Committee for Risk Assessment

RAC

Annex 1

Background document

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

pirimiphos-methyl (ISO); *O*-[2-(diethylamino)-6-methylpyrimidin-4-yl] *O*,*O*-dimethyl phosphorothioate

EC Number: 249-528-5
CAS Number: 29232-93-7

CLH-O-0000001412-86-247/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

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: Pirimiphos-methyl (ISO); O-[2-(diethylamino)-6-methylpyrimidin-4-yl] O,O-dimethyl phosphorothioate

EC Number: **249-528-5**

CAS Number: **29232-93-7**

Index Number: **015-134-00-5**

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Version number: 2 **Date: December 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PIRIMIPHOS-METHYL (ISO); O-[2-(DIETHYLAMINO)-6-METHYLPYRIMIDIN-4-YL] O,O-DIMETHYL PHOSPHOROTHIOATE

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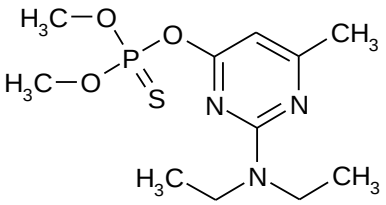
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*Phosphorothioic acid, O-[2-(diethylamino)-6-methyl-4-pyrimidinyl] O,O-dimethyl ester, O-[2-(Diethylamino)-6-methylpyrimidin-4-yl] O,O-dimethyl phosphorothioate (IUPAC)*
Other names (usual name, trade name, abbreviation)	*0-[2-(diethylamino)-6-methyl-4-pyrimidin] 0,0-dimethylphosphorothioate (CA)*
ISO common name (if available and appropriate)	*Pirimiphos-methyl*
EC number (if available and appropriate)	249-528-5
EC name (if available and appropriate)	
CAS number (if available)	29232-93-7
Other identity code (if available)	*CIPAC number: 239a*
Molecular formula	$C_{11}H_{20}N_3O_3PS$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	305.4
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)	*≥ 880 g/kg*

1.2 Composition of the substance

Pirimiphos-methyl does not contain any other constituents, isomers or additives.

There are a number of confidential impurities listed for pirimiphos-methyl, none of which are relevant to the classification of the substance.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2:

	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	015-134-00-5	Pirimiphos-methyl (ISO); O-[2-(diethylamino)-6-methylpyrimidin-4-yl] O,O-dimethyl phosphorothioate	249-528-5	29232-93-7	Acute Tox. 4* Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410			
Dossier submitters proposal	015-134-00-5	Pirimiphos-methyl (ISO); O-[2-(diethylamino)-6-methylpyrimidin-4-yl] O,O-dimethyl phosphorothioate	249-528-5	29232-93-7	**Amend**: Acute Tox. 4 **Add:** STOT-RE 1 **Retain:** Aquatic Acute 1 Aquatic Chronic 1	H302 H372 H400 H410	GHS07 GHS08 Danger	H302 H372 H410		ATE = 1414 mg/kg bw Acute M factor = 1000 Chronic M factor = 1000	
Resulting Annex VI entry if agreed by RAC and COM	015-134-00-5	Pirimiphos-methyl (ISO); O-[2-(diethylamino)-6-methylpyrimidin-4-yl] O,O-dimethyl phosphorothioate	249-528-5	29232-93-7	Acute Tox. 4 STOT-RE 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H372 H400 H410	GHS07 GHS08 GHS09 Danger	H302 H372 H410		ATE = 1414 mg/kg bw Acute M factor 1000 Chronic M factor 1000	

Table 3: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	***harmonised classification proposed***	**Yes**
Acute toxicity via dermal route	*hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/irritation	*hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*hazard class not assessed in this dossier*	No
Respiratory sensitisation	*hazard class not assessed in this dossier*	No
Skin sensitisation	*hazard class not assessed in this dossier*	No
Germ cell mutagenicity	***data conclusive but not sufficient for classification***	**Yes**
Carcinogenicity	***data conclusive but not sufficient for classification***	**Yes**
Reproductive toxicity	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	***harmonised classification proposed***	**Yes**
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	***harmonised classification proposed***	**Yes**
Hazardous to the ozone layer	*hazard class not assessed in this dossier* Not applicable as pirimiphos-methyl is not listed in Annex I to Regulation (EC) No. 1005/2009 (recognising the Montréal Protocol)	No

Hazard class	Reason for no classification	Within the scope of public consultation
	and no Ozone Depleting Potential (ODP) is reported.	

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Pirimiphos-methyl is an existing (2nd stage) pesticide active substance approved in accordance with Directive 91/414/EEC. There is an existing entry on Annex VI of CLP (translated from Annex I of Dir 67/548/EEC) which includes classification as Acute Toxicity Category 4*; H302, Aquatic Acute Category 1; H400 and Aquatic Chronic Category 1; H410.

In the original EFSA conclusion (EFSA Scientific Report, 2005) concerns were raised due to the finding of some rare pancreatic and brain tumours in an old and poorly reported study in rats. These tumours were found to be within the historical control data (HCD) but taking into account the uncertain nature of the tumour findings, it was concluded by EFSA that a carcinogenic effect could not be dismissed at the time. In order to address the uncertainties, EFSA requested further HCD. In lieu of a further assessment taking into account these data, the original EFSA conclusion included the classification carcinogenicity, category 3; R40 (equivalent to carcinogenicity, category 2 under CLP) for pirimiphos-methyl. The concern was not shared by classification and labelling experts as pirimiphos-methyl was not classified for carcinogenicity in the original submission.

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

RAC general comment
Pirimiphos-methyl is an active substance in the meaning of Regulation (EU) No 1107/2009 and is used as a broad-spectrum insecticide for use in grain stores and related industrial outlets. This substance has an existing entry in Annex VI of the CLP regulation. This CLH proposal aims at modifying the existing classification based on data submitted as part of the pesticide renewal process (partly old, partly new data when compared to the original application).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

Pirimiphos-methyl is an active substance in the meaning of Regulation (EU) No 1107/2009 and therefore, according to Article 36(2) of the CLP further justification that action is required at a Community level is not required.

In accordance with the alignment process with the renewal of the active substance under Regulation (EU) No. 1107/2009, it is necessary to prepare a targeted CLH report taking into account new data, including new historical control data pertaining to the carcinogenicity study in rats, and also to address the minimum classification indicated for Acute Toxicity Category 4*; H302. In addition to this, a change to the existing entry is proposed due to new interpretation of the existing data for specific target organ toxicity following

repeated dosing. Due to significant inhibition of brain and erythrocyte acetylcholinesterase at low doses in animal studies, classification with STOT-RE 1; H372 is also proposed. The environmental hazards and fate of pirimiphos-methyl have been considered in this proposal, taking into account studies that were previously evaluated and new studies available in the open literature. The resulting classification proposed remains the same as previously (Aquatic acute 1; H400 and Aquatic chronic 1; H410), however a new M factor of 1000 for both acute and chronic aquatic toxicity has been proposed.

