Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

azamethiphos (ISO); *S*-[(6-chloro-2-oxooxazolo[4,5-*b*]pyridin-3(2H)-yl)methyl] *O,O*-dimethyl thiophosphate

EC Number: 252-626-0

CAS Number: 35575-96-3

CLH-O-0000001412-86-290/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

13 June 2019

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

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

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

Index Number: **N/A**

Contact details for dossier submitter: **UK Competent Authority**

Chemicals Regulation Directorate

Health and Safety Executive

United Kingdom

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

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON AZAMETHIPHOS (ISO); *S*-[(6-CHLORO-2-OXOOXAZOLO[4,5-*B*]PYRIDIN-3(2*H*)-YL)METHYL]*O*,*O*-DIMETHYL THIOPHOSPHATE

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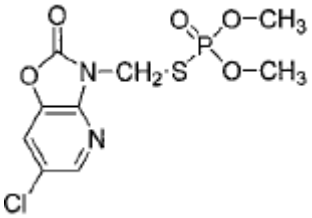
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON AZAMETHIPHOS (ISO); *S*-[(6-CHLORO-2-OXOOXAZOLO[4,5-*B*]PYRIDIN-3(2*H*)-YL)METHYL]*O,O*-DIMETHYL THIOPHOSPHATE

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:

	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		Azamethiphos	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 Danger	H302 H331 H317 H410	-	ATE (oral) = 500 mg/kg bw ATE (inhalation) = 0.5 mg/l M (acute) = 1000 M (chronic) = 1000	-
Resulting Annex VI entry if agreed by RAC and COM		Azamethiphos	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 Danger	H302 H331 H317 H410	-	ATE (oral) = 500 mg/kg bw ATE (inhalation) = 0.5 mg/l M (acute) = 1000 M (chronic) = 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

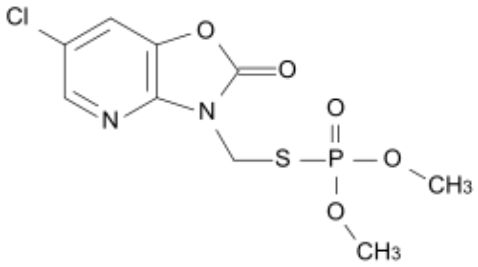
Azamethiphos is an existing biocide active substance in the review programme under Regulation 528/2012 for which the UK is the evaluating Competent Authority. It does not have an existing entry in Annex VI of CLP and the classification and labelling has not previously been considered in the harmonised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

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

RAC general comment

Azamethiphos has not previously been reviewed for harmonised classification and labelling and does not have an existing entry in Annex VI of the CLP Regulation. It is proposed to use Azamethiphos as the active substance in an insecticide in Product Type 18 of the Biocidal Products Regulation for the control of flies (*Musca domestica*) in animal houses. It is used as a veterinary substance almost exclusively for the off-animal control of houseflies and nuisance flies as well as crawling insects in livestock operations: stables, dairy premises, piggeries, poultry houses, etc. Azamethiphos is also an active ingredient of products, which are applied as a bath treatment to control pre-adult and adult sea lice (*Lepeophtheirus salmonis*) in farmed Atlantic salmon (*Salmo salar*).

Azamethiphos is an organic thiophosphate. Structurally it belongs to the class of chemical entities known as oxazolopyridines - polycyclic compounds containing an oxazole ring fused to a pyridine ring.



Organophosphate chemicals reversibly inhibit acetylcholinesterase resulting in an accumulation of the neurotransmitter acetylcholine in the central and peripheral nervous system.

It is noted that azamethiphos has been evaluated by the EMA Committee for Veterinary Medicinal Products in 1999 (EMA, 1999) and the CLH report contains a link to this report: https://www.ema.europa.eu/en/documents/mrl-report/azamethiphos-summary-report-2-committee-veterinary-medicinal-products_en.pdf. The studies supporting this report were not included or assessed in the CLH report and are not available to RAC.

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	Oudhoff K.A., 2008	EC A.10 GLP

Property	Value	Reference	Comment (e.g. measured or estimated)
	brown residue. 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.		98.8%
Explosive properties	The substance does not contain any chemical groups that are indicative of explosive properties.	-	-
Self-ignition temperature	Auto flammability = 240ºC	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.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) declared that all information for physicochemical properties were 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 (UK is the evaluating Competent Authority for azamethiphos as an existing biocide active substance in the review programme of Regulation 528/2012).

The DS summarised the physicochemical properties of azamethiphos as having been determined according to the OECD procedures, using reliable instrumental methods.

Explosives: no test data

The substance does not contain any chemical groups that are indicative of explosive properties. 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). DS proposal: no classification.

Flammable solids

Test data available, method EC A.10 – the substance did not ignite on contact with the

ignition source but melted leaving a brown residue. DS proposal: no classification.

Self-reactive substances and mixtures

No studies available. There are no groups in the molecule associated with explosive or self-reactive properties. DS proposal: no classification.

Pyrophoric solids

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

Self-heating substances and mixtures

No suitable test data available. There is no evidence to show that azamethiphos possesses self-heating properties. DS proposal: no classification.

Substances and mixtures 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. DS proposal: no classification.

Oxidising solids

No test data. The substance does not contain any chemical groups that are indicative of oxidising properties. DS proposal: no classification.

Corrosive to metals

No data available. Based on the experience in manufacture and handling, the substance does not materially damage metallic containers. DS proposal: no classification.

Overall, the Ds proposed no classification for physical hazards.

Comments received during public consultation

Self-reactive substances

One Member State Competent Authority (MSCA) disagreed with the proposed non-classification for azamethiphos as a self-reactive substance and referred to the classification procedure given in section 2.8.4.2 of Annex I to Regulation (EC) No 1272/2008 (CLP Regulation):

a) There are no chemical groups present in the molecule associated with explosive or self-reactive properties. Examples of such groups are given in Tables A6.1 and A6.2 in Appendix 6 of the UN RTDG (recommendation on the transport of dangerous goods), Manual of Tests and Criteria; or

(b) For a single organic substance or a homogeneous mixture of organic substances, the estimated self-accelerating decomposition temperature (SADT) for a 50 kg package is greater than 75 °C or the exothermic decomposition energy is less than 300 J/g. The onset temperature and decomposition energy can be estimated using a suitable calorimetric technique (see Part II, sub-section 20.3.3.3 of the UN RTDG, Manual of Tests and Criteria). The MSCA deemed that azamethiphos has chemical groups present in the molecule which are associated with explosive or self-reactive properties, taking into account information in Bretherick's Handbook of Reactive Chemical Hazards: *A number of phosphate and thiophosphate esters are of limited thermal stability and undergo highly exothermic self-accelerating decomposition reactions which may be further catalysed by impurities. The potential hazards can be reduced by appropriate thermal control measures.* The MSCA noted that for azamethiphos the oxygen balance value is -88.9, which identifies the substance to be a potential explosive, as it is greater than the limit value of -200. The MSCA recommended classification based on the experimental study and results obtained for SADT for a 50 kg package. Additionally, classification may be made through the determination of the thermal characteristics of the substance obtained by differential thermal analysis, differential scanning Calorimetry (DSC) which can provide data of the exothermic decomposition energy. The DSC should confirm that the exothermic decomposition energy is < 300 J/g and the onset of exothermic decomposition is < 500 °C, in order to conclude on the non-classification of the substance as a self-reactive. The MSCA insisted that the experimental results be made available in order to enable firm conclusions to be drawn on explosive and self-reactive properties of azametiphos.

The DS replied (a) that the structure does contain a thiophosphate ester moiety and that Brethericks refers to a number of phosphates and thiophosphates being of limited thermal stability, and (b) the DSC showed a discrete exothermic (decomposition) between 175-200°C but the heat of decomposition is not provided, therefore it is not possible to fully assess the result against the criteria.

Self-heating substances

One MSCA supported no classification based on the low melting point of the substance of 90 °C - the classification procedure for self-heating substances need not be applied, because the substance is completely molten at 160°C. Another MSCA noted that test results had not been provided to demonstrate that the active substance is not a self-heating substance. The conclusion "data lacking" is not appropriate. At least a scientific case (or a test according to the Manual of Tests and Criteria of the UN RTDG)

should be provided by the applicant to confirm this point. The DS agreed with the comment.

Corrosive to metals

One MSCA noted that test data had not been provided to demonstrate that the active substance is not corrosive to metals. The conclusion "data lacking" is not appropriate.

Assessment and comparison with the classification criteria

Comparison with the criteria

Explosives:

1. Azamethiphos does not contain any of the chemical groups listed in UN RTDS Table A6.1. The onset decomposition temperature for azamethiphos is below 500 °C, but information on the decomposition energy was not provided.
2. The oxygen balance value is below the limit (-200), but the substance has been on the market for almost 30 years without incidents.

In the Proposed Registration Decision (PRD2016-25), published by the Health Canada Pest Management Regulatory Agency it is declared that explosive properties are not expected for the product Salmosan Vet (50 % azamethiphos as an active ingredient). RAC supports the DS' proposal for no classification of azamethiphos for explosive properties.

Flammable solids

According to the test data available, method EC A.10 – the substance did not ignite on contact with the ignition source but melted, leaving a brown residue. RAC supports the DS' proposal for no classification.

Self-reactive substances and mixtures

Azamethiphos does not contain chemical groups associated with the explosive or self-reactive properties listed in Tables A6.1 and A6.2 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria and RAC supports the DS proposal for no classification.

During the PC, the following comment was received from an MSCA: *Some thiophosphates and phosphates are thermally unstable and undergo highly exothermic self-accelerating decomposition reactions, which may be further catalysed by impurities according to Bretherick's Handbook of Reactive Chemical Hazards*.

RAC's response to this comment is that this is a very general rule which includes all substances of P(III), P(V) and P(VI). The conclusion is valid mostly for compounds of P(III) as is shown in Appendix 6 of the UN RTDG and P(VI). The check of all examples for self-reactive substances of P(V) (see Additional key Elements in the BD) as presented in Bretherick's Handbook) could be also attributed to the additional self-

reactive group – azo, azide, peroxo, nitro… or to the reaction with active substance – chlorine, diazinon etc.

Additionally, the compound is stable on accelerated storage at 54 °C (Proposed Registration Decision (PRD2016-25), published by the Health Canada Pest Management Regulatory Agency for the product Salmosan Vet (50 % azamethiphos)). Overall, RAC supports the DS' proposal for no classification in the absence of additional test data.

Pyrophoric solids

Experience in handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable at room temperature for prolonged periods of time). RAC supports the DS' proposal for no classification.

Self-heating substances and mixtures

RAC considers that the classification procedure for self-heating substances need not be applied, because the substance is completely molten at 160 °C as commented by one MSCA during the PC. Therefore, RAC supports the DS' proposal for no classification, and no other tests need to be performed.

Substances and mixtures which in contact with water emit flammable gases

Azamethiphos is soluble in water and used as a stable aqueous solution, most of the studies with azamethiphos were performed in aqueous solutions and there are no reports for emission of gas. Therefore, RAC support DS' proposal for no classification.

Oxidising solids

Azamethiphos has oxygen atoms bonded to phosphorus in an organophosphate group. However, it is much easier to oxidize this substance than to reduce azametiphos taking into account the molecular structure. Therefore, azamethiphos is not considered an oxidizer, and RAC supports the DS' proposal for no classification.

Corrosive to metals

Azamethiphos is a weak acid (pKa 2.2), pH of aqueous solution is 4-7, taking into account molecular structure azamethiphos is not considered corrosive toward metals. Therefore, RAC supports the DS' proposal for no classification.

Overall, RAC agrees with the DS proposal **not to classify azamethiphos for physical hazards**.

Additional key elements

Self-reactive substances and mixtures

Examples from Bretherick's Handbook

Allyl phosphorodichloridite, 1165* – P(III) -

Bis(trimethylsilyl) phosphonite, 2607 – P(III)

O—O-tert-Butyl di(4-tolyl) monoperoxophosphate, 3757 – peroxo compound

O—O-tert-Butyl diphenyl monoperoxophosphate, 3706 - – peroxo compound

Diallyl phosphite (Di-2-propenyl phosphonite), 2450 – P(III)

Dibenzyl phosphite, 3651 – P(III)

Dibenzyl phosphorochloridate, 3643 - It is too unstable to be distilled, and the precursory phosphite also tends to decompose on distillation

Dibutyl hydrogen phosphite, 3080 – P(III)

Diethyl 4-nitrophenyl phosphate, 3323 - nitrophenyl

Diethyl 4-nitrophenyl thionophosphate, 3322 - nitrophenyl

Diethyl ethanephosphonite, 2567 – P(III)

Diethyl phosphite, 1727 – P(III)

Diethyl phosphorochloridate, 1675 - Presence of hydrogen chloride as impurity causes an uncontrollable exothermic reaction during preparation of diethyl phosphate from the title compound.

