamox, and the metabolites ^{14}C-CL312622 and ^{14}C- CL354825

Soil designation Origin	Europe (BW)	USA (CA)	Europe (GI)	Europe (Li10)	USA (NJ)
Textural class (USDA scheme)	Sandy loam	Loamy sand	Silt clay loam	Loamy sand	Loam
Soil texture [%], (USDA scheme)					
Sand	63.7	87.0	12.7	84.1	29.0
Silt	24.6	8.0	48.3	11.5	44.0
Clay	11.7	5.0	39.0	4.3	27.0
Organic carbon[1] [%]	1.37	0.28	3.84	0.97	1.33
CEC [meq /100g]	10.1	6.6	29.0[2]	5.4[2]	8.1
pH (water)	7.8	8.1	8.1	6.5	6.9

[1]organic carbon =organic matter / 1.724
[2]unit: $cmol^+/kg$

B. STUDY DESIGN

1. Experimental conditions

In order to determine the adsorption and desorption behaviour of ^{14}C-labelled imazamox, and metabolites ^{14}C-CL312622 and ^{14}C- CL354825 in test soils, soil samples were air-dried and sieved (particle size < 2 mm).

Initial experiments, conducted for 24 h with two non-sterilized soils, revealed that the optimal soil / solution ratio for the adsorption/desorption tests was 1/1 for ^{14}C-imazamox and ^{14}C-CL312622, whereas for ^{14}C- CL354825 was 1/5. Subsequent preliminary experiments indicated no interferences with the material used.

From the adsorption equilibrium tests, which was carried out with five sampling points in duplicate between 4 and 48h, equilibration times of 4h, 24 h, and 48h was chosen for the isotherms test with ^{14}C-CL 354825, ^{14}C-imazamox and ^{14}C-CL 312622, respectively. From the stability test run in parallel, it was observed that all the test items were stable during the 48 h shaking period in absence of soil.

2. Adsorption-Desorption Isotherm Determination

To determine adsorption isotherms, standard solutions of the test item in 0.01 M $CaCl_2$ were prepared at five concentrations levels, ranging from 0.01 to 1.00 µg/mL. The experiment was conducted in duplicate. Per soil type a total of 10 soil samples were dosed (duplicates per amount/concentration level dosed), plus sufficient blank controls. Aliquots of 10 mL of the test solution were added to 10 g of the test soil for ^{14}C-imazamox and ^{14}C-CL 312622. In the case of ^{14}C-CL 354825, 10 mL of the test solution were added to 2 g of the test soil. Samples were placed horizontally on a mechanical shaker at 250 rpm and shaken for the time determined at equilibrium test in darkness. Thereafter the soil/water specimens were centrifuged, decanted, and aliquots of the supernatants were assayed by LSC to determine the percentage of total applied radioactivity (%TAR) in the supernatants. Aliquots from the highest dose sample supernatants were analysed directly by Radio-HPLC to determine the nature of the radioactivity.

Desorption was carried out in two steps. For desorption step 1, the decanted supernatant from the adsorption experiment was replaced by an equal volume of $CaCl_2$ solution without test item. The new mixture was gently agitated for the same test period on a mechanical shaker at 250 rpm in a temperature controlled dark room. After shaking, the mixtures were centrifuged at appropriate rotation. Aliquots of the supernatants were assayed by LSC to estimate the radioactivity present in the desorption supernatants. Aliquots from the highest dose samples supernatants were analysed by Radio-HPLC to determine the nature of the radioactivity in the desorption solution. Desorption step 2 was performed in an analogous manner with the soil samples left from desorption step 1.

Control specimens with only the test item in aqueous 0.01 M $CaCl_2$ solution (adsorption controls) were used to show that no significant adsorption on the surface of the test vessels occurred.

2. Description of analytical procedures

To show the stability of the test item and to draw a mass balance, the soil remaining after the desorption steps was extracted. The extraction of test items was performed by shaking the soils with 20 mL of methanol/water (60/40, v/v) solution on a mechanical shaker for 30 minutes. After shaking, the samples were centrifuged appropriately. This procedure was done three times. Aliquots of the extracts were assayed by LSC to determine % TAR in the extract aliquots from the extracts were analysed by Radio-HPLC to determine the nature of the radioactivity in the extract.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Complete recovery of each analyte from each soil was achieved, with means ranging from 96.8 % (GI and Li10 soils) to 98.7 % (NJ soil) for ^{14}C-imazamox, from 95.6% (NJ soil) to 97.6% (GI soil) for ^{14}C-CL 312622 and from 94.6% (BW soil) to 99.2% (CA soil) for ^{14}C- CL354825.

B. FINDINGS

Detailed results from the adsorption and desorption tests for ^{14}C-imazamox and ^{14}C-metabolites CL312622 and CL354825 in all five soils are presented in Table 7.1.3.1.1-2 to Table 7.1.3.1.1-7 Adsorption-desorption and extraction supernatants of the high dose samples and test substance treatment solution freshly prepared and after 48 hours shaking were analysed by Radio-HPLC. The Radio-HPLC chromatograms show that all the test items were stable during the adsorption/ desorption experiments.

Table 7.1.3.1.1-2: Adsorption of ^{14}C-imazamox based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	K_F [mL/g]	1/n	K_{FOC} [mL/g]	K_d [1)] [mL/g]	K_{oc} [1)] [mL/g]
BW	Sandy loam	0.10	0.97	7.04	0.11 0.10	7.77 7.41
CA	Loamy sand	0.06	0.98	22.21	0.06 0.06	20.10 19.70
GI	Silt clay loam	0.08	0.98	1.98	0.07 0.07	1.90 1.87
Li10	Loamy sand	0.14	0.96	14.20	0.15 0.14	15.85 14.13
NJ	Loam	0.06	1.02	4.68	0.06 0.05	4.54 3.83

[1)] The respective values given in the table refer to the replicates of the higher concentration level of the test item (nominal concentration of 1.00 µg/mL).

Table 7.1.3.1.1-3: Desorption of ^{14}C-imazamox based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	K_{Fdes1} [mL/g]	1/n	$K_{FOCdes1}$ [mL/g]	K_d [1)] [mL/g]	K_{oc} [1)] [mL/g]
Desorption 1						
BW	Sandy loam	0.17	0.96	12.45	0.18 0.15	13.19 11.22
CA	Loamy sand	0.10	0.95	33.67	0.09 0.08	30.82 29.13
GI	Silt clay loam	0.14	0.98	3.53	0.14 0.14	3.60 3.55
Li10	Loamy sand	0.21	0.96	21.21	0.23 0.22	23.36 22.70
NJ	Loam	0.09	0.95	6.99	0.10 0.08	7.37 6.15
Desorption 2						
BW	Sandy loam	0.29	0.95	20.88	0.27 0.23	19.76 16.60
CA	Loamy sand	0.14	0.91	48.94	0.14 0.13	50.80 44.01
GI	Silt clay loam	0.23	0.96	5.93	0.25 0.26	6.59 6.79
Li10	Loamy sand	0.29	0.97	29.42	0.31 0.31	31.56 32.26
NJ	Loam	0.12	0.88	9.22	0.14 0.14	10.74 10.55

[1)] The respective values given in the table refer to the replicates of the higher concentration level of the test item (nominal concentration of 1.00 µg/mL).

Table 7.1.3.1.1-4: Adsorption of ^{14}C-CL 312622 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	K_F [mL/g]	1/n	K_{FOC} [mL/g]	K_d [1)] [mL/g]	K_{oc} [1)] [mL/g]
BW	Sandy loam	0.06	1.03	4.62	0.06 0.06	4.59 4.47
CA	Loamy sand	0.06	1.07	20.03	0.06 0.05	19.47 18.96
GI	Silt clay loam	0.26	1.00	6.80	0.26 0.26	6.76 6.78
Li10	Loamy sand	0.06	0.98	5.73	0.07 0.05	7.01 5.59
NJ	Loam	0.08	1.01	5.86	0.08 0.08	6.03 6.00

[1)] The respective values given in the table refer to the replicates of the higher concentration level of the test item (nominal concentration of 1.00 µg/mL).

Table 7.1.3.1.1-5: Desorption of ^{14}C-CL 312622 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	K_{Fdes1} [mL/g]	1/n	$K_{FOCdes1}$ [mL/g]	K_d [1)] [mL/g]	K_{oc} [1)] [mL/g]
Desorption 1						
BW	Sandy loam	0.09	1.00	6.67	0.09 0.10	6.90 7.11
CA	Loamy sand	0.08	1.06	26.76	0.07 0.07	25.04 25.98
GI	Silt clay loam	0.31	0.99	8.20	0.32 0.32	8.33 8.30
Li10	Loamy sand	0.13	1.08	13.59	0.13 0.11	13.45 11.12
NJ	Loam	0.14	1.11	10.29	0.12 0.12	8.75 9.08
Desorption 2						
BW	Sandy loam	0.17	1.04	12.33	0.16 0.16	11.67 11.63
CA	Loamy sand	0.12	1.10	43.52	0.11 0.10	38.29 35.93
GI	Silt clay loam	0.38	0.93	9.87	0.44 0.43	11.38 11.21
Li10	Loamy sand	0.30	1.15	31.23	0.22 0.20	23.03 20.45
NJ	Loam	0.23	1.09	17.05	0.19 0.20	14.46 15.01

[1)] The respective values given in the table refer to the replicates of the higher concentration level of the test item (nominal concentration of 1.00 µg/mL).

Table 7.1.3.1.1-6: Adsorption of ^{14}C-Cl354825 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	K_F [mL/g]	1/n	K_{FOC} [mL/g]	K_d [1)] [mL/g]	K_{oc} [1)] [mL/g]
BW	Sandy loam	5.61	0.78	409.18	5.75 5.73	419.76 418.24
CA	Loamy sand	1.28	0.71	451.59	1.31 1.33	460.44 469.28
GI	Silt clay loam	1.97	0.71	51.32	1.92 1.85	49.95 48.05
Li10	Loamy sand	8.20	0.86	845.16	8.03 8.60	827.77 886.34
NJ	Loam	4.82	0.82	361.21	5.02 5.10	376.45 382.17

[1)] The respective values given in the table refer to the replicates of the higher concentration level of the test item (nominal concentration of 1.00 µg/mL).

Table 7.1.3.1.1-7: Desorption of ^{14}C-Cl354825 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	K_{Fdes1} [mL/g]	1/n	$K_{FOCdes1}$ [mL/g]	K_d [1)] [mL/g]	K_{oc} [1)] [mL/g]
Desorption 1						
BW	Sandy loam	11.08	0.82	808.88	13.74 13.54	1002.76 988.12
CA	Loamy sand	3.24	0.82	1141.13	4.26 4.44	1499.83 1563.05
GI	Silt clay loam	4.22	0.80	109.91	5.22 5.14	135.82 133.97
Li10	Loamy sand	14.22	0.87	1466.44	16.25 17.47	1675.25 1800.53
NJ	Loam	7.95	0.86	596.03	9.41 9.55	705.10 715.96
Desorption 2						
BW	Sandy loam	16.51	0.84	1204.82	22.88 22.49	1669.81 1641.38
CA	Loamy sand	6.41	0.90	2255.34	8.45 8.43	2973.59 3037.31
GI	Silt clay loam	7.50	0.86	195.24	10.48 9.99	272.84 260.22
Li10	Loamy sand	19.31	0.88	1990.30	23.49 23.06	2421.77 2376.82
NJ	Loam	11.73	0.89	879.38	14.65 14.56	1098.27 1091.64

[1)] The respective values given in the table refer to the replicates of the higher concentration level of the test item (nominal concentration of 1.00 µg/mL).

III. CONCLUSION

The adsorption and desorption behaviour of ^{14}C-imazamox and metabolites ^{14}C-CL312622 and ^{14}C-CL354825 was determined on five US and European soils, which covered a range of pH (in water) from 6.5 to 8.1, a range of organic carbon content from 0.28% to 3.84% and four different USDA textural classes (Silt Clay Loam, Loam, Loamy sand and Sandy Loam). Complete recovery of each analyte from each soil was achieved.

Summary of imazamox adsorption values

Imazamox adsorption values from studies previously submitted, as well as the new study summarized in this supplement dossier are presented below. Corrected adsorption values are also presented where applicable.

For each soil, a correction factor was applied to the sorption coefficients of imazamox in order to address uncertainty of low sorbing compounds using a method proposed by Boesten *et al.* [*Boesten J.J.T.I., Van der Linden, A.M.A., Beltman, W.H.J., Pol, J.W. (2011): Leaching of plant protection products and transformation products: Proposals for improving the assessment of leaching to groundwater in the Netherlands. Alterra report 2264*]. This method takes into account OECD guideline 106, which states that if the product of the soil/solution ratio and K_f is below 0.3 for a given soil, inaccuracy in the K_f estimation could result. The correction procedure considers differences between actual recovery and 100% as material not adsorbed to the soil, and the K_f value is accordingly corrected. Correction of adsorption values for low sorbing compounds is an approach taken in the EFSA (2010) review for fluazifop-p [EFSA (2010) *Conclusion on the peer review of the pesticide risk assessment of the active substance fluazifop-P (evaluated variant fluazifop-P-butyl)* EFSA Journal 2010;8(11):1905].

Table 7.1.3.1.1-8: Adsorption values of imazamox

Soil designation (Origin)	Soil texture	OC [%]	pH* [-]	K_f [L kg^{-1}]	$K_{f,oc}$ [L kg^{-1}]	K_f corr.**	$K_{f,oc}$ corr.**	1/n [-]	Study (BASF DocID)
Sharkey (USA)	Clay Loam	1.9	5.3	2.45	130	2.45	130	0.97	Mangels (1994/7000931)
Tippecanoe (USA)	Silt Loam	1.1	3.9	1.22	116	1.22	116	0.94	
Sassafras (USA)	Sandy Loam	0.9	5.4	0.28	33	0.27	31.9	0.96	
Beardon (USA)	Silty Clay Loam	2.6	6.9	0.12	5	0.11	4.67	0.96	
Plano (USA)	Loam	1.4	5.3	0.21	15	0.20	14.4	0.91	
Buelah (USA)***	Loamy Sand	0.28	6.0	0.08	29				
97-669 (UK)	Clay	1.8	7.4	0.09	5	0.07	3.73	0.93	Heim &Ta (1998/7000903)
98-404 (UK)	Clay	2.4	7.0	0.25	11	0.22	9.85	0.91	
93-165 (Italy)	Silty Clay Loam	0.9	7.4	0.59	o66	0.59	66.0	0.91	Heim &Ta (1998/7000904)
95-013 (Italy)	Clay	1.0	7.7	0.48	48	0.48	48.0	0.85	
98-405 (Italy)	Silty Clay Loam	1.1	7.5	0.20	18	0.15	13.6	0.87	
Bruch West (Germany)	Sandy loam	1.4	7.3	0.10	7	0.09	6.50	0.97	Vasques (2012/3004061)
CA (USA)***	Loamy sand	0.28	7.6	0.06	22				
La Gironda Arahal (Spain)	Silty clay loam	3.8	7.6	0.08	2	0.07	1.79	0.98	
Li10 (Germany)	Loamy sand	1.0	6.0	0.14	14	0.13	13.4	0.96	
NJ (USA)	Loam	1.3	6.4	0.06	5	0.05	4.10	1.02	
Geometric mean				**0.237**	**16.5**	**0.218**	**15.1**	**0.94**§	

* Values converted from reported pH_{H2O} to pH_{CaCl2}, as the sorption study was performed in $CaCl_2$ solution. Conversion used: $pH_{CaCl2} = 1.018 \times pH_{H2O} - 0.660$ taken from Boesten al. (2011)

** Corrected for uncertainty of low sorbing compounds as discussed in Boesten et al. (2011)

*** Not considered, as organic carbon content is <0.3% and does not fulfill guideline requirements (OECD 106)

§ Arithmetic mean

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Report: CA 7.1.3.1.2/1
Vasques A.C., 2012a
Adsorption / desorption behavior of 14C-BAS 720 H and metabolites 14C-CL312622 and 14C-CL354825 on different US and European soils
2012/3004061
Guidelines: OECD 106 (2000), EPA 835.1230
GLP: Yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Summary of imazamox metabolite CL 312622 and CL 354825 adsorption values

Adsorption values for metabolite CL 312622 and CL 354825 from studies previously submitted, as well as the new study summarized in this supplement dossier (see section CA 7.1.3.1.1/1) are presented below. Corrected adsorption values are also presented where applicable.

For each soil, a correction factor was applied to the sorption coefficients of CL 312622 in order to address uncertainty of low sorbing compounds using a method proposed by Boesten *et al.* [*Boesten J.J.T.I., Van der Linden, A.M.A., Beltman, W.H.J., Pol, J.W. (2011): Leaching of plant protection products and transformation products: Proposals for improving the assessment of leaching to groundwater in the Netherlands. Alterra report 2264*]. This method takes into account OECD guideline 106, which states that if the product of the soil/solution ratio and K_f is below 0.3 for a given soil, inaccuracy in the K_f estimation could result. The correction procedure considers differences between actual recovery and 100% as material not adsorbed to the soil, and the K_f value is accordingly corrected. Correction of adsorption values for low sorbing compounds is an approach taken in the EFSA (2010) review for fluazifop-p [EFSA (2010) *Conclusion on the peer review of the pesticide risk assessment of the active substance fluazifop-P (evaluated variant fluazifop-P-butyl)* EFSA Journal 2010;8(11):1905]. Correction of sorption was not necessary for CL 354825 since this is not a low sorbing compound.

Table 7.1.3.1.2-1: Adsorption values of CL 312622

Soil designation (Origin)	Soil texture	OC [%]	pH* [-]	K_f [L kg^{-1}]	$K_{f,oc}$ [L kg^{-1}]	K_f corr.**	$K_{f,oc}$ corr.**	1/n [-]	Study (BASF DocID)
Sharkey (USA)	Clay Loam	1.9	5.3	1.05	55.9	1.05	55.9	0.90	Kuhn (1995/7000974)
Tippecanoe (USA)	Silt Loam	1.1	3.9	1.81	172.0	1.81	172	0.92	
Sassafras (USA)	Sandy Loam	0.9	5.5	0.75	88.2	0.75	88.2	0.97	
Beardon (USA)	Silty Clay Loam	2.6	6.9	0.64	24.7	0.64	24.7	0.94	
Plano (USA	Loam	1.4	5.4	0.75	54.7	0.75	54.7	0.96	
Buelah (USA)***	Loamy Sand	0.28	5.3	0.74	264				
Bruch West (Germany)	Sandy loam	1.4	7.3	0.60	4.62	0.6	4.62	1.03	Vasques (2012/3004061)
CA (USA)***	Loamy sand	0.28	7.6	0.06	20.03				
La Gironda Arahal (Spain)	Silty clay loam	3.8	7.6	0.26	6.80	0.25	6.54	1.00	
Li10 (Germany)	Loamy sand	1.0	6.0	0.06	5.73	0.05	4.92	0.98	
NJ (USA)	Loam	1.3	6.4	0.08	5.86	0.07	5.23	1.01	
Geometric mean				**0.431**	**22.0**	**0.417**	**21.3**	**0.97**§	

* Values converted from reported pH_{H2O} to pH_{CaCl2}, as the sorption study was performed in $CaCl_2$ solution. Conversion used: $pH_{CaCl2} = 1.018 \times pH_{H2O} - 0.660$ taken from Boesten al. (2011)

** Corrected for uncertainty of low sorbing compounds as discussed in Boesten et al. (2011)

*** Not considered, as organic carbon content is <0.3% and does not fulfill guideline requirements (OECD 106)

§ Arithmetic mean

Table 7.1.3.1.2-2: Adsorption values of CL 354825

Soil designation (Origin)	Soil texture	OC [%]	pH* [-]	K_f [L kg^{-1}]	$K_{f,oc}$ [L kg^{-1}]	1/n [-]	Study (BASF DocID)
Buelah (USA)**	Loamy Sand	0.28	5.3	1.97	704	0.71	Kuhn (1995/7000870)
Sharkey (USA)	Clay Loam	1.9	5.3	18.2	968	0.89	
Tippecanoe (USA)	Silt Loam	1.1	3.9	14.1	1343	0.89	
Sassafras (USA)	Sandy Loam	0.9	5.4	5.96	701	0.79	
Beardon (USA)	Silty Clay Loam	2.6	6.9	3.43	132	0.68	
Plano (USA	Loam	1.4	5.3	7.31	534	0.78	
Bruch West (Germany)	Sandy loam	1.4	7.3	5.61	409	0.78	Vasques (2012/3004061)
CA (USA)**	Loamy sand	0.28	7.6	1.28	452	0.71	
La Gironda Arahal (Spain)	Silty clay loam	3.8	7.6	1.97	51.3	0.71	
Li10 (Germany)	Loamy sand	1.0	6.0	8.20	845	0.86	
NJ (USA)	Loam	1.3	6.4	4.82	361.2	0.82	
Geometric mean				**6.35**	**421**	**0.80**§	

* Values converted from reported pH_{H2O} to pH_{CaCl2}, as the sorption study was performed in $CaCl_2$ solution. Conversion used: $pH_{CaCl2} = 1.018 \times pH_{H2O} - 0.660$ taken from Boesten al. (2011)

** Not considered, as organic carbon content is <0.3% and does not fulfill guideline requirements (OECD 106)

§ Arithmetic mean

CA 7.1.3.2 Aged sorption

No data were generated or required.

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

No data were generated or required.

CA 7.1.4.1.1 Column leaching of the active substance

No data were generated or required.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

No data were generated or required.

CA 7.1.4.2 Lysimeter studies

No additional data were generated or required. Lysimeter study information along with the Notifier's statement [*Beigel (2002a): Response to the question from ANNEX 3 to Concise Outline Report of ECCO 106 Peer Review Meeting for Imazamox, Section 2, Environmental fate and behavior, concerning unknown radioactivity and the presence of CL354825 in the leachate of imazamox lysimeter study ENV93-05 (Hassink 1997): BASF DocID 2002/5004224*] was included in the previous imazamox DAR (SANCO/4325/2000 –Final, 29 November 2002).

CA 7.1.4.3 Field leaching studies

No data were generated or required.

CA 7.2 Fate and behaviour in water and sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

No additional data were generated or required.

CA 7.2.1.2 Direct photochemical degradation

Report:	CA 7.2.1.2/1 Singh M. et al., 2013a Aqueous photolysis of 14C-BAS 720 H 2013/7001838
Guidelines:	EPA 835.2240, EPA 835.2210, EPA 161-2, OECD 316 (Photodegradation in Water)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The aqueous photolysis of BAS 720 H – imazamox was studied by using [^{14}C]-pyridine labelled imazamox (6.20 mg/L) and [^{14}C]-imidazolinone labelled imazamox (5.49 mg/L) in sterile buffer solution at pH 7. The test solutions of imazamox were continuously exposed to a Xenon arc lamp (wavelength > 290 nm), emitting a light spectrum similar to natural sunlight (>290 nm) at an average intensity of 551 mW/cm^2 (pyridine label samples) and 574 W/m^2 (imidazolinone label samples) for 15 days. Temperature was kept constant at 22°C. The headspace of the reaction vessels with the test solutions were continuously flushed with sterile CO_2-free moistened air and volatiles were collected in a series of trapping solutions (100 mL of 1N NaOH each). Samples were taken at 0, 4 hour, 8 hour, 1, 2, 3, 6, 9, 13 and 15 days after treatment (DAT). Dark control samples were incubated under the same conditions except for irradiation.

For determination of the quantum yield of imazamox, a mixture of p-nitroacetophenone (PNAP) and pyridine was used as chemical actinometer. The vessel with the actinometer solution (200 mL) was irradiated under similar conditions as the other test vessels.

All samples were measured for total radioactivity (LSC) and analysed by HPLC. The results showed that the photolysis of ^{14}C- labelled imazamox in pH 7 buffer solution resulted in a very fast decline of imazamox to less than 2 % TAR after 3 and 1 DAT for the pyridine and imidazolinone labels, respectively. Imazamox was stable in the dark control samples.

The degradation half-life (DT_{50}) of imazamox under continuous irradiation was calculated to be 0.21 days according to single first-order kinetics. The quantum yield calculation for imazamox was 4.8 x 10^{-3} mole Einstein^{-1}.

Imazamox rapidly degraded to a very large number of products under the photolytic conditions. None of the degradation products exceeded 5% TAR at the end of the study (15 DAT). All the major/significant radioactive residues including several minor degradation products, observed during the course of photolysis, were identified/characterized using HPLC co-chromatography with reference standards and/or Mass Spectrometry.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Imazamox (BAS 720 H)
Reg.No.: 4096483
Chemical name: (RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Molar mass: 305.33 g/mol (non-labelled)
Molecular formula: $C_{15}H_{19}N_3O_4$

Labelled test item
Label: imidazolinone-5-^{14}C; 3-^{15}N
Specific radioactivity: 420000 dpm/µg
Radiochemical purity: 98.7 %

Label: pyridine-3-^{14}C, imidazolone-3-^{15}N
Specific radioactivity: 399000 dpm/µg
Radiochemical purity: 99.7 %

2. Buffer solution: Boric acid, lot number A0284825 (ACROS) and HPLC grade water was used for the preparation of pH 7 test buffer solution.

B. STUDY DESIGN

1. Experimental conditions

Glass reaction vessels, containing magnetic stir bars and special quartz glass covers, were used as the test vessels. The test vessel was connected to an air flow through system. The head space of the vessel was continuously flushed with sterile CO_2-free moistened air. The outgoing air was bubbled through volatile trapping solutions of 1N NaOH.

Each vessel was filled with 200 mL of the experimental solution, which was prepared by taking 0.9 mL from the ^{14}C-imazamox stock solution and diluting with the buffer solution to get a concentration of 6.2 mg/L (pyridine label) and 5.49 mg/L (imidazolinone label). The vessels were situated in rectangular blocks to keep the temperature constantly at 22 ± 1°C.

The irradiated samples were placed in a SUNTEST CPS apparatus and continuously exposed to a Xenon arc lamp emitting a light spectrum similar to natural sunlight (> 290 nm) at an average intensity of 551 mW/cm^2 (pyridine label) and 574 W/m^2 (imidazolinone label), simulating a clear spring day at 40º N latitude.

A number of aliquots (~1.0 mL each) of the experimental solution were transferred into HPLC vials, capped and stored in dark inside an incubator maintained at 22±1º C. These samples were used as dark controls. Volatile radioactive residues for the dark control samples were not collected.

2. Sampling

The irradiated and dark control samples were sampled at 0, 4 hours, 8 hours, 24 hours (1 day), 2 days, 3 days, 6 days, 9 days, 13 days and 15 days after treatment (DAT) for both labels.

3. Description of analytical procedures

All samples were measured for total radioactivity by Liquid Scintillation Counting (LSC) and were analysed by HPLC to determine a metabolite pattern. Aliquots of the volatile trapping solutions were also measured for total radioactivity by LSC.

Characterization and identification of degradation products in aqueous samples was performed by co-chromatography with reference compounds via radio-HPLC and MS-analysis. Specifically, for the zero DAT and 1 DAT irradiated samples from both [^{14}C]-pyridine- labelled imazamox and [^{14}C]-imidazolinone labelled imazamox, a chiral HPLC analysis was also performed.

Sterility checks were performed for the experimental solutions at starting and end points of incubation. The test systems were under sterile conditions for the entire experimental period.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Total recoveries and distribution of radioactivity in the water samples and volatile traps are presented in Table 7.2.1.2-1 and Table 7.2.1.2-2. The average material balance for the dark control samples (Rep 1 and Rep 2) ranged from 98.8 – 98.9% of the total applied radioactivity (TAR) (pyridine label) and 104 - 105% TAR (imidazolinone label). The average material balance for the irradiated samples (Rep 1 and Rep 2) ranged from 96.7 – 96.8% TAR (pyridine label) and 90.1 – 96.9% TAR (imidazolinone label).

Photolysis of [^{14}C]-imidazolinone labelled imazamox produced the largest amount of volatile radioactivity (~85.5% TAR at 2 DAT). Photolysis of [^{14}C]-pyridine- labelled imazamox produced smaller amounts of volatile radioactivity and accounted for a maximum of 13.4% TAR at 15 DAT. All the volatile radioactivity for both labels was $^{14}CO_2$.