5 IDENTIFIED USES

Pirimiphos-methyl is a broad-spectrum insecticide for use in grain stores and related industrial outlets.

6 DATA SOURCES

Pirimiphos-methyl draft RAR (UKCA 2016)

Pirimiphos-methyl DAR (UKCA October 2003)

EFSA Conclusion (EFSA Scientific Report, 2005)

7 PHYSICOCHEMICAL PROPERTIES

Table 4: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	White solid and clear liquid (99.6 % purity) Pale yellow liquid (93.5 % purity)	Husband R (1997) Husband R (1998)	EPA OPPTS 830.6302
Melting/freezing point	20.8 °C (freezing point)	Husband R (1997)	EEC Method A 99.6 % purity
Boiling point	Not applicable		
Relative density	1.17 g/cm^3 at 20 °C	Husband R (1997)	EEC Method A3 99.6 % purity
Vapour pressure	2.0 x 10^{-6} kPa at 20 °C	Husband R (1997)	EEC Method A4 100 % purity
Surface tension	62.9 mN/m at 20 °C	Husband R (1998)	EEC Method A5 93.5 % purity
Water solubility	10 mg/l in purified water 11 mg/l at pH5 10 mg/l at pH7 9.7 mg/l at pH9	Husband R (1997)	CIPAC MT 157.1 Purity 99.6 %

Property	Value	Reference	Comment (e.g. measured or estimated)
Partition coefficient n-octanol/water	Log P_{ow} = 3.9 at 20 °C in water buffered at pH4 Log P_{ow} = 4.2 at 20 °C in purified water and water buffered at pH5 and 7	Husband R (1997)	^{14}C pirimiphos methyl – radiochemical purity 99.5 %
Flash point	92 ± 2 °C	Husband R (1998)	EEC Method A9 (closed cup only) Purity 93.5 %
Flammability	Not applicable as the substance is a liquid at room temperature.		
Explosive properties	Pirimiphos methyl does not contain any bond groupings known to confer explosive properties. The exothermic heat of decomposition was measured by DSC at 200 J/g.	Husband R (1998)	
Self-ignition temperature	330 ± 5 °C	Husband R (1998)	EEC Method A15 Purity 93.5 %
Oxidising properties	Not classified as an oxidising substance	Husband R (1998)	UN 0.2 Purity 93.5 %
Stability in organic solvents and identity of relevant degradation products	Readily soluble in all solvents. Solubility > 250 g/kg for xylene, 1,2 dichloroethane, methanol, acetone and ethyl acetate at 20 °C Solubility is 249 g/kg in n-heptane at 20 °C	Husband R (1998)	EEC Method A6 Purity 93.5 %
Dissociation constant	4.3 at 20 °C	Husband R (1997)	OECD 112 Purity 99.6 %

8 EVALUATION OF PHYSICAL HAZARDS

Physical hazards are not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

A series of studies is available, investigating the *in vivo* toxicokinetics of pirimiphos-methyl in rats and its *in vitro* metabolism in rat and human liver microsomes (Anon, 1997a, 1997b, 1997c, 1997d, Anon, 1998, Anon, 2002, Anon, 2003).

The results of these studies indicate that pirimiphos-methyl is rapidly and extensively absorbed and excreted in Wistar-derived rats following oral administration at 1 or 250 mg/kg bw/d.

Over 70 % of the administered dose was excreted in the urine, with 50 % of the administered dose present in the 0-12 hour urine sample. Data from bile-duct cannulated animals showed that entero-hepatic circulation is important in the toxicokinetics of pirimiphos-methyl, with a significant proportion of the faecal residue due to biliary excretion. Entero-hepatic circulation was more evident in females and in animals receiving repeated doses. Metabolism in rats is extensive, with a number of metabolites formed retaining functional groups consistent with cholinesterase inhibition. No parent compound was detected in bile or urine. At 1 mg/kg bw/d excretion and metabolism was essentially unaltered with the sex of the animals or repeat administration. Administration of 50 or 250 mg/kg bw produced evidence of saturation in phosphorothioate - pyrimidinyl esterase and *N*-dethylation in females.

Four days after dosing, less than 2 % of the administered dose remained in the carcass and tissues, though abdominal fat had particularly high concentrations in females dosed with 250 mg/kg bw (6200 times the levels in females dosed at 1 mg/kg bw).

It was shown that *in vitro* preparations of liver microsomes from rats and humans formed similar metabolites, and in the case of rats formed broadly similar metabolites to those produced *in vivo*. The route of administration (oral versus dermal) did not affect the metabolic pathway in rats.

9.2 EVALUATION OF HEALTH HAZARDS

9.3 Acute toxicity

This harmonised classification and labelling report comprises a targeted assessment of pirimiphos-methyl and as such, only acute *oral* toxicity is considered.

9.4 Acute toxicity - oral route

The acute oral toxicity of pirimiphos-methyl has been investigated in one study in rats (summarised in Table 5 below).

Table 5: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**
Oral (gavage) OECD 401 GLP (Anon. 1999)	Rat, Alpk: AP_fSD 5/sex	Pirimiphos-methyl (91.7% pure) in corn oil	500, 1000 or 2000 mg/kg bw	1414 mg/kg bw

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

The acute oral toxicity of pirimiphos-methyl was investigated in rats. Groups of Alpk:AP$_f$SD rats (5/sex) received pirimiphos-methyl in corn oil, by gavage at 500, 1000 or 2000 mg/kg bw. Clinical signs of neurotoxicity were considered to be treatment-related (piloerection, urine staining, and neurological signs such as tip toe gait, salivation and upward curvature of the spine) and were seen in all treatment groups with a dose-related increase in severity. There were no deaths at 500 or 1000 mg/kg bw. All animals receiving 2000 mg/kg bw were killed *in extremis* on days 2 - 3. The acute oral median LD_{50} of pirimiphos-methyl was calculated to be 1414 mg/kg bw (95% limits 1000 - 2000 mg/kg) [Acute Toxicity Estimate (ATE) = 1414 mg/kg bw].

9.4.2 Comparison with the CLP criteria

The oral LD_{50} value of 1414 mg/kg bw in rats is within the range $300 < LD_{50} \leq 2000$ for classification as Acute Tox 4, H302

9.4.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox 4: H302 harmful if swallowed. Data conclusive and sufficient for classification (ATE = 1414 mg/kg bw)

9.5 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

9.6 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The scope for acute toxicity was limited to the oral route, to address the current minimum classification.