Dimethyl 2-chloro-4-nitrophenyl thionophosphate, 2955 - - nitrophenyl

Dimethyl 3-chloro-4-nitrophenyl thionophosphate, 2956 - - nitrophenyl

Dimethyl 4-nitrophenyl thionophosphate, 2974 - - nitrophenyl

Dimethyl ethanephosphonite, 1726 – P(III)

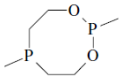
Dimethyl hydrazidophosphate, 0955 - hydrid

Dimethyl N,N-dichlorophosphoramidate, 0897 -

Dimethyl phosphoramidate, 0948 - in reaction with chlorine

O,O-Dimethyl S-methylcarbamoylmethyl phosphorodithioate, 2003 – dimethoate- aliphatic, accident, melting point 43 C, flash point 107

2,6-Dimethyl-1,3-dioxa-2,6-diphosphacyclooctane, 2543



Temperature above 120 cause explosion

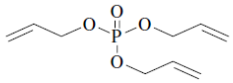
Di(O—O-tert-butyl) ethyl diperoxophosphate, 3368 – peroxo compound

Diphenyl azidophosphate, 3483 - azid

Potassium O,O-diphenyl dithiophosphate, 3475 – in reaction with Arenediazonium salts

Pyrocatecholato(2-)(quinolin-8-olato-N,O)-trioxygenido(2-)phosphorus, 3671 - hexavalent phosphorus ozonide, stable at (-20 °C), exploded in contact with air or on warming to ambient temperature

Triallyl phosphate, 3178 – aliphatic, under distillation



Alkali-washed material, stabilised with 0.25 % of pyrogallol, was distilled at 103 °C/4 mbar until slight decomposition began. The heating mantle was then removed and the still-pot temperature had fallen below its maximum value of 135 °C when the residue exploded violently [1]. The presence of solid alkali [2] or 5 % of phenolic inhibitor is recommended, together with low-temperature high-vacuum distillation, to avoid formation of acidic decomposition products, which catalyse rapid exothermic polymerisation.

Trimethyl phosphate, 1314 – explode during distillation

Trimethyl phosphite, 1311 – P(III)

Trimethyl thiophosphate, 1312 – in reaction with chlorine

* - numbers as given in Bretherick's Handbook of Reactive Chemical Hazards Seventh Edition Volume 1

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

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

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

Oral

Azamethiphos has been tested in a GLP compliant study according to OECD TG 423 (Confidential, 2008a, CAR 3.2.1). Three female Wistar rats per group were exposed by gavage to either 300 or 2 000 mg/kg bw in 1 % aqueous carboxymethyl cellulose at a purity of 96.2 %. At the high dose of 2 000 mg/kg bw all animals died. Clinical signs included hunched posture in all animals. Dark red fluid was seen in the thoracic cavity of 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 were reported in all animals on days 1 and/or 2. The DS derived an oral LD_{50} of 500 mg/kg bw for males and females, although only females were tested, based on the flow chart included in OECD TG 423.

As the LD_{50} of 500 mg/kg bw lies within the criteria for classification as Acute Tox. 4, the DS proposed to classify azamethiphos as Acute Tox. 4; H302, with an ATE of 500 mg/kg bw.

Dermal

Azamethiphos was tested in a GLP compliant study according to OECD TG 402 (Confidential, 2008b, CAR 3.2.2). Male and female Wistar rats (5/sex/dose) were exposed to 2 000 mg/kg

bw azamethiphos (purity 96.2 %, in 1 % aqueous carboxymethyl cellulose, 10 % of body surface). None of the animals died, but clinical signs were observed. They included chromodacryorrhoea in three males and one female on day 1 or 2 and hunched posture in one male on day 1. Scales were seen on the treated skin area of one male and one female between days 7 and 12.

The dermal LD_{50} for azamethiphos was determined to be > 2 000 mg/kg bw in this study. No classification was proposed by the DS.

Inhalation

Azamethiphos was tested in a GLP compliant acute inhalation study according to OECD TG 403 (Confidential, 2009a, CAR 3.2.3). Male and female Wistar rats (5/sex/group) were exposed to dust aerosol concentrations of 1.1 and 5.2 mg/L (nominal: 7.3 and 23 mg/L; MMAD: 3.2 and 2.3). Another group of 5 males was exposed to dust aerosol concentrations of 0.54 mg/L (nominal: 3.5 mg/L; MMAD: 2.9). Exposure duration was 4 hours via the nose only.

All animals died at 5.2 mg/L. Reduced respiratory rate was noted in one animal at 1¼ hours after initiation of exposure and laboured breathing at 2¼ hours. This animal was found dead at 3 hours. All other animals were found dead at the first inspection.

At 1.1 mg/L, 3 males and 1 female died. One male died during exposure and a second shortly after exposure. The two remaining animals that died were found dead the day following exposure. Clinical signs on removal from the restraining tubes included shaking heads, one male and two females showed spread hind legs and in one female gasping was reported. Subsequently hunched posture, hypothermia, laboured respiration, piloerection, rales and general tremors were observed in males and females. In addition, females also showed chromodacryorrhoea (bloody tears). All symptoms resolved by day 5.

At 0.54 mg/L, none of the males died. Clinical signs included chromodacryorrhoea (snout), hunched posture, laboured respiration and piloerection.

No precise LC_{50} was derived, but based on the results in males it was concluded that the LC_{50} in the most sensitive sex lay between 0.5 and 1 mg/L with an MMAD in the range of 2.3-2.9 µm. This range coincides with the criteria of $0.5 < LC_{50} \leq 1$ mg/L (dusts and mists) for classification as Acute Tox. 3; H331, therefore the DS proposed this classification. Since no precise LC_{50} was available the DS proposed to use the default ATE of 0.5 mg/L for dusts and mists classified in category 3 for acute inhalation toxicity.

Comments received during public consultation

One MSCA supported the classification as Acute Tox. 3; H331 and the ATE (inhalation) of 0.5 mg/L (dusts or mists). Another MSCA commented that the ATE should not be specified, in order to leave it open for companies to use potentially available data, which could allow to derive a more precise ATE.

The DS responded that if the ATE was not defined this could lead to confusion with suppliers applying differing values. RAC agrees with the DS' response and also notes that the CLP guidance states the following under section 1.5.3: Harmonised ATE values: "*From 2016 harmonised Acute Toxicity Estimates (ATE) may be included in annex VI of CLP. These values have to be used, just as any other harmonised item. ATEs are one way of expressing acute toxicity (see Annex I to CLP, 3.1.2.1)*."

In an additional oral non-guideline but GLP compliant study (located in the CAR; Confidential, 2009b, CAR 3.2.1), 16 Sprague Dawley Crl rats per sex and dose were exposed by gavage to 50 and 250 mg/kg bw azamethiphos. The purity of the test material was 96.2 % and it was dosed in aqueous carboxymethyl cellulose. At 50 mg/kg bw clinical signs included hunched posture (6/32) and piloerection (1/32). Acetylcholinesterase in erythrocytes was reduced by a maximum of 38 % in males and 43 % in females. At 250 mg/kg bw clinical signs included hunched posture (14/32), calm behaviour (6/32), lethargy (2/32) and piloerection (6/32). Acetylcholinesterase in erythrocytes was reduced by a maximum of 68 % in males and 58 % in females.

All clinical signs occurred between 1 to 4 hours after dosing, with a peak between 1 and 2 hours after dosing. No deaths were reported.

The peak of acetylcholine esterase inhibition was after 1 hour and while it recovered to pre-dose levels after 12 hours at the low dose, acetylcholine esterase activity remained reduced after 12 hours at the high dose.

Assessment and comparison with the classification criteria

Oral

RAC agrees with the DS' proposal to classify azamethiphos as Acute Tox. 4; H302, based on the reliable oral toxicity study in rats (OECD TG 423, GLP, Confidential 2008a, CAR 3.2.1). As no mortality was seen at 300 mg/kg bw and 100 % mortality was seen at 2 000 mg/kg bw, it can be concluded that the LD_{50} is in the range between 300 and 2 000 mg/kg bw. The DS derived an oral LD_{50} of 500 mg/kg bw for females, based on the flow chart included in the guideline OECD TG 423. In the absence of LD_{50} values for males, this value alone was used to derive the ATE.

No deaths were reported in a second non-guideline acute oral toxicity study (Confidential 2009c, CAR 3.2.1, see BD under additional key elements) at lower doses. The observed clinical signs seen at 300 mg/kg bw in the first study and at 50 and 250 mg/kg bw in the second study were indicative for acetylcholine esterase inhibition and increased in incidence and severity with dose. This is also supported by the dose related decrease in erythrocyte acetylcholine esterase activity at 50 and 250 mg/kg bw detected in the second study.

Based on the derived LD_{50}, RAC supports the proposed ATE of 500 mg/kg bw, which is also the converted acute toxicity point estimate for oral Acute Tox. 4 according to table 3.1.2, Annex I of the CLP regulation.

Dermal

RAC agrees with the DS that no classification for acute dermal toxicity is warranted, given that the LD_{50} value in the reliable dermal acute toxicity study conducted according to OECD TG 402 (Confidential, 2008b, CAR 3.2.2) was above the classification limit of 2 000 mg/kg bw.

Inhalation

RAC agrees with the DS' proposal to classify as Acute Tox. 3; H331, based on the reliable inhalation toxicity study in rats (OECD TG 403, GLP, Confidential 2009a, CAR 3.2.3). The results indicate an LC_{50} value for the most sensitive sex, males, in the range of 0.5-1 mg/L. As no precise LC_{50} is available, the proposal to use the default ATE of 0.5 mg/L for category 3 (dusts and mists) is supported by RAC, which is in accordance with Annex I of the CLP

regulation (table 3.1.2).

In conclusion, RAC proposes to **classify azamethiphos as Acute Tox. 4; H302 with an ATE of 500 mg/kg bw and as Acute Tox. 3; H331 with an ATE of 0.5 mg/L (dusts and mists)**.

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of azamethiphos has been tested in a standard skin irritation study, in three male New Zealand White rabbits, conducted according to OECD TG 404 and GLP (Confidential, 2009g, CAR 3.2.3). The purity of the test material was 96.2 % and 0.5 g was moistened with 0.7 mL water. Exposure was semi-occlusive for 4 hours. Neither erythema nor oedema were seen in any of the animals at any time point.

No human data are available.

The DS concluded that azamethiphos does not fulfil the classification criteria for skin irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

As no skin reactions were noted in the single guideline and GLP compliant study available, RAC agrees with the DS' proposal for **no classification for skin corrosion/irritation**.

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

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The potential of azamethiphos to induce eye irritation was tested in three male New Zealand White rabbits in an OECD TG 405 and GLP compliant study (confidential, 2008c, CAR 3.3.2). The purity of the test material was 96.2 % and it was applied as powder. No corneal or iridial lesions were seen (Cornea: 0, 0, 0; Iris: 0, 0, 0). Conjunctival redness and chemosis were seen in all animals. While redness reversed by 72 hours, slight chemosis was still visible after 72 hours (Redness: 2, 1, 0; Chemosis: 0.3, 0.3, 0.3). The calculated mean scores were below the limits for classification of ≥ 2 for redness and ≥ 2 for chemosis.

No human data were available.

The DS proposed no classification.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

As only slight eye effects were reported in the single guideline and GLP compliant study available, which resulted in scores below the limit for classification, RAC agrees with the DS' proposal for **no classification for serious eye damage/irritation**.

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

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS stated that there is no specific information on the potential of azamethiphos to induce respiratory sensitisation.

No human data are available.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC concludes on **no classification for respiratory sensitization due to lack of data.**

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	Mouse,	10%, 25%	No EC_3-value calculated.	Confidential

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, Dose levels duration of exposure**	**No. sensitised/total no.**	**Reference**
Lymph Node Assay (LLNA) OECD 429 GLP Purity 96.2%	CBA 20 animals (female) (5 groups)	and 50% in propylene glycol	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	(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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of azamethiphos was tested in a Local Lymph Node Assay (LLNA), conducted according to OECD TG 429 and GLP. Twenty CBA mice were allocated to four groups: control, 10 %, 25 %, and 50 %. The purity of the substance was 96.2 %, the vehicle was propylene glycol and 25 µL were applied per ear on days 1, 2 and 3. A reliability check was conducted using α-HCA less than 6 months prior to study (Confidential, 2008d, CAR 3.4.1).

Erythema was seen in 4/5 animals at the mid-dose and 5/5 animals at the high dose. Enlargement of lymph nodes was seen in all animals treated ≥ 25 %. At all concentrations a stimulation index (SI) of > 3 was determined (10 %: 14.1; 25 %: 18.6; 50 %: 16.4). No EC3 value could therefore be derived. On this basis, the DS concluded that a classification is clearly justified by the observed stimulation indices, however, as no reliable EC3 can be derived no sub-categorisation is possible.

Comments received during public consultation

During the public consultation one MSCA supported the proposal for classification without sub-categorisation. The reasoning was that on the basis of the available data no EC3 value could be derived and that already at the lowest dose of 10 % the SI was rather high, which did not increase significantly at 25 % and 50 %.