Table 7.2.1.2-1: Recovery and distribution of radioactivity during aqueous photolysis of [^{14}C]-pyridine labelled imazamox [% TAR]

Sampling interval	aqueous solution at pH 7	NaOH solution traps	Material Balance
irradiated			
0 h	100.00	n.a.	100.00
0 h	100.00	n.a.	100.00
4 h	97.06	2.59	99.65
4 h	96.71	2.61	99.32
8 h	96.31	4.05	100.36
8 h	96.77	2.84	99.61
1 DAT	94.89	0.48	95.37
1 DAT	95.20	0.67	95.88
2 DAT	95.78	-1.84	93.70*
2 DAT	94.38	-2.25	91.53*
3 DAT	94.41	3.91	98.32
3 DAT	92.49	7.02	99.51
6 DAT	90.50	4.76	95.26
6 DAT	87.50	10.35	97.86
9 DAT	89.25	5.46	94.72
9 DAT	86.26	7.05	93.31
13 DAT	89.00	6.97	95.97
13 DAT	86.39	7.99	94.38
15 DAT	88.69	6.11	94.80
15 DAT	82.13	13.44	95.57

Table 7.2.1.2-1: Recovery and distribution of radioactivity during aqueous photolysis of [^{14}C]-pyridine labelled imazamox [% TAR]

Sampling interval	aqueous solution at pH 7	NaOH solution traps	Material Balance
dark control			
0 h	100.00	n.a.	100.00
0 h	100.00	n.a.	100.00
4 h	100.30	n.a.	100.30
4 h	100.00	n.a.	100.00
8 h	100.57	n.a.	100.57
8 h	101.28	n.a.	101.28
1 DAT	94.97	n.a.	94.97
1 DAT	97.14	n.a.	97.14
2 DAT	97.99	n.a.	97.99
2 DAT	95.99	n.a.	95.99
3 DAT	98.88	n.a.	98.88
3 DAT	98.94	n.a.	98.94
6 DAT	99.00	n.a.	99.00
6 DAT	98.55	n.a.	98.55
9 DAT	97.36	n.a.	97.36
9 DAT	96.63	n.a.	96.63
13 DAT	100.55	n.a.	100.55
13 DAT	99.23	n.a.	99.23
15 DAT	99.74	n.a.	99.74
15 DAT	100.51	n.a.	100.51

n.a. = not applicable; *negative number %TAR volatile is negligible, value based on original %TAR
h= hour; DAT = days after treatment

Table 7.2.1.2-2: Recovery and distribution of radioactivity during aqueous photolysis of [^{14}C]- imidazolinone labelled imazamox [% TAR]

Sampling interval	aqueous solution at pH 7	NaOH solution traps	Material Balance
irradiated			
0 h	100.0	n.a.	100.00
0 h	100.0	n.a.	100.00
4 h	66.26	39.05	105.31
4 h	64.60	33.08	97.67
8 h	47.55	55.84	103.39
8 h	44.83	60.01	104.84
1 DAT	19.32	83.07	102.39
1 DAT	19.31	82.26	101.58
2 DAT	15.85	85.51	101.36
2 DAT	17.13	81.91	99.04
3 DAT	16.60	80.93	97.53
3 DAT	15.54	81.31	96.84
6 DAT	18.39	70.58	88.97
6 DAT	17.36	63.21	80.57
9 DAT	19.23	73.18	92.40
9 DAT	15.64	60.99	76.62
13 DAT	12.29	76.79	89.08
13 DAT	11.15	60.52	71.67
15 DAT	11.33	77.39	88.72
15 DAT	11.29	60.63	71.93
dark control			
0 h	100.00	n.a.	100.00
0 h	100.00	n.a.	100.00
4 h	105.43	n.a.	105.43
4 h	105.58	n.a.	105.58
8 h	104.43	n.a.	104.43
8 h	104.72	n.a.	104.72
1 DAT	104.63	n.a.	104.63
1 DAT	105.82	n.a.	105.82
2 DAT	103.72	n.a.	103.72
2 DAT	103.22	n.a.	103.22
3 DAT	104.63	n.a.	104.63
3 DAT	104.45	n.a.	104.45
6 DAT	103.81	n.a.	103.81
6 DAT	103.91	n.a.	103.91
9 DAT	107.68	n.a.	107.68
9 DAT	106.13	n.a.	106.13
13 DAT	107.79	n.a.	107.79
13 DAT	100.71	n.a.	100.71
15 DAT	107.00	n.a.	107.00
15 DAT	106.57	n.a.	106.57

n.a. = not applicable
h= hour; DAT = days after treatment

B. TRANSFORMATION OF PARENT COMPOUND

The [^{14}C]-pyridine- labelled imazamox degraded very rapidly to a large number of products under continuous irradiation. Its concentration in the pH 7 buffer solution accounted for < 2% TAR at 3 DAT. The number of the degradation products increased with time. Most of the degradation products were unknown products composed of several peaks, being none of the individual components > 5% TAR, except only one peak for the 13 DAT sample Rep 2 (~5.71% TAR). The other degradation products in the irradiated samples were identified as metabolites M5, M7, M8, M14, M16, M17 and M18. The degradation products M7 and M18 reached a maximum of 4.23% and 6.55% TAR, respectively, at a single sampling interval and then declined to <5% TAR at the end of the study (15 DAT). The major degradation products M8, M14 and M17 declined over time, accounting for < 5% TAR at 15 DAT. The other major degradation products M5 and M16 remained above 11% TAR during the course of the study duration, but were composed of multiple HPLC peaks and none accounted for > 5 %TAR at 15 DAT.

The [^{14}C]-imidazolinone labelled imazamox also degraded very rapidly to a large number of products under continuous irradiation in the pH 7 buffer solution. Most of the degradation products were a mixture of carbonates and carbamic acid salts, which were captured and identified as $^{14}CO_2$. The concentration of the parent compound (imazamox) accounted for < 2% TAR after only 1 day. The number of the degradation products increased with time. The degradation products M12, M13 and M15 in the irradiated samples, were minor and accounted for < 2% TAR at the final sampling interval (15 DAT). Other minor unknown degradation products were also detected, being none of the individual component > 1.41% TAR.

The analysis of the dark control samples demonstrated that there was no significant breakdown of imazamox in the absence of light at pH 7 and 22°C.

The chiral HPLC analysis showed that the ratio of isomers of the parent compound (imazamox) did not change during photolysis.

The major pathways of imazamox degradation in water under continuous irradiation consisted in an opening of the imidazolinone ring followed by further hydrolytic oxidation, which leads to a number of products, and an oxidation of methoxymethyl group on the pyridine ring, leading ultimately to the formation of CO_2 and NH_3.

Calculation of DT_{50} and DT_{90} values

The calculation of the aqueous photolysis endpoints (DT_{50}, DT_{75}, and DT_{90} values) for imazamox was performed by non-linear regression using the software package KinGUI version 2.2012.202.925, following the recommendations of the FOCUS Kinetics workgroup for deriving trigger endpoints. The decline of the observed concentration of imazamox (% TAR) from the different experiments was described by single first-order (SFO) kinetics. The calculated $DT_{50,}$ DT_{75}, and DT_{90} values for imazamox under continuous irradiation were 0.21, 0.43 and 0.71 days, respectively. Imazamox was stable in the dark control test systems.

III. CONCLUSION

Imazamox rapidly degraded to a very large number of products under the irradiated conditions. None of the degradation products exceeded 5% TAR at the end of the study (15 DAT).

CA 7.2.1.3 Indirect photochemical degradation

No additional data were generated or required.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

Report:	CA 7.2.2.1/1 Schwarz H., 2012a BAS 720 H (Imazamox) - Determination of the ready biodegradability in the CO2-evolution test 2012/1254659
Guidelines:	OECD 301 B, ISO 9439, EPA 835.3110, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part C.4
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The ready aerobic biodegradability of BAS 720 H - imazamox was investigated in water containing mineral salts and a microbial inoculum (activated sludge from a municipal sewage plant). Imazamox was added to the test medium and the inoculum to achieve a concentration of 20 mg TOC/L corresponding to approximately 34 mg imazamox /L.

Duplicate control systems containing the microbial inoculum without test or reference substance were used to determine the endogenous microbial CO_2 evolution. Duplicate test systems dosed with the test substance at a nominal concentration of 20 mg TOC/L were used to monitor biodegradation of the test substance. A reference substance system containing readily biodegradable aniline at a nominal concentration of 20 mg TOC/L was also tested to verify the viability of the microbial inoculum. All systems were sampled on days 0, 1, 3, 7, 10, 14, 17, 21, 24, 27, 28 and 29. The average CO_2 evolved from the control systems was subtracted from the CO_2 evolved in the test and reference substance systems.

The test substance systems yielded mean theoretical carbon dioxide ($ThCO_2$) values of <10 % CO_2/$ThCO_2$ at the end of exposure period of 28 days. Therefore, imazamox was not biodegradable under the conditions of the test. Biodegradation in the reference substance system reached 79 % CO_2/$ThCO_2$at the end of the study, verifying that the microbial inoculum was viable and active after 28 days.

From the results obtained, it was concluded that imazamox is classified as not readily biodegradable under the conditions of this test.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Imazamox BAS 720 H
CAS number:	114311-32-9
Molecular weight:	305.3 g/mol
Chemical purity:	98.9 (tolerance ± 1.0%)
2. Reference Material	Aniline
CAS number:	62-53-3
Molecular weight:	93 g/mol

3. Microbial inoculum

Municipal activated sludge from the wastewater treatment plant of Mannheim/Baden-Württemberg (Germany) was used as biological test system. The inoculum was collected on 25th June 2012 from the aeration tank of the plant and aerated in the laboratory until use. A suitable aliquot of the activated sludge suspension was sieved by a finely woven mesh with a mesh size of about 1 mm. After settling, the supernatant was discarded and the sludge suspension was filled up with drinking water. The sludge of the suspension was adjusted to a concentration of 6.0 g/L dry weight and then added to the test vessels to obtain a sludge concentration of 30 mg/L dry substance. The activated sludge suspension in the test vessels was pre-aerated for about 48 hours at a temperature of 22 ± 2°C.

4. Mineral medium

An aqueous mineral salt medium provided essential mineral nutrients and trace elements necessary to sustain the inoculum throughout the test period. The used mineral medium complied with the test guideline OECD 301B.

The final pH of the mineral salt medium was 7.4 ± 0.2. The test medium was aerated with CO_2-free air.

B. STUDY DESIGN

1. Experimental conditions

Each test system consisted of a 2 L incubation bottles containing 1.5 L of the test solution, which contained mineral medium, microbial inoculum, water and the appropriate test or reference substance. The bottles were connected to two serial scrubbing bottles (total volume 250 mL) filled with 100 mL 0.05M sodium hydroxide solution for the adsorption of carbon dioxide from biodegradation processes. Usually twice a week the total inorganic carbon (TIC) values of the adsorption solutions were determined and used for the calculation of the produced carbon dioxide. The incubation bottles were stirred on magnetic stirrers; the aeration was performed with carbon dioxide free air at a flow of approximately 800 mL per hour.

For preparation of the test assays, the required volumes of deionized water and the solutions of mineral salts were dosed to all test vessels and the pH adjusted to 7.4 Test and reference (aniline) substances were added at a concentration equal to 20 mg/L total organic carbon (TOC) to the test vessels of the test substance and test reference assays. Subsequently, these test assays were stirred for approximately 30 minutes to ensure the dissolving of the test substance in the mineral medium.. An additional assay for inhibition control test contained the test substance and the reference substance in the same concentration (20 mg/L TOC each). The test vessels were connected with an aeration unit providing carbon dioxide free air.

At the end of exposure, the pH values were measured in each test vessel. For stripping of carbon dioxide, dissolved in the test medium, each test vessel was acidified by adding 2 mL of concentrated hydrochloric acid. The concentration of dissolved organic carbon (DOC) in the blank controls and reference substance assays were determined. Since the test substance was sufficiently soluble in water, DOC measurements could be also performed from the inhibition control and test substance assays.

2. Sampling

All systems were sampled on days 0, 1, 3, 7, 10, 14, 17, 21, 24, 27, 28 and 29.

3. Analytical procedure

The TIC- and DOC-analyses were performed as repeat determination using a TOC-analyser working with a combustion/non-disperse infrared gas analysis method. For calibration of the TOC-analyser, standard samples were measured before start of measurements to prove the conformity with the calibration curve.

The samples for TIC-analysis (absorption solution) were measured without further treatment.

The samples for DOC analysis were centrifuged for about 15 minutes at 4000 rpm. The samples were analysed on the day of sampling.

The DOC was calculated using the following equation:

TC - IC = TOC

TC = total carbon
IC = inorganic carbon
TOC = total organic carbon (since samples were centrifuged TOC = DOC)

II. RESULTS AND DISCUSSION

The test substance systems yielded theoretical carbon dioxide ($ThCO_2$) values of < 10% $CO_2/ThCO_2$ at the end of the exposure (mean value from two single test assays). Therefore, imazamox was not biodegradable under the conditions of the test. The selected test concentration was tested in an additional inhibition control test assay and no toxic effects on the microorganisms were observed. Biodegradation of the reference substance system reached 79% $CO_2/ThCO_2$ at the end of the study, verifying that the microbial inoculum was viable and active after 28 days.

III. CONCLUSION

Based on the quantitative determination of the formed carbon dioxide in the test substance assays in comparison to the calculated maximal theoretical carbon dioxide production imazamox was not readily biodegradable in this carbon dioxide evolution test.

CA 7.2.2.2 Aerobic mineralisation in surface water

Report:	CA 7.2.2.2/1 Ebert D. et al., 2013a 14C-BAS 720 H (Imazamox): Aerobic mineralization in surface water 2013/1125941
Guidelines:	OECD 309 (April 2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The purpose of this study was to determine the mineralisation and degradation rate of the herbicidal active substance imazamox (BAS 720 H) in an aquatic system under dark conditions. The pelagic test system was chosen for this study. Additionally, it was investigated if a potential shift between the two enantiomers did occur under the applied test conditions.

The test was performed with two different imazamox concentrations (10 µg/L and 50 µg/L). Two differently ^{14}C labelled test items were applied: [^{14}C]-imidazolinone- and [^{14}C]-pyridine-labelled imazamox. The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 1°C in the dark. Samples for the experiment were taken at 0, 3, 7, 14, 29, 42 and 63 days after treatment (DAT).

The amount and nature of radioactivity in the water samples was determined by LSC and radio-HPLC. The enantiomer ratio was determined by using a chiral HPLC method. Volatiles were trapped in appropriate trapping solutions and also analysed by LSC. Parent substance and metabolite identification (M720H002 (CL 312622) and M720H003 (CL 336554)) was done by comparison of retention times with the corresponding reference items and/or by HPLC MS/MS.

The obtained results showed that imazamox degraded only very slowly in a pure water environment as provided in the pelagic test. More than 95% TAR was still detectable as unchanged parent in the water samples after 63 DAT under dark conditions. The results were very comparable to the sterile test vessels. The behaviour of imazamox under the pelagic test conditions was characterised by very slow hydrolysis and formation of trace amounts of cleavage products, of which M720H002 (CL 312622) and M720H003 (CL 336554) in sum accounted for a maximum of 6.8% TAR. All other peaks never exceeded 2.6% TAR. The ratio of the two imazamox enantiomers was about 1:1 in all analysed samples indicating that no enantiomer shift took place during incubation.

Overall, the degradation of imazamox was characterized by a low mineralization rate irrespective of test concentration or label position. The amount of $^{14}CO_2$ never exceeded 1.0% TAR within 63 days.

No kinetic evaluation of imazamox degradation rates was performed since no significant degradation was observed in the pelagic test.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Imazamox (BAS 720 H)
Reg.No.: 4096483
Chemical name: (RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Molar mass: 305.33 g/mol (non-labelled)
Molecular formula: $C_{15}H_{19}N_3O_4$

Labelled test item
Label: imidazolinone-5-^{14}C; 3-^{15}N
Specific radioactivity: 7.22 MBq/mg
Radiochemical purity: 98.3 %

Label: pyridine-3-^{14}C, imidazolone-3-^{15}N
Specific radioactivity: 6.65 MBq/g
Radiochemical purity: 98.6 %

2. Test system

Water was collected on September 7th, 2012 from Berghäuser Altrhein, a pond-like side arm of the river Rhine south of Speyer (Rhineland-Palatinate, Germany).

The water was filtered through a 0.2 mm mesh net directly at the field sampling site. Filling of water into the test vessels was done at the day of collection except for the 0 day samples, which were prepared, applied and sampled 6 days later on September 13th, 2012. The water was stored at 4°C under dark conditions.

Various hydrological characteristics of the test system of which pH, O_2 content, redox potential and temperature of the water as well as redox potential of the sediment were determined directly at the site of sampling. The characterization of the water is summarised in Table 7.2.2.2-1.

Table 7.2.2.2-1: River water and sediment characteristics

Name:	Berghäuser Altrhein
Origin:	Rhineland-Palatinate, Germany
Sampling date:	September 7th, 2012
Parameters measured at sampling date	
Temperature [°C]	16.4
pH (water)	7.10
Oxygen concentration [mg/L]	2.8
Redox potential water [mV]	282
Redox potential sediment [mV]	-160
Parameters measured at 0 day sample	September 13th, 2012
Temperature [°C]	17.1
pH (water)	7.53
Oxygen concentration [mg/L]	10.7
Redox potential water [mV]	238
Redox potential sediment [mV]	n.p.
Water parameters	
TOC (total organic carbon) [mg/L]	3.8
DOC (dissolved organic carbon) [mg/L]	< 5
Hardness [mmol/L]	1.60
Carbonate hardness [mmol/L]	1.51
N total [mg/L]	0.75
P total [mg/L]	0.09
Microbial plate counts [microbial counts/mL]	
Bacteria	$1.58x10^4$* $1.16x10^3$**
Fungi	116* 74.0**
Actinomycetes	70.0* 4.0**

*water sample 3 days after field sampling.
**untreated water sample at the end of incubation period.

B. STUDY DESIGN

1. Experimental conditions

A total number of 54 test vessels were prepared for incubations. The flasks were filled with about 400 mL of water. **Table 7.2.2.2-2** illustrates the experimental setup. Four test vessels (two for each imazamox label, later applied with the high test concentration) were sterilised in an autoclave (30 min, 121°C).

Table 7.2.2.2-2: Experimental setup

System	Test conc.[µg/L]	Number of test vessels
Test vessels		
imidazolinone-^{14}C	50	13
	10	11
pyridine-^{14}C	50	13
	10	11
controls		
benzoic acid	10	4
Untreated*	-	2

*For water characterization at the end of incubation

The test systems were placed on multiplate magnetic stirrers and incubated at 20 ± 1°C in a metabolism chamber providing the test vessels with a continuous flow of fresh air. A glass rod with an encapsulated small magnetic stick hanging from the test vessel screw cap slightly agitated the upper 1-2 cm water layer to keep the oxygen saturation on sufficient high level.

After application of the test item, each test vessel was connected to the air stream leading to a trapping system equipped with a total of two gas washing bottles, the first flask containing ethylene glycol (25 mL) and the second flask with about 45 mL 1 M NaOH (amended with a coloured pH indicator). The sterilized test vessels were kept closed and not connected to the trapping system, and were only opened for a short period to be treated with test item under sterile conditions.

For the ^{14}C-benzoic acid treated vessels, a third volatile trap of 0.5 M NaOH (45 mL) was connected to the air stream after the sampling at 15 days. After each sampling, the volatile traps were replaced by new traps containing fresh solutions.

Untreated control samples were used for system characterisation and for microbial plate counts at the end of the experiment.

Application
The nominal application rates of the test item were 50 µg/L and 10 µg/L for the high and the low application rate, respectively. This was achieved by pipetting 200 µL of the corresponding application solutions into the upper water layer of the test vessels. The nominal application rate for viability control (^{14}C-benzoic acid treated vessels) was 10 µg/L. The rate was attained by treating four test vessels with 200 µL of the ^{14}C-benzoic acid application solution.

2. Sampling

The test systems were sampled at 0, 3, 7, 14, 29, 42 and 63 days after treatment (DAT). The sterile vessels (only high concentration test) were sampled after 29 and 64 days. The volatile traps collected at each sampling time were disconnected from the air stream and stored at room temperature until analysis.

The untreated control vessels for system characterization were sampled after 64 days. The parameters temperature, O_2 content, pH and redox potential of the water were recorded. The water from both vessels was combined and the sample was prepared for microbial plate counts.

Considering the^{14}C-benzoic acid treated test vessels, the volatile traps were sampled at 0, 3, 8, 15, 30, 43 and 64 DAT. For water sampling, the test vessels were disconnected from the air flow system and samples were taken after 15, 30, 43 and 64 days.

3. Description of analytical procedures

At each sampling date, the respective flasks were removed from the incubator. The temperature, O_2 content, pH and redox potential of the water were determined in all water samples types. The water volume was determined by weighing and then transferred into Nalgene®-bottles. Three aliquots of 2 mL were used for determination of the ^{14}C-concentration by LSC. Aliquot of 5 mL water was subjected to HPLC analysis without further workup.

For the high application rate, aliquots of the water samples were additionally concentrated by C18-SPE work-up for chiral HPLC and selected samples for LC-MS/MS. The column was preconditioned with 5 mL of methanol and 5 mL of 0.5% formic acid. An amount of 50 mL of water (with 250 µL of formic acid) was applied to the preconditioned column. The eluate was collected and measured for radioactivity. Then 1 x 1 and 2 x 2 mL of methanol were applied to the column. The methanol eluate was collected, adjusted to volume, measured by LSC and subjected to chiral HPLC. Prior to HPLC-MS analysis the methanol eluates were concentrated, re-dissolved in acetonitrile/water (1/1, v/v), and cleaned by a centrifugation step.

Samples from ^{14}C-benzoic acid treated test vessels, aliquots (3 x 2 mL) were taken per test vessel for LSC measurements and one aliquot of 5 mL for HPLC analysis. Then the vessels were reconnected to the air flow system.

Trapping solutions were transferred into 50 mL volumetric flasks and filled up to volume with distilled water (ethylene glycol traps) or 1 N NaOH (NaOH traps) and aliquots (2 x 1 mL) were measured by LSC.

4. Calculation of the degradation/dissipation rates

No significant degradation of imazamox in the test vessels was observed in the pelagic test. Therefore no kinetic evaluation of the recorded data was performed.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

The material balance for the pelagic test system ranged from 98.0% to 103.4% of total applied radioactivity (TAR). In the sterile vessels, the material balance ranged from 101.1% to 102.0% TAR. The material balance and the distribution of radioactivity in the pelagic test for high and low concentrations are shown in Table 7.2.2.2-3 and Table 7.2.2.2-4, respectively.

Table 7.2.2.2-3: Material balance and radioactivity distribution after application of ^{14}C-imazamox to pelagic test (high dose). Values in percent of the total applied radioactivity [%TAR]

DAT	Imadazolinone label (high concentration)				Pyridine label (high concentration)			
	Water	Volatiles ethylene glycol traps	Volatiles $^{14}CO_2$	Material balance	Water	Volatiles Ethylene glycol traps	volatiles $^{14}CO_2$	Material balance
0	98.2	n.p.	n.p.	98.2	98.2	n.p.	n.p.	98.2
3	99.6	0.0	0.0	99.6	100.7	0.0	0.0	100.7
7	98.4	0.0	0.0	98.4	99.7	0.0	0.0	99.8
14	98.5	0.0	0.0	98.5	100.1	0.0	0.0	100.1
29	100.8	0.0	0.0	100.8	101.8	0.1	0.0	101.9
42	101.0	0.1	0.0	101.1	100.9	0.0	0.0	100.9
63	101.6	0.0	0.4	102.0	102.3	0.1	1.0	103.4
29 (sterile)	101.1	n.p.	n.p.	101.1	101.5	n.p.	n.p.	101.5
64 (sterile)	101.9	n.p.	n.p.	101.9	102.0	n.p.	n.p.	102.0

n.p. not performed

Table 7.2.2.2-4: Material balance and radioactivity distribution after application of ^{14}C-imazamox to pelagic test (low dose). Values in percent of the total applied radioactivity [%TAR]

DAT	Imadazolinone label (low concentration)				Pyridine label (low concentration)			
	Water	Volatiles ethylene glycol	Volatiles NaOH	Material balance	Water	Volatiles Ethylene glycol	NaOH volatiles	Material balance
0	99.0	n.p.	n.p.	99.0	98.0	n.p.	n.p.	98.0
3	100.4	0.0	0.0	100.5	99.8	0.0	0.0	99.8
7	100.6	0.0	0.0	100.6	100.2	0.1	0.0	100.3
14	99.8	0.0	0.0	99.8	99.1	0.0	0.0	99.1
29	101.7	0.0	0.0	101.7	100.6	0.3	0.0	100.9
42	102.7	0.0	0.0	102.7	100.3	0.6	0.0	100.9
63	103.3	0.0	0.0	103.3	102.6	0.3	0.0	102.9

n.p. not performed

No significant differences were found in imazamox behaviour between the high and the low concentration of the test substance applied to the water system. The amount of radioactivity in the water was found to be very stable. At the end of the study (63 DAT) it ranged from 101.6% to 103.3% TAR. For all samples and sampling time points the radioactivity in the volatile traps never exceeded 1.0% TAR indicating a low rate of mineralization.

The control vessels treated with ^{14}C-benzoic acid showed that the microbiota was active in the system Berghäuser Altrhein. After 64 days, the total $^{14}CO_2$ was 71.5 - 83.6% TAR. The material balance could be established for sampling times where water samples were radio assayed (after 15, 30, 43 and 64 days). The material balance ranged from 82.8 to and 89.5% TAR. It was assumed that the missing radioactivity probably consisted of dissolved $^{14}CO_2$ in the water which escaped during sampling and LSC measurement.

B. FINDINGS

Physicochemical parameters of the test systems

During the incubation with imazamox, the O_2 saturation in the water of system was always ≥ 95%.The redox potential in the water ranged from +210 to + 270 mV proving overall aerobic conditions. The pH in the water of the viable vessels was slightly basic with most values between 8.4 and 8.6. The lowest pH was measured at 8.1.

Considering the benzoic acid control system, at the time points of water sampling the O_2 saturation in the water was in all cases > 95%. The redox potential ranged from +204 mV to +279 mV. The pH values were measured in a range of 8.5 to 8.6.

Characterisation and identification of residues in water and sterile extracts

No significant degradation of imazamox in the pelagic test was observed. At 63 DAT, between 95.5% and 98.0% TAR was still be recovered as unchanged parent for the different concentrations and radiolabels. The detected amounts of the active substance and its metabolites in the water samples are presented in Table 7.2.2.2-5and Table 7.2.2.2-6.

The metabolites M720H002 (CL 312622) and M720H003 (CL 336554) were detected together in one peak in the HPLC system. The sum of the two components accounted for a maximum of 6.8% TAR. Separation of the two peaks during HPLC-MS/MS analysis indicated that neither peak exceeded 5% in any of the water samples. The low amount of formed metabolites and the low formation of any other degradation products (below or equal to 2.6% TAR) indicate that only negligible microbial degradation took place.

There was no difference in imazamox concentration between the sterilised incubations and the viable vessels.

Table 7.2.2.2-5: Metabolite overview for the water phase after application of ^{14}C-imazamox to pelagic test system (high dose). Values in percent of the total applied radioactivity [%TAR]

DAT	Imadazolinone label-high concentration				
	M720H002 CL312622)/ M720H003 (CL 336554)	**Unknown**	**Imazamox**	**Unknown**	**^{14}C total**
	t_{Ret} = 16.6	t_{Ret} = 18.3	t_{Ret} = 19.6	t_{Ret} = 23.9	
0			95.9	2.3	98.2
3	1.5	0.4	96.6	1.0	99.6
7	3.4		95.0		98.4
14	3.3		95.2		98.5
29	4.1		96.7		100.8
42	5.6		95.4		101.0
63	6.1		95.5		101.6
29 (sterile)	3.5		97.6		101.1
64 (sterile)	4.9		97.0		101.9
DAT	**Pyridine label-high concentration**				
0			97.5	0.7	98.2
3	0.8		99.8		100.7
7	1.1		98.6		99.7
14	2.1		98.0		100.1
29	2.7		99.2		101.8
42	3.0		97.9		100.9
63	4.3		98.0		102.3
29 (sterile)	1.9		99.6		101.5
64 (sterile)	2.4		99.6		102.0

t_{Ret} = retention time in minutes

Table 7.2.2.2-6: Metabolite overview for the water phase after application of 14C-imazamox to pelagic test system (low dose). Values in percent of the total applied radioactivity [%TAR]

DAT	Imadazolinone label-low concentration				
	M720H002 (CL 312622) and M720H003 (CL 336554)	Unknown	Imazamox	Unknown	^{14}C total
	t_{Ret} = 16.6	t_{Ret} = 18.3	t_{Ret} = 19.6	t_{Ret} = 23.9	
0			96.3	2.6	99.0
3	1.4		98.1	0.9	100.4
7	2.7		97.9		100.6
14	3.0		96.9		99.8
29	4.9		96.8		101.7
42	6.6		96.1		102.7
63	6.8		96.4		103.3
DAT	**Pyridine label-low concentration**				
0			97.1	0.9	98.0
3	0.8		99.0		99.8
7	1.1		99.1		100.2
14	1.7		97.4		99.1
29	2.1		98.5		100.6
42	3.1		97.2		100.3
63	5.4		97.3		102.6

t_{Ret} = retention time in minutes

Enantiomer-specific analyses

For all analysed samples, the enantiomer ratio was approximately 1:1 for both radiolabels at the high concentration level.

Degradation of the reference test items

No kinetic evaluation of imazamox degradation rates was performed since no significant degradation was observed under the applied test conditions.

