CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification: Azamethiphos (ISO)

EC Number: **252-626-0**

CAS Number: **35575-96-3**

Index Number: **N/A**

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Version number: 2 **Date: September 2018**

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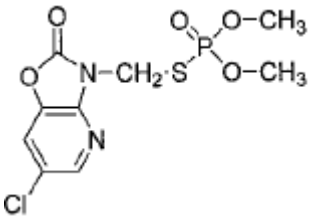
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazolo[4,5-b]pyridin-3-ylmethyl O,O-dimethyl phosphorothioate
Other names (usual name, trade name, abbreviation)	Phosphorothioic acid, S-[(6-chloro-2-oxooxazolo[4,5-b]pyridine-3(2H)-yl)methyl] O,O-dimethyl ester
ISO common name (if available and appropriate)	Azamethiphos
EC number (if available and appropriate)	252-626-0
EC name (if available and appropriate)	S-[(6-chloro-2-oxooxazolo[4,5-b]pyridin-3(2H)-yl)methyl] O,O-dimethyl thiophosphate
CAS number (if available)	35575-96-3
Other identity code (if available)	N/A
Molecular formula	$C_9H_{10}ClN_2O_5PS$
Structural formula	
SMILES notation (if available)	O=P(OC)(OC)SCN1c2ncc(Cl)cc2OC1=O
Molecular weight or molecular weight range	324.7 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 98%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Azamethiphos	≥ 98 %	Not listed	**Acute Tox 4; H302** **Acute Tox 4; H332** **Skin Sens 1; H317** **Aquatic Acute 1; H400** **Aquatic Acute 1; H410**

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Confidential				

No impurities of relevance to the classification and labelling have been identified in the technical material at the time of submission of the CLH report.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling of azamethiphos (ISO) according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No entry on Annex VI										
Dossier submitters proposal	TBD	azamethiphos (ISO); S-[(6-chloro-2-oxooxazolo[4,5-b]pyridin-3(2H)-yl)methyl] O,O-dimethyl thiophosphate	252-626-0	35575-96-3	Acute Tox. 4 Acute Tox. 3 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H331 H317 H400 H410	GHS06 GHS09 Dgr	H302 H331 H317 H410		oral: ATE = 500 mg/kg bw inhalation: ATE = 0,5 mg/L M = 1000 M = 1000	
Resulting Annex VI entry if agreed by RAC and COM	TBD	azamethiphos (ISO); S-[(6-chloro-2-oxooxazolo[4,5-b]pyridin-3(2H)-yl)methyl] O,O-dimethyl thiophosphate	252-626-0	35575-96-3	Acute Tox. 4 Acute Tox. 3 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H331 H317 H400 H410	GHS06 GHS09 Dgr	H302 H331 H317 H410		oral: ATE = 500 mg/kg bw inhalation: ATE = 0,5 mg/L M = 1000 M = 1000	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data lacking	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data lacking	Yes
Acute toxicity via oral route	**Harmonised classification proposed**	**Yes**
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	**Harmonised classification proposed**	**Yes**
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	Yes/No
Skin sensitisation	**Harmonised classification proposed**	**Yes**
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard class not applicable	No
Hazardous to the aquatic environment	**Harmonised classification proposed**	**Yes**
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Azamethiphos is an existing biocide active substance in the review programme under Regulation 528/2012 for which the UK is the evaluating Competent Authority. It does not have an existing entry in Annex VI of CLP and the classification and labelling has not previously been considered in the harmonised process.

At the time of submission the substance is not registered under REACH.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Azamethiphos is an existing biocide active substance in the review programme of Regulation 528/2012 for which the UK is the evaluating Competent Authority. It does not have an existing entry on Annex VI of CLP and is subject to harmonised classification in accordance with Article 36(2) of CLP.

5 IDENTIFIED USES

Azamethiphos is used within the EU in insecticides, acaricides and to control other arthropods (PT 18).

6 DATA SOURCES

The primary information sources for this CLH report is the draft Competent Authority Report (dCAR) prepared by the UKCA (2017). In addition, for the assessment of carcinogenicity, the UK CA has included two further carcinogenicity studies in the CLH report that are not included in the dossier assessed under Regulation (EU) No 528/2012. These studies were submitted to the UK CA and evaluated for the UK Advisory Committee on Pesticides in 2003. They were also considered by the EMEA Committee for Veterinary Medicinal Products report for Azamethiphos (EMEA/MRL/527/98-FINAL) (http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500010779.pdf).

At the time of submission, Azamethiphos is not registered under REACH.

7 PHYSICOCHEMICAL PROPERTIES

All references are taken from sections 1.3 and 1.4 of Part A of the Competent Authority Report (CAR) for Azamethiphos PT 18 – November 2017 and Section A3 of Doc IIIA to the CAR

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Beige powder	Oudhoff K.A., 2008,	Observation
Melting/freezing point	90°C	Oudhoff K.A., 2008,	EC A.1 (DSC) OECD 102 GLP 98.8%
Boiling point	Reaction and/or decomposition of the test substance above 200°C and no boiling observed below this temperature	Oudhoff K.A., 2008,	EC A.1 (DSC) OECD 102 GLP 98.8%
Relative density	1.63	Oudhoff K.A., 2008,	EC A.1 OECD 109 (gas comparison pycnometer) GLP 98.8%
Vapour pressure	2.21 x 10^{-8} Pa at 20°C (1.66 x10-10 mmHg)	Oudhoff K.A., 2008	EC A.4 OECD 104 (isothermal gravimetry) GLP 98.8%
Surface tension	68.5 mN/m at 19.8°C	Oudhoff K.A., 2008	EC A.5 OECD 115 (Harmonised ring method) GLP 98.8%
Water solubility	1.6 g/l at pH 5 and 20.1 °C 1.27 g/l at pH 7 and 20.0°C 0.881 g/l at pH 9 and 20.2°C	Oudhoff K.A., 2008	EC A.6 (flask method) OECD 105 GLP 98.8%
Partition coefficient n-octanol/water	Log Pow = 1.0 at 20.1°C and pH 7	Oudhoff K.A., 2008	EC A.8 (shake flask) OECD 107 GLP 98.8%
Flash point	Not applicable melting point is 90°C	-	-
Flammability	The substance did not ignite on contact with the ignition source but melted leaving a brown residue.	Oudhoff K.A., 2008	EC A.10 GLP 98.8%

Property	Value	Reference	Comment (e.g. measured or estimated)
	Examination of the chemical structure and experience in handling and use indicates that the substance is not pyrophoric and does not emit flammable gases on contact with water.		
Explosive properties	The substance does not contain any chemical groups that are indicative of explosive properties.	-	-
Self-ignition temperature	Auto flammability = 240°C	Oudhoff K.A., 2008	EC A.15 DIN 51794 IEC 79-4
Oxidising properties	The substance does not contain any chemical groups that are indicative of oxidising properties.	-	-
Granulometry	10% of material is 19.340 µm. 50% of material is < 56.723µm. 90% of material is < 178.942 µm. Material is a fine powder.	Brekelmans., 2008	Laser diffraction test
Stability in organic solvents and identity of relevant degradation products	No data	-	-
Dissociation constant	pKa basic: 2.2	Oudhoff K.A., 2009	EC A.4 OECD112
Viscosity	N/A - solid	-	-

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

8.1.1 Short summary and overall relevance of the information provided on explosive properties

No test data. The substance does not contain any chemical groups that are indicative of explosive properties.

8.1.2 Comparison with the CLP criteria

If there are no chemical groups associated with explosive properties present in the molecule, a substance shall not be classified as explosive (section 2.1.4.3 of Annex I to CLP).

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Not relevant, the substance is a solid.

8.3 Oxidising gases

Not relevant, the substance is a solid.

8.4 Gases under pressure

Not relevant, the substance is a solid.

8.5 Flammable liquids

Not relevant, the substance is a solid.

8.6 Flammable solids

Method	Results	Remarks	Reference
EC A.10	The substance did not ignite on contact with the ignition source but melted leaving a brown residue.	-	Oudhoff K.A., 2008

8.6.1 Short summary and overall relevance of the provided information on flammable solids

In an A10 study, the substance did not ignite on contact with the ignition source but melted leaving a brown residue.

8.6.2 Comparison with the CLP criteria

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. The substance did not ignite on contact with the ignition source but melted leaving a brown residue. Therefore, the criteria for classification as a flammable solid are not met.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified – conclusive but not sufficient for classification.

8.7 Self-reactive substances

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No studies available.

8.7.2 Comparison with the CLP criteria

A substance is considered to be self-reactive where the SADT is less than or equal to 75°C when transported in a 50 kg package.

There are no groups in the molecule associated with explosive or self reactive properties.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified – data conclusive but not sufficient for classification.

8.8 Pyrophoric liquids

Not relevant, the substance is a solid.

8.9 Pyrophoric solids

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No studies are available. However, no incidences of spontaneous ignition following contact with air have been reported during the handling and use of azamethiphos.

8.9.2 Comparison with the CLP criteria

According to Section 2.10.4.1 of Annex 1 of CLP, the classification procedure for pyrophoric solids need not be applied when experience in manufacture and handling shows that the substance does not spontaneously ignite upon coming into contact with air at normal temperatures. There are no reports in the available studies of azamethiphos spontaneously igniting when in contact with air. Therefore, azamethiphos does not meet the criteria for classification as a pyrophoric solid.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified – conclusive but not sufficient for classification.

8.10 Self-heating substances

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

No suitable test data available.

8.10.2 Comparison with the CLP criteria

A substance is classified as self-heating when a positive result is obtained in the test method outlined in subsection 33.3.1.6 of the UNRTDG Manual of Tests and Criteria. No such data are available.

There is no evidence to show that azamethiphos possess self-heating properties .

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified – data lacking

8.11 Substances which in contact with water emit flammable gases

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No data derived in accordance with the recommended test method in CLP have been provided. However, azamethiphos has been handled in water within many of the studies available and there are no reports of violent reaction or emission of gas.

8.11.2 Comparison with the CLP criteria

According to Section 2.12.4.1 of Annex I of CLP, the classification procedure for this hazard class need not be applied if experience in production or handling shows that the substance does not react with water. Therefore, classification for this class is not applicable to azamethiphos.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified – conclusive but not sufficient for classification.

8.12 Oxidising liquids

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not relevant, substance is a solid.

8.13 Oxidising solids

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

No test data. The substance does not contain any chemical groups that are indicative of oxidising properties.

8.13.2 Comparison with the CLP criteria

The substance does not contain any chemical groups that are indicative of oxidising properties therefore classification for this class is not applicable to azamethiphos.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified – conclusive but not sufficient for classification.

8.14 Organic peroxides

Not relevant, substance is not an organic peroxide.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data available.

8.15.2 Comparison with the CLP criteria

A substance is classified as corrosive to metals using the test method outlined in section 37.4 of the UN RTDG Manual of Tests and Criteria. No data are available to indicate that azamethiphos is corrosive to metals. However, based on the experience in manufacture and handling, the substance does not materially damage metallic containers.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – data lacking.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Non-human information

Azamethiphos is rapidly absorbed and extensively metabolised in the rat during single and repeat dose studies by the oral route [*Confidential (2009)*]. Metabolism of azamethiphos to 2-methylamino-3-hydroxy-5-chloropyridine and to 2-amino-3 hydroxy-5-chloropyridine and glucuronidation are the major metabolic routes followed by the production of an N-acetyl cysteinyl conjugate after low single dose and low repeated dose oral administration and for both sex groups (male and female). In addition, three other metabolites show indications of sulphation, although they could not be further identified. No qualitative difference in metabolite profile is observed between low and high single and repeated dosing. In addition, no difference in metabolism is observed between the sexes. Urine was the most important route for the excretion of azamethiphos (91 - 98%), with excretion via faeces accounting for a minor amount of the radioactivity (1.9-4.2%). No saturation in the urinary elimination pathway occurred upon repeated dosing and dose increase. Azamethiphos is rapidly excreted with the majority excreted in the urine between 0 and 8 hours post administration and almost complete excretion within 24 hours.

Human information

No human data was available on metabolism of azamethiphos. Dermal absorption of azamethiphos was investigated in an *in vitro* study with human skin from a wettable granule formulation (Badigon 10 WG) containing 10% azamethiphos. Under the current guidance it was not possible to determine a dermal absorption value from the concentrated product; however, a value of 20% could be derived for the diluted product (2.5 g/l). No additional data is available on the toxicokinetics in humans.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Azamethiphos when administrated orally is well absorbed, readily distributed into all organs and completely metabolised. The major route of excretion is via the urine. There is no evidence of bioaccumulation of azamethiphos in tissues.

Refer to section 3.1 of the CAR.

10 EVALUATION OF HEALTH HAZARDS

The technical material used for the generation of the human health data is known as azamethiphos. The purity specified throughout the dossier is 96.2% pure.

Azamethiphos is an organophosphate, a class of chemicals which reversibly inhibits acetylcholinesterase resulting in an accumulation of the neurotransmitter acetylcholine in the central and peripheral nervous system. As might be expected a key finding in many of the studies is an effect on acetylcholinesterase activity in the erythrocytes.