Additional key elements

It is noted that azamethiphos has been evaluated by EMA in 1999 (EMA, 1999) and the CLH report contains a link to this report: https://www.ema.europa.eu/en/documents/mrl-report/azamethiphos-summary-report-2-committee-veterinary-medicinal-products_en.pdf. In this report it is stated that azamethiphos was a skin sensitiser in two separate studies in guinea pigs and that there were also cases of skin sensitisation reported in a small number of pesticide spray operators. No further information on these studies or additional data were available to RAC.

Assessment and comparison with the classification criteria

RAC notes that the tested doses were too high to identify an EC_3 value. Even the lowest concentration tested (10 %) resulted in a SI of 14.1, which is considerably higher than the cut-off value of 2 % for sub-categorization, and the two highest doses seem to be in the plateau range of the response. Consequently, the calculation of an EC3 value appears to not be reliable. Nevertheless, when using the SI achieved at the lowest tested concentration of 10 % to calculate an EC3, this results in a value of 2.1 %, which is at the cut-off value of 2 % for sub-categorization. Considering that the tested doses might have been in the range where the response had already reached a plateau, which was also supported by the comment of a MSCA

during PC, the linear extrapolation towards the low dose range might result in an underestimation of the sensitising potential of azamethiphos. The use of lower doses might have allowed differentiating between sub-categories 1A and 1B.

Although the available data indicate strong skin sensitising potential, RAC agrees with the DS' proposal to **classify azamethiphos as Skin Sens. 1; H317 without sub-categorisation**, as no reliable EC3 value can be derived on the basis of the available data.

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 control:* -S9: mitomycin C (0.5 µg/ml) +S9: cyclophosphamide *Solvent control:* DMSO	**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 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.

Method	Organism/ strain	Concentrations tested	Remarks and Result
		Test material: azamethiphos (96.2% pure)	
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 37ºC 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>
<tr><td>In vivo rat liver Unscheduled DNA synthesis</td><td>Rat, Wistar:
3 males/sampling time/test group

1</td><td>Main study
Dose: 850 and 425 mg/kg bw in propylene glycol (gavage)

Positive control:</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</td></tr>
</table>

<table>
<tr><th>Method</th><th>Organism/ strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>OECD486
GLP

Confidential (2008)
CAR 3.8.2</td><td>male/sampling time/control

Sampling times 2-4 and 12-16</td><td>10 mg/kg bw dimethylnitrosamine (DMN)

50 mg/kg bw 2-acetylaminofluorene (AAF)</td><td>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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro studies

Azamethiphos was tested in four *in vitro* mutagenicity / genotoxicity tests, all of which gave positive results.

In a reliable bacterial mutation assay (OECD TG 471, GLP; Confidential, 2008e) with no indication of cytotoxicity, positive and negative controls were within the historical control range, azamethiphos showed mutagenic potential in two strains. A dose-related increase in the number of revertant colonies in tester strain TA100 of up to 2.6- and 1.9-fold was observed compared with the solvent control in the absence and presence of metabolic activation (S9), 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.

Azamethiphos was also found to be clastogenic and to induce gene mutations in mammalian cells, as demonstrated by a positive gene mutation assay (OECD TG 476, GLP; Confidential, 2008f, CAR 3.8.1) and a positive chromosome aberration test (OECD TG 473, GLP; Confidential, 2008g, CAR 3.8.1). The results of the OECD TG 476

were dose dependent, clearly above historical controls and reproducible and were positive in the absence and presence of S9 mix. 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. In the OECD TG 473 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 increase in endo-replicated chromosomes was noted.

The genotoxic potential of azamethiphos was further investigated in an *in vitro* alkaline comet assay using L5178Y mouse lymphoma cells (Confidential, 2017a; CAR 3.8.1). 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 (the test material was strongly cytotoxic at 500mg/mL, as indicated by 47.9 % survival, while at 250 µg/mL survival was 75.5 %). Azamethiphos induced statistically significant increases in the percentage of DNA in the 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 control data (HCD) for negative controls (0.53 % ± 0.06 %) under the same experimental conditions. Furthermore, a dose-response relationship was observed, as demonstrated by the Kruskal-Wallis test. Azamethiphos induced statistically and biologically (> upper bound of negative HCD) significant increases in the percentage of DNA in the tail in the absence of metabolic activation.

Overall, the DS concluded that the results from the *in vitro* studies show that azamethiphos has mutagenic potential.

In vivo studies

The CLH dossier contains 3 *in vivo* studies investigating the genotoxic/mutagenic potential of azamethiphos, a mouse micronucleus test, a rat liver UDS test and a comet assay conducted in rat stomach and duodenum.

No evidence of increased micronucleus formation was observed in male mice in the mammalian bone marrow micronucleus test (OECD TG 474, GLP, Confidential, 2008h, CAR 3.8.2). Despite azamethiphos not affecting the PCE/NCE ratio in this study, the DS concluded that the target tissue was exposed adequately as the 2 higher doses after intraperitoneal administration resulted in clear systemic toxicity and because toxicokinetic studies have demonstrated that greater than 90 % of the substance is excreted via the urine (CAR, section 9). The DS mentioned that in this study it was demonstrated that azamethiphos was well distributed to organs and tissues, including

the bone marrow. Therefore, the DS concluded that the OECD TG 474 study was robust and provided clear evidence of the absence of an *in vivo* hazard to chromosomes.

In a guideline and 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 (1 000 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 TG 489, 3 groups of 5 male mice were administered two doses of either 50, 100, 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 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 any of the concentrations tested in either the stomach or duodenum. Azamethiphos was not genotoxic in this COMET assay in the rat up to a dose causing systemic toxicity.

Despite the clear cut demonstration of mutagenicity *in vitro*, the DS concluded that based on the three robust *in vivo* studies, the classification criteria are not fulfilled.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The CLH report contains information on toxicokinetics of azamethiphos, which is obtained from a study conducted according to OECD TG 417 (Confidential, 2009f, CAR 3.1). In an EMA report on azamethiphos (EMA, 1999), two additional toxicokinetic studies are mentioned, one in the rat and the other in lactating goats. Relevant information on all three studies is described in the following section.

Azamethiphos has been tested in four *in vitro* and three *in vivo* genotoxicity/mutagenicity tests. All *in vitro* studies gave positive results.

In line with the DS, RAC is of the opinion that the available *in vitro* data clearly demonstrate that azamethiphos has mutagenic potential. Based on positive results in a bacterial mutation assay, a gene mutation and a chromosomal aberration test in mammalian cells and an *in vitro* COMET assay it could be demonstrated that azamethiphos is clastogenic and induces gene mutations.

In this regard, it is relevant to note that the alkylating potency has been demonstrated for azamethiphos by Zitko (2001). In this study from the open

literature the alkylating potency was almost half that of methyl iodide, a strong electrophile which was used as a positive control in this study and two thirds of dichlorvos, an organophosphate with a similar structure to that of azamethiphos. Other organophosphates, which were also investigated in this study, had clearly lower alkylating potency.

All three *in vivo* genotoxicity/mutagenicity studies with azamethiphos were negative, however, in contrast to the DS, RAC is not of the opinion that *in vivo* mutagenic potential can be completely excluded on the basis of these results.

The bone marrow micronucleus test (Confidential, 2008h, CAR 3.8.2) was conducted according to OECD TG 474 and GLP and met the necessary standards. However, RAC does not agree with the DS' conclusion that it was demonstrated that the target tissue, bone marrow, was adequately exposed. RAC agrees with the DS' analysis of the available OECD TG 417 study (Confidential, 2009f, CAR 3.1) that azamethiphos is well absorbed, readily metabolised after low single dose and low repeated dose oral administration, that the major route of excretion is via the urine and that there is no evidence of bioaccumulation of azamethiphos in tissues. However, RAC does not agree with the conclusion that the substance was readily distributed to all organs. The study only demonstrates that hardly any radioactivity was detectable 48 hours after the last dose in the analysed tissues, as radioactivity lower than 1 % was found in both male and female after single dose exposure.

The toxicokinetics of azamethiphos have also been investigated in another rat study and in lactating goats. The original study reports are not available to RAC, but the results are briefly summarised in the EMA report (EMA, 1999). The conclusions are similar to those from the above OECD TG 417 study (Confidential, 2009f, CAR 3.1). A relevant finding from these studies is, that the position of radiolabelling within the molecule strongly affects the results. When the molecule was labelled at the methylene group, 5 mg/kg bw orally dosed to rats resulted in 41 %, 4 % and 35 % of the administered radioactivity being recovered from the urine, the faeces and in expired air, respectively, within 24 hours. However, when the substance was labelled in the pyrimidine moiety, 85 % to 98 % was recovered from urine. This further demonstrates that the substance is heavily metabolised and that even if it is assumed that radioactivity was seen in all tissues, it might be that not the parent compound or relevant metabolites were transported to those sites. In this regard it is relevant to note that in Obe and Vijayalaxmi (2007), it is pointed out that several compounds which are metabolised to biologically active forms give a negative response in the bone marrow micronucleus assay. Some active metabolites have a very short lifespan and do not reach bone marrow at sufficient concentrations to induce micronuclei. In fact, some rodent liver carcinogens, including dialkyl nitrosamines, nitro aromatic compounds, and azo derivatives, gave negative results in a bone marrow assay.

From the bone marrow micronucleus study itself, it cannot be concluded that bone marrow was exposed, as there was no effect on the PCE/NCE ratio.

The available liver UDS test (Confidential, 2008i, CAR 3.8.2) is considered reliable and

to fulfil the requirements of OECD TG 486 and GLP. It is noted, however, that despite the relatively high doses applied in this test, which induced clear neurotoxicity in other acute tests, did not induce such effects at the low dose of 425 mg/kg bw and only in some animals of the high dose of 850 mg/kg bw. In the CAR Doc IIIA, it is described that on the slides from treated animals, no or only slight cytotoxicity (e.g. pyknosis ≤ 10 %) was observed. Therefore, the liver might not be a relevant target tissue. In addition, it should be noted that in a recent evaluation of 5 *in vivo* methods to test for *in vivo* genotoxicity/mutagenicity (Zeller *et al.*, 2018) the liver UDS test was the least sensitive for predicting carcinogenicity.

Given the high reactivity *in vitro* and the demonstration of alkylating properties of azamethiphos the BPC-WG considered it necessary to investigate genotoxicity at the site of contact *in vivo*. An *in vitro* and an *in vivo* COMET assay were therefore conducted. The *in vitro* test was deemed necessary to confirm the sensitivity of such a method for an alkylating agent like azamethiphos (see section on *in vitro* tests). As target organs for the *in vivo* COMET assay (Confidential, 2017b, CAR 3.8.2), stomach and duodenum were selected as relevant organs to assess site of contact genotoxicity. In a preliminary test, 320 and 500 mg/kg bw resulted in death of animals or resulted in animals being killed for ethical reasons. At 200 mg/kg bw no deaths occurred but slightly decreased spontaneous motor activity was observed. In the main study 50, 100*,* 200 mg/kg bw were tested. Animals were treated twice, 24 hours apart and samples of stomach and duodenum were collected 3-4 hours after the second treatment. The study was in line with OECD TG 489 and GLP.

Neither statistically nor biologically significant increases in the mean of the medians of percentage of DNA in tail were observed at any of the doses tested and the positive control gave an appropriate response. There were no relevant increases in the number of 'hedgehog' comets at any dose level. Azamethiphos did not induce site of contact genotoxicity in the stomach and the duodenum in this assay.

When comparing the results of the *in vitro* and *in vivo* tests with the classification criteria for germ cell mutagens it is obvious that classification in category 1 is not justified. There are no human data and there are no animal studies demonstrating genotoxic or mutagenic potential in germ cells nor in somatic cells.

For category 2, CLP states that a substance is regarded as a category 2 mutagen if it causes concern for humans owing to the possibility that it may induce heritable mutations in germ cells of humans. Classification is based on positive results in mammals and/or, in some cases, in *in vitro* experiments with supporting information from *in vivo* studies or chemical structure activity relationship to known germ cell mutagens. In the case of Azamethiphos, there is a clear positive signal from a total of four *in vitro* tests, however this is not supported by data from *in vivo* studies as none of the three *in vivo* studies gave positive results.

RAC concludes that also category 2 is not applicable for azamethiphos as none of the three *in vivo* studies gave positive results. RAC agrees with the conclusion of the DS that **no classification for germ cell mutagenicity is warranted**.

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 colspan="3">Leiomyoma</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></td><td></td><td></td></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)</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</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)</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Dose levels**	**Observations and remarks (effects of major toxicological significance)**
Males/females 50/sex/dose (carcinogenicity group) OECD 409* Reliability: 1 Confidential (1989) CAR 3.9	test)	↓CHER# (>20%) **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	Azamethiphos Purity not specified Dose: 0, 11, 97 & 396 ppm	**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.