In a study according to OECD TG 401 and GLP (Anon. 1999), Alpk:AP$_f$SD rats (5/sex/dose) were administered 500, 1 000 or 2 000 mg/kg bw of pirimiphos-methyl (91.7 % pure) in corn oil via gavage. Clinical signs considered to be treatment-related (including piloerection, urine staining and neurological signs such as tip toe gait, salivation and upward curvature of the spine) were seen in all treatment groups with a dose-related increase in severity. No deaths were observed at 500 and 1 000 mg/kg bw, but at 2 000 mg/kg bw all rats were killed in extremis on day 2-3. The acute oral LD_{50} was determined at 1 414 mg/kg bw. Classification in category 4 was proposed by the DS as the LD_{50} is within the limits of 300–2 000 mg/kg bw for category 4, with an oral ATE of 1 414 mg/kg bw.

Comments received during public consultation

The proposed classification was supported by two MSCAs.

Assessment and comparison with the classification criteria

The oral LD_{50} of 1 414 mg/kg bw determined in rats lies within the numeric criteria for Acute Tox. 4 (300–2 000 mg/kg bw). Therefore, RAC supports the DS proposal for **Acute Tox. 4; H302**, with an ATE of 1 414 mg/kg bw.

9.7 Skin corrosion/irritation

Hazard class not assessed in this dossier.

9.8 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

9.9 Respiratory sensitisation

Hazard class not assessed in this dossier.

9.10 Skin sensitisation

Hazard class not assessed in this dossier.

9.11 Germ cell mutagenicity

The potential of pirimiphos-methyl to induce gene mutations in bacterial cells, gene mutation/clastogenicity in mammalian cells and clastogenicity/aneuploidy in mammalian cells has been investigated in a number of *in vitro* studies.

Table 6: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any Reference	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations
Bacterial reverse mutation assay OECD 471 GLP (Sokolowski, 2015)	Pirimiphos-methyl (83.4 % pure)	Experiment I: plate incorporation test Experiment II: pre-incubation test Concentrations: 3 – 5000 µg/plate Test strains: *S. typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA100; *E. coli* WP2 *uvrA* pKM101 and WP2 pKM101. Tested in the presence and absence of metabolic activation (liver S9 mix).	**Negative ± S9** Precipitation was observed at the top two doses tested (± S9) Cytotoxic effects observed: Experiment I - strain TA 1537 (-S9), strain TA 98 (± S9) and strain WP2 pKM101 (+S9).
Bacterial reverse	Pirimiphos-methyl	Concentration: 1.6 – 5000 µg/plate	**Negative ± S9**

<table>
<tr><th>Method, guideline, deviations if any Reference</th><th>Test substance,</th><th>Relevant information about the study including rationale for dose selection (as applicable)</th><th>Observations</th></tr>
<tr><td>mutation assay
OECD 471*
GLP

(Callendar, 1984)</td><td>(88.9 % pure)</td><td>Test strains:
S. typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA100
Tested in the presence and absence of metabolic activation (liver S9 mix).</td><td>Precipitation was seen at the top dose (-S9)
Cytotoxicity data were not presented.</td></tr>
<tr><td>Mammalian cell gene mutation assay
OECD 476*
GLP

(Cross, 1986)</td><td>Pirimiphos-methyl (90.7% purity)</td><td>Concentration: 0, 12.5, 25, 50 and 100 µg/ml in DMSO
Test system: mouse lymphoma cells L5178Y (TK ± locus).
Exposure time: 2 hours with a 72 hour expression period.
Tested in the presence and absence of metabolic activation (liver S9 mix).</td><td>Negative ± S9
There was a concentration-related decrease in cell survival, reaching 0 at 200 µg/ml (-S9).
Decreased cell survival was only seen at 200µg/ml (+S9).</td></tr>
<tr><td>Chromosome aberration *in vitro*
OECD 473
GLP

(Sokolowski, 2015a)</td><td>Pirimiphos-methyl (83.4% pure)</td><td>Test system: cultured human lymphocytes
Tested in the presence and absence of metabolic activation (liver S9 mix).
Without S9 mix
Experiment IA
Exposure: 4 h
Recovery: 18 h
Preparation: 22 h

Experiment IB and II
Concentrations:
Exposure: 22 h
Preparation: 22 h

Concentrations tested:
IA: 3.9 - 2000 µg/ml
Turbidity at ≥15.6 µg/ml (- S9) and at); phase separation at concentrations of ≥ 500 µg/ml

IB: 15.6 - 250 µg/ml
Turbidity & phase separation at concentrations of ≥ 62.5 µg/ml

II: 0.5 - 250 µg/ml
No turbidity noted.

With S9 mix
Experiment IA & II
Exposure: 4 h
Recovery: 18 h
Preparation: 22 h</td><td>Negative ± S9
No clastogenicity was observed at the concentrations evaluated either with or without S9 in experiments 1A and 1B.

Experiment II:
Small increases in chromosomal aberrations noted with no clear dose-response:
<table><tr><th>Concentration (µg/mL)</th><th>% Aberrant cells (excluding gaps)</th></tr><tr><td>0</td><td>2.6 %</td></tr><tr><td>31.3</td><td>3.5 %</td></tr><tr><td>125</td><td>2.7 %</td></tr><tr><td>250</td><td>3.8 %</td></tr></table>No evidence of an increase in polyploid metaphases

In the absence and presence of S9 mix, clear cytotoxicity was observed at the highest evaluated concentrations.</td></tr>
</table>

Method, guideline, deviations if any Reference	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**
		Concentrations tested: IA: 3.9 - 2000 µg/ml *Turbidity at concentrations of ≥ 62.5 µg/ml. Phase separation at concentrations of ≥ 1000 µg/ml* II: 7.8 - 1000 µg/ml *No turbidity noted. Phase separation at concentrations of >62.5 µg/ml* Positive controls: -S9: ethylmethane sulfonate (EMS) and +S9: cyclophosphamide (CPA)	
Chromosome aberration *in vitro* OECD 473* GLP (Wildgoose, 1986)	Pirimiphos-methyl (90.7 % pure)	Concentration: 0, 12, 29, 58 and 116 µg/ml in DMSO Test system: human lymphocytes from 2 donors (1 male/1female). Exposure time: 3 hours with a 24 hour growth period. 200 cells per donor/experiment scored Tested in the presence and absence of metabolic activation (liver S9 mix). Assays performed in duplicate and repeated. Positive controls: mitomycin C and cyclophosphamide	**Negative ± S9** Cytotoxicity was evident at ≥ 58 µg/ml, with a 60 % reduction in mitotic index at 116 µg/ml.
Sister chromatid exchange OECD 479* GLP (Howard, 1986)	Pirimiphos-methyl (90.7 % pure)	Concentration: 0, 0.14, 0.29, 1.4, 2.9, 14, 29, 145 and 289 µg/ml. Test system: Chinese hamster lung fibroblasts (Don cells). Tested in the presence and absence of metabolic activation (liver S9 mix). Concentrations tested: 0, 0.14, 0.29, 1.4, 2.9, 14, 29 and 145 µg/ml 50 cells per culture scored for SCEs; 2 cultures/conc^{n} (with the exception of the 145 µg/ml culture where 25 cells were scored). Positive controls: mitomycin C and cyclophosphamide	**Negative ± S9** Significant cytotoxicity observed at 145 µg/ml (± S9) – 80 % depression of mitotic index. Positive controls gave expected results. There was considerable variation observed in the SCE frequency between cultures. No biologically significant increase in SCEs was observed, gien the observed variability.