III. CONCLUSION

It can be concluded that imazamox is not significantly degraded in a pure water environment as provided in the pelagic test. After 63 days more than 95% TAR was recovered as the unchanged active substance. Some trace amounts of the known metabolites M720H002 (CL 312622) and M720H003 (CL 336554) were detected and identified by retention time comparison and/or HPLC-MS analysis and accounted together for 6.8% TAR or less after 63 days. Other degradation products were detected only in minor amounts (≤ 2.6% TAR). Radioactivity in the volatile traps never exceeded 1.0% TAR indicating a low rate of mineralization. No differences in the imazamox behaviour were observed regarding radiolabel or concentration level.

The enantiomer ratio of imazamox was about 1:1 in all analysed samples.

No kinetic evaluation of imazamox degradation rates was performed since no significant degradation was observed under the applied test conditions.

CA 7.2.2.3 Water/sediment studies

Report: CA 7.2.2.3/1
Wu S-S. et al., 2013a
Aerobic aquatic metabolism of 14C-BAS 720 H
2013/7001809
Guidelines: OECD 308, EPA 835.4300
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The aquatic metabolism of imazamox -BAS 720 H- was investigated in two different aerobic water/sediment systems under dark conditions. The systems were treated with [^{14}C]-imidazolinone- and [^{14}C]-pyridine- labelled imazamox, which were applied at a rate of 50 µg/L. This application rate was 10 times higher than normal application rate to permit determination of the disappearance of imazamox and identification of major metabolites.

Replicate samples were collected at 0, 7, 14, 30, 59/61, and 100 days after treatment (DAT). Water and sediment were worked up separately. Water samples and sediment extracts were analysed by liquid scintillation counting (LSC). The bound residues were further characterised by NaOH extraction and humic substance fractionation. Identification of parent and its transformation products was performed by retention matching with reference standards by HPLC and confirmation by LC/MS. Selective samples from both water and sediment were analysed by chiral HPLC to show no conversion between the two isomers of the parent compound and diacid metabolite CL 312622.

Total recovery for [^{14}C]-imidazolinone labelled imazamox averaged amounts of 99.76% and 97.75% of the total applied radioactivity (TAR) in Golden Lake and Goose River water/sediment systems, respectively. Mean total recoveries for [^{14}C]-pyridine- labelled imazamox were 101.21% and 96.93% TAR in the Golden Lake and Goose River water/sediment systems, respectively.

In samples treated with [^{14}C]-imidazolinone labelled imazamox, after 100 DAT, the non-extractable ^{14}C-residues averaged amounts of 2.95% and 8.44% TAR in the systems Golden Lake and Goose River, respectively. In samples treated with [^{14}C]-pyridine labelled imazamox, after 100 DAT, the non-extractable ^{14}C-residues reached a mean value of 4.19% and 14.60%TAR in the systems Golden Lake and Goose River, respectively.

Imazamox steadily degraded and mineralized in two different water/sediment test systems under aerobic conditions. One major (> 5% TAR) metabolite and several minor metabolites (< 5% TAR) were found in the water/sediment systems tested. The diacid metabolite CL 312622 was detected at a maximum value of 10.4% and 6.4% TAR in the Golden Lake water/sediment system treated with [^{14}C]-imidazolinone and [^{14}C]-Pyridine labelled imazamox, respectively. In the Goose River water/sediment system treated with [^{14}C]-imidazolinone labelled imazamox, CL 312622 was detected at maximum levels of 6.3% TAR.

However, the levels detected for the metabolite CL 321622 were below than 5% after application of [^{14}C]-pyridine labelled imazamox to the Goose River test system.

Kinetic analysis and calculations of DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS Kinetics workgroup to derive aquatic trigger and modelling endpoints. The analysis was done by a non-linear regression method using the software package KinGUI. The selected best-fit models and the corresponding results of the DT_{50}/DT_{90} calculations for imazamox (trigger endpoints) are listed in the table below.

Table 7.2.2.3-1: Selected best-fit models and endpoints for imazamox

System	Kinetic Model	χ^2 error	DT_{50} [d]	DT_{90} [d]
Whole system				
Golden Lake	DFOP	0.87	480	1000
Goose River	HS	1.01	213	870
Water				
Golden Lake	DFOP	1.88	288	1000
Goose River	DFOP	1.52	43.4	358

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 720 H - Imazamox
Chemical name: (RS)-2-(4 -isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Molecular mass: 305.3 g/mol (non-labelled)
Molecular formula: $C_{15}H_{19}N_3O_4$

Label: imidazolinon-5-^{14}C, 3-^{15}N
Specific radioactivity: (433,200 dpm/µg)
Radiochemical purity: 98.3%

Label: Pyridine-3-^{14}C, imidazolinone-3-^{15}N
Specific radioactivity: (399,000 dpm/µg)
Radiochemical purity: 98.6%

2. Test systems

The water/sediment systems were collected from the Golden Lake (coarse sediment) and the Goose River (fine sediment) located in Eastern North Dakota, USA. The Golden Lake sediment was classified as sand and the Goose River sediment was classified as clay loam. Characterization of water and sediment is presented in Table 7.2.2.3-2.

The sediments were screened through a 2-mm sieve and shipped overnight to the laboratory with headspace. Samples were stored in a refrigerator at about 4 °C between 7 – 11 days before used for pre-incubation. The water was filtered through a coarse “Whatman filter paper” before filling in the test system bottles. Sediment microbial biomass - was determined by using the fumigation and extraction method.

Table 7.2.2.3-2: Characterisation of the water/sediment systems

Designation **Origin**		**Golden Lake** **North Dakota, USA**	**Goose River** **North Dakota, USA**
Water			
pH		8.5	8.4
hardness	[mg/L, $CaCO_3$ equivalent]	669	826
Total organic C (end of study)	[ppm]	11.2	9.9
Ca	[ppml]	99	144
Mg	[ppml]	101	111
Na	[ppml]	101	175
Hardness	[mg/L, $CaCO_3$ equivalent]	669	826
Conductivity	[mmhos/cm]	1.46	1.88
Sodium adsorption ratio	[SAR]	1.70	2.67
Total dissolved solids	[ppm]	1168	1564
Turbidity	[NTU]	4.80	16.3
bacteria	[cfu]	31	67
fungi	[cfu]	0	0
actinomycetes	[cfu]	5	11
Sediment			
textural class	(USDA)	Sand	Clay loam
sand	[%]	89	31
silt	[%]	6	34
clay	[%]	5	35
pH (H_2O)		8.3	8.4
Soluble salts	[mmhos/cm]	0.64	0.71
cation exchange capacity	[meq/100g]	8.7	21.4
Organic matter	[%]	1.05	5.6
Organic carbon	[%]	0.61	3.3
Total N	ppm	0.07	0.03
Olsen P	[ppm]	9	20
Biomass (beginning of study)	[µg/g dry mass]	48.4	141.8
Biomass (end of study)	[µg/g dry mass]	412.3	604.2

B. STUDY DESIGN

1. Experimental conditions

Approximately 80 g aliquots of the wet sediment were transferred to 250 mL-polypropylene centrifuge bottles (test systems), which were connected in series using a Tygon tubing system from the outlet of one bottle to the inlet of the next and finally to volatile traps. About 150 mL of the associated water was added to each bottle. Twelve test systems (2 series of 6 bottles, each to serve as duplicates) were prepared for each sediment/water system and for each [^{14}C]-label of imazamox. Four additional samples were dosed at the same rate to serve as backups.

During the incubation, a stream of air was gently bubbled through the water phase of each test system to maintain an aerobic state. The air existing from the series of samples passed through a series of 1 N NaOH solutions to trap any $^{14}CO_2$ evolved during samples incubation. The test systems were aerobically incubated in a chamber at 20 ± 2°C in the dark. Non treated test systems were prepared additionally and incubated exactly as the test samples. These systems were used for pH, redox potential and dissolved oxygen level measurements to determine the aerobic state and for microbial activity check at the last sampling interval.

Prior to dosing, the water/sediment systems for the [^{14}C]-imidazolinone and [^{14}C]-pyridine labels were pre-incubated at 20 ± 2°C in the dark under air atmosphere for 22 and 19 days, respectively.

Application
Application solutions of [^{14}C]-imidazolinone labelled and [^{14}C]-pyridine labelled imazamox were prepared in acetonitrile at concentrations of approximately 0.075 mg/mL. Aliquots of 100 µL of the respective application solution (7.5 µg of the test substance) were applied to the test system surface water using a pipette. Final nominal concentration of ^{14}C-imazamox was about 0.05 ppm (10 times the maximum application rate of 50 g a.i./ha), which permits measurement of the disappearance of parent compound and identification of major metabolites formed.

2. Sampling

Immediately after dosing, samples treated with the [^{14}C]-imidazolinone and [^{14}C]-pyridine labels were collected at the 0 DAT sampling interval. For [^{14}C]-imidazolinone label, duplicate samples were sampled from the chamber at 7, 14, 30, 59 and 100 days after treatment (DAT). Replicate samples treated with [^{14}C]-pyridine label were sampled from the chamber at 7, 14, 30, 61 and 100 DAT.

The NaOH solutions in the traps for the CO_2 evolved were replaced with fresh solutions at every sampling date. The pH and oxidation-reduction (redox) potential of the water and sediment as well as the dissolved oxygen (DO) of the water were measured at each sampling interval.

3. Description of analytical procedures

Samples were centrifuged (approx. 15 min at 4000 rpm) and the water layers decanted and analysed by liquid scintillation counting (LSC). If the water contained > 5% of the total applied radioactivity (TAR), it was concentrated by C-18 solid phase extraction (SPE) and analysed by high performance liquid chromatography (HPLC).

Sediment samples were extracted once with methanol (100 mL) and then twice with 100 mL of methanol: water (1:1) by shaking for 30 minutes at 300 strokes per minute. Each extract was assayed by LSC and then combined and concentrated before analysing by radio-HPLC. The solid portions were processed by air-drying and the non-extractable radioactivity (NER) was determined by oxidative combustion analysis.

All the sediment samples from each interval were further extracted with 0.5N NaOH solution (100 mL) and fractionated into fulvic and humic acids to characterize the bound residues. The fulvic acid was cleaned up by using C-18 SPE and subjected to HPLC analysis, if it contained >5% TAR. The remaining residues (humins) were combusted after bound residue analysis for material balance purposes.

Trapping solutions were analysed by LSC. Attempts were made to identify all ^{14}C-residues above of 5% TAR. Identification of parent and its transformation products was performed by retention matching with reference standards by HPLC and confirmation by LC/MS.

Representative water and sediment samples were analysed by chiral HPLC to show no conversion between the two isomers of the parent compound and the metabolite CL 312622.

4. Calculation of the degradation/dissipation rates

The kinetic analysis was carried out following the recommendations of the FOCUS workgroup on degradation kinetics in order to derive aerobic aquatic trigger endpoints. The software package KinGUII v. 2.2012.202.925 was used for parameter fitting using non-linear regression methods (Iteratively Reweighted Least Squares).

Kinetic evaluations were performed for imazamox at the P-I level (one-compartment model) considering the approach described in the FOCUS (2006) kinetics guidance. Both DT_{50} and DT_{90} endpoints are reported for the whole systems and water phases of both test systems. No endpoints were determined for the sediment phases, as imazamox sediment concentrations did not decrease by the end of the 100 day study duration.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The average total recovery for [^{14}C]-imidazolinone radiolabelled imazamox was 99.76% and 97.75% of the total applied radioactivity (TAR) in Golden Lake and Goose River water/sediment systems, respectively. The average total recovery for [^{14}C]-pyridine radiolabelled imazamox was 101.21% and 96.93% TAR in Golden Lake and Goose River water/sediment systems, respectively.

The distribution of radioactivity in the different compartments of the two water/sediment systems treated with [^{14}C]-imidazolinone labelled imazamox and [^{14}C]-pyridine labelled imazamox is presented in Table 7.2.2.3-3 and Table 7.2.2.3-4.

B. FINDINGS

Radioactivity in the water

Overall, the radioactivity in the water layer of Golden Lake and Goose River test systems decreased for both labels (Table 7.2.2.3-3 and Table 7.2.2.3-4). The ^{14}C-residues of the [^{14}C]-imidazolinone labelled imazamox in the water layer decreased from 94.89-97.75% TAR at 0 DAT to 69.43-72.78% TAR at 100 DAT in the Golden Lake test system and from 102.64-104.39% TAR at 0 DAT to 38.72-52.91% TAR at 100 DAT in the Goose River water/sediment system. In the water layer, the ^{14}C-residues of [^{14}C]-pyridine labelled imazamox decreased from 99.44-100.43% TAR at 0 DAT to 79.64-84.72% TAR at 100 DAT in the Golden Lake test system and from 101.09-102.96% TAR at 0 DAT to 40.48-46.18% TAR at 100 DAT in the Goose River water/sediment system, respectively.

Radioactivity in the sediment

Corresponding to the decline of radioactivity in the water phase, radioactivity in the sediment increased with the time in both systems. For the samples treated with [^{14}C]-imidazolinone labelled imazamox, the organic extractable ^{14}C-residues increased from 5.14-5.73% TAR at 0 DAT to 15.55-16.06% TAR at 100 DAT in the Golden Lake test system and from 1.25-2.56% TAR at 0 DAT to 18.26-22.89% TAR at 100 DAT in the Goose River test system. The organic extractable ^{14}C-residues of [^{14}C]-pyridine labelled imazamox increased from 3.52-3.90% TAR at 0 DAT to 11.32-13.02% TAR at 100 DAT in the Golden Lake test system, and from 0.85-0.95% TAR at 0 DAT to 18.12-19.15% TAR at 100 DAT in the Goose River test system.

Volatiles

At the end of the experiment, the amount of $^{14}CO_2$ evolved never exceeded 1% TAR within 100 DAT. For [^{14}C]-imidazolinone label, the maximum total evolved CO_2 was 0.44-0.45% and 0.49-0.55% TAR in Golden Lake and Goose River water/sediment systems, respectively. The maximum total amount of evolved CO_2 from the [^{14}C]-pyridine labelled test item was 0.29-0.33% and 0.32-0.37% TAR in Golden Lake and Goose River water/sediment systems, respectively.

Non-extractable radioactivity

Considering the [^{14}C]-imidazolinone labelled imazamox, the non-extractable ^{14}C-residues increased from 0.19-0.21% TAR at 0 DAT to 2.87-3.02% TAR at 100 DAT in the Golden Lake test system, and from 0.06-0.08% TAR at 0 DAT to 6.86- 10.01% TAR at 100 DAT in the Goose River test system (Table 7.2.2.3-3). In the case of [^{14}C]-pyridine labelled imazamox, the non-extractable ^{14}C-residues increased from 0.16-0.20% TAR at 0 DAT to 3.53-4.85% TAR at 100 DAT in the Golden Lake test system and from 0.09-0.11% TAR at 0 DAT to 12.70-16.49% TAR at 100 DAT in the Goose River test system (Table 7.2.2.3-4).

Table 7.2.2.3-3: Distribution of the radioactivity after application of [^{14}C]-imidazolinone imazamox to the Golden Lake and Goose River water/sediments systems [%TAR]

DAT	Water	sediment				NaOH (Trap)	Total recovery
		Total organic extracts	NaOH extract	Total extracts	NER (Humin)		
Golden Lake							
0	94.89	5.14	0.79	100.82	0.19	NA	101.01
0	97.75	5.73	0.87	104.35	0.21	NA	104.56
7	81.61	12.33	6.11	100.05	1.56	0.02	101.63
7	86.56	11.87	3.73	102.16	1.02	0.02	103.21
14	73.81	15.91	5.14	94.85	1.55	0.06	96.47
14	75.85	16.79	4.52	97.16	1.53	0.08	98.77
30	80.04	14.01	4.39	98.45	1.96	0.12	100.53
30	80.48	14.04	4.35	98.87	1.79	0.17	100.83
59	75.88	14.51	4.37	94.76	2.92	0.21	97.90
59	77.79	15.12	4.28	97.19	2.24	0.25	99.68
100	69.43	16.06	6.17	91.66	3.02	0.44	95.12
100	72.78	15.55	5.75	94.08	2.87	0.45	97.40
Goose River							
0	104.39	1.25	0.39	106.02	0.08	NA	106.10
0	102.64	2.56	0.59	105.79	0.06	NA	105.85
7	74.19	9.40	8.93	92.53	6.18	0.08	98.78
7	84.46	8.60	4.39	97.45	2.71	0.06	100.22
14	64.52	12.93	9.18	86.63	7.68	0.18	94.50
14	68.06	14.34	9.03	91.43	7.51	0.15	99.08
30	59.66	16.13	11.29	87.07	8.71	0.24	96.01
30	59.66	14.70	11.29	85.65	9.73	0.23	95.62
59	51.78	18.80	14.17	84.74	11.47	0.31	96.52
59	56.29	18.66	11.63	86.57	10.48	0.33	97.38
100	38.72	22.89	18.94	80.55	10.01	0.49	91.05
100	52.91	18.26	13.27	84.44	6.86	0.55	91.84

DAT = days after treatment
NER = non extractable residues
NA = not applicable (no sample to be analysed)

Table 7.2.2.3-4: Distribution of the radioactivity after application of [^{14}C]- pyridine imazamox to the Golden Lake and Goose River water/sediments systems [%TAR]

DAT	Water	sediment				NaOH (Trap)	Total recovery
		Total organic extracts	NaOH extract	Total extracts	NER (Humin)		
Golden Lake							
0	100.43	3.52	0.65	104.60	0.20	NA	104.80
0	99.44	3.9	0.69	104.02	0.16	NA	104.19
7	82.73	11.12	4.72	98.57	1.63	0.01	100.21
7	83.50	11.38	3.90	98.78	1.89	0.01	100.67
14	78.74	12.29	4.22	95.26	1.37	0.01	96.64
14	77.47	12.87	5.18	95.51	2.79	0.01	98.32
30	79.23	14.78	4.53	98.54	2.05	0.06	100.64
30	81.00	14.24	4.09	99.33	2.02	0.05	101.39
61	76.33	15.91	4.40	96.64	2.87	0.15	99.66
61	80.51	14.47	3.45	98.43	2.63	0.14	101.20
100	79.64	13.02	4.46	97.11	4.85	0.29	102.26
100	84.72	11.32	4.58	100.62	3.53	0.33	104.48
Goose River							
0	101.09	0.85	0.21	102.15	0.11	NA	102.26
0	102.96	0.95	0.23	104.14	0.09	NA	104.23
7	71.74	11.18	9.51	92.43	5.83	0.01	98.26
7	77.24	11.38	6.47	95.08	3.36	0.01	98.45
14	63.40	13.38	12.96	89.74	6.14	0.01	95.90
14	64.78	13.06	10.45	88.29	5.06	0.01	93.37
30	52.51	13.70	12.45	78.66	8.75	0.04	87.44
30	63.15	14.55	14.82	92.52	7.61	0.03	100.16
61	53.20	17.91	12.98	84.09	12.86	0.15	97.09
61	55.47	17.26	13.03	85.76	11.82	0.14	97.72
100	46.18	18.12	15.01	79.31	12.70	0.32	92.33
100	40.48	19.15	19.48	79.11	16.49	0.37	95.97

DAT = days after treatment
NER = non extractable residues
NA = not applicable (no sample to be analysed)

Results of bound residues characterisation for [^{14}C]-imidazolinone labelled imazamox for Golden Lake and Goose River water/sediment systems, and for [^{14}C]-pyridine labelled imazamox for both test systems are given in Table 7.2.2.3-5 and Table 7.2.2.3-6, respectively.

The characterization of [^{14}C]-imidazolinone labelled bound residues from the 0.5 N NaOH extractions showed that the activity in humic acids was only 0.04-0.11% TAR at 0 DAT, and it increased to a maximum level of 0.33-0.52% TAR at 100 DAT in the Golden Lake test system. In the Goose River water/sediment system the activity in humid acids accounted for 0.25-0.46% TAR at 0DAT and increased to 0.79-1.07% TAR at 100 DAT. Similarly, characterization of [^{14}C]-pyridine label bound residues showed only 0.8-0.14% TAR and 0.07-0.08% TAR in humic acid at 0 DAT, increasing to a maximum level of 0.43-0.59% TAR and 0.49-1.13% TAR at 100 DAT for Golden Lake and Goose River test systems, respectively.

Table 7.2.2.3-5: Distribution of the radioactivity in the bound residues from the NaOH extraction after the application of [^{14}C]-imidazolinone imazamox to the Golden Lake and Goose River water/sediment systems [%TAR]

DAT	NaOH extract	Fulvic acids	Humic acids	Humin
Golden Lake				
0	0.79	0.68	0.11	0.19
0	0.87	0.83	0.04	0.21
7	6.11	4.21	1.90	1.56
7	3.73	3.33	0.40	1.02
14	5.14	4.72	0.41	1.55
14	4.52	5.69	0.00	1.53
30	4.39	3.77	0.62	1.96
30	4.35	3.97	0.38	1.79
60	4.37	3.96	0.41	2.92
60	4.28	3.96	0.32	2.24
100	6.17	5.84	0.33	3.02
100	5.75	5.23	0.52	2.87
Goose River				
0	0.39	0.13	0.25	0.08
0	0.59	0.13	0.46	0.06
7	8.93	8.50	0.43	6.18
7	4.39	4.12	0.27	2.71
14	9.18	8.43	0.75	7.68
14	9.03	8.55	0.48	7.51
30	11.28	10.61	0.67	8.71
30	11.29	10.85	0.44	9.73
61	14.17	13.21	0.96	11.47
61	11.63	10.91	0.71	10.48
100	18.94	17.87	1.07	10.01
100	13.27	12.48	0.79	6.86

DAT = days after treatment
NER = non extractable residues
NA = not applicable (no sample to be analysed)

Table 7.2.2.3-6: Distribution of the radioactivity in the bound residues from the NaOH extraction after the application of [^{14}C]-pyridine imazamox to the Golden Lake and Goose River water/sediment systems [%TAR]

DAT	NaOH extract	Fulvic acids	Humic acids	Humin
Golden Lake				
0	0.65	0.57	0.08	0.20
0	0.69	0.55	0.14	0.16
7	4.72	4.41	0.31	1.63
7	3.90	4.15	0.00	1.89
14	4.22	3.99	0.24	1.37
14	5.18	4.90	0.28	2.79
30	4.53	4.28	0.25	2.05
30	4.09	4.04	0.05	2.02
61	4.40	3.86	0.53	2.87
61	3.45	3.26	0.19	2.63
100	4.46	4.03	0.43	4.85
100	4.58	3.99	0.59	3.53
Goose River				
0	0.21	0.14	0.07	0.11
0	0.23	0.15	0.08	0.09
7	9.51	8.97	0.54	5.83
7	6.47	6.29	0.18	3.36
14	12.96	12.27	0.69	6.14
14	10.45	9.82	0.63	5.06
30	12.45	11.74	0.71	8.75
30	14.82	14.20	0.62	7.61
61	12.98	12.52	0.46	12.86
61	13.03	12.56	0.48	11.82
100	15.01	14.06	0.94	12.70
100	19.48	18.35	1.13	16.49

DAT = days after treatment
NER = non extractable residues

Characterisation and identification of residues in water and sediment

For the Golden Lake and Goose River test systems, the concentration of [^{14}C]-imidazolinone labelled imazamox decreased from 94.5-95.6% TAR at 0 DAT to 76.5-87.1% TAR at 100 DAT, and from 98.0-98.9% TAR at 0 DAT to 65.9-72.2% TAR at 100 DAT, respectively. CL 312622 was the only major transformation product observed at a maximum value of 10.4% TAR and 6.3% TAR in both systems, respectively.

Considering [^{14}C]-pyridine labelled imazamox, its concentration decreased from 90.4-98.0% TAR at 0 DAT to 80.3-83.4% TAR at 100 DAT, and from 96.8-99.7% TAR at 0 DAT to 61.4-65.2% TAR at 100 DAT in the Golden Lake and Goose River test systems, respectively. Only CL 312622 was the major transformation product observed, with a maximum value of 6.4% TAR in the Golden Lake test system. In the Goose River test system, there was no major transformation products above 5% TAR.

For both test systems and labels, several minor transformation products were also observed, however all of them were below 5% TAR.

HPLC analysis of fulvic acid fractions from the NaOH extracts of [^{14}C]-imidazolinone labelled imazamox showed a maximum value for the parent of 5.1% TAR at 14 DAT, and a value of 13.2% TAR at 100 DAT for the Golden Lake and Goose River test systems, respectively. In both test systems no major metabolite above 5% TAR were detected. For the [^{14}C]-pyridine labelled imazamox, HPLC analysis of fulvic acid fractions showed a maximum value for the parent of 4.1% TAR at 14 DAT and 14.8% TAR at 100 DAT in the Golden Lake and Goose River test systems, respectively. No major metabolite above 5% TAR was detected in [^{14}C]-pyridine labelled imazamox treated samples.

Degradation rates

Kinetic analysis and calculations of DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS Kinetics workgroup to derive aquatic trigger and modelling endpoints. Table 7.2.2.3-7 and Table 7.2.2.3-8 present the data used for the kinetic evaluation. Data was analysed by non-linear regression methods (Iteratively Reweighted Last Squares) using KinGUII software (v. 2.2012.202.925).

Table 7.2.2.3-7: Goose River water/sediment system data used for kinetic evaluation [% TAR]

DAT	Label	Imazamox			CL 312622			CL 354825		
		System [%TAR]	Water [%TAR]	Sediment [%TAR]	System [%TAR]	Water [%TAR]	Sediment [%TAR]	System [%TAR]	Water [%TAR]	Sediment [%TAR]
0	Imi	101.43	101.43	*0*	*0*	*0*	0	0	0	0
0	Imi	101.06	101.06	*0*	*0*	*0*	0	0	0	0
0	Pyr	98.67	98.67	*0*	*0*	*0*	0	0	0	0
0	Pyr	102.05	102.05	*0*	*0*	*0*	0	0	0	0
7	Imi	86.14	69.55	16.59	4.54	4.09	0.45	--	--	--
7	Imi	87.92	79.56	8.36	5.13	4.90	0.23	0.03	--	0.025*
7	Pyr	82.14	67.96	14.18	2.68	2.45	0.24	1.00	--	1.00
7	Pyr	80.35	73.08	7.28	3.34	3.11	0.23	0.50	--	0.50
14	Imi	81.43	61.11	20.32	3.25	2.39	0.86	0.03	--	0.025*
14	Imi	85.12	63.57	21.55	3.94	3.13	0.81	0.31	--	0.31
14	Pyr	89.59	60.53	21.06	2.49	2.00	0.49	0.19	--	0.19
14	Pyr	82.51	61.72	20.79	2.90	2.60	0.30	0.05	--	0.05
30	Imi	80.18	54.21	25.97	5.22	4.44	0.77	0.08	0.025*	0.05
30	Imi	78.30	54.57	23.73	4.98	4.10	0.88	0.10	--	0.10
30	Pyr	70.23	48.32	21.91	2.78	2.44	0.33	0.32	0.025*	0.29
30	Pyr	82.24	58.27	23.97	3.48	3.09	0.39	0.23	0.025*	0.20
59	Imi	74.79	44.53	30.25	6.27	5.06	1.19	0.31	0.025*	0.28
59	Imi	75.82	48.46	27.36	6.00	4.86	1.13	0.17	--	0.17
61	Pyr	73.80	47.89	25.91	3.13	2.75	0.38	0.18	0.025*	0.15
61	Pyr	73.38	49.04	24.34	4.48	3.82	0.65	0.35	0.025*	0.32
100	Imi	65.94	30.96	34.98	5.07	3.97	1.10	3.16	0.69	2.47
100	Imi	72.17	44.92	27.25	5.47	4.53	0.94	2.05	--	2.05
100	Pyr	65.21	39.88	25.33	2.79	2.17	0.63	2.47	0.46	2.01
100	Pyr	61.37	32.50	28.87	3.29	2.35	0.93	3.05	0.93	2.12

DAT = days after application
Imi = [^{14}C]-imidazolinone radiolabel
Pyr = [^{14}C]-pyridine radiolabel
*Numbers set to ½ LOD according to FOCUS (2006)
Italic numbers set to zero, residue added to water phase according to FOCUS (2006)
-- = Not used in the kinetic fitting

Table 7.2.2.3-8: Golden Lake water/sediment system data used for kinetic evaluation [% TAR]

DAT	Label	Imazamox			CL 312622			CL 354825		
		System [%TAR]	Water [%TAR]	Sediment [%TAR]	System [%TAR]	Water [%TAR]	Sediment [%TAR]	System [%TAR]	Water [%TAR]	Sediment [%TAR]
0	Imi	97.07	97.07	*0*	*0*	*0*	0	0	0	0
0	Imi	98.05	98.05	*0*	*0*	*0*	0	0	0	0
0	Pyr	99.62	99.62	*0*	*0*	*0*	0	0	0	0
0	Pyr	92.10	92.10	*0*	*0*	*0*	0	0	0	0
7	Imi	92.56	76.91	15.66	4.46	3.74	0.72	--	--	--
7	Imi	93.07	81.78	11.29	3.65	3.23	0.42	--	--	--
7	Pyr	93.46	80.48	12.98	2.62	2.25	0.37	--	--	--
7	Pyr	88.91	80.00	8.90	2.57	2.36	0.21	0.03	--	0.025*
14	Imi	89.21	69.26	19.96	4.42	3.73	0.68	--	--	--
14	Imi	91.53	71.94	19.59	4.03	3.35	0.68	--	--	--
14	Pyr	85.91	75.12	10.79	2.82	2.80	0.025*	--	--	--
14	Pyr	86.85	74.38	12.47	2.80	2.43	0.37	0.20	--	0.20
30	Imi	86.57	73.64	12.93	4.79	4.33	0.46	--	--	--
30	Imi	88.28	75.12	13.16	5.03	4.43	0.59	--	--	--
30	Pyr	90.10	74.08	16.01	3.56	3.28	0.28	--	--	--
30	Pyr	88.96	75.49	13.47	3.72	3.54	0.18	0.03	--	0.025*
59	Imi	84.01	66.64	17.37	6.59	5.93	0.66	0.03	--	0.025*
59	Imi	87.14	69.24	17.90	6.92	6.05	0.87	0.03	--	0.025*
61	Pyr	82.47	68.93	13.54	4.91	4.21	0.70	0.03	--	0.025*
61	Pyr	85.02	72.77	12.24	5.10	4.52	0.58	0.03	--	0.025*
100	Imi	76.48	57.74	18.74	8.88	7.40	1.48	0.66	--	0.66
100	Imi	78.05	59.93	18.12	10.39	8.97	1.41	0.54	--	0.54
100	Pyr	80.30	69.12	11.18	6.45	5.77	0.68	0.53	--	0.53
100	Pyr	83.36	71.58	11.78	6.30	6.61	0.70	0.73	--	0.73

DAT = days after application
Imi = [^{14}C]-imidazolinone radiolabel
Pyr = [^{14}C]-pyridine radiolabel
*Numbers set to ½ LOD according to FOCUS (2006)
Italic numbers set to zero, residue added to water phase according to FOCUS (2006)
-- = Not used in the kinetic fitting

The selected best-fit models and the corresponding results of the DT_{50}/DT_{90} calculations for imazamox in the total system (water + sediment) and the water phase are listed in Table 7.2.2.3-9. Kinetic endpoints were not determined for imazamox in sediment since imazamox reached peak levels in sediment at or near the end of the incubation.