All references are taken from the Section 3 of Part A of the Competent Authority Report (CAR) for Azamethiphos PT 18 – November 2017 and Section A6 of Doc IIIA to the CAR.

Acute toxicity

The acute toxicity of azamethiphos has been investigated in the rat following administration by the oral, dermal and inhalation routes.

10.1 Acute toxicity - oral route

Table 8: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Test substance, Dose levels, duration of exposure**	**Observations and remarks**	**LD_{50}**
OECD 423 (Acute Toxic Class Method) GLP Rat, Wistar (3/group; females) Confidential (2008) CAR 3.2.1	2000 & 300 mg/kg bw azamethiphos in 1% aq carboxymethyl cellulose 96.2% pure	**At 2000 mg/kg bw:** all animals died. Clinical signs included hunched posture in all animals. Dark red fluid in the thoracic cavity seen in one animal at necropsy. **At 300 mg/kg bw:** None of the animals died. Clinical signs included hunched posture, piloerection, uncoordinated movements and/or shallow respiration on Days 1 and/or 2.	500 mg/kg bw

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute oral toxicity study, an oral LD_{50} of 500 mg/kg bw was derived for both male and female rats.

No human data are available.

10.1.2 Comparison with the CLP criteria

The oral LD_{50} value of 500 mg/kg bw for female rats is within the criteria of $300 < LD_{50} \leq 2000$ for classification as Acute Tox 4; H302. Based on the LD_{50} value, an Acute Toxicity Estimate (ATE) of 500 mg/kg bw is proposed.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox 4; H302 – Harmful if swallowed

ATE oral = 500 mg/kg bw

10.2 Acute toxicity - dermal route

Table 9: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Test substance, Dose levels , duration of exposure	Observations and remarks	LD_{50}
OECD 402 GLP Rats, Wistar (5/sex/group) Confidential (2008), CAR 3.2.2	2000 mg/kg bw in 1% aq carboxymethyl cellulose Purity: 96.2%	None of the animals died. Clinical signs included chromodacryorrhoea, hunched posture and scales on treated skin area	>2000 mg/kg bw

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

A dermal LD_{50} of >2000 mg/kg bw was derived for both male and female rats.

No human data are available.

10.2.2 Comparison with the CLP criteria

The LD_{50} of >2000 mg/kg bw for rats exposed to azamethiphos via the dermal exposure route is above the value for classification (2000 mg/kg). No classification is proposed.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified - conclusive but not sufficient for classification

10.3 Acute toxicity - inhalation route

Table 10: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Test substance, , form and particle size (MMAD), Dose levels, duration of exposure	Observations and remarks	LC_{50}
OECD 403 GLP Rats, Wistar (5/sex/group) (3 groups of males, 2 groups of females) Confidential (2009), CAR 3.2.3	Actual: 0.54, 1.1, 5.2 mg/l (dust aerosol) Nominal: 23.0, 7.3, 3.5 mg/l MMAD = 2.3, 3.2, 2.9 Duration 4 hours (nose only) Purity: 96.2%	**At 5.2 mg/l:** all animals died during exposure. **At 1.1 mg/l**: 4/10 animals died (3 male + 1 female). Clinical signs included shaking heads (all animals), spread hind legs (1/5 males and 2/5 females) on removal from restraining tubes. Thereafter, gasping, hunched posture, hypothermia, laboured respiration, piloerection, rales, general tremors (3/5 males and 2/5 females days 1-2) and chromodacryorrhoea. All symptoms resolved by day 5. **At 0.54 mg/l (males only)**: No animals died. Clinical signs included chromodacryorrhoea (snout), hunched posture, laboured respiration and piloerection (males)	0.5 – 1.0 mg/l

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In an acute inhalation study, the LC_{50} was measured at 0.5 – 1.0 mg/l for both male and female rats.

No human data are available.

10.3.2 Comparison with the CLP criteria

The inhalation LC_{50} value of 0.5 – 1.0 mg/l with an MMAD in the range of 2.3 - 2.9 µm is within the numeric criteria of $0.5 < LC50 \leq 1$mg/l (dusts and mists) for classification as Acute Tox 3; H331. Since no precise LC_{50} value is available, the default ATE value is proposed. In accordance with Annex I of the CLP Regulation (Table 3.1.2), an ATE of 0.5 mg/l is appropriate for dusts and mists classified in category 3 for acute toxicity via the inhalation route.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Acute Tox 3; H331 – Toxic if inhaled

ATE inhalation = 0.5mg/l

10.4 Skin corrosion/irritation

The potential of azamethiphos to cause skin and eye irritation has been investigated in the rabbit.

Table 11: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404	Rabbit, New Zealand White 3 males	Azamethiphos Batch no: 070624 96.2% pure Vehicle: Water	0.5 g moistened in 0.7 ml water Exposure: 4 hours (semi-occlusive)	Average scores in individual animals from gradings at 24, 48 and 72 hours were: Erythema: 0,0,0 Oedema: 0,0,0	Confidential (2008), CAR 3.3.1

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The skin irritation potential of Azamethiphos has been tested in a standard skin irritation study, in three male New Zealand White rabbits. Neither erythema nor oedema was seen in any of the animals.

No human data are available.

10.4.2 Comparison with the CLP criteria

Classification as a skin irritant is required when the mean score is ≥ 2.3 but < 4 for erythema or oedema in at least 2 out of 3 animals calculated from observations at 24, 48 and 72 hours after patch removal. Classification is also applicable where inflammation persists to the end of the observation period or there is a pronounced variability in response. Azamethiphos did not cause either erythema or oedema (all scores were 0) in any of the animals tested. Therefore, the criteria for classification as a skin irritant are not met.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified – conclusive but not sufficient for classification

10.5 Serious eye damage/eye irritation

Table 12: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405	Rabbit, New Zealand White 3 males	96.2% pure	Amount administered 62.1 mg	Mean scores in individual animals from gradings at 24, 48 and 72 hours were: Cornea: 0, 0, 0 Iris: 0, 0, 0 Redness: 2, 1, 0 Chemosis: 1, 0, 0 Fully reversible within 72 hours	Confidential (2008), CAR 3.3.2

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The eye irritation potential of azamethiphos has been tested in a standard eye irritation study in male New Zealand White rabbits. No corneal or iridial lesions were seen. Conjunctival redness and chemosis was seen in all animals. All ocular reactions had resolved by 72 hours post-application.

No human data are available.

10.5.2 Comparison with the CLP criteria

Azamethiphos caused mild, transient irritation of the eye in New Zealand White rabbits. Effects observed between 24 and 72 hours were conjunctival redness and swelling with average scores of 2, 1 and 0 for conjunctival redness and 1, 0 and 0 for chemosis in the 3 tested animals. This does not meet the criteria for classification (average score for iritis ≥ 1, and/or corneal opacity ≥ 1, and/or conjunctival redness ≥ 2, and/or conjunctival oedema ≥ 2, in at least 2 of 3 tested animals).

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Not classified – conclusive but not sufficient for classification

10.6 Respiratory sensitisation

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

There is no specific information on the potential of azamethiphos to induce respiratory sensitisation.

No human information is available.

10.6.2 Comparison with the CLP criteria

No data are available.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification – data lacking

10.7 Skin sensitisation

The potential of Azamethiphos to cause skin sensitisation has been investigated in the mouse.

Table 13: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, Dose levels duration of exposure	No. sensitised/total no.	Reference
Local Lymph Node Assay (LLNA) OECD 429 GLP Purity 96.2%	Mouse, CBA 20 animals (female) (5 groups)	10%, 25% and 50% in propylene glycol	No EC_3-value calculated. SI values: 10%: 14.1 25%: 18.6 50%: 16.4 Erythema seen in animals treated at 25% (4/5) and 50% (5/5). No oedema observed in any animal. Enlargement of nodes seen in all animals treated at 25% and 50%. Positive control: α-hexyl cinnamic aldehyde (HCA, SI = 13.5	Confidential (2008), CAR 3.4

The skin sensitisation potential of azamethiphos was investigated in a local lymph node assay (LLNA). Erythema and enlarged nodes were seen in the animals treated at 25 and 50%. The test concentrations were determined from a preliminary study in which erythema was observed from 25%. All nodes in the 10% treatment group were considered normal in size and no erythema was observed. As all concentrations tested gave an SI of ≥3 (>14 at all concentrations tested), an EC_3 value was not calculated.

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Azamethiphos induced a positive response in an LLNA study, with SI values of 14.1, 18.6 and 16.4 for concentrations of 10%, 25% and 50% azamethiphos, respectively.

No human data are available.

10.7.2 Comparison with the CLP criteria

Azamethiphos induced skin sensitisation in a local lymph node assay at all the concentrations tested (SI of ≥3). It therefore meets the criteria for classification for skin sensitisation Category 1. No EC_3 value could be calculated and therefore the data do not allow for sub-categorisation of azamethiphos (i.e., Category 1A is applicable where the EC3 ≤ 2 and Category 1B where the EC value is >2).

10.7.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens 1; H317 – May cause an allergic skin reaction

10.8 Germ cell mutagenicity

Table 14: Summary table of mutagenicity/genotoxicity tests in vitro

Method	Organism/ strain	Concentrations tested	Remarks and Result
Bacterial reverse mutation assay OECD 471 GLP Verspeek-Rip (2008a) CAR 3.8.1	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA	(-S9 mix 50, 100, 250, 300, 350, 400, 450, 500 µg/ml +S9-mix 5, 10, 25, 50, 75, 100, 125, 160 µg/ml Test material: azamethiphos (96.2% pure)	**Positive** In the absence of S9, a clear and reproducible dose-related increase in revertant numbers was seen in strain TA100. No increase was seen in TA100 with S9, or in any other strain with or without S9.
Mammalian cell chromosome aberration Test OECD 473 GLP Drs Buskens C.A.F. (2008b) CAR 3.8.1	Cultured peripheral human lymphocyte	*Experiment 1* (without and with S9-mix) 100, 150, 200, 250, 300, 350, 500 µg/ml Exposure time: 3h Scoring: up to 250 µg/ml *Experiment 2* Without S9 mix 10, 25, 50, 75, 100 and 150 µg/ml Exposure time: 24 and 48 h With S9-mix (5%) 100, 200, 250, 300, 350, 400, 500, 550 and 600 µg/ml Scoring: up to 400 µg/ml Exposure time: 3 hours	**Positive** *Experiment 1* *-S9 mix* Increased number of cells with structural chromosome aberrations. Dose dependent increase in number of polyploid cells *+S9 mix* Increased number of cells with structural chromosome aberrations. Dose dependent increase in number of polyploid cells. *Experiment 2* *-S9 mix* Increased number of cells with structural

Method	Organism/ strain	Concentrations tested	Remarks and Result
		Positive control: -S9: mitomycin C (0.5 µg/ml) +S9: cyclophosphamide *Solvent control:* DMSO Test material: azamethiphos (96.2% pure)	chromosome aberrations. increase in number of polyploid cells following 24 hour continuous exposure at the highest concentration *+S9 mix* Increased number of cells with structural chromosome aberrations.
Gene mutation assay in mammalian cells OECD 476 GLP Verspeek-Rip C.M. 2008c) CAR 3.8.1	L5178Y /TK+/- mouse lymphoma cells	*-S9 mix* 50, 100, 250, 300, 350, 400, 450, 500 µg/ml Exposure time: 3 hours *+S9-mix* 5, 10, 25, 50, 75, 100, 125, 160 µg/ml Exposure time: 3 hours Test material: azamethiphos (96.2% pure) Solvent control: DMSO	**Positive** *-S9 mix* Up to 8.0-fold increase in the mutation frequency at the TK locus (at 500 µg/ml) Up to 5.8- and 6.5-fold increases in the mutation frequency of the small and large colonies, respectively *+S9 mix* Up to 7.0-fold increase in the mutation frequency at the TK locus (at 160 µg/ml) Up to 4.2- and 9.4-fold increases in the mutation frequency of the small and large colonies, respectively
In vitro mammalian cell alkaline comet assay No OECD guideline available. Test carried out according to international; workshop reports/reviews defining optimal conditions for Comet assay (Tice *et al*, 2000; Hartmann *et al*, 2004) GLP Compliant Simar (2017) CAR 3.8.1	L5178Y mouse lymphoma cells	62.5, 125 and 250 µg/ml 3 replicates per concentration Exposure time: 4 hours at 37°C Test material: azamethiphos (99.68% pure) Only tested in the absence of metabolic activation **Cells evaluated:** 50 cells/slide 100 cells/culture 300 cells/concentration **Positive control:** methylmethane sulfonate (20 µg/ml)	**Positive** Azamethiphos induced statistically and biologically significant increases in the percentage of DNA in tail in the absence of metabolic activation. The test material was strongly cytotoxic at 500 µg/ml 47.9% survival; at 250 µg/ml survival was 75.5%.

Four studies have been evaluated to investigate the mutagenic potential of azamethiphos *in vitro*.