* Study was in compliance of the OECD test guideline at the time. Test guidelines have been updated since the study date.

Table 7: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations
Micronucleus Assay in mice Oral Guideline and GLP status unknown Limited reporting [Rajini et al., 1986 (published)]	Pirimiphos-methyl (90.5 % pure)	CFT Swiss mice (males, numbers unknown) Dose: 0, 100, 200 and 400 mg/kg bw for 2 days	**Negative** No changes to incidence of micronucleated PCEs and NCEs at any dose. A slight reduction in the PCE/NCE ratio in the top dose group.
In vivo UDS assay Oral (gavage) OECD 486 GLP (Anon., 1998a)	Pirimiphos-methyl (93.5 % pure)	Alpk:APfSD rats (males, 5/dose) Dose: 400 or 800 mg/kg bw in corn oil Sampling times: 2 or 16 hours Net nuclear grain counts (NNG) were determined for 60 cells/animal. Positive control: 1,2-dimethylhydrazine.	**Negative** Pirimiphos-methyl did not induce unscheduled DNA synthesis in this assay No indication of marked cytotoxicity or of an increase in net nuclear grain count. Signs of sedation and increased sensitivity to sound were seen prior to the 16 hour sampling time.
Dominant lethal study in mice Pre-dates OECD and GLP guidelines (Anon., 1975a)	Pirimiphos-methyl (purity not stated)	Charles River CD-1 mice (males, 15/dose; 30 controls) Dose: 0, 15, 80 or 150 mg/kg bw/d in corn oil for 5 days Positive controls: ethyl methanesulphonate and cyclophosphamide	**Negative** No evidence that pirimiphos-methyl caused dominant lethal mutations. Sporadic effects, but no consistent increases in the percentage of early deaths or number of early deaths per pregnancy. No evidence of increases in pre-implantation losses or late deaths.

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

9.11.1.1 *In vitro* genotoxicity

Pirimiphos-methyl was tested *in vitro* in two bacterial reverse mutation assays, a mammalian cell gene mutation assay, a clastogenicity study, two chromosomal aberration studies – all performed according to guidelines and GLP. An *in vitro* sister chromatid exchange study is also available.

Bacterial reverse mutation assays

In a recently performed study (Sokolowski, 2015), pirimiphos-methyl was tested in both a plate incorporation test (experiment I) and a pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and the *Escherichia coli* strains WP2 *uvrA* pKM101 and WP2 pKM101. The test material was assayed over a dose range of at 3 - 5000µg/plate both in the absence and presence of metabolic activation. No increase in revertant colony numbers of any of the six tester strains was observed following treatment with pirimiphos-methyl at any concentration level, either in the presence or absence of metabolic activation (S9 mix). There was no evidence of mutagenicity in this study.

In an older study (Callendar, 1984), pirimiphos-methyl was tested in a plate incorporation assay in the presence and absence of a liver S9 mix at concentrations of 1.6 – 5000 µg/plate using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA100. In the two separate experiments, pirimiphos-methyl did not induce any significant increase in the observed numbers of revertant colonies in any tester strain, either in the presence or absence of metabolic activation. Under the conditions of this study, pirimiphos-methyl was not mutagenic in this assay.

There are four bacterial reverse mutation assays available in the public literature and in a report used for the DAR for original approval (2003) (Seiller, 1972, Seiller, 1976, Shirasu, 1984 and Hanna & Dyer, 1975). All were conducted between the years 1972-1984 for non-regulatory purposes. Several positive results were obtained, however little weight is given to these on the basis that they were no conducted to current standards and the reporting available is somewhat limited.

Mammalian cell gene mutation assay

The mutagenic potential of pirimiphos-methyl was assessed in L5178Y mouse lymphoma cells at concentrations of 0, 12.5, 25, 50, 100 or 200 µg/ml (Cross, 1986). The compound was tested both in the absence and presence of metabolic activation. Exposure times were 2 hours, with a 72 hour expression period. There was a dose-related decrease in cell survival (reaching zero at 200 µg/ml) in the absence of S9 mix. In the presence of S9 mix, a decrease in cell survival was only seen at 200 µg/ml, reaching a minimum of 30 % survival. There were no significant increases in mutation frequency above the background frequency (~1 in 10^4). Appropriate results were obtained with the positive controls. Pirimiphos-methyl, in the presence and absence of metabolic activation, was not mutagenic at the $TK^{+/-}$ locus of L5178Y mouse lymphoma cells.

Chromosome aberration in vitro

A recent chromosomal aberration test was performed to assess the potential of pirimiphos-methyl to induce structural chromosomal aberrations in cultured human lymphocytes (Sokolowski, 2015a). This was carried out in both the presence and absence of liver S9 mix.

Three independent experiments were performed. In experiment 1A, the exposure period was 4 hours with and without S9 mix using concentrations of 3.9 – 2000 µg/ml. In experiment 1B, the exposure time was 4 hours, performed in the absence of S9 mix using concentrations of 15.6 – 250 µg/ml. Finally, in experiment II, the exposure period was 4 hours in the presence of S9 (concentrations: 7.8 – 1000 µg/ml) and 22 hours in the absence of S9 (concentrations: 0.5 – 250 µg/ml). In each treatment group, two parallel cultures were analysed and a minimum of 150 metaphases per culture were evaluated for structural chromosomal aberrations.

Reduced mitotic indices at the top concentrations evaluated indicated clear cytotoxicity (45 ± 5 % of control).

There was no evidence of structural chromosomal aberrations in experiment 1A or 1B. In experiment II, where cells were exposed for a period of 4 h in the presence of S9, although no clear dose-response was observed, there appeared to be a slightly increased frequency of chromosomal aberrations (excluding gaps) in the low and high dose group only. These were 2.6, 3.5, 2.7 and 3.8 % in controls, 31.3, 125.0 and 250 µg/ml groups respectively. There was no statistical significance in these data. No such effects were observed in Experiment 1A under the same conditions, despite the use of higher concentrations and the presence of increased cytotoxicity. The increases in aberrations are therefore considered toxicologically irrelevant. There was no evidence of an increase in polyploid metaphases after treatment with pirimiphos-methyl. All positive controls behaved accordingly.