Table 7.2.2.3-9: Selected best-fit models and endpoints for imazamox

System	Kinetic Model	χ^2 error	DT_{50} [d]	DT_{90} [d]
Whole system				
Golden Lake	DFOP	0.87	480	1000
Goose River	HS	1.01	213	870
Water				
Golden Lake	DFOP	1.88	288	1000
Goose River	DFOP	1.52	43.4	358

Isomerization

To investigate the isomerization of imazamox and its metabolite CL 312622 in water/sediment under aerobic conditions, the methanol/water extracts and the water layer from both the [^{14}C]-imidazolinone and [^{14}C]-pyridine labels were subjected to a chiral separation. Results indicated the ratio of R and S isomers of imazamox and its metabolite CL 312622 (1:1) was not changed under aerobic conditions suggesting that there was no conversion from one isomer to another and, therefore no preferential isomeric degradation of imazamox and its metabolite CL 312622 in water or sediment.

III. CONCLUSION

The study demonstrated that imazamox steadily degraded and mineralized in two different water/sediment test systems under aerobic conditions. For the Golden lake test system, the DT_{50} values were 480 days and 288 days for the total system (water + sediment) and the water phase, respectively. Considering the Goose river test system, the DT_{50} values were 213 and 43.4 days for the total system (water + sediment) and the water phase, respectively. There was only one major metabolite observed with a concentration > 5% TAR. The metabolite CL 312622 was detected in the Golden lake water/sediment system with [^{14}C]-imidazolinone and [^{14}C]-pyridine labelled imazamox at a maximum value of 10.4 and 6.4% TAR, respectively. Mineralisation of imazamox to CO_2 was observed, but the maximum levels of CO_2 for both labelling in the tested water/sediment systems were < 1% TAR at 100 DAT.

A degradation pathway for imazamox in water/sediment systems under aerobic conditions was proposed. Imazamox was initially oxidized to CL 312622 (the diacid metabolite), which was further converted to CL 354825 (the hydroxyl acid metabolite). Finally, imazamox and its degradation products are mineralized to CO_2 by soil microorganisms.

Report: CA 7.2.2.3/2
Donaldson F.P., 2013d
Kinetic evaluation of the aerobic aquatic metabolism of BAS 720 H (imazamox)
2013/7002692

Guidelines: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp., SANCO/10058/2005 rev. 2 (FOCUS kinetics report)

GLP: no
(certified by <none>)

Executive Summary

The aim of the study was to perform a kinetic analysis in order to derive trigger and modelling endpoints for imazamox using data obtained from two studies on degradation of imazamox in aerobic aquatic systems. In a 1997 study, pyridine ring-^{14}C-labelled imazamox was applied to two aquatic systems (Mill stream pond and Iron hatch runoff), and in a study conducted in 2013, pyridine ring or imidazolinone ring-^{14}C-labelled imazamox was applied to another two aquatic systems (Goose River and Golden Lake). Imazamox was applied at a rate of approximately 150 g a.i. ha^{-1} to the four systems.

A kinetic analysis was performed following the recommendation of the FOCUS workgroup on degradation kinetics to derive trigger and modelling endpoints at the P-I level (one-compartment approach) and the P-II level (two-compartment approach: water and sediment). It was not possible to determine P-I sediment kinetic endpoints in any of the test systems since imazamox levels increased slowly throughout the experiment and reached peak levels at or near the end of the incubations.

For the total system at the P-I level, the maximum DT_{50} and DT_{90} values for use as trigger endpoints were 480 and 1000 days, respectively. For the water phase at the P-I level, the maximum DT_{50} and DT_{90} values were 288 and 1000 days, respectively. No statistically significant ($p < 0.05$) correlations were found between DT_{50} values and system properties such as aqueous pH, sediment organic carbon, redox potential, dissolved oxygen concentration, or clay content.

At the P-II analysis level it was not possible to produce robust endpoints for $DegT_{50}$ values in water and sediment using a first-order compartment model including water/sediment partitioning. Therefore, the recommendations of the FOCUS 2012 Surface Water Scenarios Work Group were followed. The resulting geometric mean $DegT_{50}$ values for use as modelling endpoints were 233 days for the water phase and 1000 days for the sediment phase at level P-II.

There were no statistically significant ($p < 0.05$) correlations between system properties such as aqueous pH, sediment organic carbon, redox potential, clay content, and dissolved oxygen concentration and total system or water phase DT_{50} (or $DegT_{50}$) values.

Level M-I analyses could not be performed for any of the systems, as none of the systems provided enough data points for a robust kinetic fit.

I. MATERIAL AND METHODS

A. MATERIALS

The kinetic evaluation of imazamox was conducted for two studies, each with two aquatic systems [*McCullough and Lewis (1997): "AC299263: Degradation and Metabolism in Water Sediment Systems"*. BASF DocID 1997/7000986, *Wu, S-S. (2013): "Aerobic Aquatic Metabolism of ^{14}C-BAS 720 H"*. BASF DocID 2013/7001809]. In the study conducted in 1997, pyridine labelled-imazamox (^{14}C-radiolabelled imazamox) was applied at a rate of approximately 150 g a.i. ha^{-1} to the Mill stream pond and Iron hatch runoff aquatic systems originating from the River Frome in Dorset, UK. In the study conducted in 2013, ^{14}C-radiolabelled imazamox (pyridine ring or imidazolinone ring labelled) was applied at a rate of approximately 150 g a.i. ha^{-1} to the Goose River and Golden Lake aquatic systems originating from the USA. Kinetic evaluation was performed in order to derive persistence and modelling endpoints using data from the 1997 study and to derive modelling endpoints using data from the 2013 study according to the recommendation of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp.*].

Kinetic models included in the assessment

Four kinetic models were applied to the data sets: single first-order (SFO), first-order multi-compartment (FOMC), double-first-order in parallel (DFOP), and hockey stick (HS). These models were tested sequentially depending on the desired endpoint (trigger or modelling). Descriptions of each kinetic model as well as the decision trees for model selection/endpoint determination can be found in FOCUS [*FOCUS (2006)*].

The best-fit kinetic model was determined through a combination of visual and statistical analyses. Appropriate kinetic models should visually describe the data well with residuals randomly scattered about the zero line, have low χ^2 error values (preferably < 15%), and have parameters that differ significantly from zero. Parameters are considered significantly different from zero if their p-value is < 0.1 (90% confidence) for water-sediment studies [*FOCUS (2006)*].

Kinetic modelling strategy

Kinetic evaluations were carried out for imazamox according to guidance on water-sediment studies found in FOCUS [*FOCUS (2006)*]. Two levels were evaluated to derive both trigger and modelling endpoints. Level I considers the water-sediment system as individual compartments and evaluates the total system (water+sediment), water phase, and sediment phase kinetics individually. Level II considers the entire water-sediment system and models not only the degradation in each compartment but the mass transfer between compartments. The two levels are applied to parent (P-I, P-II) while only level I is applied to metabolites (M-I, no M-II approach currently exists).

At level P-II, analyses require the use of SFO kinetics for both compartments [*FOCUS (2006)*]. The model representation of the physical system was checked for validity by way of the Fsed test described in FOCUS [*FOCUS (2006)*].

Level M-I analyses require fitting the metabolite data from the peak reported concentration onward. None of the systems provided enough data points for a robust kinetic fit. Therefore, level M-I analyses were not evaluated for any of these systems.

Data handling

As the test compound was applied directly to the water phase, it was assumed that all residue at time zero would be restricted to this compartment. Therefore, any residue reported in the sediment phase at time zero was added to the water phase and subsequently fixed to zero.

In the case of study 2013, replicate samples were taken for each time point in the study. These replicates were used individually, without averaging, for the kinetic analysis. The experiment was conducted for both radiolabelled imazamox compounds (^{14}C-imidazolinone, ^{14}C-pyridine). Results from each experiment were combined and analysed as one data set (a total of four replicates per sampling time).

Values below the quantification or detection limit for parent compound and metabolites were treated as recommended by the FOCUS workgroup [*FOCUS (2006)*]. Since no information was provided regarding LOQ or LOD, the lowest reported value between imazamox, CL 312622, and CL 354825 was taken as the LOD (0.36% and 0.05% for the 1997 study and 2013 study, respectively).

Software for kinetic evaluation

The software package KinGUII (v. 2.2012.202.925) was utilized to determine the best-fit kinetic model [*Schäfer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007): "KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics". In Del Re, A.A.M. et al (Eds): Proceedings of the XIII Symposium of Pesticide Chemistry, Piacenza, 2007, p. 916-923*]. The iteratively reweighted least squares (IRLS) method was selected to optimize model parameters, estimate parameter standard deviations, determine parameter confidence (t-test), calculate correlation coefficients (r^2) and χ^2 error percentage, and calculate DT_{50} and DT_{90} endpoints. The error tolerance and maximum iterations were set to 0.000001 and 100, respectively

II. RESULTS AND DISCUSSION

Level P-I

An overview of the estimated trigger and modelling endpoints for imazamox is given in Table 7.2.2.3-10.

Table 7.2.2.3-10: Trigger and modelling endpoints for imazamox (Level P-I)

1997 Study	**Mill stream pond**					
		Trigger Endpoints			*Modelling Endpoints*	
	Compartment[1]	Kinetic Model	DT_{50} [d]	DT_{90} [d]	Kinetic Model	DT_{50}[2] [d]
	Total System	SFO	155	516	SFO	155
	Water Phase	FOMC	14.3	1000[4]	DFOP	76.4[3]
	Iron hatch runoff					
		Trigger Endpoints			*Modelling Endpoints*	
	Compartment	Kinetic Model	DT_{50} [d]	DT_{90} [d]	Kinetic Model	DT_{50} [d]
	Total System	SFO	129	429	SFO	129
	Water Phase	HS	50.5	206	HS	67.1[3]
2013 Study	**Goose River**					
		Trigger Endpoints[5]			*Modelling Endpoints*	
	Compartment	Kinetic Model	DT_{50} [d]	DT_{90} [d]	Kinetic Model	DT_{50} [d]
	Total System	HS	213	870	HS	283[3]
	Water Phase	DFOP	43.4	358	DFOP	135[3]
	Golden Lake					
		Trigger Endpoints[5]			*Modelling Endpoints*	
	Compartment[1]	Kinetic Model	DT_{50} [d]	DT_{90} [d]	Kinetic Model	DT_{50} [d]
	Total System	DFOP	480	1000	DFOP	525[3]
	Water Phase	DFOP	288	1000	DFOP	441[3]

[1] Peak sediment level of imazamox reached at or near the end of the incubation in all test systems, so no sediment kinetic endpoints were determined
[2] DT_{50} for the water phase and $DegT_{50}$ for the total system
[3] Conservatively estimated as $ln(2)/k_{slow}$
[4] 1000 day conservative default value assumed where the calculated DT_{90} >1000 days
[5] Trigger endpoints determined in a previous study

Level P-II

Due to poor confidence in parameter estimations, no endpoints were used from the level P-II analyses. However, a total system DT_{50} from the P-I analysis can be applied to the faster degrading compartment, while fixing the DT_{50} of the other compartment to 1000 days [*FOCUS (2012): "Generic guidance for FOCUS surface water Scenarios," v. 1.2.*]. Results based on this recommendation are summarised in Table 7.2.2.3-11. A geomean $DegT_{50}$ of 233 days was computed for the water compartment, while the conservative default $DegT_{50}$ of 1000 days was assigned to the sediment compartment.

Table 7.2.2.3-11: Persistence and modelling endpoints for imazamox (Level P-II)

Compartment	$DegT_{50}$* [d]			
	Study 1997		Study 2013	
	Mill stream pond	Iron hatch runoff	Goose River	Golden Lake
Water	155	129	283	525
Sediment	1000	1000	1000	1000

* estimated according to FOCUS (2012) recommendations

III. CONCLUSION

Trigger and modelling endpoints of imazamox in four aerobic aquatic systems were determined according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

For the total system at the P-I level, the maximum DT_{50} and DT_{90} values for use as trigger endpoints were 480 and 1000 days, respectively. For the water phase at the P-I level, the maximum DT_{50} and DT_{90} values were 288 and 1000 days, respectively. Kinetic endpoints were not determined for the sediment phase. No statistically significant correlations were found between DT_{50} values and system properties such as aqueous pH, sediment organic carbon, redox potential, dissolved oxygen concentration, or clay content.

At the P-II analysis level it was not possible to produce robust endpoints for $DegT_{50}$ values in water and sediment using a first-order compartment model including water/sediment partitioning. Instead, the total system $DegT_{50}$ from the P-I analysis (geomean of N = 4 values) was used as the water compartment modelling endpoint, while a conservative default $DegT_{50}$ value was assigned to the sediment compartment [*FOCUS (2012)*]. The resulting $DegT_{50}$ values from this approach were 233 days for the water compartment and 1000 days for the sediment compartment at level P-II.

There were no statistically significant ($p < 0.05$) correlations between system properties such as aqueous pH, sediment organic carbon, redox potential, clay content, and dissolved oxygen concentration and total system or water phase DT_{50} (or $DegT_{50}$) values.

Level M-I analyses could not be performed for any of the systems, as no system provided enough data points for a robust kinetic fit.

CA 7.2.2.4 Irradiated water/sediment study

No data were generated or required.

CA 7.2.3 Degradation in the saturated zone

No data were generated or required.

CA 7.3 Fate and behaviour in air

CA 7.3.1 Route and rate of degradation in air

No additional data were generated or required. See the previous DAR (SANCO/4325/2000 – Final, 29 November, 2002) for a summary of available information.

CA 7.3.2 Transport via air

No data were generated or required. The vapor pressure of imazamox was reported to be $<1.33\times10^{-5}$ Pa at 25°C (SANCO/4325/2000 –Final 29 November 2002), which is $<6.92\times10^{-5}$ Pa at 20°C calculated assuming at molar energy of vaporization of 95 kJ/mol. Therefore, imazamox is considered to have low potential to enter the atmosphere.

CA 7.3.3 Local and global effects

No data were generated or required.

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

Imazamox and metabolites CL 312622 and CL 354825 are the residues defined for risk assessment in soil, surface water, and ground water. Imazamox is the residue defined for risk assessment in air.

CA 7.4.2 Definition of the residue for monitoring

Imazamox and metabolites CL 312622 and CL 354825 are the residues defined for monitoring in soil, surface water, and ground water. Imazamox is the residue defined for monitoring in air.

CA 7.5 Monitoring data

No relevant monitoring data were available.

BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
21.10.14	CP 8.1.4 Justification for missing data	BASF DocID 2013/1348643 MCA Section 8 Version 1
03.11.14	CA 8.2: Endpoints for study on *Lemna gibba* (DocID 2013/1090997) were substituted by recalculated endpoints (based on mean measured concentrations); footnotes to Table 8.2.1 were added / modified CA 8.2.2.1: Remarks to early-life stage fish study (DocID 2013/7001357) were included. CA 8.2.6.1: Remarks to alga study (DocID 2012/1185673) were included. CA 8.2.7: Summary for study on *Lemna gibba* (DocID 2013/1090997) was updated considering mean measured concentrations; remarks to study on *Spirodela polyrhiza* (DocID 2013/1246580) were included.	
06.11.2014	CA 8.8: The summary for the activated sludge study (DocID ID-549-005) performed with imazamox was provided below as a summary which has not been submitted previously	

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Introduction

Due to a mistake, the numbering of the new and ongoing studies listed in the application form submitted for imazamox Annex I Renewal was made according to old OECD data point system. Thus, the numbering of the studies in the application does not comply with the Revised EU numbering in the following AIR 3 ecotoxicology chapters. Nevertheless, all studies listed in the application form are to be found in respective ecotoxicology chapters

CA 8.1 Effects on birds and other terrestrial vertebrates

Updated dossier parts (October, 2014) are marked; *i.e.* all changes are highlighted in yellow and deleted parts are struck through.

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

No new study available.

CA 8.1.1.2 Short-term dietary toxicity to birds

No new study available.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No new study available.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

No new study available.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

No new study available.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

No new study available.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. In the EU there is no guidance or validated regulatory protocols yet available neither on the type of regulatory testing necessary nor how to conduct a risk assessment for amphibian and reptiles. In the case of imazamox, there are no studies in the literature on the toxicity of this active ingredient on amphibians and reptiles.

According to the new aquatic guidance document (EFSA, 2013) amphibian should be included in the aquatic and terrestrial risk assessment. In absence of GLP studies the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons and the absence of standard guidelines for amphibian testing). With regard to the aquatic risk assessment several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2012; Weltje et al., 2013). Regulatory ecotoxicological information on terrestrial amphibians is scarce in general. However, in the few cases where terrestrial stages of amphibians were tested in the same study as birds and mammals the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 13 in Fryday and Thompson, 2012). This suggests that the quantitative risk assessment done for birds and mammals would be conservative for the terrestrial phase of amphibians.

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherm (i.e., do not maintain a constant body temperature) and as a result feeding activity will peak at warm days and will be zero during hibernation or at cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday & Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. This uncertain will remain until appropriate and validated testing methods are developed and validated and adequate regulatory risk assessment schemes are implemented in the EU.

Further, imazamox has been used for about 17 years in many countries worldwide. There are no publications showing a potential risk of this compound to amphibians and reptiles and despite the long term use worldwide we are not aware of a single finding that amphibians or reptiles were harmfully affected by applications of this herbicide.

Overall no data and no public literature are available on this topic. In the case of imazamox, there are no studies in the literature on the toxicity of this active ingredient on amphibians and reptiles.

References:

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Fryday S and Thompson H (2009a): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

Fryday S and Thompson, H (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK

Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013): Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994

CA 8.1.5 Endocrine disrupting properties

For mammals there is no concern for endocrine disrupting properties of imazamox as outlined in detail in chapter CA 5.8.3.

For birds there is no indication from the reproductive toxicity studies for an endocrine disrupting potential of imazamox. Please refer to chapter CP 10.1.1.

CA 8.2 Effects on aquatic organisms

Updated dossier parts (October, 2014) are marked; *i.e.* all changes are highlighted in yellow and deleted parts are crossed out.

Since Annex I inclusion of imazamox (BAS 720 H), new toxicity studies on the active substance and its metabolites have been performed and as a result there are new endpoints which are now used in the aquatic risk assessment. Summaries of these new studies are provided below. Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of imazamox are provided in the EU Review documents of imazamox (Monograph, Vol. 3, Annex B.9, July, 1999; EU Review Report, SANCO/4325/2000 -Final, November 2002).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1.

Full references used within the following chapters are given at the end of the document

Table 8.2-1: Summary of the toxicity values of the active substance imazamox and its major metabolites for aquatic organisms

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
active substance: imazamox				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 122	[redacted] ID-511-002	yes
Lepomis macrochirus	96 h LC_{50}	> 119	[redacted], ID-511-001	yes
Cyprinodon variegatus #	96 h LC_{50}	> 97	[redacted] ID-511-004	no (no data requirement in the EU) #
Oncorhynchus mykiss	28 d NOEC	122	[redacted] ID-512-001	yes
Oncorhynchus mykiss	96 d NOEC (ELS)	11.8	[redacted] ID-519-003	yes
Cyprinodon variegatus 1) #	35 d NOEC (ELS)	1.22	[redacted] 2013/7001357	no (no data requirement in the EU) #

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
Aquatic invertebrates				
Daphnia magna	96 h EC_{50}	> 122	Yurk & Wisk, ID-521-001	yes
Daphnia magna [1)]	48 h EC_{50}	> 100 [2)]	Doerner, 2012/1182323 [2)]	no (new study)
Mysidopsis bahia [#]	96 h LC_{50}	> 100	Olivieri et al., ID-521-009	no (no data requirement in the EU) [#]
Daphnia magna	21 d NOEC	137	Yurk & Wisk, ID-523-001	yes
Algae [4)]				
Selenastrum capricornutum	120 h E_rC_{50} 120 h E_bC_{50}	> 0.037 [4)] > 0.037	Hoberg et al., ID-521-003	yes
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50} 72 h E_yC_{50}	29.1 [3), ~~4)~~] 7.5 [3)]	Hoffmann, 2012/1185673 [3)]	no (new study)
Anabaena flos-aquae	120 h E_rC_{50} 120 h E_bC_{50}	> 0.038 [4)] > 0.038	Hoberg et al., ID-521-004	yes
Navicula pelliculosa	120 h E_rC_{50} 120 h E_bC_{50}	> 0.037 [4)] > 0.037	Hoberg et al., ID-521-002	yes
Skeletonema costatum [#]	120 h E_rC_{50} 120 h E_bC_{50}	> 0.039 [4)] > 0.039	Hoberg et al., ID-521-006	yes
Aquatic macrophytes [4)]				
Lemna gibba *	14 d E_bC_{50} 14 d E_bC_{50}	0.014 [5)] 0.011 [6)]	Hoberg et al., ID-521-005 *	yes
Lemna gibba [1)]	7 d E_rC_{50} 7 d E_yC_{50}	0.02131 [~~4),~~ 5), $] 0.01116 [5), $]	Dorner, 2013/1090997	no (new study)
	7 d E_rC_{50} 7 d E_yC_{50}	0.05081 [~~4),~~ 6), $] 0.01522 [6), $]		
Lemna gibba (higher-tier with sediment) [1)]	7 d E_rC_{50} 7 d E_yC_{50}	0.022 [~~4),~~ 5)] 0.0099 [5)]	Dorner, 2013/1246583	no (new study)
	7 d E_rC_{50} 7 d E_yC_{50}	0.060 [~~4),~~ 6)] 0.010 [6)]		
Myriophyllum aquaticum (higher-tier with sediment) [1)]	7 d E_rC_{50} 7 d E_yC_{50}	> 100 [~~4),~~ 6), 7)] > 100 [6), 7)]	Backfisch, 2013/1165858	no (new study)
	7 d E_rC_{50} 7 d E_yC_{50}	> 100 [~~4),~~ 8)] 54 [8)]		
Spirodela polyrhiza (higher-tier with sediment) [1)]	11 d E_rC_{50} 11 d E_yC_{50}	0.085 [~~4),~~ 5)] 0.051 [5)]	Backfisch, 2013/1246580	no (new study)
	11 d E_rC_{50} 11 d E_yC_{50}	> 1.00 [~~4),~~ 6)] > 1.00 [6)]		

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
Ceratophyllum demersum (higher-tier with sediment) [1)]	8 d E_rC_{50} 8 d E_yC_{50}	>1.00 [4), 6)] > 1.00 [6)]	Backfisch, 2013/1246581	no (new study)
	8 d E_rC_{50} 8 d E_yC_{50}	0.063 [4), 7)] 0.031 [7)]		
	8 d E_rC_{50} 8 d E_yC_{50}	0.050 [4), 8)] 0.030 [8)]		
	8 d E_rC_{50} 8 d E_yC_{50}	0.074 [4), 9)] 0.024 [9)]		
	8 d EC_{50}	0.029 [10)]		
	8 d EC_{50}	0.021 [11)]		
Glyceria maxima (higher-tier with sediment) [1)]	10 d E_rC_{50} 10 d E_yC_{50}	0.481 [4), 6)] 0.104 [6)]	Backfisch, 2013/1246582	no (new study)
	10 d E_rC_{50} 10 d E_yC_{50}	0.032 [4), 7)] 0.021 [7)]		
	10 d E_rC_{50} 10 d E_yC_{50}	0.069 [4), 8)] 0.032 [8)]		
	10 d EC_{50}	0.021 [11)]		
metabolite: CL 312622				
Aquatic macrophytes [4)]				
Lemna gibba [1)]	7 d E_rC_{50} 7 d E_yC_{50}	6.3 [4), 5)] 2.8 [5)]	Baetscher, 2006/1030257	no (new study)
	7 d E_rC_{50} 7 d E_yC_{50}	59 [4), 6)] 4.5 [6)]		
metabolite: CL 354825				
Aquatic macrophytes [4)]				
Lemna gibba [1)]	7 d E_rC_{50} 7 d E_yC_{50}	43.1 [4), 5)] 10.5 [5)]	Rzodeczko, 2011/1150030	no (new study)
	7 d E_rC_{50} 7 d E_yC_{50}	54.5 [4), 6)] 47.7 [6)]		

~~**Bold** figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and macrophytes), the relevant endpoint for the most sensitive species is used in the aquatic risk assessment.~~

ELS = early life stage

1) New study, conducted after Annex I inclusion of the active substance imazamox.

2) The (lower) endpoint obtained in a more recent study (performed after Annex I inclusion of imazamox) with the active substance imazamox on *D. magna* is used for the aquatic risk assessment since the test design of the older (EU agreed) study differs from the recommendations of current guidance (*i.e.* study duration was 96 h instead of 48 h as recommended by the OECD guideline 202 (OECD, 2004)).

3) The exact growth rate / yield endpoints obtained in the more recent study (performed after Annex I inclusion of imazamox) on the green alga *P. subcapitata* is used for the aquatic risk assessment since in previous studies on different algal species no exact endpoints could be defined for this group of organisms (*i.e.* no effects were observed at the highest tested concentrations). Furthermore, the test design of the older (EU agreed) studies differs from the recommendations of current guidance (*i.e.* study duration was 120 h instead of 72 hours as recommended by OECD guideline 201 (OECD, 2011)).

[4)] In accordance with recent guidelines for aquatic primary producers (*e.g.*, EFSA Guidance document (EFSA 2013); ~~Aquatic Guidance Document (SANCO, 2002);~~ OECD guideline 201 (OECD, 2011) and 221 (OECD, 2006)), ~~only~~ the EC_{50} values determined for the endpoint 'growth rate' (E_rC_{50}) should preferably be ~~are~~ considered for the risk assessment for algae and macrophytes if both “growth rate” and “biomass” endpoints are available. Nevertheless, TER calculations for algae and aquatic plants are provided in MCP dossiers using the endpoints based on both “growth rate” and “biomass/yield”.

[5)] based on frond no. [6)] based on dry weight

[7)] based on total (shoot) length [8)] based on wet weight

[9)] based on main shoot length [10)] based on side shoots length

[11)] based on no. of side shoots/leaves

* The results of the older (EU agreed) study on *Lemna gibba* (Hoberg et al., ID-521-005) are not considered relevant for the aquatic risk assessment anymore since the test duration in this study deviates from the recommendations of current guidelines (*i.e.* test duration of 14 days instead of 7 days as recommended by EFSA (2013) and OECD (2006). Instead, the results of the more recent studies performed on *Lemna gibba* and other macrophyte species (after Annex I inclusion of imazamox) according to current guidelines are considered in the aquatic risk assessment.

\# marine species; toxicity data are provided as additional information; however they are not considered for the risk assessment.

$ Endpoints have been recalculated based on mean measured test item concentrations; for details see updated summary provided below.