Method, guideline, deviations if any, species, strain, sex, no/group	**Dose levels**	**Observations and remarks (effects of major toxicological significance)**
(ICR) BR) Males/females 60/sex/dose (carcinogenicity group) Non-guideline Pre-GLP Reliability: 2 Confidential. (1982)	Approximately 0, 2, 14 & 57 mg/kg bw/d	pigment/amyloid deposition in a range of tissues, but no clear dose response and no individual findings were statistically significant ($p<0.05$). **<u>Neoplastic effects</u>** 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).

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

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 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

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of azamethiphos has been investigated in 5 oral carcinogenicity studies, 3 in the rat and 2 in the mouse. While the mouse and two of the rat studies were dietary studies, one rat study was via gavage. All five were presented in the CLH report, but the CAR does not provide further details of the 4 dietary studies than presented here.

Table: *Summary of the available carcinogenicity studies, table 16 from the CLH report.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Dose levels**	**Observations and remarks (effects of major toxicological significance)**
Confidential, 2011a CAR 3.9 Oral (gavage) 12/24 month combined chronic/ carcinogenicity study Rat, Crl:WI(Han) males/females 50/sex/dose (carcinogenicity group) **OECD TG 453** GLP Reliability:1	Dose: 0, 0.05, 0.5, 5 mg/kg bw (daily) Vehicle: propylene glycol	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.

	males				females			
mg/kg bw/day	**0**	**0,05**	**0,5**	**5**	**0**	**0,05**	**0,5**	**5**
Jejunum								
Leiomyoma	0	0	0	0	0	1 (2%)	2 (4%)	2 (4%)
Leiomyosarcoma	0	0	0	1 (2%)	0	0	0	0
Duodenum								
Leiomyoma	0	0	0	0	1 (2%)	0	0	0
Ileum								
Ileum examined, but no findings reported in any group								
Endometrial								
glandular hyperplasia	-	-	-	-	3 (6%)	0	0	3 (6%)
adenoma					1 (2%)	1 (2%)	1 (2%)	0
adenocarcinoma					6 (12%)	3 (4%)	6 (12%)	12 (24%)

Historical control data:

Study in Wistar (Han) rats, performed in the same laboratory

<table>
<tr><td></td><td></td><td>
<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>
</td></tr>
<tr><td>Confidential, 1989a
CAR 3.9
Oral (dietary)
Rat CD(SD)BR
Carcinogenicity: 50/sex/group
Chronic: 20/sex/group
Additional animals were sacrificed at 52 weeks (10/sex/group) and week 56 after 4 weeks on control diet (10/sex at 0 and 1 500 ppm).
OECD TG 453*
GLP compliant
Reliability:1</td><td>Azamethiphos purity 94.2 %
Dose: 0, 20, 200, 1 500 ppm
Equivalent to 0, 0.8, 8.2, 62 mg/kg bw/day males and 0, 1.1, 11, 89 mg/kg bw/day females</td><td>No treatment related increase in tumour incidence.
Survival was unaffected by azamethiphos.
Body weight gain was reduced from the beginning of the study in both sexes at the top dose and in males from week 4 onwards in the mid dose. Food consumption was reduced by about 10 % during the first month at the top dose.</td></tr>
<tr><td>Confidential, 1982a
Oral (dietary)
2 year carcinogenicity study
Rat CD(SD)BR
Males/females
60/sex/dose (carcinogenicity group)
Guideline not stated
Pre-GLP
Reliability: 2</td><td>Azamethiphos Purity: 95.6 %
Dose: 0, 15, 60, 327 ppm
Approximately 0, 0.8, 3, 16 mg/kg bw/day</td><td>There was no significant increase in mortality in any dose group.
There was an inversion of the ratio of mammary gland fibroadenoma to adenocarcinoma:
<table>
<tr><td></td><td colspan="4">Males</td><td colspan="4">Females</td></tr>
<tr><td>mg/kg bw/day</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:</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>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 malig. 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>
</td></tr>
<tr><td>Confidential, 1989b
CAR 3.9
Oral (dietary)
Mouse CD-1
51/sex/group
24 month
carcinogenicity study</td><td>Azamethiphos purity 94.2 %
Dose: 0, 50, 500, 1 500, 4 000 ppm
Equivalent to 0, 6.2, 60.2, 183.4, 491.4 mg/kg bw/day males and 0, 7.7, 76.2,</td><td>There was no significant increase in mortality in any dose group.
No treatment related increase in tumour incidence.</td></tr>
</table>

OECD TG 451 GLP compliant Reliability:1	219.7, 582.9 mg/kg bw/day females	
Confidential, 1982b Oral (dietary) Lifetime carcinogenicity study Mouse (CD-1 (ICR) BR) Males/females 60/sex/dose (carcinogenicity group) **Non-guideline** **Pre-GLP** Reliability: 2	Azamethiphos Purity not specified Dose: 0, 11, 97, 396 ppm Approximately 0, 2, 14, 57 mg/kg bw/day	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.

No treatment related neoplastic findings were reported in the dietary mouse and rat studies, but small increases in the incidences of leiomyoma and endometrial adenocarcinoma were reported in the rat gavage study.

The data from the 4 dietary rat and mouse studies have been considered previously by the UK Advisory Committee on Pesticides in 2003 and EMA (EMA, 1999). Both concluded that there were no treatment related neoplastic effects in these studies. A summary of the neoplastic findings in these studies is presented in the table above and the non-neoplastic findings are summarised in the table in the section on STOT RE.

In the recent gavage study in rat (Confidential, 2011a, CAR 3.9) an increased incidence of leiomyoma of the jejunum was observed in each group of female rats. The incidences were 0/50, 1/50, 2/50 and 2/50 at 0, 0.05, 0.5 and 5 mg/kg bw/day azamethiphos, respectively. No incidences of leiomyoma were seen in males at any part of the small intestine. HCD are limited to a single study that was carried out concurrent with the present study. In this study there were two cases of leiomyoma in the small intestine (0.7 %), one in the jejunum (female) and one in the ileum (male); thus the HCD show a low level background incidence of leiomyoma in the small intestine. When considering 3 further studies from the same laboratory, which were outside the recommended time period of ±4-5 years, the background incidence for the small intestine ranged from 0-1 % and was 0 % for the jejunum, further indicating that it is a rare type of tumour.

There was no statistical significant difference between the incidence of leiomyoma of the jejunum seen in female animals in any of the dosed groups and that from the internal control group in a pair wise comparison ($p < 0.05$). However, a positive trend in the incidence of leiomyoma in the jejunum from control to the high does group was observed when analysed according to the method of Peto *et al.*, (1980). This trend was, however, not evident when the results from the duodenum and the jejunum were combined and incidences from the entire small intestine were analysed together.

The DS stated that the approach to combine all leiomyomas of the small intestine was described by McConnell *et al.* (1986) and accepted by the US National Toxicology Program in evaluating rodent carcinogenicity studies. They further stated that this approach is also in line with the

REACH Member State Committee decision (MSC 47/48) not to specify whether the jejunum or duodenum is sampled in *in vivo* COMET assays due to the difficulty in distinguishing between the two tissues. In addition, they questioned the validity of trend testing in the absence of pairwise significance and a reported control value of zero.

Overall, the DS concluded that the HCD, though limited to a single study, demonstrate that there is a low background incidence for leiomyomas. In the absence of leiomyomas seen in any of the earlier dietary carcinogenicity studies in rat and mouse, which tested far higher doses, in the absence of a clear dose-response relationship and no statistical significance the dossier submitter concluded that the finding was incidental and not treatment related.

The recent gavage study in rat (Confidential, 2011a, CAR 3.9) also identified an increased incidence in endometrial adenocarcinoma in the high dose females above HCD (which were derived from a single study only: 21/150 (14 %)). Four additional studies, which were conducted at the same laboratory, but between 2001 and 2004, gave a background incidence range for endometrial adenocarcinoma between 0 – 6 %, whereas the HCD from the supplier of the animals from 1997 – 2009 indicated highly variable incidences ranging from 0.89 % - 14 %. The incidence in the high dose females was not statistically significantly different from the control by pairwise comparison ($p < 0.05$). No dose response relationship and a relatively high incidence in the control was observed (6/50 (12 %), 2/50 (4 %), 6/50 (12 %) and 12/50 (24 %) at 0, 0.05, 0.5 and 5 mg/kg bw/day, respectively), but there was a positive trend ($p < 0.05$) for incidental tumours alone. When all proliferative endometrial lesions, endometrial hyperplasia, endometrial adenoma and endometrial adenocarcinoma were analysed combined, there was no trend. The data on non-neoplastic lesions of the uterus/endometrium and applicable HCD were not available to RAC. The DS also noted that in none of the earlier dietary carcinogenicity studies in the rat and mouse was an increased incidence in endometrial tumours reported. Overall, they concluded that the increase in this tumour finding was incidental on a weight of evidence basis.

In conclusion, the DS was of the opinion that classification for carcinogenicity is not justified.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Leiomyomas

Leiomyomas were slightly increased in the jejunum in the dosed females (0, 1, 2, 2 in control, low, mid, and high dose respectively), but not in males, in a gavage carcinogenicity study (Confidential, 2011a, CAR 3.9). Leiomyomas are benign tumours originating from smooth muscle tissue. No leiomyosarcoma was seen in any of the females, but a single incidence was seen in the jejunum of a top dose male of this study (no HCD are provided for leiomyosarcoma). A single leiomyoma was also seen in the duodenum of a control female.

There was no statistically significant increase in incidence by pairwise comparison with the control, but a positive trend for the incidence in jejunum was observed. This was, however, not observed when the results from jejunum and duodenum were analysed together. The DS referred to McConnel *et al.* (1986) and a conclusion from the Member State Committee (MSC

47/48) to support this approach. However, while the MSC conclusion might be relevant for the COMET assay, McConnel *et al.* (1986) states that smooth muscle neoplasms are combined for all sites of the body, except the gastrointestinal and reproductive tracts, where they are evaluated independently. RAC is therefore of the opinion that the findings in the duodenum and the jejunum should be assessed separately.

Although the original study report was not available to RAC, the CLH report and the CAR stated that there were no other effects or pre-neoplastic lesions in the gastro-intestinal tract observed in this study. However, it is relevant to note that in one of the dietary mouse carcinogenicity studies (Confidential, 1989b, CAR 3.7.1) lesions were described in the stomach and the small intestine. A statistically significant and dose dependent increase in hyperplastic avillous mucosa was detected in the small intestine at doses ≥ 60 mg/kg bw/day in males and ≥ 76 mg/kg bw/day in females. In the top dose of this study, there was also a strong and statistically significant increase in erosion/ulcer in stomach as well as in the small intestine in males (491.4 mg/kg bw/day) and females (582.9 mg/kg bw/day).

These findings could be indicative of a local effect on the mucosa, however, an *in vivo* COMET assay conducted in the stomach and duodenum gave negative results (see the section on germ cell mutagenicity). However, it might be questioned whether local effects (including damage and repair) would be expected to precede a tumour arising from underlying mesenchymal structures (like smooth muscle tissue).

In addition, leiomyomas as well as leiomysarcomas are very rare tumours in humans and rodents, as also indicated by the HCD. They either occur as benign or malignant tumours and preneoplastic lesions are not necessarily expected due to the rareness of this lesion.

Endometrial adenocarcinomas

The recent gavage study in rat (Confidential, 2011a, CAR 3.9) also identified an increased incidence in endometrial adenocarcinoma in the high dose females. Endometrial adenocarcinoma is highly variable with relatively high background incidences, but the incidence in the top dose clearly exceeded the HCD. No preneoplastic lesions were observed in this study. However, in the dietary rat carcinogenicity study (Confidential, 1989a, CAR 3.9) a dose dependent statistically significant increase of hydrometra was seen at the two highest doses (11, 89 mg/kg bw/day) and a statistically significant increase in pyometra was seen at the top dose (see table in STOT RE section). The observed changes might have resulted from endometritis.

The DS stated that the relevance of the tumour findings in the gavage rat study is lowered due to the fact that the tumours were not seen in four other carcinogenicity studies with dietary exposure. For site of contact effects it can be assumed that the test material can act more effectively when applied via gavage and is not admixed with the chyme. Also for systemic effects differences between dietary and gavage exposure are likely. For this reason, it is not justified to ignore tumours seen via gavage, which were not seen in the dietary studies at even higher doses.

In this regard it is also important to note that in the assessment of EMA (1999), it was mentioned that azamethiphos is prone to degradation in animal feed and that in the earlier repeat-dose studies, achieved test substance intakes were lower than expected until allowances were made for this instability. It is not clear which of the "earlier" studies were affected by this and RAC has no access to information on stability of the test material in animal feed of the

dietary studies.

Considering the gavage study (Confidential, 2011a, CAR 3.9) alone it is important to note that the tested doses were rather low (top dose 5 mg/kg bw/day). As stated previously, there were 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, therefore it can be concluded that the maximum tolerated dose (MTD) was not achieved in this study.