Under the conditions of this study, it can be concluded that the test substance did not induce structural chromosomal aberrations in human lymphocytes *in vitro*. Therefore, pirimiphos-methyl is considered non-clastogenic in this chromosome aberration test, when tested up to cytotoxic concentrations.

The clastogenic potential of pirimiphos-methyl was also investigated *in vitro* in a older chromosomal aberration study (Wildgoose, 1986). Human lymphocytes were incubated with pirimiphos-methyl at concentrations of 0, 12, 29, 58 or 116 μg/ml in the presence and absence of rat liver S9 metabolic activation. Cytotoxicity was evident at ≥ 58 μg/ml, with a 60 % reduction in mitotic index at 116 μg/ml. A total of 200 cells per donor/experiment were scored for chromosome aberrations. The assays had a 3 hour exposure time, with 24 hours growth, used duplicate cultures and were repeated. It is noted that the exposure time in this study is shorter than specified by current guidelines. The test system was shown to be sensitive to chromosome-damaging effects, by the response given to the positive controls, mitomycin C and cyclophosphamide. However, no significant increase in chromosomal aberrations was observed in any of the cultures treated with pirimiphos-methyl, indicating the test material was not clastogenic to human lymphocytes *in vitro.*

Sister chromatid exchange

The potential of pirimiphos-methyl to induce sister chromatid exchange (SCE), was tested in Chinese hamster lung fibroblasts (Don cells) at concentrations of 0, 0.14, 0.29, 1.4, 2.9, 14, 29, 145 or 289 μg/ml in the presence and absence of metabolic activation (Howard, 1986). Since the study was carried out, the test guideline it was conducted according to has been deleted, however a summary is included to add to the weight of evidence.

In the presence of S9, there was a statistically significant increases in SCE frequency observed at the two higher dose levels, 29 and 145 μg/ml. However, there was a large degree of variability in the frequency of SCEs between cultures. These ranged from 6.8 – 13.3 SCE/cell. There was no overall consistency and therefore no real-dose response. SCE formation only reached double that of the negative control at the top concentration of 145 μg/ml, occurring in the presence of significant cytotoxicity (80 % depression in mitotic index) meaning adequate cell replication could not take place. There was no clear, dose-related increase in mean number of SCEs either in the presence or absence of S9.

9.11.1.2 *In vivo* genotoxicity

Pirimiphos-methyl was tested *in vivo* in a micronucleus assay in mice, an *in vivo* UDS assay in rats and a dominant lethal study in mice.

Micronucleus assays in mice

In a micronucleus assay (Rajini et al., 1986), pirimiphos-methyl (90.5 % purity) was administered orally to inbred male CFT Swiss mice at doses of 0, 100, 200 and 400 mg/kg bw. The test compound was administered in two equal instalments separated by a 24 hour interval. Six hours after the second treatment, the mice were killed by cervical dislocation. Bone marrow preparations were made and stained in the manner of Schmid (1975). There was a slight reduction in the PCE/NCE ratio in the top dosage group, however, the incidence of micronucleated PCEs and NCEs was not affected by the treatment at any dose level.

A second, older micronucleus assay also exists, however not enough study details were given for this to be considered as part of the assessment (Seiller 1976). No increase in the incidence of micronuclei was apparent.

In vivo UDS assay in rats

In a 1998, GLP-compliant study, the potential for pirimiphos-methyl to induce unscheduled DNA synthesis was investigated in rats (Anon., 1998a). Male Alpk:AP_fSD rats (5/dose/time point) received pirimiphos-methyl by gavage in corn oil at 400 or 800 mg/kg bw (doses based on a sighting study). After 2 or 16 hours,

animals were anaesthetised, the livers perfused, then hepatocyte cultures were prepared and incubated with ^{3}H-thymidine. Net nuclear grain counts (NNG) were determined for 60 cells/animal from autoradiograph slides. Signs of sedation and increased sensitivity to sound were seen prior to the 16 hour sampling time. There was no indication of marked cytotoxicity or of an increase in NNG in any animal treated with pirimiphos-methyl. The positive control produced a clear response. Pirimiphos-methyl did not induce unscheduled DNA synthesis in this assay.

Dominant lethal study in mice

Pirimiphos-methyl was tested for dominant lethal mutagenic activity in male Charles River CD-1 mice in a 1975 study, predating test guidelines and GLP (Anon., 1975a). Following a preliminary toxicity test, the dose levels chosen were 0, 15, 80 or 150 mg/kg bw/d. The males were mated with virgin females (1:2) weekly for 8 weeks. Females were killed approximately 13 days after mating and the uteri examined for live implants, early deaths and late deaths. There were sporadic effects, but no consistent increases in the percentage of early deaths or number of early deaths per pregnancy. There was no evidence of increases in pre-implantation losses or late deaths. Appropriate responses were obtained with the positive controls (ethyl methanesulphonate and cyclophosphamide). Thus under the conditions of this assay, there was no evidence that pirimiphos-methyl caused dominant lethal mutations.

9.11.1.3 Conclusion

Pirimiphos-methyl was tested in a number of *in vitro* and *in vivo* tests for genotoxicity. Clear, unambiguous negative results were obtained in all of the guideline *in vitro* studies available. There was no evidence of a mutagenic effect to mammalian somatic cells in the *in vivo* studies available.

9.11.2 Comparison with the CLP criteria

In order to be classified in category 1A for germ cell mutagenicity, a substance must be known to cause heritable mutations or be regarded as if they induce heritable mutations in the germ cells of humans. There is no evidence to support the classification of 2-phenoxyethanol in category 1A.

For classification with 1B, positive results must be obtained from *in vivo* heritable germ cell mutation tests in mammals alone or in combination with some evidence that the substance has potential to cause mutations to germ cells.

For classification in category 2 there should be positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

- Somatic cell mutagenicity tests *in vivo*, in mammals; or
- Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

The weight of evidence presented suggests that pirimiphos-methyl is not mutagenic *in vitro* and there was no evidence to suggest the presence of mutagenicity following *in vivo* testing. Therefore, pirimiphos-methyl should not be classified for germ cell mutagenicity.

9.11.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified – data conclusive but not sufficient for classification.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Pirimiphos-methyl was tested in a number of *in vitro* and *in vivo* studies for genotoxicity. For studies compliant with (or closely resembling) OECD test guidelines and GLP, the DS reported negative results for six *in vitro* studies and three *in vivo* studies in the CLH report. The *in vitro* studies concern two gene mutation tests in bacterial cells (Callendar, 1984; Sokolowski, 2015), one gene mutation test in L5178Y/TK± mouse lymphoma cells (Cross, 1986), two chromosome aberration tests in human lymphocytes (Wildgoose, 1986; Sokolowski, 2015a) and one SCE-test in Chinese hamster lung fibroblasts (Howard, 1986). *In vivo*, pirimiphos-methyl was tested in an UDS-assay in rats (Anon., 1998a) and in a micronucleus test (Rajini *et al*., 1986) and a dominant lethal test in mice (Anon., 1975a).