CA 8.2.1 Acute toxicity to fish

The following acute toxicity study performed with imazamox on the marine fish species *Cyprinodon variegatus* is provided in support of the aquatic risk assessment and has not been evaluated previously.

The following study is not required for registration in the EU; however, it was performed due to new U.S. data requirements.

Report:	CA 8.2.1/1 [redacted] 1998a Acute toxicity of AC 299, 263 (Imazamox) technical to the sheepshead minnow (Cyprinodon variegatus) under flow-through test conditions (in the first place amendment # 1 included) ID-511-004
Guidelines:	EPA 40 CFR 158(E), EPA 72-3(a)
GLP:	no (certified by <none>)

Executive Summary

In a flow-through acute toxicity laboratory study, sheepshead minnow were exposed to 13, 21, 35, 58 and 97 mg imazamox/L (nominal) and a dilution water control in groups of 10 animals in glass aquaria containing 15 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity at test initiation and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal. After 96 hours of exposure, no mortality and toxic effects were observed in the control and in all test item concentrations tested.

In a flow-through acute toxicity study with sheepshead minnow, the LC_{50} (96 h) for imazamox was determined to be > 97 mg a.s./L based on nominal concentrations. The NOEC (96 h) was ≥ 97 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483, CL 299263); Lot no. AC6935-63; purity: 97.1%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*), juveniles, average body weight 0.28 g at test end; supplied by "Aquatic BioSystems", Fort Collins, Colorado, USA.

Test design: Flow-through system (96 hours); 10 fish per aquarium, 2 replicates per concentration; loading rate: 0.19 g/L; assessment of mortality and symptoms of toxicity at test initiation and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 13, 21, 35, 58 and 97 mg imazamox/L).

Test conditions: 20 L glass aquaria, test volume 15 L, filtered natural seawater diluted with deionized water, salinity: 15 - 16‰; temperature: 21.9 °C - 22.5 °C; pH 6.8 - 8.1; oxygen content: 6.9 mg/L - 7.7 mg/L; flow rates: 5.8 volume additions/24 h/test vessel; photoperiod 16 h light : 8 h dark maintained by fluorescent lights providing a light intensity of 65 foot candles; no aeration, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and at test termination. Measured concentrations for imazamox ranged from 98% to 103% of nominal concentrations at test initiation and from 92% to 97% of nominal at test termination. As the measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality and toxic effects were observed in the control and in all test item concentrations tested. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1: Acute toxicity (96 h) of imazamox to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**13**	**21**	**35**	**58**	**97**
Mortality [%]	0	0	0	0	0	0
Symptoms	none	none	none	none	none	none
Endpoints [mg imazamox/L] (nominal)						
LC_{50} (96 h)	> 97					
NOEC (96 h)	≥ 97					

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow, the LC_{50} (96 h) for imazamox was determined to be > 97 mg a.s./L based on nominal concentrations. The NOEC (96 h) was ≥ 97 mg a.s./L (nominal).

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

The following fish early life stage toxicity study performed with imazamox on the marine species *Cyprinodon variegatus* is provided in support of the aquatic risk assessment and has not been evaluated previously.

The following study is not required for registration in the EU; however, it was performed due to new U.S. data requirements.

Report:	CA 8.2.2.1/1 [redacted] 2013b BAS 720 H: Early life-stage toxicity test with the Sheepshead minnow, *Cyprinodon variegatus*, under flow-through conditions 2013/7001357
Guidelines:	EPA 850.1400
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of imazamox to sheepshead minnow (*Cyprinodon variegatus*) embryos and fry was investigated in a 35-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to imazamox at nominal concentrations of 0.33, 0.65, 1.3, 2.5, 5.0 and 10 mg a.s./L (corresponding to mean measured concentrations of 0.303, 0.624, 1.22, 2.51, 4.87 and 10.1 mg a.s./L). Hatchability, survival rate and behavior of sheepshead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The biological results are based on mean measured concentrations. Egg hatch began on day 4 and ended between study days 6 and 8 in the control and all test item treatments. 95% hatch was reached in all treatments by study day 7 (day 0 post-hatch) and the overall hatching success in the control was 95%. Post-hatch survival was 97% in the control and between 94% and 99% in all test item treatments. No statistically significant effects on the hatching success, post-hatch survival, time to start of hatch and time to end of hatch was observed for any of the test item treatments as compared to the control. The mean standard fish lengths in the in the test item treatments ranged from 12.8 mm to 13.9 mm compared to 13.9 mm in the control treatment. A statistically significant reduction in fish length was observed in the three highest mean measured concentrations of 2.51, 4.87, and 10.1 mg a.s./L as compared to the control. The mean blotted wet weight was 0.0842 g in the control and ranged from 0.0698 g to 0.0848 g in the test item treatments. Fish weights were statistically significantly reduced compared to the control at the highest test item concentration of 10.1 mg a.s./L after 35 days of exposure. Morphological and behavioral abnormalities were observed between study days 8 and 21 in the control and the 0.303, 0.624, 1.22, and 10.1 mg a.s./L test treatments and included spinal curvature, fish laying on the bottom of the test chamber, and a conjoined twin.

However, these abnormalities did not appear to be dose-dependent because the number of test substance treatment fish with abnormalities was not greater than those observed in the control. No morphological or behavioral abnormalities were noted after day 21.

In an early life stage study with sheepshead minnow (*Cyprinodon variegatus*), the overall NOEC (35 d) for imazamox was determined to be 1.22 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483); batch no. COD-001579; purity: 98.9%.

B. STUDY DESIGN

Test species: Sheepshead Minnow (*Cyprinodon variegatus*); unfertilized eggs and milt were collected from mature sheepshead minnow reared in-house less than 24 hours before test initiation; male sheepshead minnows were sacrificed to obtain testes for fertilization.

Test design: Flow-through system (35 d); 6 test item concentrations plus a dilution water control; 4 replicate test chambers per treatment with 20 fertilized eggs in each; a proportional diluter system was used for the preparation of test solutions and intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. On study day 10 (*i.e.*, day 3 post-hatch), all live fry were counted and released into their respective replicate growth chamber. Survival of the fry was monitored daily following hatch by visually inspecting each test chamber, and any behavioral or physical changes were recorded, including abnormalities. On day 35 (28 days post hatch) surviving animals were sacrificed and measured for length and weight.

Endpoints: NOEC values based on hatchability, survival, toxic signs and growth.

Test concentrations: Control (dilution water), 0.33, 0.65, 1.3, 2.5, 5.0, and 10.0 mg a.s./L (nominal); corresponding to mean measured concentrations of 0, 0.303, 0.624, 1.22, 2.51, 4.87 and 10.1 mg a.s./L.

Test conditions:	Test vessels: glass aquaria (15 x 21 x 24 cm) with a test volume of approx. 5.0 L; 4 replicate test chambers; glass incubation cups (used during embryo stage) with 9 cm diameter and Nitex® screen replacing the bottom; one incubation cup per test chamber; test medium: laboratory saltwater added to demineralized laboratory freshwater; water temperature 24.1 °C - 25.6 °C; pH 7.90 - 8.75; dissolved oxygen: 5.61 mg/L - 7.26 mg/L ; salinity at test initiation: 19.5 - 20.9‰ (except for salinity of 24.5% on study day 0 measured in one replicate in the control); light intensity: ranged from 555 to 670 lux (day 16); photoperiod: 16 h light : 8 h dark (30-minute simulated dawn and dusk transition period); flow rate: approx. 2.9 cycles per hour with 0.5 L per diluter cycle in each replicate test chamber (resulting in 6.9 volume additions (*i.e.* 35 L) per test chamber over a 24-h period); feeding: fish larvae were fed times daily *ad libitum* brine shrimp nauplii (*Artemia*) from day 4 onwards (start of hatch), standard commercial fish food was added to the daily food beginning on day 20; fish were fed *ad libitum* at least three times daily during the week and at least twice a day on weekends.
Analytics:	Analytical verification of imazamox concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; Fisher's exact test and/or ANOVA followed by Dunnett's test ($p = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements of imazamox concentrations were conducted in samples collected four days prior to initiation and in the control and in all test item concentrations at test initiation, on study days 1, 7, 11, 14, 21, 28 and at test termination after 35 d of exposure. Measured concentrations of imazamox in the test item treatments prior to initiation of the definitive test ranged from 70% to 99% of the nominal concentrations. Measured concentrations of imazamox in the test item treatments on day 0 of the exposure ranged from 58% to 101% of the nominal concentrations. The 0.33 mg a.s./L nominal treatment recoveries were suspected to be < 80% of nominal due to a diluter malfunction. However, one day after application, measured water concentrations of imazamox in all test item treatments were between 90% and 102% of nominal concentrations. On days 7 through 35, measured values were between 92% and 106% of nominal. The mean measured concentrations of imazamox in the test-substance treatments for the 35-day exposure ranged from 92% to 101% of the nominal concentrations. Overall, the concentrations imazamox in all but the lowest test substance treatment on study day 0 was maintained within 20% of the mean measured concentrations throughout the exposure period. The following biological results are based on mean measured concentrations.

Biological results: Egg hatch began on day 4 and ended between study days 6 and 8 in the control and all test item treatments. 95% hatch was reached in all treatments by study day 7 (day 0 post-hatch) and the overall hatching success in the control was 95%. Post-hatch survival was 97% in the control and between 94% and 99% in all test item treatments. No statistically significant effects on the hatching success, post-hatch survival, time to start of hatch and time to end of hatch was observed for any of the test item treatments as compared to the control (Dunnett`s test, $p = 0.05$).
The mean standard fish lengths in the test item treatments ranged from 12.8 mm to 13.9 mm compared to 13.9 mm in the control treatment. A statistically significant reduction in fish length was observed in the three highest mean measured concentrations of 2.51, 4.87, and 10.1 mg a.s./L as compared to the control (Dunnett`s test, $p = 0.05$). The mean blotted wet weight was 0.0842 g in the control and ranged from 0.0698 g to 0.0848 g in the test item treatments. Fish weights were statistically significantly reduced compared to the control at the highest test item concentration of 10.1 mg a.s./L after 35 days of exposure (Dunnett`s test, $p = 0.05$).
Morphological and behavioral abnormalities were observed between study days 8 and 21 in the control and the 0.303, 0.624, 1.22, and 10.1 mg a.s./L test treatments and included spinal curvature, fish laying on the bottom of the test chamber, and a conjoined twin. However, these abnormalities did not appear to be dose-dependent because the number of test substance treatment fish with abnormalities was not greater than those observed in the control. No morphological or behavioral abnormalities were noted after day 21.

The results are summarized in Table 8.2.2.1-1.

Table 8.2.2.1-1: Chronic toxicity of imazamox to sheepshead minnow (*Cyprinodon variegatus*) in an fish early life-stage test (35 d)

Concentration [mg a.s./L] (nominal)	**Control**	**0.33**	**0.65**	**1.3**	**2.5**	**5.0**	**10.0**
Concentration [mg a.s./L] (mean measured)	**Control**	**0.303**	**0.624**	**1.22**	**2.51**	**4.87**	**10.1**
Hatching success [%]	95	86	90	95	84	94	90
Start of hatch [d]	5	4	4	4	4	4	4
Time to 95% hatch [d]	7	7	7	7	7	7	7
End of hatch [d]	7	7	7	7	7	7	7
28-day post-hatch survival [%]	97	96	96	97	94	99	96
Mean standard length on day 35 [mm]	13.9	13.8	13.8	13.6	13.5 *	13.5 *	12.8 *
% of control #	100.0	99.3	99.3	97.8	97.1	97.1	92.1
Mean blotted wet weight on day 35 [mm]	0.084	0.081	0.085	0.080	0.079	0.080	0.070 *
% of control #	100.0	96.4	101.2	95.2	94.0	95.2	83.3
	Endpoints [mg imazamox/L] (mean measured)						
$NOEC_{overall}$ (35 d)	1.22						

* Statistically significant differences compared to the control (Dunnett's test; p = 0.05).
\# Calculated on the basis of the mean values.

III. CONCLUSION

In an early life stage study with sheepshead minnow (*Cyprinodon variegatus*), the overall NOEC (35 d) for imazamox was determined to be 1.22 mg a.s./L, based on mean measured concentrations.

Remarks: On page three the "Statement of Compliance" can be found. US EPA does not issue GLP Certificates to laboratories as the European countries do. As the study was conducted for US registrations, the report was built up according to US requirements. The analytical validation and results are described on pages 19-20 and numerical results are listed in Table 1.

CA 8.2.2.2 Fish full life cycle test

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.2.3 Bioconcentration in fish

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.3 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of imazamox as well as based results of available studies there is no indication of endocrine disrupting properties of this active substance. Thus, no studies are required.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

The following acute toxicity study on *Daphnia magna* performed with imazamox is provided in support of the aquatic risk assessment and has not been evaluated previously.

The following study is required for registration in the EU. The new study was performed due to a request of the Japanese authority. As a new study according to the current guidance is available, the new endpoint should be used in the risk assessment.

Report:	CA 8.2.4.1/1 Dorner S., 2012b Acute toxicity of Reg.No. 4096483 to Daphnia magna STRAUS in a 48 hour static test 2012/1182323
Guidelines:	OECD 202, EPA 850.1010
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to imazamox at a single nominal concentration of 100 mg a.s./L and a water control in 4 replicates per concentration, containing 5 daphnids each. The daphnids were observed for immobility 24 and 48 hours after start of exposure.

The biological results are based on nominal concentrations. After 48 hours of exposure, no immobility of the daphnids was observed in the control and at the tested concentration of 100 mg imazamox/L.

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of imazamox was > 100 mg a.s./L based on nominal concentrations. The NOEC was determined to be ≥ 100 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483), batch no. COD-001579; purity: 98.9%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France), >2 < 24 h old at test initiation.

Test design: Static system (48 hours), limit test with 1 test item concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: 0 (control) and 100 mg imazamox/L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water "M4" (Elendt medium); pH 7.73 - 7.95; oxygen concentration: 8.5 mg/L - 9.1 mg/L; temperature: 20.7 °C - 21.3 °C; total hardness: 2.52 mmol/L at test initiation; conductivity: 667 µS/cm ate test initiation; photoperiod: 16 h light : 8 h dark; no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted at the beginning and at the end of the test. The analyzed content of imazamox was 100.6% of nominal at test initiation and 99.0% of nominal at test termination. As the measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 48 hours of exposure, no immobility of daphnids was observed in the control and at the tested concentration of 100 mg imazamox/L. For results see Table 8.2.4.1-1.

Table 8.2.4.1-1: Effects of imazamox on *Daphnia magna* immobility

Concentration [mg a.s./L] (nominal)	**Control**	**100**
Immobility (24 h) [%]	0	0
Immobility (48 h) [%]	0	0
Endpoints [mg imazamox/L] (nominal)		
EC_{50} (48 h)	> 100	
NOEC (48 h)	≥ 100	

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of imazamox was > 100 mg a.s./L based on nominal concentrations. The NOEC was determined to be ≥ 100 mg a.s./L (nominal).

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute toxicity study performed with imazamox on the marine invertebrate species *Mysidopsis bahia* is provided in support of the aquatic risk assessment and has not been evaluated previously.

The following study is not required for registration in the EU; however, it was performed due to new U.S. data requirements.

Report:	CA 8.2.4.2/1 Olivieri C.E. et al., 1998b Acute toxicity of AC 299, 263 (Imazamox) technical to the mysid (Mysidopsis bahia) under flow-through test conditions (Amendment included, in the first place) ID-521-009
Guidelines:	EPA 40 CFR 158(E), EPA 72-3(c)
GLP:	no

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, saltwater mysids were exposed to 0 (control), 13, 22, 36, 60 and 100 mg imazamox/L (nominal) in groups of 10 animals in glass beakers containing 15 L water with 2 replicates per concentration. Saltwater mysids were observed for survival and symptoms of toxicity at test initiation and after 4, 24, 48, 72 and 96 hours of exposure.

The biological results are based on nominal concentrations. After 96 hours of exposure, no mortality and toxic effects were observed in the control and in all test item concentrations tested.

In a flow-through acute toxicity study with saltwater mysids (*Mysidopsis bahia*), the LC_{50} (96 h) for imazamox was determined to be > 100 mg a.s./L based on nominal concentrations. The NOEC (96 h) was ≥ 100 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483; CL 299263), Lot. no. AC6935-63; purity: 97.1%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures; originally obtained from "Aquatic BioSystems", Fort Collins, Colorado, USA.

Test design: Flow-through system (96 hours); 10 mysids per test chamber, 2 replicates per concentration; loading: 0.0080 g/L at and time and 0.00013 g/L/24 h; assessment of mortality and symptoms of toxicity at test initiation and 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (96 h), NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 13, 22, 36, 60 and 100 mg imazamox/L (nominal)

Test conditions: 20 L glass aquaria, test volume 15 L, filtered natural seawater mixed with deionized water, salinity: 15 - 16‰; temperature: 22.0 °C - 22.4 °C; pH 6.8 - 8.0; oxygen content: 7.4 mg/L - 7.6 mg/L; flow rates: 6.0 volume additions/24 h/test vessel; photoperiod 16 h light : 8 h dark maintained by fluorescent lights providing a light intensity of about 65 foot candles; mysids were fed with live brine shrimps (*Artemia nauplii*) at least once per day.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at test termination. Measured concentrations for the imazamox ranged from 85% to 94% of nominal concentrations at test initiation and from 84% to 97% of nominal at test termination. As the measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality and toxic effects were observed in the control and in all test item concentrations tested. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Acute toxicity (96 h) of imazamox to saltwater mysids (*Mysidopsis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**13**	**22**	**36**	**60**	**100**
Mortality [%]	0	0	0	0	0	0
Symptoms	none	none	none	none	none	none
Endpoints [mg imazamox/L] (nominal)						
LC_{50} (96 h)	> 100					
NOEC (96 h)	≥ 100					

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Mysidopsis bahia*), the LC_{50} (96 h) for imazamox was determined to be > 100 mg a.s./L based on nominal concentrations. The NOEC (96 h) was ≥ 100 mg a.s./L (nominal).

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

No further studies required; thus, this point is not addressed *via* new toxicity studies.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.5.3 Development and emergence in Chironomus riparius

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.5.4 Sediment dwelling organisms

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

The following algal toxicity study performed with imazamox is provided in support of the aquatic risk assessment and has not been evaluated previously.

The following study is required for registration in the EU. The new study was performed due to a request of the Japanese authority. As a new study according to the current guidance is available, the new endpoint should be used in the risk assessment.

Report:	CA 8.2.6.1/1 Hoffmann F., 2012b Effect of BAS 720 H (Imazamox, Reg.No. 4096483) on the growth of the green alga Pseudokirchneriella subcapitata 2012/1185673
Guidelines:	OECD 201, EPA 850.5400
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 96 hour static acute toxicity laboratory study, the effect of imazamox on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0.30, 0.75, 1.9, 4.7, 11.7, 29.3 and 73.2 mg imazamox/L. Assessment of growth was conducted 24, 48, 72 and 96 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at all test item concentrations tested.

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) for imazamox was determined to be 29.1 mg a.s./L and the E_yC_{50} (96 h) was 6.3 mg a.s./L, based on nominal concentrations. After 72 hour of exposure, the respective E_rC_{50} and E_yC_{50} values were determined to be 29.1 and 7.5 mg a.s./L, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483), batch no. COD-001579; purity: 98.9%, (tolerance: ± 1.0%)

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; stock obtained from the "The Culture Collection of Algae" University of Texas at Austin, USA.

Test design: Static system; test duration 96 hours; 7 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours and 96 hours.

Test concentrations: Control, 0.30, 0.75, 1.9, 4.7, 11.7, 29.3 and 73.2 mg imazamox/L (nominal).

Test conditions: 100 mL glass Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 (at test initiation); temperature: 21.0 °C - 23.0 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at 8000 lux; constant shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of imazamox ranged from 97.5% to 107.8% of nominal at test initiation and from 86.9% to 95.8% of nominal at test termination. As the measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control and at all test item concentrations tested. The effects on algal growth are summarized in Table 8.2.6.1-1.

Table 8.2.6.1-1: Effect of imazamox on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	**0.30**	**0.75**	**1.9**	**4.7**	**11.7**	**29.3**	**73.2**
Inhibition in 72 h (growth rate) [%]*	-0.6	2.1	4.4	13.2	24.0	45.4	79.9
Inhibition in 72 h (yield) [%]*	-2.5	7.6	15.4	39.3	60.0	83.2	97.1
Inhibition in 96 h (growth rate) [%]*	-0.1	1.6	5.9	8.3	26.6	47.7	76.2
Inhibition in 96 h (yield) [%]*	-0.7	7.1	24.5	32.2	71.8	90.1	98.1

	Endpoints [mg imazamox/L] (nominal)
E_rC_{50} (72 h)	29.1 (95% confidence limits 27.0 - 31.3)
E_rC_{10} (72 h)	5.1 (95% confidence limits 4.3 - 5.9)
E_yC_{50} (72 h)	7.5 (95% confidence limits 7.0 - 8.0)
E_yC_{10} (72 h)	1.2 (95% confidence limits 1.0 - 1.4)
E_rC_{50} (96 h)	29.3 (95% confidence limits 28.1 - 30.5)
E_rC_{10} (96 h)	4.9 (95% confidence limits 4.5 - 5.3)
E_yC_{50} (96 h)	6.3 (95% confidence limits 5.7 - 6.8)
E_yC_{10} (96 h)	1.2 (95% confidence limits 1.0 - 1.4)

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) for imazamox was determined to be 29.1 mg a.s./L and the E_yC_{50} (96 h) was 6.3 mg a.s./L, based on nominal concentrations. After 72 hour of exposure, the respective E_rC_{50} and E_yC_{50} values were determined to be 29.1 and 7.5 mg a.s./L, respectively.

Remarks: In Table 3 of App. 1 for the time 0 h (T0) cell numbers are given (1 x 10000); the values for 24 h, 48 h and 72 h (T24 – 72) are given as photometer extinction values. For comparison and endpoint calculations photometer extinction values need to be transferred to cell numbers. For this transformation a calibration curve is needed giving cell numbers versus extinction values. This curve is given on page 15 of the report. On page 23, table 4, the yield values for t24h, t48h and t72h are presented. The original cell number for each time period can be calculated by adding the cell numbers from T0h (inoculum of 10 000).

CA 8.2.6.2 Effects on growth of an additional algal species

No further studies required; thus, this point is not addressed *via* new toxicity studies.

CA 8.2.7 Effects on aquatic macrophytes

The following toxicity studies on aquatic primary producers performed with imazamox and its major metabolites are provided in support of the aquatic risk assessment and have not been evaluated previously.

The following study was conducted, as additional data was required for the refined risk assessment for aquatic plants.

Report:	CA 8.2.7/1 Backfisch K., 2013e Effect of BAS 720 H (Imazamox) on the growth of the aquatic plant Myriophyllum aquaticum 2013/1165858
Guidelines:	OECD 221, OECD 219, ASTM E 1913-04
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of imazamox on the growth of the rooted dicotyledonous aquatic plant species *Myriophyllum aquaticum* was investigated in the presence of sediment. The following nominal concentrations were applied: 0 (control), 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 mg imazamox/L. At test termination, plant growth (total length, wet weight and dry weight), root development and visual observations were recorded. Determination of total length and visual observations was conducted additionally at test initiation and once during the 7 day exposure period. The starting dry weight was determined by calculating a mean factor based on the ratios of the final dry weights and lengths of the control replicates, which is then multiplied by the initial length data for each plant in all treatments. The percentage inhibition relative to the control was calculated for each test concentration based upon growth rates and final yield for all parameters.

The biological results are based on nominal concentrations. No statistically significant differences compared to the control were observed at any test item concentration for all measured parameters (wet weight, total length, dry weight and root development). Furthermore, no other particular visual findings, discolorations or other unusual observations were recorded over the whole study duration.

In a 7-day aquatic-plant test with *Myriophyllum aquaticum*, the E_rC_{50} of imazamox was determined to be > 100 mg a.s./L based on total length, wet weight and dry weight (nominal). The E_yC_{50} was > 100 mg a.s./L based on total length and dry weight and 54 mg a.s./L based on wet weight (nominal). The overall NOEC based on all measured parameters was ≥ 100 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483); batch no. COD-001579; purity 98.9%

B. STUDY DESIGN

Test species: *Myriophyllum aquaticum* (Haloragaceae), a dicotyledonous aquatic plant species, cultivated in-house (non-GLP) after purchase from the nursery "Petrowsky", Eschede, Germany.

Test design: Static system including sediment; 3 day rooting phase (five plants per replicate) prior to the exposure period; thereafter reduction to three plants per replicate followed by a 7 day exposure phase; application *via* water phase; 8 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; assessment of growth and visual effects during the study and at study termination. .

Endpoints: EC_{50} and NOEC with respect to growth rate and yield related to wet weight, dry weight, total length and root development (visual observations) after 7 days of exposure.

Test concentrations: 0 (control), 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 mg imazamox/L (nominal).

Test conditions: 2.0 L glass beakers, standard artificial sediment (OECD 219, pH 7.41) and 1.8 L Smart & Barko medium; pH of test solutions 7.64 - 7.68 at test initiation and pH 7.55 - 7.77 at test termination; water temperature: 20.6 °C - 22.0 °C; oxygen saturation: 66.1% - 92.6%; conductivity: 305 µS/cm; photoperiod: 16 h light : 8 h dark, light intensity: 8900 - 10200 lux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics; probit analysis using linear max. likelihood regression for EC_{50} calculations; William's Multiple Sequential t-test Procedure or Welch-t test (α = 0.05) for determination of the NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the concentrations of the active substance was conducted in each concentration at the beginning and at the end of the test. The analytically determined concentrations of imazamox ranged from 98% to 113% of nominal concentrations at test initiation and from 102% to 118% of nominal at test termination except for the lowest concentration where about twice of the nominal concentration was measured at test start and test end. As the lowest treatment did not influence the EC_x determination and the measured concentrations in all the other test treatments confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No statistically significant differences compared to the control were observed at any test item concentration for all measured parameters (wet weight, total length, dry weight and root development). Furthermore, no other particular visual findings, discolorations or other unusual observations were recorded over the whole study duration. The results are summarized below (see Table 8.2.7-1).

Table 8.2.7-1: Effect of imazamox on the growth of the aquatic plant *Myriophyllum aquaticum*

Concentration [mg a.s./L] (nominal)	**0.03**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
Inhibition after 7 d [%] (growth rate based on total length)	8.6	4.7	15.9	15.1	17.6	20.0	26.8	39.5
Inhibition after 7 d [%] (yield based on total length)	20.0	24.2	32.9	30.9	41.8	36.2	40.3	51.9
Inhibition after 7 d [%] (growth rate based on wet weight)	5.2	5.3	16.8	28.7	27.3	28.9	30.8	38.6
Inhibition after 7 d [%] [#] (yield based on wet weight)	17.9	18.4	35.3	39.7	46.0	44.4	41.6	50.1
Inhibition after 7 d [%] [#] (growth rate based on dry weight)	10.6	- 11.7	9.1	9.5	1.1	22.1	17.6	23.6
Inhibition after 7 d [%] (yield based on dry weight)	19.2	4.4	24.8	22.6	24.7	37.3	29.1	34.0

	Endpoints [mg imazamox/L] (nominal)
E_rC_{50} (7 d) based on total length, wet weight and dry weight	> 100.0
E_yC_{50} (7 d) based on total length and dry weight	> 100.0
E_yC_{50} (7 d) based on wet weight	54.0 (95% confidence limits: 10 - 5574)
NOE_rC / NOE_yC (7 d) based on total length, wet weight and dry weight	≥ 100.0
NOEC (7 d) root development [+]	≥ 100.0

[#] Negative values indicate stimulated growth compared to the control.
[+] Based on visual observations.

III. CONCLUSION

In a 7-day aquatic-plant test with *Myriophyllum aquaticum*, the E_rC_{50} of imazamox was determined to be > 100 mg a.s./L based on total length, wet weight and dry weight (nominal). The E_yC_{50} was > 100 mg a.s./L based on total length and dry weight and 54 mg a.s./L based on wet weight (nominal). The overall NOEC based on all measured parameters was ≥ 100 mg a.s./L (nominal).

The following study is required, as the test design of the older (EU agreed) study partly differs from the recommendations of current guidance.

The following summary was updated, considering the mean measured test item concentrations for determination of the toxicity endpoints. The results of updated statistics are presented below the study summary.

Report:	CA 8.2.7/2 Dorner S., 2013b Effect of Reg.No. 4096483 on the growth of Lemna gibba 2013/1090997
Guidelines:	OECD 221, EPA 850.4400, ASTM E 1415-91
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of imazamox (BAS 720 H) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0.0025, 0.0050, 0.010, 0.020, 0.040 and 0.080 mg a.s./L (corresponding to mean measured concentrations of 0, 0.0018, 0.0030, 0.0061, 0.014, 0.028, 0.047 mg a.s./L). Assessment of growth and other effects were conducted 2, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rates and final yield for the parameters frond number and dry weight (biomass).