In a bacterial reverse mutation assay, azamethiphos induced a dose-related increase in the number of revertant colonies in tester strain TA100 of up to 2.6- and 1.9-fold compared with the solvent control in the absence and presence of S9-mix respectively. In tester strain WP2uvrA increases in the number of revertant colonies were up to 1.5- and 1.7-fold in the absence and presence of S9-mix respectively. According to the laboratory criteria and based on the results of this study, azamethiphos was positive *in vitro* for mutagenicity in *Salmonella typhimurium TA100* both in the presence and absence of S9 while the result was equivocal in WP2uvrA in the absence of S9-mix.

In the mammalian gene mutation assay with L5178Y /$TK^{+/-}$ mouse lymphoma cells, azamethiphos induced 8.0-fold dose-related increases in mutation frequency at the TK locus in the absence of S9-mix and a 7-fold increase in the presence of S9-mix. These increases are more than three-fold the historical control range and dose-related and are therefore considered biologically relevant. Increases in the mutation frequency of both small and large colonies were observed when compared with the mean mutation values from the solvent controls, indicating chromosome aberrations and gene mutations. Based on the results of this it was concluded that azamethiphos is mutagenic in the mouse lymphoma L51878Y test system in this study.

In an *in vitro* mammalian chromosomal aberration study, azamethiphos induced statistically significant ($p<0.05$) and biologically relevant increases in the number of cells with chromosome aberrations at the highest concentration tested both in the absence and presence of S9-mix. An increase in the number of polyploid cells was also noted. This occurred in a dose-dependent manner in the absence and presence of S9 mix in the first experiment while in a second experiment it was only seen in the absence of S9-mix following 24 hour continuous exposure at the highest concentration. No effects on the number of cells with endoreplicated chromosomes were observed either in the absence or presence of S9-mix.

The genotoxic potential of azamethiphos was investigated in an *in vitro* alkaline comet assay using L5178Y mouse lymphoma cells (Simar, 2017). Cells were exposed to concentrations of 62.5, 125 and 250 µg/ml azamethiphos for 4 hours in the absence of metabolic activation only. The top concentration tested was determined by the cytotoxicity of the test item.

Azamethiphos induced statistically significant increases in the percentage of DNA in tail at 125 and 250 µg/mL when compared to the negative control, with means of the medians of percentages of DNA in tail of 1.13 and 3.06 % vs. 0.34% in the corresponding negative control. These values were outside the highest value from the historical data for negative controls (0.53% ± 0.06%) under the same experimental conditions. Furthermore, a dose-response relationship was observed, as demonstrated by Kruskall-Wallis assessment.

Results from the comet assay (performed without exogenous metabolic activation)

	Conc^n (µg/ml)	**% survival**	**% DNA in tail**			**Percentage hedgehogs**	**Statistical significance**
			Mean of medians per culture (3/concentration)	**Non-parametric statistical assessment**			
				p Kruskall-Wallis	**p Mann-Whitney**		
Negative control	0	100	0.34	<0.05	-	0.00	-
Azamethiphos	62.5	100.6	0.26		<0.05	1.91	<0.05
	125	87.1	1.13		<0.05	1.60	N.S.
	250	75.7	3.06		N.S.	0.65	N.S.
Methylmethane sulfonate	20	95.9	17.25	-	<0.05	1.92	<0.05

Azamethiphos induced statistically and biologically (> upper bound of negative historical data) significant increases in the percentage of DNA in tail in absence of metabolic activation.

Overall, results from the *in vitro* studies show that azamethiphos has a mutagenic potential. The *in vitro* gene mutation study showed a mutagenic potential in bacteria in the absence of metabolic activation but gave an equivocal result in the presence of metabolic activation. Azamethiphos was found to be clastogenic and mutagenic in mammalian cells under the experimental conditions of each study. In an *in vitro* alkaline comet assay, azamethiphos was shown to be genotoxic in L5178Y mouse lymphoma cells. On the basis of these results azamethiphos is considered to be mutagenic *in vitro*.

Table 15: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

<table>
<tr><th>Method</th><th>Organism/ strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>Mouse bone marrow micronucleus test
OECD 474

GLP

Confidential (2008)
CAR 3.8.2</td><td>Mouse NMRI BR
30 males/group</td><td>Dose: 125, 60 and 30 mg/kg bw in arachis oil (intraperitoneal)

Sampling time: 24 and 48 hours

Positive control: 40 mg/kg bw cyclophosphamide (CP)</td><td>Negative
No increase in the mean MPE per 2000 PCE in azamethiphos-treated animals compared with the vehicle control.

At 125 or 60 mg/kg bw: lethargy, ataxia, tremors, rough coat, ventral recumbency and hunched posture were seen. At 30 mg/kg bw clinical signs included lethargy, ataxia, rough coat and hunched posture. No treatment-related clinical signs or deaths were noted in either control group.

<table>
<tr><th>Dose (mg/kg bw)</th><th>Sampling Time (h)</th><th>No of MPE per 2000 PCE (mean±SD)</th><th>Ratio PCE/NCE (mean±SD)</th></tr>
<tr><td rowspan="2">125</td><td>24</td><td>1.6±2.6</td><td>0.98±0.14</td></tr>
<tr><td>48</td><td>0.6±0.5</td><td>1.00±0.07</td></tr>
<tr><td>60</td><td>24</td><td>1.0±1.0</td><td>0.97±0.10</td></tr>
<tr><td>30</td><td>24</td><td>1.2±1.8</td><td>0.94±0.10</td></tr>
<tr><td>0</td><td>24</td><td>0.0±0.0</td><td>0.86±0.07</td></tr>
<tr><td>CP</td><td>48</td><td>35.8±10.8</td><td>0.34±0.10</td></tr>
</table>

MPE – micronucleated polychromatic erythrocytes
PCE – polychromatic erythrocytes
NCE – normochromatic erythrocytes</td></tr>
</table>

<table>
<tr><th>Method</th><th>Organism/ strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr>
<td>In vivo rat liver Unscheduled DNA synthesis

OECD486
GLP

Confidential (2008)
CAR 3.8.2</td>
<td>Rat, Wistar:
3 males/sampling time/test group

1 male/sampling time/control

Sampling times 2-4 and 12-16</td>
<td>Main study
Dose: 850 and 425 mg/kg bw in propylene glycol (gavage)

Positive control:
10 mg/kg bw dimethylnitrosamine (DMN)

50 mg/kg bw 2-acetylaminofluorene (AAF)</td>
<td>Negative
Main study: Clinical signs included: lethargy and hunched posture at 850 mg/kg bw. No treatment-related clinical signs were seen at 425 mg/kg or in the control groups.
At the 12 - 16 hours’ treatment time one additional animal was treated with 850 mg/kg body weight to correct for possible death.
<table>
<tr><th rowspan="2">Dose (mg/kg bw)</th><th rowspan="2">Sampling time (h)</th><th colspan="2">Net Nuclear Grain count (NNG)</th><th>% of cells in repair</th></tr>
<tr><th>Mean per animal</th><th>Group average</th><th>Mean per animal</th></tr>
<tr><td>0</td><td>2-4(1)</td><td>-0.8±1.0</td><td>-</td><td>0.0</td></tr>
<tr><td></td><td>12-16(1)</td><td>-0.8±0.8</td><td>-</td><td>0.0</td></tr>
<tr><td>850</td><td>2-4(3)</td><td>-0.6±0.9
-1.0±0.7
-0.8±0.9</td><td>-0.8</td><td>0.0</td></tr>
<tr><td></td><td>12-16(3)</td><td>-0.8±0.9
-0.8±0.8
-0.8±0.9</td><td>-0.8</td><td>0.0</td></tr>
<tr><td>425</td><td>2-4(3)</td><td>-0.8±0.8
-0.7±0.8
-0.9±0.8</td><td>-0.8</td><td>0.0</td></tr>
<tr><td></td><td>12-16(3)</td><td>-0.7±0.8
-0.7±0.9
-0.7±1.0</td><td>-0.07</td><td>0.0</td></tr>
<tr><td>10 (DMN)</td><td>2-4(1)</td><td>30.5±13.4</td><td>-</td><td>99.0</td></tr>
<tr><td>50 (AAF)</td><td>12-16(1)</td><td>23.3±11.6</td><td>-</td><td>97.0</td></tr>
</table>
</td>
</tr>
<tr>
<td>Rat stomach and duodenum comet assay

OECD 489

GLP

Confidential (2017)
CAR 3.8.2</td>
<td>Rat,
OFA Sprague-Dawley

Males

5/group</td>
<td>Dose (gavage)
50, 100 and 200 mg/kg bw in peanut oil

Test material: azamethiphos (99.68% pure)

Negative control:
Peanut oil

Positive control:
methylmethane sulfonate (20 mg/kg bw)</td>
<td>Negative
Preliminary toxicity and confirmatory toxicity assays were carried out. A MTD of 200 mg/kg bw was identified.

No statistically or biologically significant increases in the mean of medians of percentage of DNA in tail were observed at the 3 tested doses in samples taken from the stomach and duodenum

Conclusion
Not genotoxic under the experimental conditions.</td>
</tr>
</table>

Three *in vivo* studies have been evaluated: a mouse micronucleus test, a rat liver UDS test and an unconventional test for comet damage in DNA from rat stomach and duodenum.

No evidence of increased micronucleus formation was observed in male mice in the mammalian bone marrow micronucleus test. Given that azamethiphos was administered by the intra-peritoneal route, it induced systemic toxicity at both of the 2 higher doses used in this study, and toxicokinetic studies have demonstrated that greater than 90% of the substance is excreted via the urine (section 9), the target tissue was exposed adequately. Azamethiphos was well distributed to organs and tissues, including the bone marrow. The study was therefore considered to be robust and to provide clear evidence of the absence of an in vivo hazard to chromosomes.

In a guideline, GLP-compliant UDS assay, there was no evidence of any induction of UDS by azamethiphos. In a range-finding test, animals dosed at 850 mg/kg by gavage showed clinical signs of toxicity, while lethality was observed at the next dose level (1000 mg/kg bw). The viability of hepatocytes from animals treated with 850 mg/kg bw was demonstrated to be acceptable. Results from the negative and positive controls were within the expected range. It can therefore be concluded that the results from this study are reliable.

In an *in vivo* alkaline comet assay following OECD guideline 489, 3 groups of 5 male mice were administered two doses of either 50, 100 and 200 mg/kg bw azamethiphos in peanut oil 24 hours apart. A negative control group received vehicle only while a positive control group was given 100 mg/kg bw/d of methylmethane sulfonate. Samples for analysis were collected 3-4 hours after the second treatment. Neither statistically nor biologically significant increases in the mean of medians of percentage of DNA in tail were observed at the any of the concentrations tested in either stomach or duodenum (see table below)

Results for in vivo Comet Assay: rat stomach and duodenum

Test item	Dose (mg/kg bw/d) (x2)	% of DNA in tail			Percentage hedgehogs	Statistical significance
		Mean of medians per animals (5/group)	Non-parametric statistical assessment			
			p Kruskall-Wallis	p Mann-Whitney		
Stomach						
Peanut oil	0	4.52	N.S.	-	-	-
Azamethiphos	50	3.60		<0.5	0.87	N.S.
	100	3.76		N.S.	0.41	<0.01
	200	2.67		N.S.	0.66	N.S.
Methylmethane sulfonate	100	30.81	-	<0.01		<0.001
Duodenum						
Peanut oil	0	1.13	N.S.	-	-	-
Azamethiphos	50	0.72		N.S.	0.98	N.S.
	100	1.36		N.S.	1.14	N.S.
	200	1.31		N.S.	0.81	N.S.
Methylmethane sulfonate	100	18.02	-	<0.01		0.05

Azamethiphos was not genotoxic under the conditions of this *in vivo* alkaline comet study.

Overall, the results of these studies provide reassurance that azamethiphos has no *in vivo* mutagenic potential on somatic cells.

No studies on germ cells are available.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The mutagenicity of azamethiphos has been investigated in seven studies. Four *in vitro* studies were positive for mutagenicity (reverse mutation in bacteria, gene mutation in mammalian cells, chromosome aberration and *in vitro* alkaline comet). The three robust *in vivo* studies showed that azamethiphos did not induce micronuclei or DNA damage and thus it is considered as not mutagenic *in vivo* in the test systems used.

No human information is available.

10.8.2 Comparison with the CLP criteria

Substances classified as Cat 1A are known to induce heritable changes or are regarded as if they induce heritable changes in germ cells of humans. There is no human data to suggest that azamethiphos causes heritable mutations and therefore it is not a Cat 1A mutagen.

Classification in category 1B can be based on: positive results from at least one *in vivo* heritable germ cell mutagenicity test in mammals; or, positive results from tests showing mutagenic effects in the germ cells of humans but without transmission to progeny; or, positive results from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence to suggest that the substance has the potential to cause mutations in germ cells. Based on the available data in mammals, azamethiphos does not meet the criteria for classification as a Cat 1B mutagen.

To attain category 2, the substance needs to show positive results in at least one *in vivo* somatic cell mutagenicity test in mammals indicating mutagenic effects in somatic cells or positive results in at least one *in vivo* somatic cell genotoxicity test, supported by *in vitro* mutagenicity results. Positive results in *in vitro* studies can only lead to classification as a Category 2 mutagen where there is support by chemical activity relationship to known germ cell mutagens. Based on all the available data, in particular the absence of genotoxicity in three *in vivo* studies, azamethiphos does not meet the criteria for classification as a category 2 mutagen.