The DS further referred to four *in vitro* bacterial reverse mutation assays reported in public literature (Seiller, 1972; Seiller, 1976; Shirasu, 1984 and Hanna & Dyer, 1975). Several positive results were described for these studies that were conducted in the period 1972-1984. As they were not performed according to current standards and had limitations in the reporting, the DS considered them of low relevance.

With all guideline *in vitro* studies providing a clear unambiguous negative result, and noting further that the *in vivo* studies conducted did not provide evidence for a mutagenic effect, the DS argued that pirimiphos-methyl should not be classified for germ cell mutagenicity.

Comments received during public consultation

One MSCA supported the proposed classification in general. A second MSCA pointed to some discrepancies between the evaluation of studies in the DAR and in the CLH report. They indicated that the DAR presents a 5th *in vitro* bacterial mutation genotoxicity study reported in public literature (Moriya, 1983), also with positive results. They further noticed that the conclusion on the SCE assay (Howard, 1986) was equivocal in the DAR but negative in the CLH dossier. IND pointed to the existence of two new mutagenicity studies with pirimiphos-methyl (an *in vitro* HPRT test and an *in vivo* micronucleus test), both of which negative in their view. These studies were conducted for the renewal process, but were not available to the DS when drafting the CLH report. Full study reports of these two new studies have been provided to RAC, and they are summarised under Additional key elements.

Additional key elements

Pirimiphos-methyl tech. – Gene Mutation Assay in Chinese Hamster V79 Cells In Vitro (V79/HPRT) - Anon., 2017.

The *in vitro* mutagenicity of pirimiphos-methyl was tested according to OECD TG 476 (2016) and GLP (claimed but not confirmed by the quality assurance auditor) by detection of forward mutations in the HPRT gene of Chinese hamster V79 cells in the presence and absence of metabolic activation. In a pre-test with and without metabolic activation with

4-hour exposure duration, cytotoxicity was observed at 68.8 µg/mL and above and phase separation at 137.5 µg/mL and above. pH and osmolarity were not affected. All tests were performed in duplicate including appropriate negative and positive controls. The period to allow expression of mutant phenotypes was 7 days.

Two experiments were performed without metabolic activation because the optimal cytotoxic cut-off range was not achieved in the first experiment. The lowest relative survival in the second experiment was 22.7 %. All other acceptability criteria were fulfilled. A clearly negative result was obtained in both experiments.

Three experiments were performed with metabolic activation. The first experiment was repeated with adjusted concentration ranges because the cytotoxicity (Relative Survival = relative adjusted cloning efficiency I) at the two highest concentrations was below 10 %. However, the mutant frequency was statistically significantly increased and above the 95 % control limit also at concentrations with a relative survival above 50 %. Also, there was a statistically significant dose-effect relation. The second experiment was repeated because only 3 concentrations fulfilled the acceptance criteria (relative survival above 10 %) and no concentration with a relative survival between 10-20 %. The mutant frequency was statistically increased at one concentration and above the 95 % control limit with a relative survival above 20 %. However, there was no statistically significant dose-effect relation. The third experiment (same concentrations) included one concentration with a relative survival between 10 and 20 % and 5 acceptable concentrations. An increase outside the 95 % control limit was observed in four concentrations of which one concentration was also statistically significant. However, there was no statistically significant dose-effect relation. The author concluded that overall, the increases in mutant frequency were sporadic and not concentration related and therefore not indicative of mutagenicity.

RAC considers the outcome of the first experiment as clearly positive as a statistically significant increase was observed at two concentrations, three concentrations were outside the 95 % control limit and the dose effect relation was statistically significant. That no relative survival between 10 and 20 % was observed does not disqualify the experiment in case of a positive result. The second and third experiment were not clearly positive and not clearly negative as significant increases outside the 95 % control limit were observed but not a statistically significant dose effect relation. Overall, RAC considers this test positive with metabolic activation and clearly negative without metabolic activation.

Pirimiphos-methyl - Micronucleus Test in the Mouse, Anon., 2017.

The capacity of pirimiphos-methyl to induce chromosomal damage and chromosomal numerical changes in bone marrow erythrocytes of mice was tested according to OECD TG 474 (2016). The dose levels were based on a preceding range test and also showed the absence of a difference in toxicity between males and females. The exposure of the bone marrow was considered likely based on the detection of pirimiphos-methyl in the blood of treated mice. Groups of 7 male mice were exposed once only to 0, 175, 350 or 700 mg/kg bw/day of pirimiphos-methyl by gavage (solvent 0.5 % CMC in water). Animals were killed 24 or 48 (highest dose only) hours later, the bone marrow extracted, and smear preparations made and stained. 4 000 polychromatic (PCE) and the normochromatic (NCE) erythrocytes within 1 000 erythrocytes per animal were scored for the presence of micronuclei. The PCE/NCE ratio was determined from 1 000 erythrocytes per animal. No scientific justification was provided for the once only treatment.

No mortality was observed in the main study. Clinical effects were observed at 350 and 700 mg/kg bw/day and more severe at the highest dose. The criteria for an acceptable test were fulfilled. No decrease in the PCE/NCE ratio was observed for any of the exposed groups. No statistically significant increase or increase above the 95 % control limits of the incidence of the number of cells with micronuclei was observed. Also there was no dose-effect relation for the dose groups terminated after 24-hours. Overall, pirimiphos-methyl did not produce an increase in the number of erythrocytes with micronuclei in mice. RAC therefore considers this study clearly negative.

Assessment and comparison with the classification criteria

In vitro

Two negative reverse gene mutagenicity tests in bacteria were available that were performed according to validated test guidelines. For the reasons specified by the DS, RAC agrees that the (partly positive) results of the four (or five; although according to the DAR the results of the Moriya study appear to have been presented in the Shirasu paper) additional bacterial tests from public literature are given little weight. Overall, pirimiphos-methyl is considered negative for reverse gene mutagenicity in bacteria.

RAC considers the SCE test (whether equivocal or negative) of low relevance, as it is no longer a standard test for regulatory purposes and more relevant *in vitro* tests on chromosome aberration in mammalian cells are available that showed pirimiphos-methyl to be negative for this effect.

RAC however considers pirimiphos-methyl to be positive for the induction of gene mutations in mammalian cells *in vitro*, based on the result obtained in the new HPRT study in the presence of metabolic activation. More weight is attached to this study than to the earlier negative L5178Y/TK± study, as that study deviated from the current OECD TG in expression period (72 hours is below the minimal required expression period of 7 to 9 days) and exposure duration (2 hours is below the suitable period of time of 3 to 6 hours).