The biological results are based on ~~nominal~~ mean measured concentrations. The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 180 fronds per vessel, corresponding to a 16.3 x multiplication. The dry weight increased from 1.5 mg to an average of 16.3 mg per vessel in the control at test termination. No morphological effects on algae were observed at ~~nominal~~ mean measured test concentrations of up to and including ~~0.010~~ 0.0061 mg a.s./L. Morphological abnormalities, including smaller and deformed fronds, were observed in the ~~0.020~~ 0.014 and ~~0.040~~ 0.028 mg a.s./L test treatments. In the highest test item concentration of ~~0.080~~ 0.047 mg a.s./L, about 50% of the fronds were yellowish.

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} for imazamox was determined to be ~~0.031~~ 0.021 mg a.s./L based on frond number and ~~0.081~~ 0.050 mg a.s./L based on dry weight (~~nominal~~ mean measured). The E_yC_{50} of imazamox was determined to be ~~0.016~~ 0.011 mg/L based on frond number and ~~0.022~~ 0.015 mg/L based on dry weight (~~nominal~~ mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. No. 4 096 483), batch no. COD-001579, purity: 98.9%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula from 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system (7 days); 7 treatment groups (6 test item concentrations plus control) with 4 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 2, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control, 0.0025, 0.0050, 0.010, 0.020, 0.040 and 0.080 mg imazamox/L (nominal); corresponding to mean measured concentrations of 0, 0.0018, 0.0030, 0.0061, 0.014, 0.028, 0.047 mg a.s./L.

Test conditions: 400 mL glass beakers, test volume 160 mL, 20x-AAP nutrient medium, pH 7.51 - 7.64 at test initiation and pH 8.17 - 8.55 at test termination; mean water temperature: 24.9 °C, continuous light, average light intensity: about 8300 lux.

Analytics: Analytical verification of the test item was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics, probit analysis for determination of the EC_x values (the results of updated statistics considering mean measured concentrations are presented below this study summary).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for imazamox ranged from 99% to 106% of nominal concentrations at test initiation and from 33% to 51% of nominal at test termination. The reduced recoveries at test termination are due to the degradation of the active substance in water under test conditions. ~~However, as the initially measures concentrations confirmed the correct application of the test item, t~~The following biological results are based on ~~nominal~~ mean measured concentrations.

Biological results: The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 180 fronds per vessel, corresponding to a 16.3 x multiplication. The dry weight increased from 1.5 mg to an average of 16.3 mg per vessel in the control at test termination. No morphological effects on algae were observed at ~~nominal~~ mean measured test concentrations of up to and including ~~0.010~~ 0.0061 mg a.s./L. Morphological abnormalities, including smaller and deformed fronds, were observed in the ~~0.020~~ 0.014 and ~~0.040~~ 0.028 mg a.s./L test treatments. In the highest test item concentration of ~~0.080~~ 0.047 mg a.s./L, about 50% of the fronds were yellowish. Effects on growth rate and yield are summarized in Table 8.2.7-2.

Table 8.2.7-2: Effect of imazamox on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**0.0025**	**0.0050**	**0.010**	**0.020**	**0.040**	**0.080**
Concentration [mg a.s./L] (mean measured)	**0.0018**	**0.0030**	**0.0061**	**0.014**	**0.028**	**0.047**
Inhibition after 7 d [%] * (growth rate based on frond no.)	0.8	0.1	10.1	30.3	65.7	79.5
Inhibition after 7 d [%] * (growth rate based on dry weight)	0.0 #	2.6	12.8	31.7	40.8	43.8
Inhibition after 7 d [%] * (yield based on frond no.)	2.5	0.4	26.2	60.9	89.5	95.0
Inhibition after 7 d [%] * (yield based on dry weight)	0.0 #	6.7	29.1	58.5	68.6	71.3

	Endpoints [mg imazamox/L] (~~nominal~~ mean measured)
E_rC_{50} (7 d) based on frond no	~~0.031 (95% confidence limits: 0.026 - 0.037)~~ 0.021 (95% confidence limits: 0.019 - 0.023)
E_rC_{10} (7 d) based on frond no	~~0.0095 (95% confidence limits: 0.0060 - 0.013)~~ 0.0067 (95% confidence limits: 0.0051 - 0.0082)
E_yC_{50} (7 d) based on frond no	~~0.016 (95% confidence limits: 0.015 - 0.019)~~ 0.011 (95% confidence limits:0.0096 - 0.012)
E_yC_{10} (7 d) based on frond no	~~0.0066 (95% confidence limits: 0.0048 - 0.0080)~~ 0.0040 (95% confidence limits: 0.0029 - 0.0049)
E_rC_{50} (7 d) based on dry weight	~~0.081 (95% confidence limits: 0.046 - 0.362)~~ 0.050 (95% confidence limits: 0.032 - 0.129)
E_rC_{10} (7 d) based on dry weight	~~0.0063 (95% confidence limits: 0.00050 - 0.013)~~ 0.0044 (95% confidence limits: 0.00087 - 0.0082)
E_yC_{50} (7 d) based on dry weight	~~0.022 (95% confidence limits: 0.013 - 0.041)~~ 0.015 (95% confidence limits: 0.0092 - 0.024)
E_yC_{10} (7 d) based on dry weight	~~0.0037 (95% confidence limits: 0.00040 - 0.0077)~~ 0.0025 (95% confidence limits: 0.00049 - 0.0048)

* Negative values indicate stimulated growth compared to the control.
\# Inhibition greater than 100% or lower than 0% were replaced by 100% and 0%, respectively.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} for imazamox was determined to be ~~0.031~~ 0.021 mg a.s./L based on frond number and ~~0.081~~ 0.050 mg a.s./L based on dry weight (~~nominal~~ mean measured). The E_yC_{50} of imazamox was determined to be ~~0.016~~ 0.011 mg/L based on frond number and ~~0.022~~ 0.015 mg/L based on dry weight (~~nominal~~ mean measured).

The results of updated statistics considering mean measured concentrations are presented in the following:

Evaluation of a Metric Response: Test Project

Effective Concentrations (ECx) for frond number, yield at 7.0 d

based on mean measured concentrations

%Inhibition of frond number, yield as caused by the test item after 7.0 d.

Treatm.[µg/L]	Mean	Std. Dev.	n	%Inhibition
1.81	2.500	n.d.	1	97.5
2.98	0.400	n.d.	1	99.6
6.15	26.200	n.d.	1	73.8
14.12	60.900	n.d.	1	39.1
28.23	89.500	n.d.	1	10.5
47.09	95.000	n.d.	1	5.0

Probit analysis using linear max. likelihood regression

Probit analysis using linear max. likelihood regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of replicates; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [µg/L]	Log(x)	%Inhibition	n	Emp. Probit	Weight	Reg. Probit
1.81	0.258	2.50	1	-1.1906	0.006	-2.259
2.98	0.475	0.40	1	-1.2433	0.069	-1.633
6.15	0.789	26.20	1	-0.5966	0.589	-0.727
14.12	1.150	60.90	1	0.273	0.906	0.315
28.23	1.451	89.50	1	0.990	0.246	1.184
47.09	1.673	95.00	1	1.128	0.036	1.825

excluded: value not in line with the chosen function

Parameters of the probit analysis

Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	5.0000
Slope b:	2.8869
Intercept a:	-3.00417
Variance of b:	7.7311
Goodness of Fit	
Chi²:	0.0242
)egrees of freedom:	4.0000
p(Chi²):	0.9999
Log EC50:	1.0406
SE Log EC50:	0.2550
g-Criterion:	0.0433
Variance (Chi²/df):	0.0061
r²:	0.9780
F:	178.0570
p(F) (df. 1;4):	0.0000

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).
The coefficient of determination, r^2 ($0 <= r^2 <= 1$), gives the proportion of variance explained by the dose/response function.
F-Test for regression: Ho: Slope = 0; hence, if p(F) <= alpha, the selected significance level, (e.g., alpha = 0.05) the regression revealed significant results (= slope is significantly different from zero).

Results of the probit analysis

Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller`s theorem).

Parameter	EC10	EC20	EC50
Value [µg/L]	3.95	5.61	10.98
lower 95%-cl	2.89	4.44	9.63
upper 95%-cl	4.90	6.65	12.48
lower 99%-cl	2.43	3.89	8.84
upper 99%-cl	5.84	7.59	13.59

n.d.: not determined due to mathematical reasons or inappropriate data
Computation of variances and confidence limits was adjusted to metric data (Christensen & Nyholm 1984).
Slope function after Litchfield and Wilcoxon: 2.220

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

Evaluation of a Metric Response: Test Project

Effective Concentrations (ECx) for frond number, growth rate at 7.0 d

based on mean measured concentrations

%Inhibition of frond number, growth rate as caused by the test item after 7.0 d.

Treatm.[µg/L]	Mean	Std. Dev.	n	%Inhibition
1.81	0.800	n.d.	1	99.2
2.98	0.100	n.d.	1	99.9
6.15	10.100	n.d.	1	89.9
14.12	30.300	n.d.	1	69.7
28.23	65.700	n.d.	1	34.3
47.09	79.500	n.d.	1	20.5

Probit analysis using linear max. likelihood regression

Probit analysis using linear max. likelihood regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of replicates; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [µg/L]	Log(x)	%Inhibition	n	Emp. Probit	Weight	Reg. Probit
1.81	0.258	0.80	1	-1.2333	0.001	-2.745
2.98	0.475	0.10	1	-1.2508	0.008	-2.187
6.15	0.789	10.10	1	-1.0001	0.149	-1.38
14.12	1.150	30.30	1	-0.4938	0.816	-0.451
28.23	1.451	65.70	1	0.394	0.900	0.324
47.09	1.673	79.50	1	0.740	0.448	0.895

excluded: value not in line with the chosen function

Parameters of the probit analysis

Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	6.0000
Slope b:	2.5732
Intercept a:	-3.40934
Variance of b:	7.0189
Goodness of Fit	
Chi²:	0.0146
Degrees of freedom:	4.0000
p(Chi²):	1.0000
Log EC50:	1.3249
SE Log EC50:	0.2556
g-Criterion:	0.0298
Variance (Chi²/df):	0.0037
r²:	0.9850
F:	258.5790
p(F) (df: 1;4):	0.0000

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).
The coefficient of determination, r^2 ($0 <= r^2 <= 1$), gives the proportion of variance explained by the dose/response function.
F-Test for regression: Ho: Slope = 0; hence, if p(F) <= alpha, the selected significance level, (e.g., alpha = 0.05) the regression revealed significant results (= slope is significantly different from zero).

Results of the probit analysis

Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC10	EC20	EC50
Value [µg/L]	6.71	9.95	21.13
lower 95%-cl	5.13	8.17	19.09
upper 95%-cl	8.17	11.54	23.33
lower 99%-cl	4.40	7.29	17.88
upper 99%-cl	9.53	12.93	24.92

n d.: not determined due to mathematical reasons or inappropriate data

Computation of variances and confidence limits was adjusted to metric data (Christensen & Nyholm 1984).
Slope function after Litchfield and Wilcoxon: 2.447

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

Evaluation of a Metric Response: Test Project

Effective Concentrations (ECx) for dry weight, yield at 7.0 d

%Inhibition of dry weight, yield as caused by the test item after 7.0 d.

Treatm.[µg/L]	Mean	Std. Dev.	n	%Inhibition
1.81	0.000	n.d.	1	100.0
2.98	6.700	n.d.	1	93.3
6.15	29.100	n.d.	1	70.9
14.12	58.500	n.d.	1	41.5
28.23	68.600	n.d.	1	31.4
47.09	71.300	n.d.	1	28.7

Probit analysis using linear max. likelihood regression

Probit analysis using linear max. likelihood regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of replicates; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [µg/L]	Log(x)	%Inhibition	n	Emp. Probit	Weight	Reg. Probit
1.81	0.258	0.00	1	-1.2533	0.103	-1.507
2.98	0.475	6.70	1	-1.0854	0.269	-1.146
6.15	0.789	29.10	1	-0.5239	0.679	-0.622
14.12	1.150	58.50	1	0.213	1.000	-0.021
28.23	1.451	68.60	1	0.466	0.793	0.481
47.09	1.673	71.30	1	0.534	0.484	0.851

excluded: value not in line with the chosen function

Parameters of the probit analysis

Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	7.0000
Slope b:	1.6669
Intercept a:	-1.93721
Variance of b:	2.0107
Goodness of Fit	
Chi²:	0.1581
Degrees of freedom:	4.0000
p(Chi²):	0.9970
Log EC50:	1.1622
SE Log EC50:	0.3293
g-Criterion:	0.2204
Variance (Chi²/df):	0.0395
r²:	0.8970
F:	34.9650
p(F) (df: 1;4):	0.0040

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).
The coefficient of determination, r^2 ($0 <= r^2 <= 1$), gives the proportion of variance explained by the dose/response function.
F-Test for regression: Ho: Slope = 0; hence, if p(F) <= alpha, the selected significance level, (e.g., alpha = 0.05) the regression revealed significant results (= slope is significantly different from zero).

Results of the probit analysis

Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC10	EC20	EC50
Value [µg/L]	2.47	4.54	14.53
lower 95%-cl	0.49	1.46	9.16
upper 95%-cl	4.75	7.54	23.65
lower 99%-cl	0.23	0.85	6.71
upper 99%-cl	10.08	12.95	32.31

Computation of variances and confidence limits was adjusted to metric data (Christensen & Nyholm 1984).
Slope function after Litchfield and Wilcoxon: 3.980

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

Evaluation of a Metric Response: Test Project

Effective Concentrations (ECx) for dry weight, growth rate at 7.0 d

%Inhibition of dry weight, growth rate as caused by the test item after 7.0 d.

Treatm.[µg/L]	Mean	Std. Dev.	n	%Inhibition
1.81	0.000	n.d.	1	100.0
2.98	2.600	n.d.	1	97.4
6.15	12.800	n.d.	1	87.2
14.12	31.700	n.d.	1	68.3
28.23	40.800	n.d.	1	59.2
47.09	43.800	n.d.	1	56.2

Probit analysis using linear max. likelihood regression

Probit analysis using linear max. likelihood regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of replicates; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [µg/L]	Log(x)	%Inhibition	n	Emp. Probit	Weight	Reg. Probit
1.81	0.258	0.00	1	-1.2533	0.047	-1.749
2.98	0.475	2.60	1	-1.1881	0.110	-1.486
6.15	0.789	12.80	1	-0.9325	0.295	-1.105
14.12	1.150	31.70	1	-0.4587	0.642	-0.666
28.23	1.451	40.80	1	-0.2306	0.914	-0.3
47.09	1.673	43.80	1	-0.1554	0.999	-0.03

excluded: value not in line with the chosen function

Parameters of the probit analysis

Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	6.0000
Slope b:	1.2148
Intercept a:	-2.06268
Variance of b:	2.6877
Goodness of Fit	
Chi²:	0.0677
Degrees of freedom:	4.0000
p(Chi²):	0.9994
Log EC50:	1.6979
SE Log EC50:	0.6762
g-Criterion:	0.2375
Variance (Chi²/df):	0.0169
r²:	0.8900
F:	32.4420
p(F) (df: 1;4):	0.0050

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).
The coefficient of determination, r^2 ($0 <= r^2 <= 1$), gives the proportion of variance explained by the dose/response function.
F-Test for regression: Ho: Slope = 0; hence, if p(F) <= alpha, the selected significance level, (e.g., alpha = 0.05) the regression revealed significant results (= slope is significantly different from zero).

Results of the probit analysis

Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC10	EC20	EC50
Value [µg/L]	4.40	10.12	49.88
lower 95%-cl	0.87	4.07	32.22
upper 95%-cl	8.19	15.54	128.85
lower 99%-cl	0.41	2.62	20.41
upper 99%-cl	17.14	24.16	203.36

n d.: not determined due to mathematical reasons or inappropriate data
Computation of variances and confidence limits was adjusted to metric data (Christensen & Nyholm 1984).
Slope function after Litchfield and Wilcoxon: 6.655

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

The following study was conducted, as additional data was required for the refined risk assessment for aquatic plants.

Report:	CA 8.2.7/3 Dorner S., 2013c Effect of Reg.No. 4096483 (BAS 720 H, Imazamox) on the growth of Lemna gibba in presence of sediment 2013/1246583
Guidelines:	OECD 221, EPA 850.4400, ASTM E 1415-91
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of imazamox on the growth of the aquatic plant *Lemna gibba* was studied in a 7-day static toxicity test in the presence of sediment. The following nominal concentrations were applied: 0 (control), 0.0030, 0.0060, 0.012, 0.024 and 0.048 mg imazamox/L. Assessment of plant growth (counting visible fronds) was conducted on days 2, 5 and 7. Percent growth inhibition relative to the control was calculated for each test concentration based upon growth rates and final yield for the parameters frond number and plant dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 575 fronds per vessel after 7 days, corresponding to a 52 x multiplication. The dry weight increased from 1.6 mg to an average of 75 mg per vessel in the control at test termination. No morphological changes were observed in the control group and at imazamox concentrations of up to and including 0.0060 mg/L. At 0.012, 0.024 and 0.048 mg/L, about 50% of the fronds showed discoloration. At 0.024 and 0.048 mg/L additionally, bulging was observed.

In a 7-day aquatic-plant test with *Lemna gibba* in the presence of sediment, the E_rC_{50} of imazamox was determined to be 0.022 mg a.s./L based on frond numbers and 0.061 mg a.s./L based on dry weight (nominal). The E_yC_{50} was 0.0099 mg a.s./L based on frond no. and 0.010 mg a.s./L based on dry weight (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483), batch no. COD-001579, purity 98.9%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system (including sediment); test duration 7 days; 5 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 2, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield related to frond numbers and dry weight after exposure over 7 days.

Test concentrations: Control, 0.0030, 0.0060, 0.012, 0.024 and 0.048 mg imazamox/L (nominal).

Test conditions: 400 mL glass beakers, test volume: 160 mL 20x-AAP nutrient medium and 100 g standard artificial sediment (OECD 219), pH of nutrient solution 7.51 - 7.56 at test initiation and pH 8.58 - 8.71 at test termination; pH of sediment 7.47 at test initiation; water temperature: 23.8 °C - 24.1 °C, continuous light, light intensity: about 8300 lux.

Analytics: Analytical verification of the test item was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics; probit analysis for EC_x calculations.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for imazamox ranged from 94% to 100% of nominal concentrations at test initiation and from 74% to 80% of nominal at test termination. As the initially measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 575 fronds per vessel after 7 days, corresponding to a 52 x multiplication. The dry weight increased from 1.6 mg to an average of 75 mg per vessel in the control at test termination. No morphological changes were observed in the control group and at imazamox concentrations of up to and including 0.0060 mg/L. At 0.012, 0.024 and 0.048 mg/L about 50% of the fronds showed discoloration. At 0.024 and 0.048 mg/L additionally, bulging was observed. Effects on growth rate and yield are summarized in Table 8.2.7-3.

Table 8.2.7-3: Effect of imazamox on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**0.0030**	**0.0060**	**0.012**	**0.024**	**0.048**
Inhibition after 7 d [%] * (growth rate based on frond no.)	-0.5	0.5	37.8	58.1	68.5
Inhibition after 7 d [%] * (yield based on frond no.)	-2.1	2.0	79.0	91.4	95.2
Inhibition after 7 d [%] (growth rate based on dry weight)	3.8	6.6	29.3	35.0	41.6
Inhibition after 7 d [%] (yield based on dry weight)	14.4	23.2	68.9	75.5	81.6
	Endpoints [mg imazamox/L] (nominal)				
E_rC_{50} (7 d) based on frond no	0.022 (95% confidence limits: 0.010 - > 0.048)				
E_rC_{10} (7 d) based on frond no	0.0054 (95% confidence limits:0.000 - 0.011)				
E_yC_{50} (7 d) based on frond no	0.0099 (95% confidence limits: n.d)				
E_yC_{10} (7 d) based on frond no	0.0072 (95% confidence limits: n.d)				
E_rC_{50} (7 d) based on dry weight	0.060 (95% confidence limits: 0.030 - > 0.048)				
E_rC_{10} (7 d) based on dry weight	0.0045 (95% confidence limits: 0.000 - 0.011)				
E_yC_{50} (7 d) based on dry weight	0.010 (95% confidence limits: 0.0036 - 0.026)				
E_yC_{10} (7 d) based on dry weight	0.0023 (95% confidence limits: 0.0000 - 0.0052)				

* Negative values indicate stimulated growth compared to the control.
n.d = confidence limits could not be determined

III. CONCLUSION

In a 7-day aquatic-plant test with *Lemna gibba* in the presence of sediment, the E_rC_{50} of imazamox was determined to be 0.022 mg a.s./L based on frond numbers and 0.060 mg a.s./L based on dry weight (nominal). The E_yC_{50} was 0.0099 mg a.s./L based on frond no. and 0.010 mg a.s./L based on dry weight (nominal).

The following study was conducted, as additional data was required for the refined risk assessment for aquatic plants.

Report:	CA 8.2.7/4 Backfisch K., 2013f Effect of BAS 720 H (Imazamox) on the growth of the aquatic plant *Spirodela polyrhiza* 2013/1246580
Guidelines:	OECD 221, OECD 219, ASTM E 1913-04
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of imazamox on the growth of the aquatic plant *Spirodela polyrhiza* was studied in a 11-day static toxicity test in the presence of sediment. The following nominal concentrations were applied: 0 (control), 0.010, 0.030, 0.10, 0.30 and 1.0 mg a.s./L. Assessment of plant growth based on frond numbers and visual observations were conducted twice during the study and at the end of the test. Additionally, plant dry weight was recorded at test termination. Percent growth inhibition relative to the control was calculated for each test concentration based upon growth rates and final yield for the parameters frond number and plant dry weight.

The biological results are based on nominal concentrations. The aquatic plant populations in the control vessels showed sufficient growth, increasing from 10 fronds per vessel to an average of 40.7 fronds per vessel after 11 days, corresponding to a 4 x multiplication and a doubling time of 5.6 days. The dry weight increased from 9.3 mg to an average of 18.6 mg per vessel in the control at test termination. No morphological changes were observed in the control group and at any of the test item concentrations tested.

In a 11-day aquatic-plant test with *Spirodela polyrhiza,* the E_rC_{50} of imazamox was determined to be 0.085 mg a.s./L based on frond numbers and > 1.0 mg a.s./L based on dry weight (nominal). The E_yC_{50} was 0.051 mg a.s./L based on frond no. and > 1.0 mg a.s./L based on dry weight (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H; Reg. no.: 4 096 483), batch no. COD-001579, purity: 98.9%.

B. STUDY DESIGN

Test species:	*Spirodela polyrhiza* (Araceae), a monocotyledonous aquatic plant species, cultivated in-house after purchase from the plant nursery "Petrowsky" Eschede, Germany.
Test design:	Static system (including sediment); test duration 11 days; 5 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; 1 plant with 4 fronds and 2 plants with 3 fronds, total number of fronds at test initiation: 10 per replicate; assessment of growth and other effects on days 4, 7 and 11; plant dry weight was recorded at test termination.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield related to frond numbers and dry weight after exposure over 11 days.
Test concentrations:	Control, 0.010, 0.030, 0.10, 0.30, 1.0 mg imazamox/L (nominal).
Test conditions:	1000 mL glass beakers and petri-dishes (Ø 8.5 cm), test volume: 600 mL 5 x-AAP nutrient medium and standard artificial sediment (OECD 219), pH of nutrient solution 7.0 at test initiation and 9.0 - 9.8 at test termination; pH of sediment 6.76 at test initiation; water temperature: 20.6 °C - 23.2 °C, oxygen saturation: 85% - 143%; light : dark - rhythm 16 : 8 h , light intensity: about 9450 - 10610 lux.
Analytics:	Analytical verification of the test item was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics; probit analysis for EC_{50} calculations, determination of NOEC values using appropriate statistical tests (*e.g.* Welch-t-test etc.).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for imazamox ranged from 105% to 132% of nominal concentrations at test initiation and from 117% to 155% of nominal at test termination. As the initially measured concentrations confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The aquatic plant populations in the control vessels showed sufficient growth, increasing from 10 fronds per vessel to an average of 40.7 fronds per vessel after 11 days, corresponding to a 4 x multiplication and a doubling time of 5.6 days. The dry weight increased from 9.3 mg to an average of 18.6 mg per vessel in the control at test termination. No morphological changes were observed in the control group and at any of the test item concentrations tested. Effects on growth rate and yield are summarized in Table 8.2.7-4.

Table 8.2.7-4: Effect of imazamox on the growth of the aquatic plant *Spirodela polyrhiza*

Concentration [mg a.s./L](nominal)	**0.010**	**0.030**	**0.10**	**0.30**	**1.0**
Inhibition after 11 d [%] * (growth rate based on frond no.)	-4.2	19.3	59.5	83.1	86.9
Inhibition after 11 d [%] * (yield based on frond no.)	-4.3	33.7	75.0	91.3	93.5
Inhibition after 11 d [%] * (growth rate based on dry weight)	-37.8	-35.7	-9.8	3.3	46.6
Inhibition after 11 d [%] * (yield based on dry weight)	-47.7	-42.1	-6.6	10.8	52.1

	Endpoints [µmg imazamox/L] (nominal)
E_rC_{50} (11 d) based on frond no	0.085 (95% confidence limits: 0.045 - 0.16)
E_rC_{10} (11 d) based on frond no	0.016 (95% confidence limits: 0.0015 - 0.034)
E_yC_{50} (11 d) based on frond no	0.051 (95% confidence limits: 0.032 - 0.079)
E_yC_{10} (11 d) based on frond no	0.013 (95% confidence limits: 0.0030 - 0.023)
E_rC_{50} / E_yC_{50} (11 d) based on dry weight	> 1.0 [1)]
E_rC_{10} (11 d) based on dry weight	0.10 (95% confidence limits: 0.0 - 0.53)
E_yC_{10} (11 d) based on dry weight	0.088 (95% confidence limits: 0.0037 - 0.25)

* Negative values indicate stimulated growth compared to the control.
1) Confidence limits could not be calculated.

III. CONCLUSION

In a 11-day aquatic-plant test with *Spirodela polyrhiza,* the E_rC_{50} of imazamox was determined to be 0.085 mg a.s./L based on frond numbers and > 1.0 mg a.s./L based on dry weight (nominal). The E_yC_{50} was 0.051 mg a.s./L based on frond no. and > 1.0 mg a.s./L based on dry weight (nominal).

Remarks: The study on the non-standard species *Spirodela polyrhiza* was performed considering the general recommendations given in the OECD Guideline 221 which, however, specially refers to freshwater aquatic plants of the genus *Lemna* (duckweed; *e.g. the standard species Lemna gibba* and *Lemna minor*). This guideline is not explicitly referenced as "one of the guideline covered" (*i.e.* see page 9 of the study report: "*The test design is partly based on existing guidelines* (*OECD 221, etc…*"). Moreover, this guideline cannot be adopted "one-to-one" for non-standard species as especially the intrinsic growth rate can differ significantly among different aquatic plant species. Thus, the doubling time of *Spirodela polyrhiza* can be widely different from the value given in the guideline for *Lemna* sp. and cannot be used as validity criteria for other species. The study is thus considered to be valid and it is considered justified to use the results of the study for higher tier risk assessment.

The following study was conducted, as additional data was required for the refined risk assessment for aquatic plants.

Report:	CA 8.2.7/5 Backfisch K., 2013g Effect of BAS 720 H (Imazamox) on the growth of the aquatic plant Ceratophyllum demersum 2013/1246581
Guidelines:	OECD 221, OECD 219, ASTM E 1913-04
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of imazamox on the growth of the aquatic plant *Ceratophyllum demersum* was studied in an 8-day static toxicity test. The following nominal concentrations were applied: 0 (control), 0.010, 0.030, 0.10, 0.30 and 1.0 mg a.s./L. At test initiation the total length and the fresh weight of the plants were recorded. In addition, determination of total length, shoot length, side shoot length, number of side shoots and visual observations were conducted twice during the 8 d exposure period and at test end. Plant wet weight and dry weight was recorded at test termination. The starting dry weight was obtained by mathematical calculation. The percentage inhibition relative to the control was calculated for each test concentration based upon growth rates and final yield for all parameters.

The biological results are based on nominal concentrations. Statistically significant differences in total shoot length and wet weight based on growth rate and yield and in main shoot length based on growth rate were observed at the four highest tested concentrations of 0.030, 0.10, 0.30 and 1.0 mg a.s./L. Main shoot length based on yield was statistically significantly reduced in all tested concentrations. Statistically significant differences in yield related to side shoot length and number of side shoots occurred at the three highest test item concentrations. No morphological changes, discolorations or other unusual observations were noted during and at the end of the test. From day seven on, growth of filamentous algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study.