For the reasons explained above, the relevance of the observed tumours in rats, leiomyomas in the jejunum of female rats at and above doses of 0.05 mg/kg bw/day, with a single leiomyosarcoma in male rat of the top dose of 5 mg/kg bw/day and an increase in endometrial adenocarcinoma at the top dose cannot be excluded. There is no information in humans and not sufficient evidence from animal studies to indicate Carc. 1A or 1B respectively. The increase in tumour incidence was only slight and tumours were only seen in one species, in one study and in one sex. However, there were two types of tumours, one clearly malignant. In both organs, which were affected by the tumour increase, the small intestine and the endometrium, inflammation and hyperplastic lesions were described. Although these findings were in different studies and, for the effects on the gastrointestinal tract, in a different species (mouse), they demonstrate that endometrium and small intestine are targets of azamethiphos toxicity. All available *in vitro* genotoxicity / mutagenicity tests were positive and it was demonstrated that azamethiphos has strong alkylating properties, but all four *in vivo* genotoxicity / mutagenicity tests gave negative results. As there are some deficiencies in the *in vivo* data base (see section on germ cell mutagenicity) an *in vivo* mutagenic potential cannot be completely excluded. On this basis RAC concludes that there is limited evidence for carcinogenic potential thus supporting **classification as Carc. 2; H351 for azamethiphos**.

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	**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	**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

Method	**Dose levels**	**Observations and remarks** **(effects of major toxicological significance)**
GLP Confidential (2009) CAR 3.10.2	mg/kg bw **F_1 generation** 0.05, 0.5 and 5 mg/kg bw Vehicle: propylene glycol	**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 **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.
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

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicology of azamethiphos has been investigated in three OECD TG 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.

Fertility

Table: *Table 17 from CLH report, slightly adapted.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Dose levels**	**Observations and remarks (effects of major toxicological significance)**
Confidential, 2009g **CAR 3.10.2** Two-generation reproductive toxicity study Oral (gavage) Rat, Sprague Dawley 24/sex/group **OECD TG 416** GLP	**F0 generation** <u>Days 1 – 9:</u> 1, 10 and 100 mg/kg bw/day <u>Day 10 – 16</u>: 0.01, 0.1 and 1 mg/kg bw/day <u>Day 17 onwards:</u> 0.05, 0.5 and 5 mg/kg bw/day Dosing commenced at least 70 days prior to mating and continued until termination. **F1 generation** 0.05, 0.5, 5 mg/kg bw Vehicle: propylene glycol	**At 5 mg/kg bw/day:** 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 acetylcholinesterase (35 % and 31 % in males and females respectively). Reproduction and developmental toxicity: no findings. **At 0.5 mg/kg bw/day:** ↓ body weight (< 10 %) from day 8 onwards. No effects on reproduction or development. **At 0.05 mg/kg bw/day:** 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. *Other dose levels (before Day 17):* **100 mg/kg bw/day**: ↓ body weight at day 8. Inhibition of acetylcholinesterase on day 9 (63 % in males and 60 % in females). **At 10 mg/kg bw/day:** Inhibition of acetylcholinesterase on day 9 (64 % and 51 % in males and females respectively). **At 1 mg/kg bw/day:** 1 female died on day 16 approximately 3h after dosing. No cause of death could be established; the only finding before death

		was slight salivation, and at necropsy, enlarged liver correlating with congestion at microscopic examination. This was not considered to be a contributory factor to death. Inhibition of acetylcholinesterase in both sexes on day 9 (45 % and 41 % in males and females respectively) and on day 16 (28 % in males), and it was not considered treatment related. **F1-GENERATION** **At 5 mg/kg bw/day:** 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 kidneys, 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 acetylcholinesterase at the end treatment (30 % and 32 % in males and females). No effects on reproduction or development. **At 0.5 mg/kg bw/day:** No effects on parents, reproduction or development. **At 0.05 mg/kg bw/day:** 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 **F2-GENERATION** No treatment-related effects at any dose level.

The DS concluded that based on the lack of effects on mating performance, number of pregnant animals, number of implantations or post-implantation losses and a lack of effects on offspring parameters in F1 and F2 that no classification is justified for fertility.

Development

Azamethiphos was tested in two guideline pre-natal developmental toxicity studies according to GLP in rat and rabbit. The results are summarised in the table below.

Table: *Table 18 from CLH report.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Dose levels**	**Observations and remarks (effects of major toxicological significance)**
Confidential, 2009h **CAR 3.10.1** Prenatal developmental Oral (gavage) Rat, Sprague Dawley Female 24 for control, low and high dose 25 for mid dose **OECD TG 414** GLP	0, 0.1, 1 and 10 mg/kg bw (daily) Vehicle: propylene glycol Dosing days 6 – 20 post-coitum. Purity: 96.2 %	<u>*Maternal toxicity*</u> No treatment-related effect on mortality, clinical signs, body weight, food consumption or necropsy. **At 10 mg/kg bw/d:** Inhibition of acetylcholinesterase activity of 34.8 and 9.7 % in erythrocytes and brain respectively. **At 1 mg/kg bw/d and below:** Inhibition of acetylcholinesterase activity of 3.4 and 7.9 % in erythrocytes and brain respectively. **At 0.1 mg/kg bw/d:** ↑ body weight during days 16 – 20. Inhibition of acetylcholinesterase activity of 0.3 and 6.7 % in erythrocytes and brain respectively. <u>*Foetal toxicity*</u> No evidence of an effect on embryo-foetal development at any dose tested.
Confidential, 2009i **CAR 3.10.1** Prenatal developmental Oral (gavage) Rabbits New Zealand White Female 24/ control, low and mid dose groups 25 high dose group **OECD TG 414** GLP	0, 0.05, 0.5 and 5 mg/kg bw in (daily) Vehicle: Arachis oil Dosing days 7 – 29 post-coitum Purity:96.2 %	<u>*Maternal toxicity*</u> No treatment-related effect on mortality, clinical signs, body weight, food consumption or necropsy. **At 5 mg/kg bw/d**: Inhibition of acetylcholinesterase activity of 69 and 11 % in erythrocytes and brain respectively. **At 0.5 mg/kg bw/d and below**: No treatment-related effects <u>*Foetal toxicity*</u> No treatment-related effects at the highest dose tested

In the rat study, there were no effects on body weight, food consumption or necropsy findings and maternal toxicity was restricted to an inhibition of cholinesterase in erythrocytes of 43.8 % at the top dose. One death in the high dose group was reported to be a gavage error. There was no evidence of an effect on embryo-foetal development at any dose level. Malformations and developmental variations occurred at similar incidences as in all dose groups, including the controls.

In rabbits, azamethiphos administered from day 7 to 29 post-insemination did not have any effect on mortality, body weight, food consumption or necropsy findings. Maternal toxicity was

demonstrated by 69 % inhibition of cholinesterase activity in erythrocytes. There was no evidence of embryo-foetal toxicity.

The DS proposed no classification for developmental toxicity based on two negative studies in rats and rabbits, supported by the absence of developmental findings in the 2-generation study.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Fertility

RAC agrees with the DS, that based on the absence of adverse effects on sexual function and fertility no classification is supported.

RAC notes that the dosing regime was changed twice during the premating phase of the F0 generation. There is no explanation for this included in the CLH report. It appears that the change in dose was made in order to lower the effect on cholinesterase activity. From the CAR Doc IIIA document the following information on the inhibition of acetylcholinesterase in plasma, erythrocytes and brain was obtained.

Inhibition (% change from mean control values)			Treatment		
			1 mg/kg (Days 1-9)	10 mg/kg (Days 1-9)	100 mg/kg (Days 1-9)
Day 9	*Males*	*CHEP*	14%	40%*	73%*
		CHER	45%*	64%*	63%*
	Females	*CHEP*	11%	27%	66%*
		CHER	41%*	51%*	60%*

			0.01 mg/kg (Days 10-16)	0.1 mg/kg (Days 10-16)	1 mg/kg (Days 10-16)
Day 16	*Males*	*CHEP*	16%	12%	13%
		CHER	18%*	14%*	28%*
	Females	*CHEP*	ns	ns	ns
		CHER	ns	ns	19%

			0.05 mg/kg (Day 17-onwards)	0.5 mg/kg (Day 17-onwards)	5 mg/kg (Day 17-onwards)
End of Treatment	*Males*	*CHEP*	20%	15%	30%*
		CHER	ns	ns	35%*
		CHEBR	ns	ns	10%
	Females	*CHEP*	11%	13%	29%*
		CHER	ns	10%	31%*
		CHEBR	ns	11%	11%

CHEP: acetylcholinesterase activity in plasma
CHER: acetylcholinesterase activity in erythrocytes
CHEBR: acetylcholinesterase activity in brain

No effects on clinical chemistry parameters other than on acetylcholinesterase activity were reported. No related clinical signs were described. Slightly reduced body weight and body weight gain in F0 males of the top dose, on day 8, which was not seen any longer after reduction of dose. Females of that group had slightly higher body weights at day 4 post-coitum and from day 4 of lactation onwards. All differences in the body weight were less than 10 % and so are not considered adverse. No effect on body weight was recorded in the F1 generation.

It is assumed that higher doses could have been tolerated and that the MTD was not reached in this study. There is no information on whether a range finding study had been conducted, either in the CLH report or in the DAR.

Development

RAC agrees with the DS, that based on the absence of adverse effects on development in rat and mouse, no classification for developmental toxicity is supported.

RAC notes however, that the doses used in the rat study were too low, and it is likely that higher doses would have been tolerated. No data on dose range finding studies are presented.

In the rabbit study considerable and statistically significant reduction of acetylcholinesterase in erythrocytes was seen after 29 days at the top dose. The reduction was also significant at the mid dose, but was < 20 %. Also, the reduction in brain acetylcholinesterase was statistically significant at the mid and high doses, but did not reach 20 %.

Dose	**% inhibition of mean control values**	
	Erythrocytes (CHER)	**Brain (CHEBR)**
0.05 mg/kg bw/day	8	5
0.5 mg/kg bw/day	15 *	12 **
5 mg/kg bw/day	69 **	11 **

* Statistically significant at p < 0.05 level
** Statistically significant at p < 0.01 level

However, no clinical signs related to acetylcholinesterase inhibition were reported in this study.

Although no relevant effects were seen in the available studies, the full developmental toxicity potential could not be assessed due to too low doses having been used, at least in the rat developmental toxicity study.

Lactation

As no effects were reported, RAC agrees with DS's no classification proposal.

Overall, based on the data provided, **no classification is warranted for reproductive toxicity, but in the case of adverse effects on development, this is due to inconclusive data**.

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

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Three acute toxicity studies were available, one for each route (oral, dermal, inhalation), which were conducted according to the guidelines and GLP. An additional oral study with single exposure in the rat, not following a specific guideline but conducted under GLP conditions is also available. The results of these studies have been described in detail in the section on 'Acute toxicity' above.

Based on the results of the available acute studies with azamethiphos, the DS considered that no specific toxic effects on organs were noted in rats, via the oral, dermal or inhalation routes and therefore no classification as STOT SE was justified. As to neurotoxic effects, the DS considered the effects as not supportive for classification. The DS concluded that although clear neurotoxic effects were reported after oral and inhalation exposure, these effects were either transient, or, where they persisted for more than 1 day, they occurred in animals that died later on.

The DS did not present the range finding acute oral rat study (Confidential, 2009b, CAR 3.2.1) and did not discuss the effects on acetylcholine esterase observed in that study.

In conclusion the DS proposed no classification for STOT SE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that clear neurotoxic effects were seen after single oral and inhalation exposures.

After inhalation exposure to 1.1 mg/L (the mid dose of the acute inhalation toxicity study) the following effects relevant for neurotoxicity and possibly indicative for acetylcholinesterase inhibition were reported: on removal from restraining tubes, shaking head and spread hind legs (1 of 5 males, 2 of 5 females) and in one female gasping were reported. Subsequently, hunched posture, hypothermia, piloerection and general tremors were observed in males and females. As it is reported that one male died during exposure, that a second was found dead at termination of exposure and a male and a female died on the day after exposure to this dose, it is assumed that the neurotoxic findings were seen in all animals that survived after the dosing period. At the top dose of 5.2 mg/L, all animals were reported dead during exposure, but at the low dose of 0.54 mg/L, which consisted of 5 males only, none of the animals died and no neurotoxic effects were described.