In vivo

Regarding somatic cells, pirimiphos-methyl did not induce unscheduled DNA synthesis in rat liver cells or an increase in mouse bone marrow erythrocytes with micronuclei. Regarding germ cells, pirimiphos-methyl did not induce mutagenic effects in a mouse dominant lethal test.

Overall, the available data on pirimiphos-methyl do not meet the criteria and RAC agrees with the DS that **no classification for germ cell mutagenicity is warranted**.

9.12 Carcinogenicity

Table 8: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method Guideline, Deviation(s) from the guideline (if any)</th><th>Species Strain Sex no/group</th><th>Dose levels duration of exposure</th><th>Results</th></tr>
<tr>
<td>Two-year combined chronic toxicity / carcinogenicity

Oral (dietary)

Pre-dates OECD and GLP guidelines

(Anon., 1974)</td>
<td>Rat (Alpk:APfsd Wistar BABU)

Main group: 48/sex/dose

Of these, 40/sex/dose were killed after 104 wks of treatment.

The remaining 8/sex/dose were continued for 4-8 weeks and observed for recovery

Satellite group: 24/sex/dose, 8/sex sacrificed at 12, 26 and 52 weeks to provide interim data on cholinesterase and clotting function.</td>
<td>Pirimiphos-methyl (86.8% pure)

Dose: 0, 10, 50 & 300 ppm

Equivalent to: 0, 0.4, 2.1 and 12.6 mg/kg bw/day (mean value across both sexes)

Exposure: 104 weeks with interim kills at 12, 26 and 52 weeks</td>
<td>
Non-neoplastic findings:
Non-neoplastic findings are presented with the specific target organ toxicity data (Table 9)

Neoplastic findings:
Marginally increased incidences of pancreatic and brain tumours were observed.
<table>
<tr><th></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><th>Dose (ppm)</th><td>0</td><td>10</td><td>50</td><td>300</td><td>0</td><td>10</td><td>50</td><td>300</td></tr>
<tr><th>Total number of animals</th><td>48</td><td>48</td><td>48</td><td>48</td><td>48</td><td>48</td><td>48</td><td>48</td></tr>
<tr><th>Animals investigated*</th><td>42</td><td>43</td><td>45</td><td>42</td><td>43</td><td>45</td><td>46</td><td>47</td></tr>
<tr><td>Pancreas, islet cell adenoma</td><td>0</td><td>0</td><td>0</td><td>4 (9.5 %)</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Pancreas, islet cell carcinoma</td><td>0</td><td>0</td><td>0</td><td>1 (2.4 %)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Brain, meningioma (B)</td><td>1 (2.4 %)</td><td>1 (2.3 %)</td><td>2 (4.4 %)</td><td>2 (4.8 %)</td><td>0</td><td>0</td><td>1 (2.2 %)</td><td>0</td></tr>
<tr><td>Brain, ependymoma (B/M)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1 (2.1 %)</td></tr>
<tr><td>Brain, ganglioneuroma (B)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1 (2.1 %)</td></tr>
</table>
* Uncertainty lies in why the numbers of animals investigated for carcinogenic findings is lower than the total number of animals in the study. It is possible that the lower numbers account for the animals that died due to a respiratory infection that occurred earlier in the study; however there is no information in the study report to support this.
In the available study reports, tumour data specifically for the 40 male and 40 females sacrificed after 104 weeks was not available.
B – Benign
M – Malignant

Historical control data:
A direct comparison is not possible for each of the HCD studies available as it is not clear if the studies terminated at 104 weeks or like this study, if there was a 4-8 week recovery period before termination occurred. Notwithstanding, the HCD relating to this study is as follows:
<table>
<tr><th></th><th>HCD originally presented in the DAR.
- 6 studies</th><th>HCD provided more recently.
- 23 studies
- terminology unknown</th></tr>
</table>
</td>
</tr>
</table>

<table>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td></td><td colspan="2">- varying terminology – study dates 1965 - 1973</td><td colspan="2">- study dates 1984 - 2004</td></tr>
<tr><td>Finding</td><td>Males</td><td>Females</td><td>Males</td><td>Females</td></tr>
<tr><td>Pancreas, islet cell adenoma</td><td>(0 – 6 %)</td><td>0 – 4 %</td><td>0 – 5/52 (0 – 9.6 %)</td><td>0 – 2/52 (0 – 3.8 %)</td></tr>
<tr><td>Pancreas, islet cell carcinoma</td><td>0 – 7%</td><td>0 – 2 %</td><td>0 – 2/52 (0 – 3.8 %)</td><td>0 – 1/52 (0 – 1.9 %)</td></tr>
<tr><td>Brain, meningioma (B)</td><td>0 – 1/24 (0 - 4 %)</td><td>0</td><td>0 – 2/52 (0 – 3.8 %)</td><td>0 – 1/52 (0 – 1.9 %)</td></tr>
<tr><td>Brain, ependymoma (B/M)</td><td>0 – 5 %</td><td>0</td><td>0 – 1/52 (0 – 1.9 %)</td><td>0 – 1/52 (0 – 1.9 %)</td></tr>
<tr><td>Brain, ganglioneuroma (B)#</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
ND = No Data
# - Additionally, ganglioneuroma have been observed in the adrenal glands of males [1/52 (1.9 %)] and in the thyroid gland of males [1/104 (1.0 %)].

Full details of the more recently provided HCD can be found in Annex I to this document.</td></tr>
<tr><td>78 week carcinogenicity
Oral (dietary)
OECD 451
GLP

(Anon., et al., 1996)</td><td>Mouse (CD-1)
50/sex/dose
Satellite group (sacrificed at 52 weeks): 10/sex/dose</td><td>Pirimiphos-methyl (purity 86.7 or 89.8% (2 batches)
Dose: 0, 50, 200 & 300 ppm
Equivalent to: 0, 9, 36 and 57 mg/kg bw/d (mean value across both sexes)
Exposure: 78 weeks with satellite group killed at 52 weeks</td><td>Non-neoplastic findings:
Non-neoplastic findings are presented with the specific target organ toxicity data (Table 9)

Neoplastic findings:
There were no neoplastic findings at any dose.</td></tr>
</table>

9.12.1 Chronic/carcinogenicity study in rats

The chronic toxicity and carcinogenicity of pirimiphos-methyl was investigated in a study in rats. The study was conducted in 1974, prior to GLP, and whilst the level of detail in the report is not to current standards, it still contains sufficient information for the purposes of hazard assessment and classification.