No germ cell mutagenicity classification of azamethiphos is proposed.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified – conclusive but not sufficient for classification.

10.9 Carcinogenicity

The carcinogenic potential of azamethiphos has been investigated by the oral route in rats and mice.

Table 16: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Oral (gavage)
12/24 month combined chronic/ carcinogenicity study
Rat, Crl:WI(Han)
Males/females
50/sex/dose (carcinogenicity group)
OECD 453
Study compliant with GLP and OECD guidelines
Reliability:1

Confidential (2011a),
CAR 3.9</td><td>Dose: 0, 0.05, 0.5 and 5 mg/kg bw (daily)
Vehicle: propylene glycol</td><td><u>Non-neoplastic findings</u>

No treatment-related effects on mortality / survival rates, clinical signs, or body weight, functional observations, ophthalmoscopy, haematology, urinalysis or organ weight at any dose tested.

Treatment-related effects on cholinesterase activity are reported in Section 10.12.

<u>Neoplastic findings</u>.

<table>
<tr><th></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>0.05</td><td>0.5</td><td>5</td><td>0</td><td>0.05</td><td>0.5</td><td>5</td></tr>
<tr><td colspan="9">Jejunum</td></tr>
<tr><td>Leiomyoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1 (2%)</td><td>2 (4%)</td><td>2 (4%)</td></tr>
<tr><td>Leiomyosarcoma</td><td>0</td><td>0</td><td>0</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Duodenum</td></tr>
<tr><td>Leiomyoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Ileum</td></tr>
<tr><td colspan="9">Ileum examined, but no findings reported in any groups</td></tr>
</table>

<table>
<tr><th>Dose (mg/kg bw/d)</th><th>0</th><th>0.05</th><th>0.5</th><th>5</th></tr>
<tr><td>Endometrial glandular hyperplasia</td><td>3 (6%)</td><td>0</td><td>0</td><td>3 (6%)</td></tr>
<tr><td>Endometrial adenoma</td><td>1 (2%)</td><td>1 (2%)</td><td>1 (2%)</td><td>0</td></tr>
<tr><td>Endometrial adenocarcinoma</td><td>6 (12%)</td><td>2 (4%)</td><td>6 (12%)</td><td>12 (24%)</td></tr>
</table>

Historical Control Data*
<table>
<tr><td>Start/end dates</td><td colspan="2">19.11.2008–5.11.2010</td></tr>
<tr><td>Sex</td><td>M</td><td>F</td></tr>
<tr><td>Number of rats examined</td><td>150</td><td>150</td></tr>
<tr><td>Leiomyoma</td><td></td><td></td></tr>
<tr><td>duodenum</td><td>0</td><td>0</td></tr>
<tr><td>jejunum</td><td>0</td><td>1 (0.7%)</td></tr>
<tr><td>ileum</td><td>1 (0.7%)</td><td>0</td></tr>
<tr><td>Total Small intestine</td><td>1 (0.7%)</td><td>1 (0.7%)</td></tr>
<tr><td>Uterine Endometrial adenocarcinoma</td><td>-</td><td>21 (14%)</td></tr>
</table>
*study in Wistar (Han) rats and performed in the same laboratory.</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Oral (dietary)
2 year carcinogenicity study
Rat CD(SD)BR
Males/females
60/sex/dose (carcinogenicity group)
Guideline not stated.
Pre-GLP
Reliability: 2

Confidential, (1982)</td><td>Azamethiphos
Purity: 95.6%
Dose: 0, 15, 60 & 327 ppm
Approximately 0, 0.8, 3 & 16 mg/kg bw/d</td><td><u>Non-neoplastic findings</u>
There were no significant increases in mortality in any dose group.

327 ppm (equivalent to 16 mg/kg bw/d)
Significantly↓ body weight((12.1% (males) and (15.7% females))
Significant ↑kidney lesions (unspecified) in males (9/60 vs 3/60 in controls)
Significantly ↑mammary gland cyst in females (18/60 vs 5/60 in controls)
Adverse effects on CHER (>20% reduction) were reported at 3 and 6 months in all dose groups (both sexes).

↓CHER# (>20%)

60 ppm (equivalent to 3 mg/kg bw/d)
↓CHER# (>20%)

15 ppm (equivalent to 0.8 mg/kg bw/d)
↑mammary cist (not significant 16/60)
↓CHER# (>20%)

<u>Neoplastic findings</u>
No treatment related increase in tumour incidence. An inversion of ratio of mammary gland fibroadenoma to adenocarcinoma.

<table>
<tr><td></td><td colspan="4">Males</td><td colspan="4">Females</td></tr>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>0.8</td><td>3</td><td>16</td><td>0</td><td>0.8</td><td>3</td><td>16</td></tr>
<tr><td>Mammary gland Cyst</td><td>0</td><td>0</td><td>0</td><td>2</td><td>5</td><td>16</td><td>7</td><td>18*</td></tr>
<tr><td>Fibroadenoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>19</td><td>14</td><td>12</td><td>11</td></tr>
<tr><td>Adenocarcinoma</td><td>2</td><td>1</td><td>0</td><td>0</td><td>6</td><td>8</td><td>10</td><td>14</td></tr>
<tr><td>Animals with malignant neoplasms</td><td>11</td><td>7</td><td>7</td><td>4</td><td>15</td><td>11</td><td>18</td><td>16</td></tr>
<tr><td>Animals with any neoplasm</td><td>40</td><td>42</td><td>36</td><td>39</td><td>56</td><td>50</td><td>56</td><td>50</td></tr>
</table>
* p<0.05

This study was carried out between 1977 and 1979. It was subject to a validation audit (by Ciba Geigy). The final report was issued in 1982. The overall standard of the investigation and reporting was considered acceptable for a rat chronic/carcinogenicity study.</td></tr>
<tr><td>Oral (dietary)
2 year carcinogenicity study (with interim kill at 52 weeks + 4 week post exposure group)
Rat (Sprague-Dawley)
Males/females
50/sex/dose</td><td>Dose: 0, 20, 200 & 1500 ppm
(Equivalent to 0, 0.8, 8.2 and 62.2 mg/kg bw/d (males); 0, 1.1, 11.2 and 88.7 mg/kg bw/d (females) mg/kg bw/d) for the carcinogenicity test)</td><td><u>Non-neoplastic findings</u>
There were no significant increases in mortality in any dose group.

1500 ppm (62.2 mg/kg bw/d (males) and 88.7 mg/kg bw/d (females))
↓ body weight (20.8% (males) and 27.8% (females))
Significantly ↑ relative kidney weight at 52 weeks (29% in females). Significantly ↑pyometra at week 52 (6/9 females), ↑hydrometra (23/90 females) ↑biliary proliferation in liver (21/90 males) and gastritis (7/90 females).
↓ serum potassium at 3 and 6 months (11% and 15% respectively); not seen at 18 months (females only)
↓CHER# (>20%)
200 and 20 ppm (8.2 and 11.2 mg/kg bw/d (males) and 0.8 and 1.1 mg/kg bw/d (females)

↓CHER# (>20%)</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Dose levels	Observations and remarks (effects of major toxicological significance)
(carcinogenicity group) OECD 409* Reliability: 1 Confidential (1989) CAR 3.9		**Neoplastic findings** No treatment related increase in tumour incidence. *Study guideline reported as OECD 409 (90-day study in non-rodents). However, the study protocol covers the essential elements of OECD 453 for a combined chronic/carcinogenicity study in rodents. Group sizes and duration complied with OECD 453. Observations performed are also those listed in OECD 453 and included the following. • Experimental observations on morbidity and mortality, clinical signs, body weight, food consumption and ophthalmoscopy. • Laboratory investigations on haematology, clinical chemistry, urine analysis and brain cholinesterase. • Pathology investigations including a full investigation of all lesions (internal and external), organ weights and histopathology (as listed in OECD 453).
Oral (dietary) Lifetime carcinogenicity study Mouse (Crl:CD-1 (ICR) BR) Males/females 51/sex/dose (carcinogenicity group) OECD 451 Study compliant with GLP and OECD guidelines and considered to be reliable Reliability: 1 Confidential (1989) CAR 3.9	Azamethiphos Purity Dose: 0, 50, 500, 1500 & 4000 ppm Equivalent to: 0, 6.2, 60.2, 183.4 and 491.4 mg/kg bw/d (males) 0, 7.7, 76.2, 219.7 and 582.9 mg/kg bw/d (females)	**Non neoplastic effects** 4000 ppm (491 mg/kg bw/d (males) and 582.9 mg/kg bw/d (females)) ↓survival from week 60 (males) and week 80 (females). Survival at termination is (11/51 vs 16/51 in controls (males); and 18/51 vs 22/51 in controls (females)). Small intestine hyperplastic/avillous mucosa (38/51 males & 41/51 females). ↓body weight 1500 ppm (183.4 mg/kg bw/d (males) and 219.7 mg/kg bw/d (females)) Small intestine hyperplastic/avillous mucosa (34/51 males & 36/51 females) 500 ppm (60.2 mg/kg bw/d (males) and 76.2 mg/kg bw/d (females)) Small intestine hyperplastic/avillous mucosa (9/51 males & 25/51 females) **Neoplastic effects** No treatment related increase in tumour incidence.
Oral (dietary) Lifetime carcinogenicity study Mouse (CD-1 (ICR) BR) Males/females	Azamethiphos Purity not specified Dose: 0, 11, 97 & 396 ppm Approximately 0, 2, 14 & 57	**Non neoplastic effects** There was no significant increase in mortality. Clinical signs and body weights were similar in all groups. No consistent pattern of findings following gross examination. Microscopic examination identified a range of lesions typical of aged mice in all groups. pigment/amyloid deposition in a range of tissues, but no clear dose response and no individual findings were statistically significant ($p<0.05$).

Method, guideline, deviations if any, species, strain, sex, no/group	Dose levels	Observations and remarks (effects of major toxicological significance)
60/sex/dose (carcinogenicity group) Non-guideline Pre-GLP Reliability: 2 Confidential. (1982)	mg/kg bw/d	**Neoplastic effects** Hepatocellular adenoma in males 4, 7, 6 and 11 at 0, 11, 97 and 396 ppm. No statistical difference in level between control animals and any treated group (p=0.053, one way Fisher exact test). Pathologists description reported adenoma were of similar appearance in all groups. Not seen in females (1, 1, 0 and 1hepatoceullar adenoma in females at 0, 11, 97 and 396 ppm, respectively. Hepatocellular carcinoma: 4, 2, 7 and 4 males at 0, 11, 97 and 396 ppm, respectively (i.e. no dose-response). No hepatocellular carcinomas in females of any group. This study was carried out between 1977 and 1979, and was subject to a validation audit performed by Ciba Geigy. A final report was issued in 1982. The audit found no evidence of malpractice. The overall standard of the investigation and reporting was considered acceptable for a mouse chronic/carcinogenicity study.

#CHER: acetylcholinesterase activity in erythrocytes

The carcinogenic potential of azamethiphos has been investigated in five studies; a 24-month combined chronic/carcinogenicity study, two 2-year carcinogenicity studies in rats and two lifetime carcinogenicity studies in mice.

Rats

An increased incidence of leiomyoma of the jejunum was observed in each group of female rats administered azamethiphos in the combined chronic/carcinogenicity study. The incidence rates were: 0/50, 1/50, 2/50 and 2/50 at 0, 0.05, 0.5 and 5 mg/kg azamethiphos, respectively. No incidences of leiomyoma were seen in males in any part of the small intestine. Historical control data are limited to only one study that was concurrent with the combined chronic/ carcinogenicity study. In this study there were two cases of leiomyoma of the small intestine (0.7%), one in the jejunum (female) and one in the ileum (male); thus the historical control data show a low level background incidence of leiomyoma in the small intestine. There is no statistically significant difference between the incidence of leiomyoma of the jejunum seen in female animals in the high-dose azamethiphos group and that from the internal control group in a pair wise comparison ($p<0.05$).

The combined chronic/ carcinogenicity study also identified an increased incidence of endometrial adenocarcinoma in the high dose group females. The incidence in the highest dose group (12 incidences compared with 6 in the control group) is not statistically different from that seen in the control group by pair-wise analysis ($p<0.05$). Similarly, there is no statistically significant difference between the incidence of uterine adenocarcinoma in the animals treated with azamethiphos and in the internal control group ($p<0.05$).

There were no treatment-related tumour findings in the earlier studies in rats or in the studies in mice carried out at much higher doses than those used in the combined chronic/ carcinogenicity study.