In a 8-day aquatic-plant test with *Ceratophyllum demersum*, the E_rC_{50} of imazamox was determined to be 0.063 mg a.s./L based on total shoot length, 0.050 mg a.s./L based on wet weight, > 1.0 mg a.s./L based on dry weight and 0.074 mg a.s./L based on main shoot length (nominal). The E_yC_{50} was 0.031 mg a.s./L based on total shoot length, 0.030 mg a.s./L based on wet weight, > 1.0 mg a.s./L based on dry weight, 0.024 mg a.s./L based on main shoot length, 0.029 mg a.s./L based on side shoots length and 0.021 mg a.s./L based on number of side shoots (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H; Reg. no.: 4 096 483), batch no. COD-001579, purity: 98.9%.

B. STUDY DESIGN

Test species: *Ceratophyllum demersum* (Ceratophyllaceae), a dicotyledonous aquatic plant species, cultivated in-house (non-GLP) after purchase from the plant nursery "Petrowsky" Eschede, Germany.

Test design: Static system (including sediment); test duration 8 days; 5 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; three uniform plants (same size) per replicate; application via water phase; assessment of plant growth and visual effects were conducted at test initiation, twice during the study and at the end of the study; plant wet weight and dry weight were recorded at test termination.

Endpoints: EC_{50} and NOEC with respect to growth rate and yield related to wet weight, dry weight, shoot length, side shoot length and number of side shoots after 8 days of exposure.

Test concentrations: Control, 0.010, 0.030, 0.10, 0.30 and 1.0 mg imazamox/L (nominal).

Test conditions: 2.0 L glass beakers and flower pots (Ø 9 cm), standard artificial sediment (OECD 219) and 1.8 L Smart & Barko medium (pH 7.72 at test initiation); oxygen saturation: 87.1% - 148.2%; pH 7.63 - 7.70 at test initiation and 9.36 - 9.82 at test termination; conductivity: 285 µS/cm at test initiation; water temperature: 20.9 °C - 22.6 °C; light : dark - rhythm 16 : 8 h, light intensity: 9.43 - 10.51 klux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics; probit analysis using linear max. likelihood regression for EC_{50} calculations; appropriate statistical tests (Welch t-test, Williams-test,) for determination of the NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in each concentration at the beginning and at the end of the test. At test initiation the analytical samples were taken from the respective bulk solutions and at the end from mixed samples (pooled replicates of each treatment). The analytically determined concentrations of imazamox ranged from 108% to 113% of nominal concentrations at test initiation and from 106% to 119% nominal at test termination. As the analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: Statistically significant differences in total shoot length and wet weight based on growth rate and yield and in main shoot length based on growth rate were observed at the four highest tested concentrations of 0.030, 0.10, 0.30 and 1.0 mg a.s./L (Welch t-test, $\alpha = 0.05$). Main shoot length based on yield was statistically significantly reduced in all tested concentrations. Statistically significant differences in yield related to side shoot length and number of side shoots occurred at the three highest test item concentrations. No morphological changes, discolorations or other unusual observations were noted during and at the end of the test. From day seven on, growth of filamentous algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study. The results are summarized in Table 8.2.7-5.

Table 8.2.7-5: Effect of imazamox on the growth of the aquatic plant *Ceratophyllum demersum*

Concentration [mg imazamox/L] (nominal)	**0.010**	**0.030**	**0.10**	**0.30**	**1.0**
Inhibition in 8 d [%] (growth rate based on total shoot length)	16.6	46.6 *	72.4 *	59.5 *	74.7 *
Inhibition in 8 d [%] (yield based on total shoot length)	24.2	54.5 #	79.5 #	72.0 #	85.1 #
Inhibition in 8 d [%] (growth rate based on wet weight)	18.6	57.9 *	59.5 *	72.5 *	72.3 *
Inhibition in 8 d [%] (yield based on wet weight)	19.5	66.8 #	69.5 #	79.0 #	76.5 #
Inhibition in 8 d [%] 1) (growth rate based on dry weight)	-11.9	21.4	-6.2	45.1	-11.2
Inhibition in 8 d [%] 1) (yield based on dry weight)	-24.1	23.1	-8.6	43.6	6.3
Inhibition in 8 d [%] (growth rate based on main shoot length)	29.2	46.5 *	65.9 *	50.6 *	66.2 *
Inhibition in 8 d [%] (yield based on main shoot length)	37.1 *	54.3 *	73.0 *	62.2 *	76.5 *
Inhibition in 8 d [%] (yield based on side shoot length)	5.8	54.9	88.7 *	85.9 *	97.2 *
Inhibition in 8 d [%] (yield based on number of side shoots)	32.9	56.5	91.3 *	82.6 *	91.3 *

	Endpoints [mg imazamox/L] (nominal)
$E_rC_{50\ total\ shoot\ length}$ (8 d)	0.063 [2)]
$E_yC_{50\ total\ shoot\ length}$ (8 d)	0.031 (95% confidence limits: 0.0 - 0.17)
NOE_rC / $NOE_yC_{\ total\ shoot\ length}$ (8 d)	0.010
$E_rC_{50\ wet\ weight}$ (8 d)	0.050 [2)]
$E_yC_{50\ wet\ weight}$ (8 d)	0.030 [2)]
NOE_rC / $NOE_yC_{\ wet\ weight}$ (8 d)	0.010
$E_rC_{50\ dry\ weight}$ (8 d)	> 1.0 [2)]
$E_yC_{50\ dry\ weight}$ (8 d)	> 1.0 [2)]
NOE_rC / $NOE_yC_{\ dry\ weight}$ (8 d)	≥ 1.0
$E_rC_{50\ main\ shoot\ length}$ (8 d)	0.074 [2)]
$E_yC_{50\ main\ shoot\ length}$ (8 d)	0.024 [2)]
$NOE_rC_{\ main\ shoot\ length}$ (8 d)	0.010
$NOE_yC_{\ main\ shoot\ length}$ (8 d)	≤ 0.010
$E_yC_{50\ side\ shoot\ length}$ (8 d)	0.029 (95% confidence limits: 0.0080 - 0.17)
$NOE_yC_{\ side\ shoots\ length}$ (8 d)	0.030
$E_yC_{50\ number\ of\ side\ shoots}$ (8 d)	0.021 (95% confidence limits: 0.0010 - 0.06)
$NOE_yC_{\ number\ of\ side\ shoots}$ (8 d)	0.030

* Statistically significant differences compared to control (Welch t-test with Bonferroni-Holm Adjustment, multiple comparison, $\alpha = 0.05$).

[#] Statistically significant differences compared to control (Welch t-test, pairwise comparison, $\alpha = 0.05$).

1) Negative values indicate stimulated growth compared to the control.

2) Confidence limits could not be calculated.

III. CONCLUSION

In a 8-day aquatic-plant test with *Ceratophyllum demersum*, the E_rC_{50} of imazamox was determined to be 0.063 mg a.s./L based on total shoot length, 0.050 mg a.s./L based on wet weight, > 1.0 mg a.s./L based on dry weight and 0.074 mg a.s./L based on main shoot length (nominal). The E_yC_{50} was 0.031 mg a.s./L based on total shoot length, 0.030 mg a.s./L based on wet weight, > 1.0 mg a.s./L based on dry weight, 0.024 mg a.s./L based on main shoot length, 0.029 mg a.s./L based on side shoots length and 0.021 mg a.s./L based on number of side shoots (nominal).

The following study was conducted, as additional data was required for the refined risk assessment for aquatic plants.

Report:	CA 8.2.7/6 Backfisch K., 2013h Effect of BAS 720 H (Imazamox) on the growth of the aquatic plant Glyceria maxima 2013/1246582
Guidelines:	OECD 221, OECD 219, ASTM E 1913-04
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of imazamox on the growth of the aquatic plant *Glyceria maxima* was studied in a 10-days static toxicity test. The following nominal concentrations were applied: 0 (control), 0.010, 0.030, 0.10, 0.30 and 1.0 mg a.s./L. Assessment of growth based on the number and length of the plant leaves was conducted at the beginning of the test, twice during the test and at test end. Plant wet weight was recorded on day 0 and day 10. Visual observations were performed on days 4, 7 and 10. At test termination, plant dry weight was determined and the development of plant roots was assessed. The percentage inhibition relative to the control was calculated for each test concentration based upon growth rates and final yield for the measured parameters.

The biological results are based on nominal concentrations. Statistically significant differences in yield based on total length and wet weight and in growth rate based on wet weight and in number of leaves compared to the control were observed at the tested concentration of 0.030 mg a.s./L and at all higher concentrations. Total length based on growth rate was statistically significantly reduced compared to the control in all test item treatments except for the lowest and the highest tested concentration. Statistically significant differences in growth rate based on dry weight occurred only at the test item concentration of 0.30 mg a.s./L. Yield based on dry weight was statistically significantly reduced at the two highest tested concentrations of 0.30 and 1.0 mg a.s./L. At the end of the test, all plants showed partial necrosis in the tested concentrations of 0.10 and 0.30 mg a.s./L and in the highest test item treatment, all plants were necrotic.

In a 10-day aquatic-plant test with *Glyceria maxima*, the E_rC_{50} of imazamox was determined to be 0.032 mg a.s./L based on total length, 0.069 mg a.s./L based on wet weight and 0.48 mg a.s./L based on dry weight (nominal). The E_yC_{50} was 0.021 mg a.s./L based on total length, 0.032 mg a.s./L based on wet weight, 0.10 based on dry weight and 0.021 mg a.s./L based on number of leaves (nominal). The overall NOEC was 0.010 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Imazamox (BAS 720 H, Reg. no. 4 096 483); batch no. COD-001579; purity 98.9%.

B. STUDY DESIGN

Test species: *Glyceria maxima* (Poaceae), a monocotyledonous aquatic plant species, cultivated in-house (non-GLP) after purchase from the plant nursery "Petrowsky" Eschede, Germany.

Test design: Static system (including sediment); test duration 10 days; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; one grass blade with 2 - 4 leaves per replicate; assessment of plant growth and visual observations during the test and at test end; plant wet weight was recorded at the beginning of the test and at test termination; dry weight and development of plant roots was recorded at test termination.

Endpoints: EC_{50} and NOEC with respect to growth rate and yield related to wet weight, dry weight, total length as well as number of leaves after 10 days of exposure.

Test concentrations: Control, 0.010, 0.030, 0.10, 0.30 and 1.0 mg imazamox/L (nominal).

Test conditions: 2.0 L glass beakers and flower pots (Ø 9 cm), standard artificial sediment (OECD 219, pH 6.76) and 800 mL Smart & Barko medium (pH 7.37 at test initiation); oxygen saturation: 95.0% - 96.7% at test initiation and 105.5% - 112.8% at test termination; pH 7.37 - 7.44 at test initiation and 6.94 - 7.06 at test termination; conductivity: 279 - 285 µS/cm; water temperature: 20.4 °C - 23.4 °C; light : dark - rhythm 16 : 8 h, light intensity: 10 klux ± 2 klux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection. At test initiation, the analytical samples were taken from the bulk solutions of each test concentration and at the end from mixed samples (pooled replicates of each treatment).

Statistics: Descriptive statistics; probit analysis using linear max. likelihood regression for EC_{50} calculations; appropriate statistical tests depending on respective data sets (*i.e.*, Student t-test, Welch t-test or William's Multiple Sequential t-test Procedure; $\alpha = 0.05$) for determination of the NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of active substance concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically determined concentrations of imazamox ranged from 96% to 109% (average 104%) of nominal concentrations at test initiation and from 103% to 118% (average 111%) nominal at test termination. As analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: Statistically significant differences in yield based on total length and wet weight (Welch t-test, $\alpha = 0.05$) and in growth rate based on wet weight and in number of leaves (William's Multiple Sequential t-test Procedure, $\alpha = 0.05$) compared to the control were observed at the tested concentration of 0.030 mg a.s./L and at all higher concentrations. Total length based on growth rate was statistically significantly reduced compared to the control in all test item treatments except for the lowest and the highest tested concentration (Student t-test, $\alpha = 0.05$). Statistically significant differences in growth rate based on dry weight occurred only at the test item concentration of 0.30 mg a.s./L (Student t-test, $\alpha = 0.05$). Yield based on dry weight was statistically significantly reduced at the two highest tested concentrations of 0.30 and 1.0 mg a.s./L (William's Multiple Sequential t-test Procedure, $\alpha = 0.05$). At the end of the test, all plants showed partial necrosis in the tested concentrations of 0.10 and 0.30 mg a.s./L and in the highest test item treatment, all plants were necrotic. The effects on growth rate and yield for all measured parameters are summarized in Table 8.2.7-6.

Table 8.2.7-6: Effect of imazamox on the growth of the aquatic plant *Glyceria maxima*

Concentration [mg imazamox/L] (nominal)	**0.010**	**0.030**	**0.10**	**0.30**	**1.0**
Inhibition in 10 d [%] [1)] (growth rate based on total length)	-10.9	77.9 *	71.5 *	79.9 *	88.4
Inhibition in 10 d [%] (yield based on total length)	5.7	87.7 #	82.2 #	89.9 #	96.1 #
Inhibition in 10 d [%] [1)] (growth rate based on wet weight)	-14.5	39.3 +	52.1 +	95.8 +	75.6 +
Inhibition in 10 d [%] (yield based on wet weight)	6.2	59.1 #	75.8 #	97.2 #	87.0 #
Inhibition in 10 d [%] [1)] (growth rate based on dry weight)	-14.7	22.1	32.9	56.0 *	48.9
Inhibition in 10 d [%] [1)] (yield based on dry weight)	-11.8	39.1	50.9	72.9 +	74.5 +
Inhibition in 10 d [%] (yield based on number of leaves)	5.9	88.2 +	88.2 +	76.5 +	100 +

	Endpoints [mg imazamox/L] (nominal)
$E_rC_{50\ total\ length}$ (10 d)	0.032 (95% confidence limits: n.d.)
$E_yC_{50\ total\ length}$ (10 d)	0.021 (95% confidence limits: n.d.)
NOE_rC / $NOE_yC_{\ total\ length}$ (10 d)	0.010
$E_rC_{50\ wet\ weight}$ (10 d)	0.069 (95% confidence limits: n.d.)
$E_yC_{50\ wet\ weight}$ (10 d)	0.032 (95% confidence limits: n.d.)
NOE_rC / $NOE_yC_{\ wet\ weight}$ (10 d)	0.010
$E_rC_{50\ dry\ weight}$ (10 d)	0.48 (95% confidence limits: n.d.)
$E_yC_{50\ dry\ weight}$ (10 d)	0.10 (95% confidence limits: 0.0060 - > 1.0)
$NOE_rC_{\ dry\ weight}$ (10 d)	≥ 1.0
$NOE_yC_{\ dry\ weight}$ (10 d)	0.10
$E_yC_{50\ no\ of\ leaves}$ (10 d)	0.021 (95% confidence limits: n.d.)
$NOE_yC_{\ no\ of\ leaves}$ (10 d)	0.010

n.d. =confidence limits could not be determined.

* Statistically significant differences compared to control (Student t-test, $\alpha = 0.05$)

\# Statistically significant differences compared to control (Welch t-test, $\alpha = 0.05$)

\+ Statistically significant differences compared to control (William's Multiple Sequential t-test Procedure, $\alpha = 0.05$)

1) Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 10-day aquatic-plant test with *Glyceria maxima*, the E_rC_{50} of imazamox was determined to be 0.032 mg a.s./L based on total length, 0.069 mg a.s./L based on wet weight and 0.48 mg a.s./L based on dry weight (nominal). The E_yC_{50} was 0.021 mg a.s./L based on total length, 0.032 mg a.s./L based on wet weight, 0.10 based on dry weight and 0.021 mg a.s./L based on number of leaves (nominal). The overall NOEC was 0.010 mg a.s./L, based on nominal concentrations.

The following study is required, due to new data requirements.

Report:	CA 8.2.7/7 Baetscher R., 2007b CL 312622 (metabolite of BAS 720 H): Toxicity to the aquatic higher plant Lemna Gibba in a 7-day static growth inhibition test 2006/1030257
Guidelines:	OECD 221
GLP:	Yes (certified by Swiss Agency for the environment, forests, and landscape; Berne, Switzerland)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of CL 312622 (metabolite of imazamox) on the growth of the duckweed *Lemna gibba* was investigated. The following concentrations were applied: 0 (control), 0.30, 0.89, 2.8, 8.8, 28 and 92 mg CL 312622/L (mean measured). Assessment of growth and other effects was conducted 2, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and mean biomass data for the parameters frond number and dry weight.

The biological results are based on mean measured concentrations. The growth rates calculated on the basis of frond numbers and dry weights of the plants were statistically significantly reduced compared to the control at the test item concentration of 0.89 mg/L and at all higher tested concentrations. The yield calculated on the basis of frond numbers and dry weights was statistically significantly lower compared to the control at all tested concentrations. No morphological effects on the test plants were recorded in the control and at concentrations of up to and including 0.89 mg CL 312622/L. At the test item concentrations of 2.8 and 8.8 mg/L, the fronds were smaller and stunted. At 28 and 92 mg/L, chlorosis was determined in addition to the effects recorded at the lower tested concentrations.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} of CL 312622 (metabolite of imazamox) based on frond no. was determined to be the 6.3 mg/L and the E_yC_{50} was 2.8 mg/L (mean measured). The E_rC_{50} of CL 312622 based on dry weight was determined to be the 59.0 mg/L and the E_yC_{50} was 4.5 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: CL 312622 (metabolite of imazamox, Reg. No. 4 110 542), batch no. AC10194-40A, purity: 86%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 days old cultures; cultures maintained in-house; stock obtained from "Syngenta AG, Ecological Science", Basel, Switzerland.

Test design: Static system (7 days); 7 treatment groups (6 test item concentrations, control) with 3 replicates for the test item treatments and the control; 3 plants with 4 fronds, total number of fronds at test initiation: 12 per replicate; assessment of growth and other effects on days 2, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control, 0 (control), 0.30, 0.89, 2.8, 8.8, 28 and 92 mg CL 312622/L (mean measured); equivalent to dilution 1:320, 1:100, 1:32, 1:10, 1:3.2 and the undiluted filtrate.

Test conditions: 250 mL glass dish, test volume 150 mL, 20x-AAP nutrient medium, pH 7.4 - 8.9; water temperature: 23 °C, continuous light, average light intensity: about 7200 lux - 8320 lux.

Analytics: Analytical verification of the test item concentrations was conducted using an HPLC-method with UV/VIS detection.

Statistics: Descriptive statistics; multiple Dunnett-test for determination of the NOEC (one-sided, $\alpha = 0.05$); probit analysis for determination of the EC_X values.

II. RESULTS AND DISCUSSION

Analytical measurements: The analytically determined concentrations of the test item in the test media at the start of the study were 0.31 mg/L (dilution 1:320), 0.95 mg/L (dilution 1:100), 3.1 mg/L (dilution 1:32), 9.5 mg/L (dilution 1:10), 30 mg/L (dilution 1:3.2) and 97 mg/L (undiluted filtrate). Thus, almost the whole amount of test item was dissolved in the undiluted filtrate. At the end of the test, 86% to 94% of the initially measured values were found in these test media. Thus, the test item was satisfactorily stable during the study under the test conditions. The following biological results are based on mean measured concentrations calculated as geometric means of the concentrations measured at the start and the end of the test.

Biological results: The growth rates calculated on the basis of frond numbers and dry weights of the plants were statistically significantly reduced compared to the control at the test item concentration of 0.89 mg/L and at all higher tested concentrations (Dunnett-tests, $\alpha = 0.05$). The yield calculated on the basis of frond numbers and dry weights was statistically significantly lower compared to the control at all tested concentrations. No morphological effects on the test plants were recorded in the control and at concentrations of up to and including 0.89 mg CL 312622/L. At the test item concentrations of 2.8 and 8.8 mg/L, the fronds were smaller and stunted. At 28 and 92 mg/L, chlorosis was determined in addition to the effects recorded at the lower tested concentrations. Effects on growth rate and yield are summarized in Table 8.2.7-7.

Table 8.2.7-7: Effect of CL 312622 (metabolite of imazamox) on the growth of duckweed *Lemna gibba*

Concentration [mg/L] (mean measured)	**0.30**	**0.89**	**2.8**	**8.8**	**28**	**92**
Inhibition after 7 d [%] (growth rate based on frond no.)	3.2	5.1*	21.9*	70.6*	80.9*	84.9*
Inhibition after 7 d [%] (growth rate based on dry weight)	3.0	6.9*	17.9*	35.2*	41.8*	52.0*
Inhibition after 7 d [%] (yield based on frond no.)	8.8*	13.6*	47.4*	91.2*	95.1*	96.3*
Inhibition after 7 d [%] (yield based on dry weight)	9.1*	20.0*	44.3*	69.3*	75.7*	83.5*
	Endpoints [mg CL 312622/L] (mean measured)					
E_rC_{50} (7 d) based on frond no	6.3 (95% confidence limits: 2.9 - 14)					
E_rC_{10} (7 d) based on frond no	1.1 (95% confidence limits: 0.04 - 2.5)					
E_yC_{50} (7 d) based on frond no	2.8 (95% confidence limits: 2.0 - 3.8)					
E_yC_{10} (7 d) based on frond no	0.76 (95% confidence limits: 0.29 - 1.2)					
E_rC_{50} (7 d) based on dry weight	59 (95% confidence limits: 32 - 159)					
E_rC_{10} (7 d) based on dry weight	0.79 (95% confidence limits: 0.12 - 2.0)					
E_yC_{50} (7 d) based on dry weight	4.5 (95% confidence limits: 2.6 - 7.9)					
E_yC_{10} (7 d) based on dry weight	0.24 (95% confidence limits: 0.04 - 0.58)					
$NOEC_r$ (7 d) based on frond no & dry weight	0.30					
$NOEC_y$ (7 d) based on frond no & dry weight	< 0.30					

* Statistically significant differences compared to control ((Dunnett-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} of CL 312622 (metabolite of imazamox) based on frond no. was determined to be the 6.3 mg/L and the E_yC_{50} was 2.8 mg/L (mean measured). The E_rC_{50} of CL 312622 based on dry weight was determined to be the 59 mg/L and the E_yC_{50} was 4.5 mg/L (mean measured).

The following study is required, due to new data requirements.

Report:	CA 8.2.7/8 Rzodeczko H., 2011b Reg.No. 4110603 (metabolite of BAS 720 H, Imazamox, CL 354825) - Lemna gibba L. CPCC 310 - Growth inhibition test 2011/1150030
Guidelines:	OECD 221
GLP:	Yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 7-day semi-static toxicity laboratory study, the effect of CL 354825 (metabolite of imazamox) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0.4, 1.0, 2.6, 6.4, 16.0, 40.0 and 100.0 mg CL 354825/L (equivalent to time-weighted mean concentrations of 0.17, 0.37, 1.00, 2.89, 8.02, 22.38, 54.50 mg/L). Assessment of growth and other effects was conducted 2, 5 and 7 days after test initiation. The percentage of growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and final yield for the parameters frond number and dry weight (biomass).

The biological results are based on time-weighted mean concentrations. After 7 days of exposure no morphological changes were observed at time-weighted mean test item concentrations up to and including 2.89 mg CL 354825/L. At 8.02 mg/L, gibbosity spots of chlorosis were observed. At the two highest tested concentrations, gibbosity of fronds, overlapping fronds, smaller size of fronds and spots of chlorosis were of smaller size.

In a 7-day semi-static aquatic plant test with *Lemna gibba* the E_rC_{50} of CL 354825 (metabolite of imazamox) was 43.1 mg/L based on frond number and > 54.5 mg/L based on dry weight (based on time-weighted mean concentrations). The E_yC_{50} of CL 354825 was determined to be 10.5 mg/L based on frond number and 47.7 mg/L based on dry weight (time-weighted mean).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: CL 354825 (Reg. No. 4 110 603; metabolite of imazamox); batch no. L67-144; purity: 98.2%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 days old cultures; cultures maintained in-house; stock obtained from University of Waterloo, Canadian Phycological Culture Centre, Ontario, Canada.

Test design: Semi-static system (7 days); water renewal after 2 and 5 days; 8 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control, 0.4, 1.0, 2.6, 6.4, 16.0, 40.0 and100.0 mg CL 354825/L (nominal); corresponding to time-weighted mean concentrations of 0.17, 0.37, 1.00, 2.89, 8.02, 22.38, 54.50 mg/L.

Test conditions: 150 mL glass vessels, test volume 100 mL, 20x-AAP nutrient medium, pH 7.21 - 8.07 in fresh solutions and pH 8.79 - 9.33 in old solutions; water temperature: 23.6 °C - 24.5 °C, continuous light, light intensity: 8900 lux - 10550 klux.

Analytics: Analytical verification of the test item was conducted using a HPLC-method with DAD detection.

Statistics: Descriptive statistics; Probit analysis for determination of EC_x values; William's Multiple Sequential t-test Procedure for determination of NOEC ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in samples of fresh and spent solutions at test initiation, at each renewal and at test termination for all test concentrations. In fresh solutions, the measured concentrations of CL 354825 were between 97.0% and 107.5% of nominal concentrations. In spent solutions the CL 354825 content ranged from values below the LoQ to up to 36.3% of nominal. The following biological results are based on time-weighted mean concentrations.

Biological results: After 7 days of exposure no morphological changes were observed at time-weighted mean test item concentrations up to and including 2.89 mg CL 354825/L. At 8.02 mg/L, gibbosity spots of chlorosis were observed. At the two highest tested concentrations, gibbosity of fronds, overlapping fronds, smaller size of fronds and spots of chlorosis were of smaller size. Effects on growth rate and yield are summarized in Table 8.2.7-8.

Table 8.2.7-8: Effect of CL 354825 (metabolite of imazamox) on the growth of duckweed *Lemna gibba*

Concentration [mg/L] (nominal)	**0.4**	**1.0**	**2.6**	**6.4**	**16.0**	**40.0**	**100.0**
Concentration [mg/L] (time-weighted mean)	**0.17**	**0.37**	**1.00**	**2.89**	**8.02**	**22.38**	**54.50**
Inhibition after 7 d [%] * (growth rate based on frond no.)	0.9	6.9	6.2	12.5	19.7	34.7	57.8
Inhibition after 7 d [%] (growth rate based on dry weight)	0.0	4.7	1.4	4.0	4.7	12.3	26.1
Inhibition after 7 d [%] * (yield based on frond no.)	2.4	16.5	14.9	27.8	40.7	61.3	81.9
Inhibition after 7 d [%] (yield based on dry weight)	0.0	13.9	4.0	10.8	14.0	31.9	56.0
Endpoints [mg CL 354825/L] (time weighted mean)							
E_rC_{50} (7 d) based on frond no	43.1 (95% confidence limits: 30.7 - 70.8)						
E_rC_{10} (7 d) based on frond no	2.6 (95% confidence limits: 1.0 - 4.4)						
E_yC_{50} (7 d) based on frond no	10.5 (95% confidence limits: 6.9 - 16.7)						
E_yC_{10} (7 d) based on frond no	0.5 (95% confidence limits: 0.2 - 1.1)						
E_rC_{50} (7 d) based on dry weight	> 54.5						
E_rC_{10} (7 d) based on dry weight	15.3 (95% confidence limits: 6.4 - 22.5)						
E_yC_{50} (7 d) based on dry weight	47.7 (95% confidence limits: 27.7 - 178.5)						
E_yC_{10} (7 d) based on dry weight	4.0 (95% confidence limits: 0.3 - 8.8)						

III. CONCLUSION

In a 7-day semi-static aquatic plant test with *Lemna gibba* the E_rC_{50} of CL 354825 (metabolite of imazamox) was 43.1 mg/L based on frond number and > 54.5 mg/L based on dry weight (based on time-weighted mean concentrations). The E_yC_{50} of CL 354825 was determined to be 10.5 mg/L based on frond number and 47.7 mg/L based on dry weight (time-weighted mean).

CA 8.2.8 Further testing on aquatic organisms

No further studies required; thus, this point is not addressed *via* new toxicity studies.

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CA 8.3 Effects on arthropods

Table 8.3-1 Toxicity to arthropods of imazamox

Substance	Endpoint	Value	Reference	Study EU agreed?
studies on honeybee larvae				
imazamox	72 h oral LD_{50} 72 h oral LC_{50}	> 99.4 µg a.s./larva > 2.932 g a.s./kg food	Kleebaum, 2013/1355066 [1)]	No, new study
studies on adult honeybees				
imazamox	48 h oral LD_{50}	> 40 µg a.s./bee	Weyman, 1997 ID-541-003	Yes
	48 h contact LD_{50}	> 58.0 µg a.s./bee		

1) This is the study listed under Data point CA 8.7.4 in the Application with BASF DocID 2014/1000361. The former study ID was wrongfully assigned to this study.

CA 8.3.1 Effects on bees

CA 8.3.1.1 Acute toxicity to bees

No new studies are available.

CA 8.3.1.1.1 Acute oral toxicity

No new studies are available.

CA 8.3.1.1.2 Acute contact toxicity

No new studies are available.

CA 8.3.1.2 Chronic toxicity to bees

No new studies are available.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

This is the study listed under Data point CA 8.7.4 in the Application with BASF DocID 2014/1000361. The former study ID was wrongfully assigned to this study.