After oral exposure, the effects relevant for neurotoxicity included hunched posture, uncoordinated movements, piloerection and/or shallow respiration on days 1 and/or 2 at 300 mg/kg bw, at the low dose of the OECD TG 423 study (Confidential, 2008a, CAR 3.2.1) and hunched posture (14/32), calm behaviour (6/32), piloerection (6/32) and lethargy (2/32)

1 to 4 hours after dosing at 250 mg/kg bw, the high dose of an acute non-guideline study (Confidential, 2009c, CAR 3.2.1), which was summarised in the CAR but not presented in the CLH report. At the low dose of 50 mg/kg bw hunched posture (6/32) and piloerection (1/32) was described. In this study, also acetylcholinesterase activity was measured in plasma and erythrocytes. Only the levels in erythrocytes were reported in the study summary included in the CAR, as they were considered more relevant. A clear dose dependent reduction in acetylcholinesterase activity is reported, with maximum reductions of 38 % in males and 43 % in females at the low dose of 50 mg/kg bw and 68 % in males and 58 % in females at the high dose of 250 mg/kg bw. The peak of acetylcholinesterase inhibition was after 1 hour and while it recovered to pre-dose levels after 12 hours at the low dose, it remained reduced after 12 hours at the high dose.

As acetylcholinesterase in brain (CHEBR) was not measured in the available acute toxicity studies.

The DS disregarded the effects on erythrocyte acetylcholinesterase and the neurotoxic effects, as they were of a transient nature or, where they persisted for more than 1 day, they occurred in animals that died later on. RAC notes that reversibility of effects is not an exclusion criterion for classification as STOT SE. Regarding the observed neurotoxic effects and their relationship to subsequent lethality, it is noted that clear neurotoxic effects also occurred at doses which did not result in death of those animals and these effects are therefore considered relevant for classification.

RAC notes that the WHO JMPR guidance (JMPR, 1999) considers inhibition CHEBR activity (≥ 20 %, statistically significant and fitting a dose related trend) and clinical signs to be the primary end-points of concern in toxicological studies on compounds that inhibit acetylcholinesterase. Inhibition (≥ 20 %, statistically significant and fitting a dose related trend) of acetylcholinesterase in erythrocyte (CHER) is also considered to be an adverse effect, which can be used as a surrogate for CHEBR inhibition when data on this enzyme are not available. Inhibition of plasma acetylcholinesterase (CHEP) is only considered as an indication of adversity.

RAC notes that based on the information available it is not possible to find out whether in the acute studies the inhibition of acetylcholinesterase activity was statistically significant. However, taking into account that the effect was dose dependent and that the degree of acetylcholinesterase inhibition that can be tolerated without clinical symptoms can vary between individuals and substances (JMPR, 1999), RAC considers the observed effects to meet the criteria for classification (in particular CLP Annex I 3.8.2.1.7.3(c)).

Also, in several repeated dose toxicity studies inhibition of acetylcholinesterase > 20 % was observed, reaching statistical significance, which was related to the clinical signs observed. There are indications that also in the repeated dose studies these effects were of an acute nature: effects are described to be intermittent in the 28 days rat study (Confidential, 2009d, CAR 3.5.1), did not increase over time in the 90 days rat study (Confidential, 2009e, CAR 3.6.1) or in the combined chronic/carcinogenicity rat study (Confidential, 2011a, CAR 3.7.1) and peaked 1-2 hours after exposure in the 90 days rat study (Confidential, 2011b, CAR 3.6.1) for 13 days and subsequently, salivation was recorded directly after treatment (see section on STOT RE). This further supports the classification as STOT SE 1. Though these effects were seen at quite low doses after repeated exposure (5 mg/kg bw/day) it is not possible to directly compare acute and repeated exposure at such low exposure levels, as the lowest dose tested in an oral acute study was 50 mg/kg bw. Already at that dose acetylcholinesterase activity was

inhibited by 38 % and 43 % in males and females, respectively (Confidential, 2009b, CAR 3.2.1). From the repeated dose toxicity studies there are no measurements of acetylcholinesterase activity directly after the first dosing or after only a few doses, the earliest time-point was after 6 weeks of repeated exposure. The acetylcholinesterase inhibition, as well as the related neurotoxic effects did not increase with the duration of exposure, but in some instances showed a slight decrease (see section on STOT RE).

CHEBR was affected in some of the chronic studies where it was measured. Sometimes inhibition was dose-dependent and statistically significantly different from controls, but never exceeded 20 % (though 19 % were reached in a 2 years rat study (Confidential, 1989a, CAR 3.9). In this regard it is relevant to note that WHO JMPR (JMPR, 1999) states that in the absence of data on acetylcholinesterase activity in peripheral nervous tissues, acetylcholinesterase in erythrocytes (CHER) can be used as a surrogate for peripheral effects for acute exposure resulting in greater inhibition of CHER than CHEBR. A few cholinesterase inhibitors, which e.g. do not pass the blood-brain barrier to an appreciable extent cause peripheral cholinergic signs associated with inhibition of erythrocyte but not CHEBR activity.

As the effects were seen at doses clearly below the guidance value of 300 mg/kg bw for STOT SE 1 via the oral route (≥ 50 mg/kg bw) and just above the guidance value of 1 mg/L for STOT SE 1 via the inhalation route (at 1.1 mg/L), RAC proposes to classify as STOT SE 1; H370 with the nervous system as the target organ.

Clear neurotoxic symptoms were seen after acute oral and inhalation exposure, but only slight effects were seen after acute dermal exposure. However, as a value of 20 % for dermal absorption has been derived from an *in vitro* test using human skin (Confidential, 2009f, CAR 3.1), effects after dermal exposure cannot be completely excluded. In conclusion, RAC does not recommend specifying a specific route of exposure.

Classification for STOT SE is not warranted, as for Category 3, no signs of respiratory tract irritation were observed in the acute studies available, and the observed transient neurotoxicity did not fulfil the criteria for narcotic effects.

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

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS described 4 repeated dose toxicity oral (gavage) studies in the STOT RE section of the CLH report, 3 studies in the rat (28 days, 90 days, 2 years) and 1 study in the dog (90 days).

In addition, the CLH report also includes 5 oral carcinogenicity studies, three in the rat and two in the mouse. While the two mouse studies and two of the rat studies were dietary studies, in the most recent rat study dosing was via gavage. However, the information on non-neoplastic findings from these studies is scarce, except for the rat carcinogenicity study via gavage (Confidential, 2011, CAR 3.7.1). Additional information can be extracted from the studies investigating reproductive toxicity.

There are no studies conducted via the inhalation or dermal routes of exposure.

Table: *Summary of repeated dose studies relevant for STOT RE. This table is table 19 from the CLH report, slightly modified with additional information on the single studies from the CAR Doc IIIA documents and the guidance values for both category 1 and 2 are included.*

Method	Dose levels	Observations and Remarks	Dose relevant for classification
Confidential, 2009d **CAR 3.5.1** 28 days oral (gavage) Rat, Sprague Dawley 3/sex/group **OECD TG 407** (not fully compliant: less than 5 animals per sex and group, brain samples for CHEBR) GLP	0, 0.5, 5 and 50 mg/kg bw/day 96.2 % pure Vehicle: propylene glycol	**50 mg/kg bw/day:** 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) CHEBR[$] appeared unaffected at any dose level.	STOT RE 1: 30 mg/kg bw/day STOT RE 2: 300 mg/kg bw/day

Confidential, 2009e **CAR 3.6.1** 90 days combined repeated-dose / neurotoxicity oral (gavage) study Rats, Sprague Dawley 15/sex/group **OECD TG 408/424** GLP	0, 0.05, 0.5 and 5 mg/kg bw/d 96.2 % pure Vehicle: propylene glycol	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[$] (12 % males).	STOT RE 1: 10 mg/kg bw/day STOT RE 2: 100 mg/kg bw/day
Confidential, 2011a **CAR 3.9** 12/24 months oral (gavage) combined chronic/ carcinogenicity study Rat, Crl:WI(Han) <u>Chronic:</u> 10/sex/group, 20/sex/group in the top dose <u>Satellite group (24 months):</u> 10/sex/group (acetylcholinesterase measurements) <u>Carc:</u> 50/sex/group **OECD TG 453** GLP	0, 0.05, 0.5 and 5 mg/kg bw (daily) 96.2 % pure Vehicle: propylene glycol	No treatment-related effects on mortality, clinical signs, haematology or body weight at any dose tested. **At 5 mg/kg bw/d** ↓ CHER[#] (-34 to -48 % (males), -27 to -41 % (females) compared to controls). **0.5 and 0.05 mg/kg bw/d** No toxicologically relevant effects. CHEBR[$] was measured but not affected to a relevant extent at any dose level.	STOT RE 1: <u>12 months:</u> 2.4 mg/kg bw/day <u>24 months:</u> 1.2 mg/kg bw/day STOT RE 2: <u>12 months:</u> 24 mg/kg bw/day <u>24 months:</u> 12 mg/kg bw/day

Confidential, 2011c **CAR 3.6.1** 90 days oral (gavage) study Dog, Beagle 4/sex/group **OECD TG 409** GLP	0, 0.2, 2 and 20 mg/kg bw/d 96.2 % pure Vehicle: propylene glycol	No treatment related mortality. **At 20 mg/kg bw/day** ↓ 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 mucus 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/day** ↓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/day** ↑ head shaking (1/4 males (incidence: 7) vs 0 in controls) CHEBR[$] was measured but not affected to a relevant extent at any dose level.	STOT RE 1: 10 mg/kg bw/day STOT RE 2: 100 mg/kg bw/day

[#] 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

The DS concluded that the only treatment related finding was a reduction of cholinesterase activity in red blood cells in both rats and dogs, which was considered consistent with azamethiphos mode of action. The dossier submitter concluded that the behavioural effects were transient and spasmodic in nature and that the effect on cholinesterase was relevant to humans. They concluded that no other consistent and treatment related effects were seen in rat or dog.

On comparison with the classification criteria for STOT RE, the DS noted that the above effects were seen below the guidance value for STOT RE 2, but considered them as not severe enough to support classification. They concluded that there were no significant functional effects associated with the changes in cholinesterase activity observed in the functional observation battery and where clinical signs indicative of neurotoxicity were reported they were transient. As lethality was seen associated with inhibited cholinesterase activity after acute exposure, this should be covered by a classification for acute toxicity.

On that basis the DS did not propose classification for STOT RE.

Comments received during public consultation

No comments were received.

Additional key elements

It is noted that azamethiphos has been evaluated by EMA in 1999 (EMA, 1999) and the CLH report contains a link to this report: https://www.ema.europa.eu/en/documents/mrl-report/azamethiphos-summary-report-2-committee-veterinary-medicinal-products_en.pdf.
Some additional repeated dose studies are described in this report. No details of these studies were available to RAC, but the report described 3 additional dietary dog studies; two 90 day studies and a one year study. While clear effects on CHER accompanied by clinical signs are described in one of the 90 day studies (which used clearly higher doses than the second 90 days study) also relevant effects on CHEBR are described in the 1 year dog study, which were not seen in any of the studies included in the CLH report. This could be an indication of a repeated dose effect, however, as the studies were were requested by ECHA but not available, they could not be included in the assessment.

Assessment and comparison with the classification criteria

The available repeated dose toxicity studies consistently showed inhibition of cholinesterase, an effect expected to be induced by organophosphate substances. In many of these studies also clinical signs, indicative of neurotoxicity were observed.

Clinical signs typical for cholinergic effects were seen in a rat gavage 28 day study (Confidential, 2009d, CAR 3.5.1). At all dose levels (0.5, 5, 50 mg/kg bw/day) lethargy, calm behaviour, tremors, flat/hunched posture, uncoordinated movement, piloerection and/or salivation were reported. For incidences per dose see the table above. The incidence of hunched posture was higher among females at 5 and 50 mg/kg bw/day compared to other groups. The incidence of the other clinical signs was not clearly related to the dose and none of the clinical signs observed during treatment appeared related to the duration of treatment. These signs were of an intermittent nature and were generally shown by individual animals only. One female death at the top dose was considered not treatment related by the study

authors. No clinical signs were noted for control animals. The lack of dose response in this study for most of the effects might be explained by the low number of animals (3/dose) used, but the effects were typical for acetylcholinesterase inhibition. In the top dose males and females CHER was reduced by 20 % and 48 %, respectively, while in the mid dose a relevant reduction of 37 % was only seen in females. No effect on CHEBR was detected, although it should be noted that brains were collected only 3 hours after the last dose, while sampling should take place between 1-2 hours after dosing. *Ex vivo* reactivation of organophosphorus-inhibited cholinesterase is fairly rapid in the case of dimethyl organophosphates, to which azamethiphos belongs (WHO JMPR guidance). Such reactivation could result in underestimation of the actual inhibition of acetylcholinesterase in the brain. However, in a preliminary study (NOTOX project 487981) stability of acetylcholinesterase inhibition by azamethiphos was demonstrated for up to 3 hours (study report not available to RAC).

In a combined oral (gavage) 90 day / neurotoxicity study (OECD TG 408/424) in rats (Confidential, 2009e, CAR 3.6.1), no clinical signs related to acetylcholinesterase inhibition were reported, at doses up to 5 mg/kg bw/day (0, 0.05, 0.5, 5 mg/kg bw/day). No neurotoxic effects were reported, including no effects on motor-activity. A dose related increase in salivation shortly after treatment in most animals of the mid and high dose was not related to inhibition of acetylcholinesterase in plasma (CHEP) or in erythrocytes in individual animals. Statistically significant inhibition of > 20 % of CHER was mainly seen at the top dose in males and females. The effect was more pronounced at 8 weeks (males: -60 %, females: -50 %) than at 13 weeks (males: 25 %, females: 28 %). No effect on CHEBR was seen, except for low dose males, which was not considered treatment related.