Other relevant information

The data from some of the studies [the four studies reported in 1982 and 1989] have been considered previously by the UK Advisory Committee on Pesticides (ACP) in 2003 and the EMEA Committee for Veterinary Medicinal Products (EMEA/MRL/527/98-FINAL)[1] both of whom concluded that there were no treatment related neoplastic effects in these studies. A summary of these studies is provided in table 16 above.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Five studies are available to inform on the carcinogenic potential of azamethiphos, three in rats and two in mice. No treatment-related neoplastic findings were reported in two of the rat studies or either of the mouse studies. Small increases in the incidences of leiomyoma and endometrial adenocarcinoma were reported in another rat study in which azamethiphos was administered at doses up to 5 mg/kg/d, neither of which showed a clear dose-response relationship; these findings are discussed further below.

Leiomyoma

A leiomyoma is a benign tumour of the smooth muscle that can occur in any organ, but the most common forms occur in the uterus, small bowel, and the oesophagus. In the small intestine leiomyomas are most commonly found in the jejunum and ileum. In one of the three available rat studies only, there were incidences of 0/50, 1/50, 2/50 and 2/50 leiomyoma of the jejunum in female WI(Han) rats at 0, 0.05, 0.5 and 5 mg/kg. No such tumours were seen in male rats in this study or in either of the other two rat studies, in both of which the doses of azamethiphos were higher. Although there was a positive trend in the incidence of leiomyoma of the jejunum observed from the control to the highest dose group, when the results from the duodenum and jejunum are combined and incidences in the entire small intestine are analysed according to the method of Peto *et al* (1980) this positive trend was not observed. There was no clear dose-response associated with the finding (over a dose range of 0.05 to 5 mg/kg bw/d) and no statistically significant difference between the incidence seen in the control group and that seen in any of the test groups ($p<0.05$ pair-wise comparison). This approach is described by McConnell *et al* (1986) and was accepted by the US National Toxicology Program in evaluating rodent carcinogenicity studies. It is also consistent with the REACH Member State Committee decision (MSC 47/48) not to specify whether the jejunum and duodenum is sampled in *in vivo* comet assays due to the difficulty in distinguishing between the two tissues. Furthermore, the validity of trend testing in the absence of pairwise significance and a reported control value of zero is questionable.

Contemporary historical control data from the testing laboratory is limited to one study; this showed a low-level background incidence of leiomyoma in the small intestine (1/150 females and 1/150 males). Leiomyoma was not identified in any of the earlier carcinogenicity studies that each employed far higher doses, two in rats (up to 16 mg/kg bw/d and 88.6 mg/kg bw/d) and two in mice (up to 57 mg/kg bw/d and 582.9 mg/kg bw/d).

Overall, in view of the absence of a clear dose-response, the absence of a statistically significant difference between the treated animals and the controls and the absence of similar findings in earlier

[1]http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500010779.pdf

studies in rats and in mice (at much higher dose levels), it is concluded that the observed leiomyoma was an incidental finding and is not a treatment-related effect.

Uterine endometrial adenocarcinoma

The reported incidences of uterine endometrial adenocarcinoma were variable across the test groups, with no dose-response relationship and a high incidence in the control animals (i.e., 6/50 (12%), 2/50 (4%), 6/50 (12%) and 12/50 (24%) at 0, 0.05, 0.5 and 5 mg/kg bw/d respectively). Contemporary historical control data from the testing laboratory is limited to a single study, in which the incidence of endometrial adenocarcinoma was reported to be 21/150 animals (14%). There were no changes in any other uterine pathology; adenoma and hyperplasia were comparable in all groups and there was no increase in the occurrence of pre-neoplastic lesions. Similarly in the 90-day and 12 month studies there was no evidence of pre-neoplastic lesions of the uterus. Historical control data are available from three earlier studies (conducted between 1999 - 2004) in which the background incidence of endometrial adenocarcinoma ranged from 0-6%. Historical control data from the supplier of the test animals shows a highly variable incidence of uterine endometrial adenocarcinoma, with a background incidence in the range of 0.89 to 14% (Giknis and Clifford, 2011); although it is noted that this data is drawn from studies initiated over a long time span (1997-2009).

The two earlier studies in rats treated with azamethiphos did not report any incidences of endometrial adenocarcinoma at dose levels up to 16 and 88.6 mg/kg bw/d; nor was it reported in mice exposed to much higher doses of azamethiphos. Overall, the increased incidence of uterine endometrial adenocarcinoma was observed in the top dose group in one study only. Whilst the incidence was above that seen in historical controls, it should be noted that there was no dose response and the background incidence in the concurrent control group was already high. Furthermore, it was not seen in two additional studies in rats at higher doses, nor was it seen in two studies in mice at much higher doses. Considering the weight of evidence, it is concluded that the increase in this tumour type was an incidental finding not a treatment-related effect.

Overall, it is concluded that there were no treatment-related tumours.

10.9.2 Comparison with the CLP criteria

Classification in category 1A or 1B is not appropriate as there is no human evidence establishing a causal relationship between exposure to azamethiphos and the development of cancer nor is there sufficient evidence of carcinogenicity in experimental animals.

A substance can be classified in Category 2 for carcinogenicity on the basis of limited evidence of carcinogenicity in experimental animals. From the available body of evidence on azamethiphos, drawn from five studies in which the substance was administered orally at doses up to 88.6 mg/kg bw/d in rats and 582.9 mg/kg bw/d in mice, there was no clear evidence of a consistent, treatment-related neoplastic effect. Therefore, taking a weight of evidence approach, it is concluded that there are insufficient grounds to classify in category 2.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Not classified – conclusive but not sufficient for classification

10.10 Reproductive toxicity

The reproductive toxicology of azamethiphos has been investigated in three OECD and GLP compliant studies. The potential for azamethiphos to affect development has been investigated in rats and rabbits, while effects on fertility were investigated in rats in a two-generation reproduction study.

10.10.1 Adverse effects on sexual function and fertility

Table 17: Summary table of animal studies on adverse effects on sexual function and fertility

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Two-generation reproductive toxicity study Oral (gavage) Rat, Sprague Dawley 24/sex/group OECD 416 GLP Confidential (2009) CAR 3.10.2	**F_0 generation** *Days 1-9:* 1, 10 and 100 mg/kg bw/d *Day 10 – 16:* 0.01, 0.1 and 1 mg/kg bw *Day 17 onwards:* 0.05, 0.5 and 5 mg/kg bw **F_1 generation** 0.05, 0.5 and 5 mg/kg bw Vehicle: propylene glycol	**At 5 mg/kg:** 1 female killed in extremis on day 25 post-coitum due to suspected early delivery; adhesions of the left horn of the uterus noted at necropsy. Not considered treatment related. Parental toxicity: ↑body wt at day 4 post-coitum and day 4 lactation onwards (<10%). Inhibition of CHER 35% and 31% in males and females respectively) Reproduction and developmental toxicity: no findings. **At 0.5 mg/kg:**_↓body weight (<10%) from day 8 onwards. No effects on reproduction or development **At 0.05 mg/kg:** 1 male killed in extremis on day 28: abnormal gait/swelling and general erythema of the left hind leg prior to sacrifice and an oedematous subcutis, reddish discolouration and a thickened left hind leg at necropsy Not considered treatment related, No other parental effects reported. Reproduction and developmental toxicity: No findings. <u>*Other dose levels (before Day 17):*</u> **100 mg/kg**: ↓body wt at day 8. Inhibition CHER (63% in males and 60% in females) on Day 9. **At 10 mg/kg:** Inhibition of CHER (64% and 51% in males and females respectively) on day 9 **At 1 mg/kg:** 1 female died on day 16 approximately 3 h after dosing. No cause of death could be established; the only finding before death was slight salivation, at necropsy enlarged liver correlating with congestion at microscopic examination. This was not considered to be a contributory factor to death. Not considered treatment related, Inhibition of CHER in both sexes on Day 9 (45% and 41% in males and females respectively) and on Day 16 (28% in males). **F_1-GENERATION** **At 5 mg/kg:** 1 female killed in extremis on day 1 of lactation due to difficulties during/just after delivery of pups. At necropsy pale discolouration of the stomach and kidney, foci on the liver, kidney and adrenal glands and an enlarged adrenal gland; coagulative necrosis of the liver, kidneys and adrenal glands at microscopic examination. Not considered treatment related, Parental toxicity: inhibition of CHER at the end treatment (30% and 32% in males and females). No effects on reproduction or development. **At 0.5 mg/kg:** No effects on parents, reproduction or development

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
		At 0.05 mg/kg: 1 female died on day 1 of lactation due to difficulties during/just after delivery of pups. At necropsy black-brown discolouration and an accentuated lobular pattern of liver, a gelatinous pancreas, dark red foci and discolouration of the kidneys and adrenal glands, alopecia at necropsy and coagulative necrosis of kidneys and the adrenal glands at microscopic examination. No effects on parental, reproduction or developmental parameters **F_2-GENERATION** No treatment-related effects at any dose level

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

The effects on fertility have been investigated in a multigeneration study in Sprague Dawley rats. No effects were seen on mating performance, number of pregnant animals, number of implantations or post-implantation loss.

No human information is available.

10.10.3 Comparison with the CLP criteria

No effects were observed that provide evidence to suggest that azamethiphos adversely affects sexual function or fertility. Therefore, it does not meet the criteria for classification.

10.10.4 Adverse effects on development

Table 18: Summary table of animal studies on adverse effects on development

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Prenatal developmental Oral (gavage) Rat, Sprague Dawley Female 24 for control, low and high dose 25 for mid dose OECD 414 GLP Confidential (2009) CAR 3.10.1	0, 0.1, 1 and 10 mg/kg bw (daily) Vehicle: propylene glycol Dosing days 6 – 20 post-coitum. Purity: 96.2%	*Maternal toxicity* No treatment -related effect on mortality, clinical signs, body weight, food consumption or necropsy. **At 10mg/kg bw/d:** Inhibition of CHER activity of 34.8 and 9.7% in erythrocytes and brain respectively. **At 1 mg/kg bw/d and below:** Inhibition of CHER activity of 3.4 and 7.9% in erythrocytes and brain respectively. **At 0.1 mg/kg bw/d:** ↑bd wt during days 16-20. Inhibition of CHER activity of 0.3 and 6.7% in erythrocytes and brain respectively. *Fetal toxicity* No evidence of an effect on embryo-fetal development at any dose tested.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Prenatal developmental Oral (gavage) Rabbits New Zealand White Female 24/ control, low and mid dose groups 25 high dose group OECD 414 GLP Confidential (2009) CAR 3.10.1	0, 0.05, 0.5 and 5 mg/kg bw in (daily) Vehicle: Arachis oil Dosing days 7 – 29 post-coitum Purity:96.2%	*Maternal toxicity* No treatment-related effect on mortality, clinical signs, body weight, food consumption or necropsy. **At 5 mg/kg bw/d**: Inhibition of CHER activity of 69 and 11% in erythrocytes and brain respectively. **At 0.5 mg/kg bw/d and below**: No treatment-related effects *Fetal toxicity* No treatment-related effects at the highest dose tested

The potential for azamethiphos to cause developmental toxicity has been investigated in rats and rabbits in two developmental toxicity studies and one multigeneration study in rats (see Section 10.10.1).

Rats

Azamethiphos was administered by gavage to groups of female Sprague Dawley rats from days 6 to 20 of gestation to investigate the effects on dams and embryo-fetal development. One death occurred in the high dose group as the result of a gavage error. There was no effect on body weight, food consumption or necropsy findings. Effects on maternal toxicity were restricted to an inhibition of cholinesterase activity in the erythrocytes of 34.8% at the top dose.

There was no evidence of an effect on embryo-fetal development at any doses tested. Malformations and developmental variations occurred at similar incidences in the control and dose groups, with no evidence of a treatment-related increase in any individual or total malformation(s) and variation(s). Similarly in the multigeneration study, no fetal malformations or abnormalities were reported up to the top dose.

Rabbits

Azamethiphos was administered by gavage to groups of female New Zealand White rabbits from days 7 to 29 post-insemination. There were no treatment-related effects on mortality, body weight, food consumption or necropsy findings. Effects on maternal toxicity were restricted a 69% inhibition of cholinesterase activity in the erythrocytes. There was no evidence of an effect on embryo-fetal toxicity.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

No effects were seen on developmental parameters. No treatment-related fetal malformations or abnormalities of concern were noted in any of the studies.

10.10.6 Comparison with the CLP criteria

No effects were observed that provide evidence to suggest that azamethiphos adversely affects development. Therefore, it does not meet the criteria for classification.

10.10.7 Adverse effects on or via lactation

No effects were reported in pups in either the reproduction or developmental toxicity studies.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

10.10.9 Comparison with the CLP criteria

No effects were reported in pups that provide evidence to suggest that azamethiphos has adverse effects on or via lactation. Therefore it does not meet the criteria for classification.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Not classified – conclusive but not sufficient for classification

10.11 Specific target organ toxicity-single exposure

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Data from the acute oral and inhalation studies indicate that exposure to azamethiphos results in neurotoxicity after a single exposure. Refer to sections 10.1 and 10.3 for full details.

10.11.2 Comparison with the CLP criteria

Classification as either STOT-SE1 or 2 is applicable to substances that have produced non-lethal toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following a single exposure.

Classification as STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

Animals when exposed to azamethiphos via the oral route showed no organ-specific effects at necropsy at any dose tested. Signs of neurotoxicity following a single exposure at 300 mg/kg bw were confined to a transient observation of uncoordinated movements in 1 out of 3 animals following treatment. These were only observed on the day of treatment. At the next dose level all animals died.