Report:	CA 8.3.1.3/1 Kleebaum K., 2013a Acute toxicity of BAS 720 H (Reg.No. 4096483) to honeybee larvae (Apis mellifera L.) under laboratory conditions (in vitro) 2013/1355066
Guidelines:	OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landesentwicklung, Dresden, Germany)

Executive Summary

In an acute toxicity test, honey bee larvae (*Apis mellifera* carnica P.) were exposed to BAS 720 H (Reg.No. 4 096 483). The toxicity of the test item was determined at concentrations of, 6.2, 12.4, 24.9, 49.7 and 99.4µg a.s./larva (corresponding to , 6.3, 12.6, 25.1, 50.3 and 100.5 µg product/larva). The concentrations of test item in the diet were 0.2, 0.4, 0.7, 1.5 and 2.9 g a.s./kg.
Additionally, honey bee larvae were treated with Dimethoate tech. as toxic standard at concentrations ranging from 8.8 to 1.1 µg dimethoate/larva or with an untreated diet C as a control.

After 72 hours of oral exposure, a mortality of 8.3 % was observed in the control. In the test item group, mortalities ranged between 0.0 % and 8.3 % at the same time. Statistically significant effects on survival occurred at none of the applied test item doses.

Other observations as smaller body size of surviving larvae or/and remaining food on D7 occurred on rare occasions. (The rate of affected larvae was below 10% of the surviving individuals in all cases.)

In an acute larval toxicity study with BAS 720 H (Reg.No. 4 096 483), the LD_{50} (72 h) was determined to be > 99.4 µg a.s./larva, which is equivalent to a LC_{50} (72 h) of > 2.932 g a.s./kg food.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 720 H (Reg.No. 4 096 483) batch no.: COD-001579; content of a.s.: imazamox (BAS 720 H): 98.9 % analyzed (tolerance ± 1.0 %).

Test species: *Apis mellifera* carnica P. (honeybee), first instar larvae; deriving from three healthy and queen-right colonies; source: Bienenfarm Kern GmbH, , 04249 Leipzig, Germany.

B. STUDY DESIGN

Test design: In a 72 hour acute test, first instar larvae of *Apis mellifera* carnica P. were exposed to 5 concentrations of BAS 720 H (Reg.No. 4 096 483) in treated food (aqueous sugar solution mixed with gelee royal). In total, 3 treatment groups were set up: 5 concentrations of the test item, one untreated control group and 4 concentrations of the toxic standard with 3 replicates per concentration and 12 larvae per replicate. Assessments of larval mortality were done after 24, 48 and 72 hours (respectively D5, D6, D7). Additionally other observations as small body size or large quantities of remaining food after 72 hours (on D7) were noted. In an analytical phase of the study the concentration of the active substance in the test item stock solution A was determined.

Endpoint: Mortality, other observations.

Reference item: Dimethoate tech. (99.8 % w/w analyzed).

Test concentrations: control (untreated diet: aqueous sugar solution with gelee royal); BAS 720 H: 6.2, 12.4, 24.9, 49.7 and 99.4 µg/larva, corresponding to 0.2, 0.4, 0.7, 1.5 and 2.9 g a.s./kg food (nominal); reference item: 1.1, 2.2, 4.4 and 8.8 µg dimethoate/larva.

Test conditions: Temperature: 34.0 °C – 34.5 °C, relative humidity: 85 - 96 % with two short periods of lower humidity, photoperiod: darkness (except during assessments), food: aqueous sugar solution mixed with gelee royal.

Statistics: Descriptive statistics; Fisher's Exact Binomial test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$); Probit analysis for calculation of the LD_{50} value of the reference item.

II. RESULTS AND DISCUSSION

After 72 hours of oral exposure, a mortality of 8.3 % was observed in the control. In the test item group, mortalities ranged between 0.0 % and 8.3 % at the same time. Statistically significant effects on survival occurred at none of the applied test item doses.

Other observations as smaller body size of surviving larvae or/and remaining food on D7 occurred on rare occasions. (The rate of affected larvae was below 10% of the surviving individuals in all cases.)

The LD_{50} (72 h) was determined to be > 99.4 µg a.s./larva, which is equivalent to a LC_{50} (72 h) > 2.932 g a.s./kg food

The results are summarized in Table 8.3.1.3-1.

Table 8.3.1.3-1: Toxicity of BAS 720 H to *Apis mellifera* carnica P. in an acute larval toxicity test

Treatment		**Mortality after 72 h[%]**	
[µg a.s./larva]	**g a.s./kg food**	**Mortality**	**Corrected mortality**
Control		8.3	--
6.213	0.183	8.3	0.0
12.426	0.367	2.8	0.0
24.851	0.733	2.8	0.0
49.702	1.466	0.0	0.0
99.405	2.932	0.0	0.0
Endpoint after 72 h			
Endpoint expressed as dose		LD_{50} > 99.4 µg a.s./larva	
		NOAEL ≥ 99.4 µg a.s./larva	
Endpoint expressed as concentration		LC_{50} > 2.932 g a.s./kg food	
		NOEC ≥ 2.932 g a.s./kg food	

The LD_{50} value (72 h) for the reference item was 4.421 µg dimethoate/larva.

III. CONCLUSION

In an acute larval toxicity study with BAS 720 H (Reg.No. 4096483), the LD_{50} (72 h) was determined to be > 99.4 µg a.s./larva, which is equivalent to a LC_{50} (72 h) > 2.932 g a.s./kg

CA 8.3.1.4 Sub-lethal effects

No new studies are available.

CA 8.3.2 Effects on non-target arthropods other than bees

No new studies are available.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No new studies are available.

CA 8.3.2.2 Effects on Typhlodromus pyri

No new studies are available.

CA 8.4 Effects on non-target soil meso- and macrofauna

Table 8.4-1 Toxicity to arthropods of imazamox

Substance	Species	Endpoint	Value [mg/kg dry soil]	Reference	Study EU agreed?
imazamox	*Eisenia fetida*	LC_{50}	> 901	England, 1995 ID-531-001	Yes [1)]
CL 312622	*Eisenia fetida*	NOEC	0.963	Grossmann, 1997 ID-570-006	Yes
CL 354825	*Eisenia fetida*	NOEC	0.963	Grossmann, 1997 ID-570-005	Yes
CL 354825	*Eisenia fetida*	NOEC	≥ 3.04	Friedrich, 2010/1110722	No, new study
CL 354825	*Folsomia candida*	NOEC	≥ 500	Friedrich, 2013/1177567	No, new study
CL 354825	organic matter decomposition	no unacceptable effects up to 1 x 60 g/ha		Mack, 2010/1126097	No, new study [2)]

1) The endpoint is presented as additional information.
2) A study summary is provided in chapter CA 8.7 as additional information.

CA 8.4.1 Earthworms – sub-lethal effects

Report: CA 8.4.1/1
Friedrich S., 2010a
Sublethal toxicity of Reg.No. 4110603 (metabolite of BAS 720 H, CL 354825) to the earthworm Eisenia fetida in artificial soil with 5% peat
2010/1110722
Guidelines: OECD 222 (2004)
GLP: Yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of CL 354825, a metabolite of imazamox, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (0.19, 0.38, 0.76, 1.52, and 3.04 mg CL 354825/kg dry soil) were incorporated into the soil (5% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality, biomass development, and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure no mortality was observed in any of the treatment groups, except of the control, where two worms died. Body weight and reproduction rate of earthworms exposed to CL 354825 were not statistically significantly different compared to the control up to the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with CL 354825 (a metabolite of imazamox) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be ≥ 3.04 mg CL 354825/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: CL 354,825 (Metabolite of imazamox, Reg. No. 411 060 3) batch no. L67-144; purity: 98.2% (tolerance ± 1.0%).

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 256 mg – 465 mg), age: approx. 3 months old; source: in-house culture.

B. STUDY DESIGN

Test design: 56-day test in treated artificial soil according to OECD 222 (5% peat only); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into glass vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).

Endpoints: NOEC; effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Nutdazim 50 FLOW (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 0.19, 0.38, 0.76, 1.52, and 3.04 mg CL 354825/kg dry soil (nominal).

Test conditions: Artificial soil according to OECD 222 (with reduced content of peat: 5%); pH 5.84 – 5.95 at test initiation, pH 5.74 - 5.82 at test termination; maximum water holding capacity (WHC): 58.2% - 58.7% at test initiation, 58.0% - 58.5% at test termination; temperature: 18.0 °C - 22.8 °C; photoperiod: 16 h light : 8 h dark, light intensity: 730 lux; food: horse manure.

Statistics: Descriptive statistics. Fisher's Exact test with Bonferroni Correction for mortality data and Dunnett's t-test for weight change and reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure no mortality was observed in any of the treatment groups, except of the control, where two worms died. Body weight and reproduction rate of earthworms exposed to CL 354825 was not statistically significantly different compared to the control (Dunnett's-t-test, $\alpha = 0.05$) up to the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized below (see Table 8.4.1-1).

Table 8.4.1-1: Effects of CL 354825, a metabolite of imazamox, on earthworms (*Eisenia fetida)* in a 56-day reproduction study

CL 354825 [mg/kg dry soil]	**Control**	**0.19**	**0.38**	**0.76**	**1.52**	**3.04**
Mortality (day 28) [%]	2.5	0.0	0.0	0.0	0.0	0.0
Weight change (day 28) [%]	84.6	92.8	82.1	87.7	80.5	81.6
No. of juveniles (day 56)	69.9	67.0	73.8	63.0	81.3	67.0
Reproduction (day 56) [%]	100.0	95.9	105.5	90.2	116.3	95.9
Feeding activity [%]	100.0	100.0	100.0	100.0	100.0	100.0
Endpoints [mg CL 354825/kg dry soil]						
$NOEC_{mortality, weight}$ (day 28)	≥ 3.04					
$NOEC_{reproduction}$ (day 56)	≥ 3.04					

III. CONCLUSION

In a 56-day earthworm reproduction study with CL 354825 (a metabolite of imazamox) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be ≥ 3.04 mg CL 354825/kg dry soil.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

Report:	CA 8.4.2.1/1 Friedrich S., 2013a Effects of Reg.No. 4110603 (metabolite of BAS 720 H, Imazamox) on the reproduction of the collembolan Folsomia candida 2013/1177567
Guidelines:	OECD 232 (2009), ISO 11267 (1999)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 4 110 603 (metabolite of BAS 720 H, imazamox) on mortality and reproduction of Collembola *(Folsomia candida)* were investigated in a laboratory study over 28 days. Five application rates (31.25, 62.5, 125, 250 and 500 mg Reg. No. 4 110 603/kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control and a solvent control treated with acetone, each with 8 replicates, were included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behaviour was carried out after 28 days.

No statistically significant effects on parental mortality were found for any concentration tested. Mortality rates of 2.5 % - 5.0 % in the test item treatment groups and 2.5 % in both control groups were recorded. No statistically significant effects on the number of juveniles compared to the solvent control were found for any concentration tested. The mean reproduction ranged between 1107 - 1195 juveniles in the test item treatments. The mean reproduction in the untreated and the solvent control reached 1063 and 1160 juveniles, respectively.

In a 28-day Collembola reproduction study with Reg. No. 4 110 603 (metabolite of BAS 720 H, imazamox) the NOEC based on mortality and reproduction was determined to be ≥ 500 mg Reg. No. 4 110 603/kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} was determined to be > 500 mg Reg. No. 4 110 603/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 4 110 603 (metabolite of BAS 720 H, imazamox), batch No. L67-144, analyzed purity: 98.2 % (tolerance ± 1.0 %).

Test species: Collembola (*Folsomia candida)*, juveniles (9 - 12 days old); source: in-house culture.

B. STUDY DESIGN

Test design: 28-day chronic laboratory test in treated artificial soil according to OECD 232 and ISO 11267; different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 7 treatment groups (5 test item concentrations, control, solvent control); 4 replicates for each test item treatment and 8 replicates for the control groups, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.

Endpoints: Mortality, reproduction rate after 28 days.

Reference item: Boric acid (100 % analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Control, solvent control, 31.25, 62.5, 125, 250 and 500 mg Reg. No. 4 110 603/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (peat: 5 %); pH 6.05 - 6.11 at test initiation, pH 5.80 - 5.88 at test termination; water content at study initiation 56.8 % - 57.0 % of maximum water holding capacity and 55.9 % - 56.8 % of maximum WHC at test termination; temperature: 18.4 C – 21.9 C; photoperiod: 16 h light : 8 h dark, light intensity: 540 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics; Fisher’s Exact Binominal Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for reproduction data ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

No statistically significant effects on parental mortality were found for any concentration tested (Fisher's Exact Binominal Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Mortality rates of 2.5 % - 5.0 % in the test item treatment groups and 2.5 % in both control groups were recorded. No statistically significant effects on the number of juveniles compared to the solvent control were found for any concentration tested (Williams-t-test, $\alpha = 0.05$, one-sided smaller). The mean reproduction ranged between 1107 - 1195 juveniles in the test item treatments. The mean reproduction in the untreated and the solvent control reached 1063 and 1160 juveniles, respectively. The results are summarized in Table 8.4.2.1-1.

Table 8.4.2.1-1: Effect of Reg. No. 4 110 603 on Collembola (*Folsomia candida)* in a 28-day reproduction study

Reg. No. 4 110 603 [mg/kg dry soil]	**Control**	**Solvent control**	**32.25**	**62.5**	**125**	**250**	**500**
Mortality (day 28) [%]	2.5	2.5	2.5	2.5	2.5	5.0	2.5
No. of juveniles (day 28)	1063	1160	1109	1152	1195	1107	1173
Reproduction in [%] of control (day 28)	--	100	96	99	103	95	101
Endpoints [mg Reg. No. 4 110 603/kg dry soil]							
$NOEC_{mortality + reproduction}$ (day 28)	≥ 500						
LC_{50}	> 500						
EC_{50}	> 500						

III. CONCLUSION

In a 28-day Collembola reproduction study with Reg. No. 4 110 603 (metabolite of BAS 720 H, imazamox) the NOEC based on mortality and reproduction was determined to be ≥ 500 mg Reg. No. 4 110 603/kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} was determined to be > 500 mg Reg. No. 4 110 603/kg dry soil.

CA 8.5 Effects on nitrogen transformation

Table 8.5-1 Toxicity to nitrogen transformation of imazamox

Substance	Endpoint	NOEC [mg/kg dry soil]	Reference	Study EU agreed?
Imazamox	Effects on nitrogen transformation	0.2	Seyfried 1996, ID-625-001	Yes
CL 312622	Effects on nitrogen transformation	0.5	Seyfried 1997, ID-570-008	Yes
CL 354825	Effects on nitrogen transformation	0.5	Seyfried 1997, ID-570-007	Yes

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

No new studies are available.

CA 8.6.2 Testing on non-target plants

No new studies are available.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

The following study is presented as additional information to the existing and already reviewed EU Dossier.

Report:	CA 8.7/1 Mack P., 2011c EXP 4110603 H (formulated product of CL 354825): Effects of the decomposition of organic matter in the field 2010/1126097
Guidelines:	Roembke et al. (2003), OECD 56 (2006)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The test item EXP 411 06 03 H (containing the active ingredient CL 354825, a metabolite of imazamox) was studied for its potential effects on the degradation of buried organic wheat straw compared to a water control after exposure of about 1, 4.5, 6, 9 and 12 months. The study was set up as randomized block design with an application directly on the bare soil (1269.57 g product/ha, equivalent to 60 g a.s./ha).

The mass loss of the straw material in the untreated control was 82.5 % at the end of the experiment after 12 months. In the treatment group, the mean mass loss after the exposure phases of about 1, 4.5, 6, 9 and 12 months were only slightly different from those in the control, resulting in effects of -2.1 %, 1.1 %, -0.6 %, 1.2 % and 0.0 %. There were no statistically significant differences between the treatment group and the water treated control.

The results of this field study with EXP 411 06 03 H (containing the active ingredient CL 354825, a metabolite of imazamox) in a 12-month monitoring program on an arable field site emphasize that CL 354825 proved to have no treatment-related ecologically relevant effects on the organic matter breakdown at a simulated plateau application rate of 60 g a.s./ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: EXP 411 06 03 H, batch no. 400001; content of a.s.: CL 354825 (metabolite of imazamox, Reg. no. 411 060 3): 48.3 g a.s./L (nominal: 50.0 g/L), density: 1.022 g/cm^3.

Test species: Naturally occurring non-target soil organisms.

B. STUDY DESIGN

Test site: Arable field site (white clover/grassland) near St. Mont (south-west of France); total size: 71 m x 27 m, the site had not received chemical application during the study apart from the test item.

Test design: Randomized block design with two treatments (test item group, water treated control) and six replicates per treatment. For the application the test item was applied directly to bare soil and incorporated into the soil to a depth of approximately 10 cm. The litter bags were buried one day after the application. Within the plots (6 x 6 m plot size) the litter bags were randomly distributed in the upper soil layer (depth of about 5 cm). The treatments were assigned randomly to the plots within each replicate. The test item was applied in a water volume equivalent to 400 L/ha using a calibrated boom sprayer.

Endpoints: Mean weight loss based on ash-free dry weight per plot per treatment.

Test rates: Treatment group 1: Untreated control (tap water); Treatment group 2: 60 g a.s./ha (equivalent to the calculated plateau concentration of 0.04 mg a.s./kg incorporated into the upper 10 cm layer of soil.

Application date: 07.10.2010; burying of litter bags: 08.10.2010.

Test conditions: Natural field conditions, soil: sandy loam, mean pH: 5.4, mean total organic carbon content: 0.7 %, mean maximum water holding capacity (WHC): 28.6 % (w/w), mean microbial biomass: 18.6 mg C/100 g dry weight.

Litter bags: Litter bags consisted of mesh material (glass fibre fabric) with a mesh size of about 5 mm. The size of a bag was about 10 cm x 20 cm. Bags were filled with 4.0 g of untreated dried wheat straw. The litter bags were buried on 08.10.2010 (one day after the application) horizontally in the plots to a soil depth of approximately 5 cm.

Sampling dates: 1st sampling on 08.11.2010; 2nd sampling on 28.02.2011; 3rd sampling on 11.04.2011; 4th sampling on 11.07.2011, 5th sampling on 11.10.2011

Sample processing: Sampling was done at 5 different time intervals and samples were immediately transported to the laboratory and stored deep frozen until further processing. The enclosed straw material was cleaned and dried for at least 12 hours at 30 °C - 35 °C. Subsequently, the straw was combusted at 600 °C for 30 minutes in order to determine the ash-free dry weight.

Analytics: Soil samples were analyzed for the active substance CL 354825.

Statistics: Descriptive statistics, t-test, $\alpha \leq 0.05$; Wilcoxon test, two-sided, $\alpha \leq 0.05$.

II. RESULTS AND DISCUSSION

The recovery rate for the active substance CL 354825 after application was 80 % of the expected soil concentration.

The mass loss of the straw material in the untreated control was 82.5 % at the end of the experiment after 12 months. In the treatment group, the mean mass loss after the exposure phases of about 1, 4.5, 6, 9 and 12 months were only slightly different from those in the control, resulting in effects of -2.1 %, 1.1 %, -0.6 %, 1.2 % and 0.0 %. There were no statistically significant differences between the treatment group and the water treated control (t-test, $\alpha \leq 0.05$; Wilcoxon test, two-sided, $\alpha \leq 0.05$).

Effects of the treatment on the degradation of buried wheat straw are summarized in Table 8.7-1.

Table 8.7-1: Mass loss [%] of wheat straw following exposure to CL 354825, a metabolite of imazamox

Treatment	Mean mass loss	Months after litter bag incorporation				
		1	4.5	6	9	12
Control	[%]	21.3	38.9	45.7	56.9	82.5
60 g a.s./ha	[%]	21.7	38.5	45.9	56.2	82.5
	% deviation from control	-2.1	1.1	-0.6	1.2	0.0

III. CONCLUSION

The results of this field study with EXP 411 06 03 H (containing the active ingredient CL 354825, a metabolite of imazamox) in a 12-month monitoring program on an arable field site emphasize that CL 354825 proved to have no treatment-related ecologically relevant effects on the organic matter breakdown at a simulated plateau application rate of 60 g a.s./ha.

CA 8.8 Effects on biological methods for sewage treatment

Updated dossier parts (October, 2014) are marked; *i.e.* all changes are highlighted in yellow and deleted parts are struck through.

Since Annex I inclusion of imazamox (BAS 720 H), no new activated sludge study has been performed, thus the results of the already submitted study are still valid. For better transparency and traceability of the active substance history, the results of already submitted and accepted study are summarized in Table 8.8-1.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	Study type	Endpoint [mg/L]	Reference	EU agreed
BAS 720 H (imazamox)	Respiration inhibition test (activated sludge from wastewater plant)	EC_{50} > 1000	Heim, D. & V. Canez, ID-549-005	Yes

Following summary of the activated sludge study (respiration inhibition test) performed with imazamox is provided below as a summary which has not been submitted previously. Actually, the original monograph does not include the summary of this study, which is presented by the review report in the list of provided study during the evaluation process but not referenced in the DAR.

Report:	CA 8.8/1 Heim D.,Canez V., 2002a BAS 720 H (Imazamox): Activated sludge, respiration inhibition test ID-549-005
Guidelines:	EEC 96/12, EEC 91/414 Annex II 8.7, OECD 209
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The effect of imazamox on the respiration rate of activated sludge collected from a wastewater treatment plant was determined. Imazamox was tested at a concentration of 100, 250, 500, 750 and 1000 mg a.s./L. Oxygen consumption rate of aerobic micro-organisms was assessed after a contact period of 180 min under aeration.

The biological results are based on the nominal concentrations. No significant inhibition of respiration was measured up to the highest tested concentration of 1000 mg a.s./L (nominal).

The EC_{50} value of imazamox in the activated sludge respiration inhibition test is > 1000 mg a.s./L. Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Imazamox (BAS 720 H; Reg. No. 4 096 483), lot no. AC 6935-63, purity: 97.1%.

B. STUDY DESIGN

Test species:	Activated sludge from Columbia Wastewater Treatment Plant in Columbia (USA).

Test design:	Assessment of the inhibitory effect of the test item on the oxygen consumption rate of aerobic micro-organisms (activated sludge) after short-term exposure of 180 min; the inoculum was aerated during the contact period; 1 replicate for the test item and the reference item; 2 replicates for the control.
Test concentrations:	Control, 100, 250, 500, 750 and 1000 mg a.s./L.
Reference item:	3.5-dichlorophenol. The reference item was applied at 3.2, 10 and 32 mg/L.
Test conditions:	Temperature: 20 °C ± 2 °C; pH 6.72 - 8.64; 1000 mL glass flasks, 500 mL of test mixture per vessel.
Analytics:	Not applicable
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

No significant inhibition of respiration was measured up to the highest tested concentration of 1000 mg a.s./L (nominal).

III. CONCLUSION

The EC_{50} value of imazamox in the activated sludge respiration inhibition test is > 1000 mg a.s./L. Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

CA 8.9 Monitoring data

No monitoring studies assessing ecotoxicological effects of imazamox are available.

BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section 9

LITERATURE DATA

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 9 LITERATURE DATA

The RMS requested to have access to foreign Evaluation Reports of imazamox, should these ones contain relevant information not part of this dossier.

Main countries having issued an Evaluation Report at some point of time are listed below, with the corresponding reference.

US (EPA)

There has not been much regulatory action in the US with imazamox that required a Risk Assessment. Registration Review of Imazamox is planned to begin in 2014 but EPA has not released any Risk Assessment in preparation of this review.

An overview of existing scientific reviews is given in the link and overview below:

http://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:7:0::NO:1,3,31,7,12,25:P3_XCHEMICAL_ID:2565

09/24/2008	Ecological risk assessment evaluating Imazamox (PC 129171) for the proposed new use on Clearfield rice (imidazolonone-tolerant rice). DP Barcode 348399 (PDF, 85 pp, 5MB)	Ibrahim Abdel-Saheb	ERB II/EFED	-
09/27/2001	PP# 0F06088. ADDENDUM TO "IMAZAMOX IN/ON ALFALFA, CANOLA, LEGUME VEGETABLES, AND WHEAT. HED Risk Assessment, D276570, 13-AUG-2001." Barcode D278055. PC Code 129171. Case 292469. Submission S573269. (PDF, 2 pp, 82K)	William H. Donovan	Registration Action Branch 1	-
08/13/2001	PP# 0F06088. IMAZAMOX IN/ON ALFALFA, CANOLA, LEGUME VEGETABLES, AND WHEAT. HED Risk Assessment. Barcode D276570. PC Code 129171. Case 292469. Submission S573269. (PDF, 2 pp, 65K)	William H. Donovan, P. V. Shah et. al	Registration Action Branch 1	-

07/11/2001	IMAZAMOX– Report of the Hazard Identification Assessment Review Committee. Tox review No. 014611 (PDF, 13 pp, 433K)	P. V. Shah	Registration Action Branch	–
05/09/1997	Imazamox – 129171: Health Effects Division Risk Characterization for Use of the New Chemical Imazamox in/on Soybeans (6F4649). MRIDs 43193201 through 43193203. (PDF, 24 pp, 1MB)	Barbara Madden	Risk Characterization & Analysis Branch	43193201... [More]

-Imazamox is a tolerance-exempt active ingredient in the US, since it was defined as safe compound, so no Health Affects Risk Assessments.

-Imazamox was registered for aquatic use a few years ago and the E fate Risk Assessment from EPA supporting the aquatic use can be found in Doc KCA.

CANADA (PMRA)

Imazamox is currently under review in Canada. The link for imazamox re-evaluation project plan is given below for information, and the document can be found in Doc KCA:

www.hc-sc.gc.ca/cps-spc/pubs/pest/_decisions/rev2012-19/index-eng.php

. A proposed re-evaluation decision for imazamox was supposed to be published for consultation in 2013 ; however at the time of writing this dossier, the document is not available yet.

So far PMRA has not identified any concerns for imazamox.

AUSTRALIA (APVMA)

Imazamox was evaluated in 2000 in the context of two formulations (RAPTOR Herbicide and RAPTOR WG Herbicide)

The report can be found in :

http://www.apvma.gov.au/registration/assessment/docs/prs_imazamox.pdf

and in corresponding Doc KCA.

This report did not conclude to any particular concern.

JAPAN

Imazamox is registered in Japan. Its ADI is actually of 3 mg/kg bw/d, based on the developmental toxicity study in rabbits (Hoberman A.M. 1995, Argus Report 101-021; see Draft Assessment Report, France Rapporteur member State 1999.
The ADI of 3 mg/kgbw/day is derived from the NOAEL of maternal toxicity: 300mg/kg/day and a SFof 100).

However, in the context of a revision program set up in 2006, it was decided to have this ADI re-evaluated to have temporary MRLs transformed into permanent MRLs. This re-evaluation was given to three Ministries:

FSC (Food Safety Commission) for evaluation of the ADI,
MHLW Ministry of Health, Labor and Welfare), for possible MRL revision or new MRL establishment and,
MAFF (Ministry of Agriculture, Forestry and Fisheries) for possible revision of current registrations, based on above evaluation.

BASF submitted a dossier to MAFF to enable the ADI re-evaluation in 2012 and decision by FSC in August 2013.

As of today, the review at FSC has not started yet, and should be delayed , probably waiting for the outcome of the imazamox JMPR Dossier in 2014.

No Evaluation report is published from any of the Ministries.

BASF
The Chemical Company

Imazamox

DOCUMENT M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

[redacted]

Telephone: [redacted]
Telefax:
e-Mail: [redacted]

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

Physico-chemical properties

Table 10-1: Physico-chemical properties of BAS 720 H

Study type	Results	Reference
Flammability	Not flammable	2013/1065841
Self heating	Not self-heating	2013/1065841
Flash point	Not applicable (melting > 40 °C)	
Explosivity	Not explosive	2013/1065841
Oxidizing properties	Not oxidizing	2013/1065841
Content of hydrocarbon	Content of hydrocarbon <10 %	Composition see doc J

The following is proposed in accordance with Directive 99/45/EC in combination with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

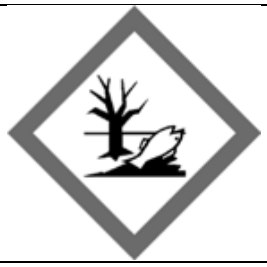
Hazard Symbol: **N**

Indication of danger: **-**

Risk Phrases: **R50/53**
Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment.

Safety Phrases: **S 35** This material and its container must be disposed of in a safe way.
S61 Avoid release to the environment. Refer to special instructions/safety data sheet.

The following is proposed in accordance with Regulation (EC) No 1272/2008:

	Imazamox
GHS pictogram	
Signal word	Warning
Hazard statement	H410: Very toxic to aquatic life with long lasting effects
Precautionary Statement Prevention	P273
Precautionary Statement Response	P391
Precautionary Statement Storage	-
Precautionary Statement Disposal	P501

Other safety/precautionary phrases:

According to Article 10 (No. 1.2) of Directive 1999/45/EC:
'To avoid risks to man and the environment, comply with the instructions for use'

According to Regulation (EC) No. 1272/2008:
EUH401 To avoid risks to human health and the environment, comply with the instructions for use.