Three males in this study died (1 each in control, low and mid dose), but as the observation did not follow a dose-response relationship, it was not considered treatment related.

The tested doses were relatively low, considering that almost no treatment related clinical signs and no mortality were reported, and that a dose up to 50 mg/kg bw/day was well tolerated for an exposure period of 28 days. The top dose of 5 mg/kg bw/day in this study is below the guidance value for STOT RE 1.

In an oral (gavage) combined chronic and carcinogenicity study (OECD TG 453) in rats (Confidential, 2011a, CAR 3.7.1), doses of 0, 0.05, 0.5 and 5 mg/kg bw/day were applied. CHER was reduced at all doses throughout the study. At the low and mid doses the inhibition remained below the level of 20 %, but reached statistical significance occasionally. At the top dose all values clearly exceeded the level of 20 % and the effect was statistically significant. Blood samples were analysed after 8, 13, 26 and 52 weeks and at the end of the study. The inhibition was between 31 % and 48 % in males and females, and remained more or less constant over time (no increase in severity with exposure duration).

As in the 90 days study, no treatment related mortality, clinical signs or effects in the functional observation test were reported, indicating that the selected doses in this study might have been too low.

In an oral (gavage) 90 day study (OECD TG 409) in dogs (Confidential, 2011c, CAR 3.6.1) the following doses were tested: 0, 0.2, 2 and 20 mg/kg bw/day. Clinical signs typical for cholinergic effects were reported in all dose groups and a dose related increase in incidence and type of effects was noted (see table above). Clinical signs were recorded from 1-2 hours after dosing up until day 13, subsequently salivation was recorded immediately after dosing. Statistically significant inhibition of CHER > 20 % was seen at most time points in mid dose males and reached up to 43 %. At the top dose, statistically significant inhibition of CHER was

seen in males and females reaching up to 87 %. No increase over time (samples were taken after 6 weeks and at the end of the study, pre- and post-treatment) was observed and the values ranged between 74 % and 87 % with no clear difference between pre- and post-treatment samples or between males and females.

In addition to the above described studies, RAC also considers further four dietary carcinogenicity studies, two in the rat and two in the mouse, to contain relevant information for the decision on classification as STOT RE. The results are summarised in the table below and are extracted from the CLH report (carcinogenicity section) and the CAR (3.7.1). No further details of these studies other than those presented in the table were available to RAC.

Table: *Dietary carcinogenicity studies – non-neoplastic repeated dose effects.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Dose levels**	**Observations and remarks** **(effects of major toxicological significance)**	**Dose relevant for classification**
Confidential, 1989a **CAR 3.7.1** Oral (dietary) Rat CD(SD)BR Carcinogenicity: 50/sex/group Chronic: 20/sex/group Additional animals were sacrificed at 52 weeks (10/sex/group) and week 56 after 4 weeks on control diet (10/sex at 0 and 1500 ppm) = recovery group. **OECD TG 453*** GLP compliant Reliability:1 Blood samples were collected at 13, 26, 52, 78 and 103 weeks. Brain samples were collected at 12 and 24 months.	Azamethiphos purity 94.2 % Dose: 0, 20, 200, 1 500 ppm Equivalent to 0, 0.8, 8.2, 62 mg/kg bw/day males and 0, 1.1, 11, 89 mg/kg bw/day females	**1 500 ppm (62/89 mg/kg bw/d males/females**) ↓ body weight (20 % males; 26 % females) and week 114 (16 % males) and 104 (37 % females). Significantly ↓ body weight gain (35 % males; 45 % females) at week 104. 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 females) and gastritis (7/90 females); Significantly ↓ serum potassium at 3 and 6 months (11 % and 15 % respectively); not seen at 18 months (females only) Significantly ↓ CHER[#]: 6 months: 35 % (males), 43 % (females) 12 months: 61 % (males), 58 % (females) 18 months: 33 % (males), 35 % (females, not statistically different) CHEBR[$ §]: 12 months ↓ 10 % (males), ↓ 19.4 % (females) 24 months ↓ 19 % (males), ↓ 15.6 % (females) **200 ppm (8.2/11 mg/kg bw/d males/females)** Significantly ↑ hydrometra (15/90 females); Significantly ↓ serum potassium at 3 and 6 months (5.4 % and 10 % respectively); not seen at 18 months (females only); Significantly ↓ CHE[#]: 6 months: 24 % (males), 26 % (females)	STOT RE 1: 12 months: 2.4 mg/kg bw/day 24 months: 1.2 mg/kg bw/day STOT RE 2: 12 months: 24 mg/kg bw/day 24 months: 12 mg/kg bw/day

		12 months: 40 % (males), 43 % females) not seen at 18 months (females only). CHEBR[$ §]: 12 months ↑ 10 % (males), ↓ 7 % (females) 24 months ↓ 5.2 % (males), no effect (females) 20 ppm (0.8/1.1 mg/kg bw/d males/females) Significantly ↓ serum potassium at 3 and 6 months (8 % and 13 % respectively); not seen at 18 months (females only). Significantly ↓ CHER[#] at 3 and 6 months (8 % and 13 % respectively), in females only; not seen at 18 months CHEBR[$ §]: 12 months: ↑ 10 % (males), ↓ 15 % (females) 24 months: ↓ 5.2 % (males), ↑ 6 % (females) Recovery group: CHER[#] and CHEBR[$] similar to control. CHEP – still decreased in males but not in females.	
Confidential, 1982a Oral (dietary) 2 year carcinogenicity study Rat CD(SD)BR Males/females 60/sex/dose (carcinogenicity group) **Guideline not stated** **Pre-GLP** Reliability: 2 No information on whether CHEBR[$] was measured.	Azamethiphos purity: 95.6 % Dose: 0, 15, 60, 327 ppm Approximately 0, 0.8, 3, 16 mg/kg bw/day	There were no significant increases in mortality in any dose group. **327 ppm (equivalent to 16 mg/kg bw/day)** 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) ↓ CHER[#] (> 20 %) **60 ppm (equivalent to 3 mg/kg bw/d)** ↓ CHER[#] (> 20 %) **15 ppm (equivalent to 0.8 mg/kg bw/d)** ↑ mammary cyst (not significant 16/60) ↓ CHER[#] (> 20 %)	STOT RE 1: 24 months: 1.2 mg/kg bw/day STOT RE 2: 24 months: 12 mg/kg bw/day
Confidential, 1989b **CAR 3.7.1** Oral (dietary) Mouse CD-1 51/sex/group 24 month carcinogenicity	Azamethiphos purity 94.2 % Dose: 0, 50, 500, 1 500, 4 000 ppm Equivalent to 0, 6.2, 60.2, 183.4, 491.4 mg/kg bw/day	**4 000 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: 11/51 vs 16/51 in control males, 18/51 vs 22/51 in control females Statistically significant findings included: ↓ body weight (20 % in males; 15 % in	STOT RE 1: 12 months: 2.4 mg/kg bw/day 24 months: 1.2 mg/kg bw/day

study **OECD TG 451** GLP compliant Reliability: 1 No measurement of acetylcholinesterase was performed in this study.	males and 0, 7.7, 76.2, 219.7, 582.9 mg/kg bw/day females	females); ↓ RBC count (23 % males, 17 % females); haemoglobin (27 % males and 25 % females); and %PCV (28 % males and 24 % females); ↑ polychromasia (20/51 vs 0/51 in the control males and 21/51 vs 2/51 in control females); ↑ in relative liver and kidney weight in females (32 % and 29 % respectively); ↑ liver haematopoiesis (18/51 vs 1/51 in control males and 12/51 vs 4/51 control females); ↑ Hepatocyte eosinophilia (9/51 and 13/51 males and females and liver centrilobular atrophy 7/51 and 5/51 males and females); neither finding seen in controls. ↑ Spleen haematopoiesis in both sexes (31/51 vs 18/51 in control males and 34/51 vs 20/51 in control females); ↑ thymus involution in males (9/51 not seen in controls) and pancreatic oedema in females (9/51 vs 1/51 in controls); ↑ stomach erosion/ulcer (30/51 and 31/51 in males/females); ↑ small intestine chronic erosion/ulcer (26/51 and 17/51 in males/females) ↑ hyperplastic/avillous mucosa in the small intestine (38/51 males and 41/51 females); **1 500 ppm (183.4 mg/kg bw/d males and 219.7 mg/kg bw/d females)** Significant ↑ small intestine hyperplastic/avillous mucosa (34/51 males and 36/51 females) **500 ppm (60.2 mg/kg bw/d males and 76.2 mg/kg bw/d females)** Significant ↑ small intestine hyperplastic/avillous mucosa (9/51 males and 25/51 females); **50 ppm (6.2 mg/kg bw/d males and 7.7 mg/kg bw/d females)** No treatment-related effects	STOT RE 2: <u>12 months:</u> 24 mg/kg bw/day <u>24 months:</u> 12 mg/kg bw/day
Confidential, 1982b Oral (dietary) Lifetime carcinogenicity study Mouse CD-1 (ICR) BR Males/females 60/sex/dose	Azamethiphos Purity not specified Dose: 0, 11, 97, 396 ppm Approximately 0, 2, 14, 57 mg/kg bw/day	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 ($p < 0.05$).	STOT RE 1: <u>24 months:</u> 1.2 mg/kg bw/day STOT RE 2: <u>24 months:</u> 12 mg/kg bw/day

(carcinogenicity group) **Non-guideline** **Pre-GLP** Reliability: 2 No information on whether acetylcholinesterase was measured.			

[#] CHER: acetylcholinesterase activity in erythrocytes
[$] CHEBR: acetylcholinesterase activity in brain
* Study was by mistake titled OECD TG 409.
[§] No information on whether this effect was statistically significant.

The studies summarised in the table further support the inhibiting effect of azamethiphos on the acetylcholinesterase in the rat, with statistically significant reductions in activity > 20 %. Again the effect did not deteriorate with chronic treatment. Clinical signs were not reported for these studies. In contrast, no effect was reported in the two mouse studies, however, at least for the first mouse study (Confidential, 1989b, CAR 3.7.1), it is stated that acetylcholinesterase was not measured. In the mouse, effects on the kidneys, liver and the blood system were observed, however, at doses exceeding the guidance values for classification as STOT RE. In a second study in the mouse, which tested lower doses than the first mouse study, no effects were reported.

It should be noted that the carcinogenicity studies summarised in the table above are all dietary studies. In the EMA assessment of azamethiphos (EMA, 1999), it was stated that azametiphos is prone to degradation in animal feed and that in earlier studies, achieved test substance intakes were on the low side until allowances were made for this instability. As no information on stability of the test material in the diet was available to RAC, it could not be judged whether the dosing was adequate. It is, however, noted that considerable effects were seen in the rat and mouse carcinogenicity study from 1989 (Confidential, 1989a,b), although the doses were clearly higher than those used in the gavage studies. While some effects were seen in the rat carcinogenicity study from 1982 (Confidential, 1982a), no effects were seen in the mouse study from 1982 (Confidential, 1982b), leaving doubts on whether the dosing was adequate.

In the 2-generation and the pre-natal development rat studies, similar effects on acetylcholinesterase activity were noted as in the remaining repeated dose toxicity studies. Also, in the rabbit pre-natal development study statistically significant inhibition > 20 % was seen at the top dose (5 mg/kg bw/day: 69 %). In this study also CHEBR was statistically significantly inhibited at the mid and top doses, though it did not reach 20 % (12 % and 11 % at mid and top dose, respectively).

Overall, RAC concludes that clear adverse effects were induced by azamethiphos treatment in rats and dogs. Though not always following a dose response relationship and not always showing consistency between biochemical changes (acetylcholinesterase) and clinical neurotoxic effects, it was clearly demonstrated that adverse effects related to acetylcholinesterase inhibition were induced by this substance, and that the effects were not only seen at doses below the guidance value for STOT RE 2, but also below the value relevant for category 1. RAC notes, however, that no increase in severity was reported with exposure duration, and in fact, in one study (90 day rat study) even a decrease in severity of the effects

was noted over time. It is also noted that several of the effects appeared directly after treatment and were therefore considered acute effects. Although acetylcholinesterase inhibition was similar prior or post treatment in the 90 day dog study, this did not demonstrate that repeated exposure is needed to induce the effect. Similar effects were also seen after acute toxicity (see section on acute toxicity and STOT SE).

Effects not related to inhibition of acetylcholinesterase were also seen in some of the studies. The most relevant findings are summarised in the table below.