Similarly, a single exposure via the inhalation route gave no indication of organ-specific toxicity at any dose tested. At doses of 1.1 mg/l, signs indicative of acute neurotoxicity were observed. These included shaking heads and spread hind legs on removal from the restraining tubes and tremors (3/5 males and all females). In most animals these were only seen on day 1; where they persisted to day 2 the animals affected were reported dead on day 3. All other symptoms were reversible. At the

next dose level (5.2 mg/l) all animals died. No neurotoxic effects were observed at lower dose levels.

Overall, based on the clinical signs seen in the acute oral and inhalation studies, no classification is proposed for STOT-SE. STOT-SE categories 1 or 2 are not justified as no effects were reported on specific organs at necropsy. STOT-SE3 is not considered applicable as, although signs of neurotoxicity were observed, these were transient and were seen in the presence of animal deaths, which are accounted for in the proposed classifications for acute toxicity (sections 10.1-10.3).

10.11.3 Conclusion on classification and labelling for STOT SE

Not classified – conclusive but not sufficient for classification

10.12 Specific target organ toxicity-repeated exposure

The repeated dose toxicity of azamethiphos has been investigated by the oral route in 3 studies in the rat (28 day, 90 day and 12/24 months) and 1 study in the dog (90 day). There are no repeated dose studies via the inhalation or dermal routes of exposure.

Table 19: Summary table of animal studies on STOT RE

Method	Dose levels	Observations and Remarks	Reference
28 day oral (gavage) Rat, Sprague Dawley 3/sex/group OECD 407 (not fully compliant) GLP	0, 0.5, 5 and 50 mg/kg bw/d 96.2% pure Vehicle: propylene glycol Guidance value for classification is 300 mg/kg bw/d	**50 mg/kg bw/d:** 1 female died on day 6 (not treatment related). ↓CHER[#] in both sexes (20%/48% male/female respectively). Clinical signs included intermittent lethargy (1 female), tremors (2 males/1 female), uncoordinated movements (1 female) and salivation (1 male) ↑ Ca (females). **5 mg/kg bw/d:** ↓CHER[#] in females (37%). Clinical signs included intermittent tremors (3 males/2 females). **0.5 mg/kg bw/d:** Clinical signs included intermittent lethargy (1 male), tremors (2 males/3 females), uncoordinated movement (1 male)	Confidential (2009) CAR 3.5.1
90 day combined repeated-dose / neurotoxicity oral (gavage) study Rats, Sprague Dawley 15/sex/group OECD 408/424 GLP	0, 0.05, 0.5 and 5 mg/kg bw/d 96.2% pure Vehicle: propylene glycol Guidance value for classification is <100 mg/kg bw/d	No treatment-related mortality **At 5 mg/kg bw/d** ↓ CHER[#] (60%/50% males/females at 8 weeks and 25/28% at 13 weeks). ↑ salivation (15 males on 216 days and 12 females on 71 days) ↑tremors (11 males on 12 days and 2 females on 2 days) **At 0.5 mg/kg bw/d** ↓ CHER[#] (22%/4.9% at 8/13) (females) weeks respectively. ↑salivation (15 males on 39 days and 8 females on 11 days) ↑ tremors (2 males on 2 days and 3 females on 5 days). **At 0.05 mg/kg bw/d** ↓ CHEBR[$], (12% males).	Confidential (2009b) CAR 3.6.1

12/24 month oral (gavage) combined chronic/ carcinogenicity study Rat, Crl:WI(Han) Satellite group: 40/sex/dose Chronic: 50/sex/group OECD 453	0, 0.05, 0.5 and 5 mg/kg bw (daily) 96.2% pure Vehicle: propylene glycol Guidance value for classification (≤ 24/12 mg/kg bw/d for 12 and 24 months respectively, calculated from the values for the 90 day rat study)	No treatment-related effects on mortality, clinical signs, haematology or body weight at any dose tested. **At 5 mg/kg bw/d** ↓ CHER[#] (-34 to -48% (males), -27 to -41% (females) compared to controls). **0.5 and 0.05 mg/kg bw/d** No toxicologically relevant effects.	Confidential (2011a), CAR 3.9
90 day oral (gavage) study Dog, Beagle 4/sex/group OECD 409	0, 0.2, 2 and 20 mg/kg bw/d 96.2% pure Vehicle: propylene glycol A guideline value of 100 mg/kg/d is considered for classification based on the value defined for the rat 90 day study.	No treatment related mortality. **At 20 mg/kg bw/d** ↓CHER[#] (up to 87%). ↑Tremors (4/4 males (incidence*: 88) and 4/4 females (incidence 43 vs 0 in controls)) ↑ salivation 3/4 males and 4/4 females (incidence: 231 and 309 respectively) vs 1 (incidence: 1) and 0 in controls ↑ head shaking (4/4 males and 4/4 females (incidence: 164 and 314 days respectively) vs 0 in controls) ↑ vomiting of food 4/4 males and 4/4 females (incidence: 27 and 64 respectively) vs 2 males (incidence: 12 days and 1) in controls ↑ vomiting of mucous 4/4 males and 4/4 females (incidence: 5 and 40) vs 1 and 1 (incidence 5 and 2) in controls ↑liver to body weight ratio (2.7% vs 3.2% (females only). **At 2 mg/kg bw/d** ↓CHER[#] in males (43%). ↑Tremors (2/4 males (incidence 2) and 4/4 females (incidence 11) vs 0 in controls) ↑ salivation 2/4 males (incidence 2) and 1/4 females (incidence 3) vs 1 (incidence 1) and 0 in controls ↑head shaking (1/4 males (incidence 1) and 2/4 females (incidence 4) vs 0 in controls) **At 0.2 mg/kg bw/d** ↑head shaking (1/4 males (incidence 7) vs 0 in controls)	Confidential (2011) CAR 3.6.1

[#]CHER: acetylcholinesterase activity in erythrocytes
[$]CHEBR: acetylcholinesterase activity in brain
*incidence is the total number of days across all animals when the effect was seen

Repeated dose toxicity: oral

Rat

There are three studies investigating the repeated-dose toxicity of azamethiphos in the rat via the oral route: a 28-day study, a 90-day repeated-dose/neurotoxicity study and a combined 12/24-month study.

In a 28-day non-guideline range-finding study to investigate the short term toxicity of azamethiphos, the main effect was on cholinesterase inhibition which became adverse (i.e., ≥ 20% - JMPR Report, 1998) in females at doses of 5 mg/kg bw/d and both sexes at the top dose. Clinical signs included lethargy, calm behaviour, tremors, flat/hunched posture, uncoordinated movement,

piloerection and/or salivation. These were seen to some extent at all dose levels, were intermittent in nature, were generally shown by individual animals only, did not appear to be related to the duration of treatment and showed no clear correlation to the administered dose.

The 90-day study included a battery of neurotoxicity tests. Three animals were found dead prior to sacrifice, but as there was no correlation with dose, these deaths are considered to be unrelated to treatment. The main effect was on cholinesterase inhibition which was >20% in both sexes at the top dose. It was also found to be slightly above the level considered adverse in mid-dose group females at 8 weeks (22%), however this finding was not present at 13 weeks (4.9%).

The long-term toxicity of Azamethiphos was tested in a 12/24 month combined chronic/carcinogenicity study in accordance with OECD 453. Animals were dosed with Azamethiphos at 0.05, 0.5 and 50 mg/kg bw/d. As with the short-term and sub-chronic study the key finding was on cholinesterase inhibition, with CHER decreased by > 20% in males and females at 5 mg/kg bw/d. However, this was seen in the absence of treatment-related clinical signs.

Dogs

In a 90 day study in Beagle dogs, animals were exposed to Azamethiphos at doses of 0.2, 2 and 20 mg/kg bw/d. All doses are below the guideline value of 100 mg/kg bw/d considered for classification based on the value defined for the rat (90 day study).

At 20 mg/kg/day, inhibition of cholinesterase activity that reached adverse levels (i.e. 87%) was observed. Transient clinical signs including tremors, salivation, shaking of the head and vomiting of food (and mucus in females) were frequently noted in all animals after dosing. At 2 mg/kg/day, inhibition of cholinesterase activity reached adverse levels in males only (43%). Clinical observations were observed spasmodically throughout the study and included tremors, salivation and shaking of the head. These effects are commonly seen following organophosphate exposure, however they were only reported incidentally at this dose level so are not considered to be adverse.

Human information

No human information available.

Repeated dose toxicity: inhalation

No data available.

Repeated dose toxicity: dermal

No data available.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The short-term repeated-dose toxicity of azamethiphos was investigated in rats and dogs. The long-term repeated-dose toxicity was addressed in a combined chronic/carcinogenicity study in rats. The carcinogenicity phase of this study is reported at Section 10.9.

At all doses, the only treatment-related finding was a reduction of cholinesterase activity in red blood cells in both rats and dogs, which was consistent with the mode of action of azamethiphos. Cholinesterase in erythrocytes was reduced by up to 60% in rats and 87% in dogs. The reported behavioural effects were transient and spasmodic in nature. The effect on cholinesterase is considered relevant to humans. No other consistent, treatment-related effects were reported in either the rat or the dog.

10.12.2 Comparison with the CLP criteria

Classification as either STOT-RE1 or 2 is applicable to substances that have produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.

Although the key finding in both rats and dogs, inhibition of cholinesterase activity in erythrocytes, was reported at doses below the guidance values for classification for STOT-RE Category 2 (<10 C ≤ 100 mg/kg bw/d based on a 90-day study in rats or ≤300 mg/kg bw/d in a 28-day study), this effect does not meet the criteria of significant or severe toxicity. There were no significant functional effects associated with the changes in cholinesterase activity observed in the FOB; where clinical signs indicative of neurotoxicity were observed (salivation, tremor and head shaking), these were transient in nature. These transient clinical observations and changes in cholinesterase activity do not indicate significant toxicity. Furthermore, lethality associated with cholinesterase inhibition was observed in the acute toxicity studies, classification and it is proposed to classify for acute toxicity accordingly.

It is thus concluded that azamethiphos does not require classification for specific target organ toxicity following repeated exposure.

10.12.3 Conclusion on classification and labelling for STOT RE

Not classified – conclusive but not sufficient for classification

10.13 Aspiration hazard

Not relevant, the substance is a solid.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

All references are taken from Section 4.1 and 4.2 of Part A of the Competent Authority Report (CAR) for azamethiphos – November 2017 (and Section A7 of Doc IIIA to the CAR).

11.1 Rapid degradability of organic substances

11.1.1 Ready biodegradability

Table 20: Available studies on the biodegradation of azamethiphos

Guideline / Test method	Test type[1]	Test substance conc. (mg l^{-1})	Degradation		Reference
			Incubation period (days)	% degradation (mineralisation) at day 28	
OECD 301B Purity 96.2%	Ready	36	28	17	Desmares-Koopman (2008) CAR 4.1.1.2
OECD 314B Purity 99.4%	Aerobic	25	28	44	Schaefer and Carpente (2014a) CAR 4.1.1.3
OECD 314C Purity 99.4%	Anaerobic	25	56	8	Schaefer and Carpente (2014b) CAR 4.1.1.3

No predictive biodegradation estimates are available.

Screening tests

A standard ready biodegradation study monitoring CO_2 evolution [modified Sturm Test] is available, conducted in accordance with OECD Guideline 301B and in conformity with GLP (Desmares-Koopman, 2008). The purity of the azamethiphos used was 96.2%. Activated sludge freshly obtained from a municipal sewage treatment plant was used, under appropriate test conditions including appropriate control responses. The extent of biodegradation (mineralisation) was 17% at the end of the 28-day study. The result indicates that azamethiphos did not undergo "rapid degradation" in this study.

Simulation tests

A test to simulate the *aerobic* biodegradation of azamethiphos in activated sludge is available, performed in accordance with OECD Guideline 314B and GLP principles (Schaefer and Carpente, 2014a). ^{14}C-radiolabelled azamethiphos (purity 99.4%) at a concentration of 25µg/l was incubated for 28 days with biotic sludge in a closed system and with abiotic sludge in an open system. In the biotic mixture, azamethiphos disappeared very rapidly, such that after 5 hours only 1.5% of the parent compound remained; metabolites more polar than azamethiphos were produced. At the end of the 28-day study the extent of transformation of azamethiphos to CO_2 was 44%. This outcome does not satisfy the criteria for "rapid degradation" (to full mineralization). Azamethiphos also

disappeared quite quickly from the abiotic mixture, only 18% of the parent compound remaining after 7 days and 4% after 28 days.

A test to simulate the *anaerobic* biodegradation of azamethiphos in activated sludge is available, performed in accordance with OECD Guideline 314C (Schaefer and Carpente, 2014b). ^{14}C-radiolabelled azamethiphos (purity 99.4%) at a concentration of 25 µg/l was incubated for 56 days with anaerobic digester sludge; the effects of an abiotic sludge were also investigated. Again, azamethiphos disappeared very rapidly from these test systems and metabolites more polar than azamethiphos were produced. At the end of the 56-day study, in the biotic mixture the extent of transformation of azamethiphos to CO_2 + methane was only 8%. This outcome does not satisfy the criteria for "rapid degradation" (to full mineralization).

11.1.2 BOD_5/COD

For the purpose of classification, data generated by the ready biodegradability study supersede direct BOD_5 and COD measurements.

11.1.3 Hydrolysis

Table 21: Hydrolysis results for azamethiphos

Guideline / Test method	pH*	Temp [°C]	Initial TS conc., C_0 [g l^{-1}]	Reaction rate constant, K_h	Half-life, DT_{50}	Coefficient of correlation, r^2	Reference
OECD 111 / Method C7 (EEC) Purity 99.4%	4	20	0.2	1.0 x10^{-3}	56.65 d	0.941	Riefer, P. (2015) CAR 4.1.1.1.1
	4	50		1.5 x 10^{-2}	1.95 d	0.996	
	4	60		3.8 x10^{-2}	0.75 d	0.990	
	7	**20**		**2.00 x 10^{-3}**	**14.0 d**	**0.991**	
	7	40	0.2	2.10 x 10^{-3}	1.40 d	0.994	
	7	50		7.70 x 10^{-2}	0.38 d	0.999	
	9	20		1.19 x 10^{-1}	0.24 d	0.994	
	9	25	0.2	1.98 x 10^{-1}	0.15 d	0.989	
	9	30		3.83 x 10^{-1}	0.08 d	0.980	

In a study conducted to OECD 111 in accordance with GLP, hydrolysis rates and half-lives of Azamethiphos at three environmentally relevant pHs were determined. The purity of azamethiphos used was 99.4%. In the preliminary test the samples were incubated at 50 ± 5 ˚C in the dark. In the main test (refer to table 21) the samples were incubated at pH 4, 7 and 9; at 20, 40, 50, and 60 °C for different periods of time until 90 % degradation of the parent compound was observed or the test had run for a maximum of 30 days; whichever came first. Samples were taken at specific intervals and the remaining percentage of the applied radioactivity (AR) was measured. All transformation products detected in excess of 5 % AR were identified by NMR and HR LC-MS/MS. In this study, the hydrolysis half-life was 14 days at pH7 and 20 ˚C or 26.6 days when converted to the average EU outdoor temperature (12°C).

11.1.4 Other convincing scientific evidence

No information.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No information.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Guideline / Test method	Test type[1]	Test substance conc. (mg l^{-1})	Degradation		Reference
			Incubation period (days)	% degradation (mineralisation) at day 28	
OECD 302B Purity 99.03%	Inherent	150	28	37.7	Hammesfahr (2016) CAR 4.1.1.2.2

An inherent biodegradation study was performed according to OECD 302 B. This GLP compliant study (Hammesfahr, 2016) was performed on Azamethiphos of 99.03% purity. Activated sludge from a domestic waste water treatment plant was used as an inoculum, and was mixed with water to give a final test concentration of 0.2 g suspended solids per litre. Azamethiphos was added at a concentration of 150 mg l^{-1} (corresponding to a DOC of 50 mg l^{-1}). A second set of flasks using diethylene glycol as a substrate (at a DOC of 50 mg l^{-1}) was used as a procedural control. A third set of flasks containing both Azamethiphos and diethylene glycol was used as a toxicity control, and an untreated control was also included. The flasks were kept at an aeration of 1 mg l^{-1} dissolved oxygen throughout the duration of the test, at a test temperature of 20 °C, and within a pH range of 7.2 to 7.7. Filtered samples were analysed for DOC by means of catalytic combustion, TOC-V CPH analyser and ASI-V autosampler. The samples were analysed for DOC at least in duplicate, excluding the toxicity control and the reference item. The test was carried out for 28 d, by which point Azamethiphos had reached 37.7% degradation. Diethylene glycol reached 103.5 % degradation by 28 d in the procedural control, and 69.76 % degradation in the toxicity control. As the test was carried out at a pH where Azamethiphos rapidly hydrolyses and no sterile control was included, it is not possible to distinguish biodegradation from abiotic degradation. This study does not support the classification of Azamethiphos as being inherently biodegradable, in terms of ultimate biodegradation.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Guideline / Test method	Test type[1]	Test substance conc. (mg kg^{-1})	Degradation		Reference
			Incubation period (days)	DT_{50}	
Proposal for a technical Protocol (draft version) – Anaerobic Transformation in Liquid Bovine and Pig Manures 38/2010B Purity 98.8 %	Simulation Tests to Assess the Biodegradability of Chemicals in Manure	0.3 mg kg^{-1} fresh cattle manure (Additional experiments carried out at 3 and 30 mg kg^{-1} fresh manure)	103	5.71 h (at 22 °C) 7040 d when NER treated as parent	Meinerling (2017) CAR 4.1.1.3.3
Proposal for a technical Protocol (draft version) – Anaerobic Transformation in Liquid Bovine and Pig	Simulation Tests to Assess the Biodegradability	0.3 mg kg-1 fresh pig manure	103	5.98 h (at 22 °C) 522 d when NER treated as parent	Meinerling (2017) CAR 4.1.1.3.3

Manures 38/2010B Purity 98.8 %	of Chemicals in Manure	(Additional experiments carried out at 3 and 30 mg kg-1 fresh manure)			

Two manure degradation studies using cattle manure (Meinerling, 2017) and pig manure (Meinerling, 2016) as substrate are available. Both "biodegradation in manure" studies (cattle manure and pig manure) were undertaken according to the Proposal for a technical Protocol (draft version) – Anaerobic Transformation in Liquid Bovine and Pig Manures 38/2010B.

The cattle manure was sampled from a cattle breeding farm and the pig manure was sampled from a pig breeding farm. Both farms had no use of veterinary medicines, biocides and other material that can alter the study. About 10 l was sampled from each farm, and the manure was homogenised and stored for four under a nitrogen atmosphere until the start of the pre-incubation phase. Sample systems were glass 500 ml flasks containing Around 50 g wwt manure, connected to a series of two CO_2 traps. Flasks were purged with nitrogen gas, closed, and incubated under test conditions for 21 - 23 days before being spiked with ^{14}C–labelled Azamethiphos. A sterile control group was created by autoclaving flasks at 121 °C for 15 min six times after addition of manure, but before addition of the test item. A parameter control group containing non-labelled test item was created, in addition to a control group with no test item. No abiotic controls were performed. Flasks were stored at 22 ± 2 °C in diffuse light or darkness. Samples were tested for methane (using a combustion unit), carbon dioxide (traps) and dissolved carbon dioxide (a manure subsample was acidified and resulting CO_2 trapped in NaOH solution). Manure samples were subject to a four stage extraction process using first acetonitrile, then methanol, then dichloromethane, and finally n-hexane. Extracts were analysed for total extractable radioactivity by liquid scintillation counting (LSC), and samples were also analysed using LC-MS/MS and HPLC. Non-extractable residues (NER) were determined by combustion of the solid matter using a sample oxidizer. In order to aid the identification of transformation products, the experiments were repeated using higher doses of test item.

Azamethiphos rapidly hydrolyses and no abiotic control was included. It is therefore not possible to distinguish biodegradation from abiotic degradation. Additionally, high levels of NER in a degradable medium prevent the deduction of reliable degradation rates from these studies.

11.1.4.4 Photochemical degradation

Photolysis

Table 22: Phototransformation of azamethiphos in water

Method, Guideline, GLP status, Reliability	Initial molar TS concentration	Total recovery of test substance [% of appl. AS]	Photolysis rate constant (k^c_p)	Direct photolysis sunlight rate constant (k_{pE})	Reaction quantum yield (φ^cE)	Half-life (DT_{50})	Remarks	Ref.
CD 91/414 /EEC (Part A, 7.2.1-1991). CD 95/36/EC (Part 7.2.1.2-1995).	$3.33*10^{-6}$ M (1.08 mg l^{-1}) Purity 98.8%	Dark controls: 94.7-103.8%. Test solutions: 71.8 – 98.4% of applied	k_{irr}=13.4619 ± 0.05396. k_{dark}=0.01416 ± 0.001139.	$5.43*10^{12}$	0.0272 reacted molecules/absorbed photons	0.051 d in samples. 49.0 d in dark controls.	Degradation products detected in the irradiated solutions > 10% (13.9 - 54.2 %) but	Brands C., 2009 CAR 4.1.1.1.2

SETAC - (Section 10-1995).		radioactivity.					not identified.	
OECD 316	3.20*10^{-6} M (1.04 mg l^{-1})	Dark controls: 99.7-106.6%. Test solutions: 98.2 – 104.0% of applied radioactivity.	k_{irr}=0.768 h^{-1} k_{dark}<0.0001 h^{-1}	10.4 d^{-1}	Xenon arc lamp: 0.064 Sunlight (40°N, summer): 0.066	Environmental:0.067 days	Two major degradation products were found at mean maximum ARs of 42.95 % and 37.3 % respectively (Could not identify compounds by NMR and HR LC-MS.)	Riefer (2017) CAR 4.1.1.1.2

A study is available investigating the aqueous photolysis of azamethiphos, conducted in accordance with OECD Guideline 316 and following the principles of GLP (Brands, 2009). The purity of the azamethiphos used was 98.8%. The degradation half-time (DT_{50}) under irradiated conditions, adjusted for 40°N sunlight, was 0.1 days (compared to 49 days in darkness). The results show that azamethiphos is subject to rapid photolysis in aqueous conditions.

A second study (Riefer, March 2017) was also performed according to OECD Guideline 316. Azamethiphos solutions at concentrations of 1mg l^{-1} were continuously irradiated for 5 hours at pH 4, 25 ± 2 °C under a sunlight-simulating light source (Xenon lamp). Samples were prepared in duplicate and dark controls were included to distinguish between photolytic degradation rates and degradation by other processes. Sacrificial concentrations of Azamethiphos in samples and dark controls were subject to LSC and HPLC analysis, and the results plotted as a function of time. In order to aid the identification of transformation products, the experiments were repeated using higher doses of test item. Two photolytic constants (k_{irr} , k_{dark}) were calculated by performing a linear regression on log transformed data. Seven degradation products were detected in the irradiated solutions, two of which can be classified as major degradation products. These two were formed at maximum mean concentrations of 42.95% AR and 37.3% AR. Identification was attempted using co-chromatography, NMR and HR LC-MS. These tests indicated that both transformation products in fact consisted of several compounds which could be associated to highly degraded transformation products. However, a separation of the specific transformation products was not achieved.

A prediction has been made using the computer programme AOPWIN for the photo-degradation of azamethiphos in air as a result of reactivity with hydroxyl radicals (Willems, 2009). The degradation half-time (DT_{50}) was predicted to be 1.3 hours. This shows that azamethiphos is also susceptible to photolytic degradation in air.

11.1.5 Summary and discussion of degradation

The hydrolysis rate of azamethiphos was pH dependent with rapid hydrolysis under alkaline conditions. However, hydrolysis was more moderate at environmentally relevant pH, with a half-life of 26.6 days at pH 7 and 12˚C. Azamethiphos is susceptible to rapid photolytic breakdown in water and is predicted to do so in air. However, it is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, there is insufficient information to evaluate photodegradation in the European environment in terms of mineralisation or transformation to non-classifiable substances. Therefore aquatic photolysis is not considered to meet the criteria for rapid degradation'

In a screening test and two simulation tests for biodegradation, all the results clearly show that the extent of full mineralisation do not meet the criteria for "rapid degradation". An inherent biodegradability test does not support the classification of Azamethiphos as being inherently biodegradable, and two degradation in manure studies do not yield reliable degradation rates.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this substance

11.3 Environmental fate and other relevant information

11.3.1 Adsorption/Desorption

An adsorption/desorption screening test – estimating the adsorption coefficient (K_{oc}) on soil and sewage sludge using High Performance Liquid Chromatography (HPLC) – has been reported (Oudhoff, 2008). The study followed OECD Guideline 121. The calculated K_{oc} and log K_{oc} values at neutral pH using this method were 99 l/kg and 2 respectively.

11.4 Bioaccumulation

11.4.1 Estimated bioaccumulation

No information.

11.4.2 Measured partition coefficient and bioaccumulation test data

The experimental octanol: water partition coefficient log K_{OW} measured for azamethiphos is 1.0 at 20 ^{o}C.

11.4.3 Summary and discussion of aquatic bioaccumulation

A study to assess the bioaccumulation of azamethiphos in fish has not been performed as the log Kow was concluded to be 1.0 (at 20°C and pH 7). This is below the trigger value for concern (i.e.,4) given in the CLP Regulation and discussed in the ECHA Guidance on the Application of the CLP Criteria. It indicates a low potential for bioaccumulation of azamethiphos.