Table: *In the dietary* ***rat*** *study (Confidential, 1989a, CAR 3.7.1) adverse effects on the uterus were reported (see also table above). For a better overview the incidences of these effects from all dose groups are summarised in the table below.*

mg/kg bw/day:	**0**	**1.1**	**11**	**89**
Pyometra	0/10	2/10	1/10	6/9 *
Hydrometra	6/90	9/90	15/90 *	23/90 *

Table: *In the dietary* ***mouse*** *study (Confidential, 1989b, CAR 3.7.1) adverse effects on the stomach and small intestine were reported.*

ppm	**0**	**50**	**500**	**1 500**	**4 000**
Males, mg/kg bw/day	**0**	**6.2**	**60**	**183**	**491**
Stomach erosion/ ulcer	0	0	0	2	30 *
Small intestine chronic erosion/ ulcer	0	0	0	0	26 *
Small intestine hyperplastic/ avillous mucosa	2	4	9 *	34 *	38 *
Females, mg/kg bw/day	**0**	**7.7**	**76**	**220**	**583**
Stomach erosion/ ulcer	0	0	0	1	31 *
Small intestine chronic erosion/ ulcer	0	0	0	0	17
Small intestine hyperplastic/ avillous mucosa	3	6	25 *	36 *	41 *

* Statistically significant increase

Some of these effects are clearly considered adverse, however, as they occurred above the relevant guidance values for STOT RE classification, they do not support classification. They might, however, be relevant for the interpretation of tumour data (see section on carcinogenicity).

RAC concludes that **no classification as STOT RE is justified** on the basis of the available data.

10.13 Aspiration hazard

Not relevant, the substance is a solid.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

Liquid substances and mixtures which contain hydrocarbons ≥ 10 % and which show kinematic viscosity < 20.5 mm^2/s should be classified. Azamethiphos is a solid, therefore the classification criteria are not met.

Comments received during public consultation

No comments were received

Assessment and comparison with the classification criteria

RAC agrees with the DS that azamethiphos **does not require classification for aspiration toxicity**.

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	0.2	1.19 x 10^{-1}	0.24 d	0.994	
	9	25		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^{o}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	103	5.71 h (at 22 °C) 7040 d when NER treated as parent	Meinerling (2017) CAR 4.1.1.3.3

		manure)			
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 pig manure (Additional experiments carried out at 3 and 30 mg kg-1 fresh manure)	103	5.98 h (at 22 °C) 522 d when NER treated as parent	Meinerling (2017) CAR 4.1.1.3.3

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

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON AZAMETHIPHOS (ISO); *S*-[(6-CHLORO-2-OXOOXAZOLO[4,5-*B*]PYRIDIN-3(2*H*)-YL)METHYL]*O*,*O*-DIMETHYL THIOPHOSPHATE

Method, Guideline, GLP status, Reliability	**Initial molar TS concentra-tion**	**Total recovery of test substance [% of appl. AS]**	**Photolysis rate constant (k^c_p)**	**Direct photolysis sunlight rate constant (k_{pE})**	**Reaction quantum yield ($\varphi^c E$)**	**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). SETAC - (Section 10-1995).	$3.33*10^{-6}$ M (1.08 mg l^{-1}) Purity 98.8%	Dark controls: 94.7-103.8%. Test solutions: 71.8 – 98.4% of applied radioactivity.	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 not identified.	Brands C., 2009 CAR 4.1.1.1.2
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 ≤ 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

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed an environmental hazard classification as Aquatic Acute 1; H400 with an M-factor of 1 000, based on acute aquatic toxicity to the crustacea *Daphnia magna* (static 48 h EC_{50} = 0.33 µg/L), and Aquatic Chronic 1; H410 with an M-factor of 1 000, based on the use of the surrogate approach, acute aquatic toxicity to the crustacea Daphnia magna (static 48 h EC_{50} = 0.33 µg/L) and a lack of rapid degradation.

Degradation

The DS concluded that azamethiphos is not rapidly degradable based on several screening tests:

Ready biodegradation of azamethiphos was studied by monitoring of CO_2 evolution (modified Sturm Test) in a test conducted in accordance with OECD TG 301B and in conformity with GLP (Desmares-Koopman, 2008). The azamethiphos purity was 96.2 %. Activated sludge freshly obtained from a municipal sewage treatment plant was used, under appropriate test conditions including adequate control response. 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.

The *aerobic* biodegradation of azamethiphos was followed in activated sludge. The test was performed in accordance with OECD TG 314B and GLP procedure (Schaefer and Carpente, 2014a). ^{14}C-radiolabelled azamethiphos (purity 99.4 %) at a concentration of 25 µg/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 %, while only 18 % and 4 % of the parent compound remained after 7 and 28 days, respectively.

The *anaerobic* biodegradation of azamethiphos in activated sludge was studied. The test was performed in accordance with OECD TG 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. As in the aerobic system, 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 and methane was only 8 %.

The DS concluded that the outcome of both tests confirmed that azamethiphos is not rapidly degradable.

An inherent biodegradation study (OECD TG 302B, GLP, 99.03 % purity, at concentration 150 mg/L, Hammesfahr, 2016) showed 37.7 % degradation in the presence of activated sludge from a domestic wastewater treatment plant, used as an inoculum, and aeration of 1 mg/L of dissolved oxygen. Three controls were included, an untreated control, the procedural control (where diethylene glycol reached 103.5 % degradation by day 28), and the toxicity control (where the mixture of azamethiphos and diethylene glycol reached 69.76 % degradation). The DS concluded that at test pH of 7.2-7.7 azamethiphos rapidly hydrolyses and the distinction between biodegradation and abiotic degradation is not possible. The study does not support the categorisation of azamethiphos as being inherently biodegradable, in terms of ultimate biodegradation.

Hydrolysis

Hydrolysis rates and half-lives of azamethiphos (purity 99.4 %) at three environmentally relevant pH values were determined (OECD TG 111, GLP compliant, Reifer, 2015). In the preliminary test, the samples were incubated at 50 ± 5 °C in the dark. In the main test, 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 reached or the test had run for a maximum of 30 days; whichever came first. The hydrolysis process (followed by measurements of applied radioactivity) and transformation products were identified by NMR and LC-MS/MS. The hydrolysis half-life was 14 days at pH 7 and 20 °C or 26.6 days when converted to the average EU outdoor temperature (12 °C).

Photochemical degradation

Phototransformation of azamethiphos in water: azamethiphos degradation half-time (DT_{50}) under irradiated conditions, adjusted for 40 °N sunlight was 0.1 days, compared to 49 days in darkness (purity 98.8 %, OECD TG 316, GLP, Brands, 2009). DS concluded that azamethiphos is subject to rapid photolysis in aqueous conditions.

In a second study (OECD TG 316, Riefer, 2017), azamethiphos photodegraded to several highly degraded transformation products, not identified. Two photolytic constants (k_{irr}, k_{dark}) were calculated.

Phototransformation of azamethiphos in air: The photo-degradation half-time (DT_{50}) in air was predicted to be 1.3 hours (Willems, 2009) as a result of reactivity with hydroxyl radicals,

using computer programme AOPWIN. The DS concluded that azamethiphos is also susceptible to photolytic degradation in air.

DS' conclusion on degradation

The DS concluded that azamethiphos is not rapidly degradable because:

- *"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 characterisation of azamethiphos as being inherently biodegradable, and the two degradation studies in manure do not yield reliable degradation rates."*

- Aquatic photolysis half-life did not to meet the criteria for rapid degradation. 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.

- Aquatic hydrolysis was more moderate at environmentally relevant pH, with a half-life of 26.6 days at pH 7 and 12 °C.

Adsorption/Desorption

The adsorption coefficient (K_{OC}) on soil and sewage sludge was determined using HPLC (OECD TG 121, Oudhoff, 2008). The calculated K_{OC} value at neutral pH was 99 L/kg.

Bioaccumulation

Measured partition coefficient and bioaccumulation test data

The experimentally determined octanol: water partition coefficient, logK_{OW}, is 1.0 at 20 °C.

The DS concluded that logK_{OW} is below the trigger value (4), which indicates a low potential for bioaccumulation of azamethiphos. Additionally, the MSCA evaluating the active substance, calculated a BCF_{fish} of 1.16 based on the logK_{OW} using equation 74 in the Technical Guidance Document (TGD, 2003).

Acute aquatic hazard

The acute toxicity of azamethiphos was studied for fish, invertebrates and algae and all three studies are of reliable quality.

***Table**: Summary of the toxicity studies on fish, aquatic invertebrates and algae.*

Species	***Guideline/ GLP status***	***Endpoint***	***Exposure/ duration***	***Results***	***Reference***
Fish *Oncorhynchus mykiss*	OECD TG 203, GLP compliant, purity 96.2 %	LC_{50} (mortality)	Static 96 hours	LC_{50} = 0.19 mg/L	Confidential, 2008j CAR 4.2.3
Invertebrate; *Daphnia magna*	OECD TG 202, GLP compliant, purity 96.2 %	EC_{50} (Immobilisation)	Static 48 hours	EC_{50} = 0.00033 mg/L	Migchielsen, 2008 CAR 4.2.3

Algae *Pseudokirchneriella subcapitata*	OECD TG 201, GLP compliant, purity 96.2 %	E_rC_{50} (growth rate inhibition) E_yC_{50} (reduction in yield)	Static 72 hours	E_rC_{50}[1] = 74 mg/L E_yC_{50}[2] = 18 mg/L NOE_rC could not be determined.	Migchielsen, 2008 CAR 4.2.3

[1] calculated from growth rate
[2] calculated from the recorded cell density

Acute (short-term) aquatic toxicity

The DS included in the CLH report one acute toxicity good quality study, performed according to OECD TG 203 and GLP compliant. Azamethiphos (96.2 % purity) toxicity toward rainbow trout *(Oncorhynchus mykiss)* resulted in an LC_{50} value of 0.19 mg/L based on mean measured concentrations.

Only one study of acceptable quality, performed according to OECD TG 202, in compliance with GLP was included in the CLH report. In the study, the acute toxicity of azamethiphos (96.2 % purity) to crustacea (*Daphnia magna*) was investigated in concentration range 0.05-1.1 µg/L for 48 h. The results were provided as nominal concentrations, however, the plant protection evaluator MSCA reported that, at the lowest concentration, the measured concentration was not within the ±20 % nominal concentration as per OECD TG. However, the DS considered that, as this deviation from the guideline regarded one concentration only, it was insignificant and accepted the calculated EC_{50} value of 0.33 µg/L based on measured concentrations as valid and as a base for final classification.

One study of acceptable quality, performed according to OECD TG 201, and GLP compliant is available to evaluate the acute toxicity of azamethiphos (96.2 % purity) to algae (*Pseudokirchneriella subcapitata,* standard 72 h, growth rate test, static system, concentrations between 4 and 87 mg/L). The growth rate reduction E_rC_{50} value of 74 mg/L was calculated based on mean measured concentrations. The study authors noted that a NOE_rC for growth rate reduction and yield inhibition could not be determined.

In the CLH no data for acute toxicity toward any other species were presented.

In the CLH no data for chronic toxicity toward any species were presented.

Comments received during public consultation

One MSCA supported classification Aquatic Acute 1, M-factor 1 000, and Aquatic Chronic 1, M-factor 1 000.

Assessment and comparison with the classification criteria

Degradation

The results obtained from several biotic degradation test performed showed that azamethiphos is not rapidly degradable and RAC supported the conclusions of the DS.

A ready biodegradations test, achieved only 17 % mineralization in 28d, while an aerobic biotic sludge study (open and closed) as well as an anaerobic digester sludge study yielded 44 % and 8 % mineralization in 28d under inherent conditions.

In two aqueous photolysis studies (OECD TG 316), azamethiphos underwent rapid photolysis under irradiated conditions and alkaline conditions (DT_{50} ~0.1 day in both two studies).

Azamethiphos was predicted to undergo photolytic degradation in air (AOPWIN). However, the extent of full mineralisation do not meet the criteria for rapid degradation.

Bioaccumulation and bioconcentration (BCF)

RAC agrees with DS about the expected low bioaccumulation potential for azamethiphos, based on a calculated BCF_{fish} of 1.41 L/kg and an estimated $logK_{OW}$ value of 1.0.

Acute aquatic hazard

RAC agrees with the DS that the results for acute toxicity toward crustacea *Daphnia magna* (EC_{50} = 0.00033 mg/L) lead to classification as Aquatic Acute 1, with an M-factor of 1 000.

Chronic aquatic hazard

Data from chronic toxicity studies are not presented in CLH. This being the case, RAC agrees with DS' classification proposal for chronic aquatic toxicity i.e., Aquatic Chronic 1, M = 1 000, based on the surrogate approach taking into account that azamethiphos is not rapidly degradable.

Overall, RAC considers that azamethiphos should be classified as **Aquatic Acute 1; H400 with an M-factor of 1 000 and Aquatic Chronic 1; H410 with an M-factor of 1 000**.

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).

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annexes

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

Annex II: Full reference list (CONFIDENTIAL)