For the aquatic compartment the CA has calculated the BCF_{fish} based on the log Kow using equation 74 in the TGD (2003). The inputs and results are summarised in the following table:

BCF calculation; inputs and results

Input	**Value**	**Source**
Log Kow	1.0	
BCF calculation		
Equation 74 (see TGD Part II, 2003)	$\text{Log } BCF_{fish} = 0.85 \times \text{Log Kow} - 0.7$	
Log BCF_{fish}	0.15 L / $kg_{wet\ fish}$	
BCF_{fish}	1.16 L / $kg_{wet\ fish}$	

The BCF_{fish} of 1.16 L / $kg_{wet\ fish}$ supports the argument that bioaccumulation is not expected.

11.5 Acute aquatic hazard

Only acute toxicity studies are available, investigating the effects of azamethiphos on fish, invertebrates and algae. All three studies were of reliable quality. The results are summarised in Table 23.

Table 23: Summary of relevant information on aquatic toxicity

Guideline/ GLP status	Species	Endpoint	Exposure		Results (calculated from measured concentrations)	Reference
			Design	Duration		
OECD 203, GLP compliant Purity 96.2%	Fish *Oncorhynchus mykiss*	LC_{50} (mortality)	Static	96 hours	LC_{50} = 0.19 mg a.s./l	Confidential (2008) CAR 4.2.3
OECD 202, GLP compliant Purity 96.2%	Invertebrate; crustacea *Daphnia magna*	EC_{50} (Immobilisation)	Static	48 hours	EC_{50} = 0.00033 mg a.s/l	Ing. Migchielsen M.H.J (2008) CAR 4.2.3
OECD 201, GLP compliant Purity 96.2%	algae *Pseudokirchneriella subcapitata*	E_rC_{50} (growth rate inhibition) E_yC_{50} (reduction in yield)	Static	72 hours	E_rC_{50}[1] = 74 mg a.s/l E_YC_{50}[2] = 18 mg a.s/l NOErC could not be determined.	Ing. Migchielsen M.H.J (2008) CAR 4.2.3

[1] calculated from growth rate

[2] calculated from the recorded cell density

11.5.1 Acute (short-term) toxicity to fish

In the one report available the acute toxicity of azamethiphos (96.2% purity) in rainbow trout *(Oncorhynchus mykiss)* was assessed in a reliable, good quality study performed according to OECD Guideline 203 and in compliance with GLP. Exposures were for 96 hours in a static system at concentrations of 0.01, 0.1 or 1 mg/litre. From the results, an LC_{50} value of 0.19 mg/l was calculated based on mean measured concentrations.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Only one study is available, in which the acute toxicity of azamethiphos (96.2% purity) in crustacea *(Daphnia magna)* was investigated. Exposures were for 48 hours in a static system at concentrations ranging between 0.00005 and 0.0011 mg/l. The study was of an acceptable standard, performed according to OECD Guideline 202 and in compliance with GLP. However, it is noted that the analytical verification of the lowest treatment concentration (nominal 0.046 µg /L) could not be confirmed by the evaluator as within +/- 20% of the nominal treatment concentration. The available data

indicates that at 0 and 48 hours respectively the measured concentration was 140 to 148% of the nominal (mean of the two replicate analytical sample measurements). This was not considered to significantly affect the study endpoints as the % immobilisation at the 0.046 µg /L (nominal) and next highest treatment concentration (0.12 µg /L) was 0%. Additionally, the study only included a solvent control and as such could not distinguish between effects of the solvent or test item. As no immobilisation was reported in the solvent control the study was considered acceptable.

From the results obtained, an immobilisation EC_{50} value of 0.00033 mg/l was calculated based on mean measured concentrations.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

A standard 72-hour growth rate test in algae *(Pseudokirchneriella subcapitata)* has been reported. The study was of an acceptable standard, performed according to OECD Guideline 201) and in compliance with GLP. Exposures were for 72 hours in a static system at concentrations between 4 and 87 mg/l. From the results obtained, a growth rate reduction E_rC_{50} value of 74 mg/l was calculated based on mean measured concentrations. The study authors noted that a NOErC for growth rate reduction and yield inhibition could not be determined.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No relevant data.

11.6 Long-term aquatic hazard

11.6.1 Chronic toxicity to fish

No data are available.

11.6.2 Chronic toxicity to aquatic invertebrates

No data are available.

11.6.3 Chronic toxicity to algae or other aquatic plants

No data are available.

11.6.4 Chronic toxicity to other aquatic organisms

No relevant data

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Aquatic acute toxicity data on azamethiphos are available for fish, invertebrates and algae. Significant acute toxicity was seen in the fish study, and azamethiphos was particularly potent in invertebrates (*Daphnia)*. The acute aquatic toxicity studies in fish and *Daphnia* gave results (LC_{50} = 0.19 mg/l and EC_{50} = 0.00033 mg/l respectively) that meet the criteria for classification with Aquatic Acute Category 1 (i.e. 96 hour LC50 for fish and 48 hr EC50 for crustacea $\leq$ 1 mg/l), In addition, the EC_{50} value in *Daphnia* lies in the range for application of an M factor of 1000 (i.e., $0.0001 < EC50 \leq 0.001$).

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

For the purposes of classification, azamethiphos is not considered to undergo “rapid degradation” and does not have significant potential to accumulate in the environment.

There are no chronic aquatic toxicity data available for azamethiphos. However, based on the available acute data (LC50 in fish = 0.19 mg/l) and *Daphnia* (EC50 = 0.00033 mg/l) and the fact that azamethiphos is not considered to be rapidly degradable, the criteria for classification with Aquatic Chronic Category 1; H410, are satisfied. A chronic M factor of 1000 is applicable.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1; H400: Very toxic to aquatic life
Acute M factor = 1000
Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects
Chronic M factor = 1000

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

No data.

12.1.2 Comparison with the CLP criteria

Azamethiphos is not mentioned as a controlled substance in the Annexes to the Montréal Protocol. Furthermore, it is not expected to enter into contact with stratospheric ozone molecules given its physico-chemical parameters and molecular structure.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified – conclusive but not sufficient for classification

13 ADDITIONAL LABELLING

None required.

14 REFERENCES

References are taken from the Competent Authority Report (CAR) for Azamethiphos (PT18) – November 2017

A full reference list (including non-confidential information is provided in Annex II to the CLP report).

Physicochemical Properties
Oudhoff K.A. (2008) Determination of Physico-Chemical Properties of Azamethiphos: Thermal Stability, melting and boiling, Density, Vapour Pressure, Surface tension, Water solubility, Solubility in Organic solvents, Partition coefficients, Flammability, Explosive properties, Self-ignition temperature, Oxidizing properties, UV-Vis absorption spectra, Mass spectra, Notox B.V., Project 488156 GLP, Unpublished

Oudhoff K.A. (2009) Determination of Physico-Chemical Properties of Azamethiphos Pestanal®: Henry's Law constant, Dissociation constant, Flammability, Pyrophoric properties,
Notox B.V., Project 490434 GLP, Unpublished

Toxicokinetics
Confidential (2009), Absorption, distribution, metabolism and excretion of [oxazolopyridine-3A-^{14}C] Azamethiphos in the Sprague dawley Rat, Unpublished

Health Hazards
Confidential (2008), Assessment of acute oral toxicity with Azamethiphos in the rat (Acute toxic class method), GLP, Unpublished

Confidential (2008), Assessment of acute dermal toxicity with Azamethiphos in the rat, , GLP, Unpublished

Confidential (2009), Assessment of acute inhalatory toxicity with Azamethiphos in the rat, GLP, Unpublished

Confidential (2008), Primary skin irritation/corrosion study with Azamethiphos in the rabbit (4-hour semi-occlusive application), GLP, Unpublished

Confidential (2008), Acute eye irritation/corrosion study with Azamethiphos in the rabbit, GLP, Unpublished

Confidential (2008), Assessment of contact hypersensitivity to Azamethiphos in the mouse (local lymph node assay), GLP, Unpublished

Verspeek-Rip C.M.. (2008a) Evaluation of the mutagenic activity of Azamethiphos in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat), Project 487962 GLP, Unpublished

Drs Buskens C.A.F. (2008b) Evaluation of the ability of Azamethiphos to induce chromosome aberrations in cultured peripheral human lymphocytes (with repeat experiment), 487963 GLP, Unpublished

Verspeek-Rip C.M. (2008c) Evaluation of the mutagenic activity of Azamethiphos in an *in vitro* mammalian cell gene mutation test with L5178Y Mouse Lymphoma cells, Project 487964 GLP, Unpublished

Simar (2017) In vitro ALKALINE COMET ASSAY ON L5178Y CELLS, Test performed without metabolic activation; GLP, Unpublished

Confidential (2008), Micronucleus test in bone marrow cells of the mouse with Azamethiphos, GLP, Unpublished

Confidential (2008), Evaluation of DNA repair inducing ability of Azamethiphos in male rat hepatocytes (*in vivo* rat hepatocyte), GLP, Unpublished

Confidential (2017) In Vivo MAMMALIAN ALKALINE COMET ASSAY IN THE MALE RAT Study Performed on stomach and duodenum (Two treatments, one sampling time); GLP, Unpublished

Confidential (2011a), A combined chronic toxicity/carcinogenicity study with Azamethiphos in the Wistar Rat, GLP, Unpublished

Confidential, (1982) Azamethiphos: two-year chronic oral toxicity study with CGA 18809 in albino rats

Confidential (1989) Lifetime oral (dietary administration) oncogenicity and toxicity study in the rat with an interim kill after 52 weeks and a 4 week treatment-free period. GLP, Unpublished

Confidential (1989) Lifetime oral (dietary administration) oncogenicity study in the mouse. GLP, Unpublished

Confidential. (1982) Azamethiphos: carcinogenicity evaluation with CGA 18809 technical in albino mice,

Peto R *et al* Guidelines for simple sensitive significance tests for carcinogenic effects in long0term animal expermeints. In: Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Suppl. 2. International Agency for Research on Cancer, Lyon (1980)

McConnell et al. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76 283-289.

Confidential (2009), A two-generation reproduction toxicity study of Azamethiphos in rats by oral gavage, GLP, Unpublished

Confidential (2009) A prenatal developmental toxicity study of Azamethiphos in rats by oral gavage, GLP, Unpublished

Confidential (2009), A prenatal developmental toxicity study of Azamethiphos in rabbits by oral gavage, GLP, Unpublished

Confidential (2009), 28-day oral finding study with Azamethiphos by daily gavage in Sprague Dawley rats, GLP, Unpublished

Confidential (2009). Repeated dose 90-day oral neurotoxicity study with azamethiphos by daily gavage in Sprague Dawley rats.

Confidential (2011). 90-day oral toxicity study with azamethiphos by daily gavage in male and female beagle dogs.

JMPR Report 1998
http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Reports_1991-2006/REPORT1998.pdf or http://www.who.int/foodsafety/publications/JMPR-guidance-document/en/

Environmental Hazards

Ing. Desmares-Koopmans M.J.E. (2008) Determination of „ready“ biodegradability: carbon dioxide (CO_2) evolution test (modified sturm test) of Azamethiphos Notox B.V., Project 487973 GLP, Unpublished

Schaefer, Edward & Carpenter, Karolina (2014a) {14C}Azamethiphos: biodegradation aerobic in activated sludge – OECD 314B Study 724E-101

Schaefer, Edward & Carpenter, Karolina (2014b) {14C}Azamethiphos: biodegradation anaerobic in activated sludge – OECD 314C Study 724E-102

Riefer, P. (2015) Azamethiphos: Hydrolysis as a Function of pH, Ibacon GmbH, Project: 77911293, GLP, Unpublished.

Hammesfahr, U., (2016), Azamethiphos: Inherent Biodegradability in a Zahn-Wellens/EMPA Test, ibacon GmbH, Germany, Project n°: 77911165

Meinerling, M., (2016), Anaerobic Transformation of Azamethiphos in Pig Manure, Institut für Biologische Analytik und Consulting IBACON GmbH, Germany, Project n°: 77913177

Meinerling M., (2017), Anaerobic Transformation of Azamethiphos in Cattle Manure, Institut für Biologische Analytik und Consulting IBACON GmbH, Germany, Project n°: 77912177

Brands C. (2009) Photochemical degradation of Azamethiphos in water Notox B.V., Project 487972 GLP, Unpublished

Riefer, P., (2015), Azamethiphos: Phototransformation in Water - Direct Photolysis [OECD 316], ibacon GmbH, report number 77911176

Willems H.P.L, (2009) Azamethiphos : Atkinson calculation Notox B.V., Project 490530 Not GLP, Unpublished

Oudhoff K.A.(2008) Estimation of the adsorption coefficient (K_{oc}) of Azamethiphos Pestanal® on soil and on sewage sludge using high performance liquid chromatography (HPLC), GLP, Unpublished

Confidential (2008), 96-hour acute toxicity study in rainbow trout with Azamethiphos (static), GLP, Unpublished

Ing. Migchielsen M.H.J. (2008) Acute toxicity study in *Daphnia magna* with Azamethiphos (static) Project 487976 GLP, Unpublished

Ing. Migchielsen M.H.J. (2008) Fresh water algal growth inhibition test with Azamethiphos Project 487977, GLP, Unpublished

15 ANNEXES

Annex I: Competent Authority Report (CAR) for Azamethiphos (PT18) – November 2017 (CONFIDENTIAL)

Annex II: Full reference list (CONFIDENTIAL)