Groups of Wistar-derived rats (48/sex/dose) were fed diets containing 0, 10, 50 or 300 ppm pirimiphos-methyl (corresponding to mean intakes of 0, 0.4, 2.1 and 12.6 mg/kg bw/d) for 104 weeks. At the end of this period

8 rats/sex/dose were maintained on control diet for 4-8 weeks to assess recovery whilst the others were killed. Towards the end of the study, animals became ill with a respiratory infection and some died. All surviving animals received oxytetracycline (18 mg/kg bw) daily for 5 days during week 86.

Regarding the number of animals investigated in the study and the uncertainty as to why they are less than the total number of animals used, this is not considered to be a significant problem in terms of the interpretation of the results. Importantly, the number of animals investigated in the top dose groups were not lower than in the control and low dose groups.

An additional 8 rats/sex/dose level were fed the same diets and killed at interim periods of 12, 26 and 52 weeks to investigate effects on brain cholinesterase inhibition and clotting function, but they did not receive pathology examinations. No tests for statistical significance were performed.

9.12.1.1 Non-neoplastic findings

Survival was similar in all groups and > 50 % at week 90 in males and week 96 in females. At its peak, the respiratory infection was responsible for the deaths of 7 animals from the control group, 3 animals from the low-dose group, 7 animals from the mid-dose group and 6 animals from the top-dose group in one week.

There were no clinical signs of toxicity and no adverse effects on body weight gain and food consumption. Clinical chemistry and haematology investigations revealed no treatment-related effects.

Inhibition of brain and erythrocyte cholinesterase activity was observed at 300 ppm in males and females and to a smaller and less consistent extent at 50 ppm; the degree of inhibition did not increase with duration of dosing. There was evidence of recovery in males, after 4 weeks; in females, erythrocyte activity normalised, but brain activity remained depressed.

9.12.1.2 Neoplastic findings

The pathology data indicate findings in the pancreas and brain.

Two lots of historical control data (HCD) were provided by the laboratory for the tumour incidences observed. The first HCD was provided in the original DAR (October 2003) and covers 6 studies of varying terminology between the years 1965 – 1973. The more recently provided HCD covers 23 studies in the same strain of rat as the main study, from the same laboratory, over a period of 20 years, between 1984 and 2004. There is no information on the study lengths. The current carcinogenicity study in rats was carried out in 1974 and it is assumed that the animals investigated included the animals that were sacrificed at 104 weeks and also those that were killed after a recovery period of 4 – 8 weeks (total study length 108 or 112 weeks). There were no data available in the study report specifically looking at the 40 male and female animals killed after 104 weeks only. The dossier submitter considers that a direct comparison can not be made between the HCD and the current study as there is no information to indicate whether the studies ended at 104 weeks or whether they had a recovery period as with the current study. On the basis that many of the findings were considered rare, the extended HCD still provides useful information. It is noted that the pattern of findings in the HCD presented did not seem to change over the 20 year period.

Pancreatic tumours

There was an increased incidence of pancreatic islet cell adenoma in the top dose males 0/42, 0/43, 0/45 and 4/42 (9.5 %) at 0, 10 ,50 and 300 ppm. In addition, one male of the top group was found to have pancreatic islet cell carcinoma. This male was found to have multiple tumours, however the pathology description does not indicate whether there was a primary tumour giving rise to metastases.

The incidence of pancreatic islet cell adenoma in the first set of HCD (1965 – 1973), provided in the DAR (October 2003) was found to range between 0 – 6 % in control males and in the new HCD (1984 – 2004) the incidence ranged between 0 - 5/52 (0 - 9.6 %). Whilst the adenoma observed are above the concurrent controls, according to both sets of HCD provided, it is not unsual to see this number of adenoma naturally occuring in

a single study. The incidence of pancreatic islet cell carcinoma in the first HCD provided was 0 – 7 % and in the newer HCD the incidence was 0 – 2/52 animals (0 - 3.8 %). The finding of 1/42 males (2.4 %) was well within these ranges.

Brain tumours

There was an increase in benign meningioma in the brains of males treated with 50 ppm and above (incidences: 1/42, 1/43, 2/45 and 2/42, percentages: 2.4, 2.3, 4.4 and 4.8 at 0, 10, 50 and 300 ppm). In the older HCD, the range of males with this findings was 0 – 4 % and out of 23 studies in the new HCD provided, there was one incidence of 2/52 (3.8 %) males in the same study with this tumour type. Therefore, it is highly possible that the increase in incidence observed in the mid- and top-dosed rats was not treatment-related.

In females, 1 top dose animal was found to have an ependymoma in the brain (incidences: 0, 0, 0 and 1/47 (2.1 %) at 0, 10, 50 and 300 ppm). This finding was not observed in the 6 studies provided in the old HCD and out of the 23 studies in the new HCD provided by the applicant, there was one study with 1/52 animals spontaneously developing this tumour type (1.9 %). Whilst the percentage of females with this finding was above the HCD, the finding of a single untreated animal with this tumour type has been seen.

One female of the top dose was found to have a ganglioneuroma, associated with the pituitary gland (a rare, benign tumour of the autonomic nervous system). This female was also found to have a pituitary tumour. The tumour finding was not observed in any of the other treatment or control groups and neither the old, nor the new HCD showed any incidence of females presenting with this tumour type. However, tumours with the same aetiology have been found to occur spontaneously in other tissues of this species of rat. Out of 23 studies, carried out over a 20 year period, ganglioneuroma have been observed in the adrenal glands [1/52 (1.9 %)] and thyroid gland of untreated males [1/104 (1.0 %)].

9.12.2 Chronic/carcinogenicity study in mice

The chronic toxicity and carcinogenicity of pirimiphos-methyl was investigated in a 1996, GLP-compliant study in mice. Groups of CD-1 mice (50/sex/dose level) were fed diets containing 0, 50, 200 or 300 ppm (equivalent to 0, 9, 36 and 57 mg/kg bw/day) pirimiphos-methyl (purity 86.7%, batch RS492/B or 89.8%, batch 20307-005) for 78 weeks - the top dose level was initially 400 ppm but this was reduced after the first week due to body weight loss. A satellite group (10/sex/dose level) was killed after 52 weeks.

Survival to week 78 was > 60 % in all groups, and was similar in all female groups over most of the study, but there was an increase in mortality at week 60 (percentage not specified). An initial increased level of early deaths was evident at 200 ppm and 300 ppm in males; the likely causes were anticholinesterase effects, nephropathy or urinary bladder obstruction.

Organ weights and incidences of neoplastic lesions were not adversely affected by treatment; overall tumour incidences (particularly common lung and liver tumours) were lower in animals receiving 300 ppm pirimiphos-methyl than in control animals.

There were increases in neoplastic lesions in any of the treated animals.

9.12.3 Comparison with the CLP criteria

Two studies are available to inform on the carcinogenic potential of pirimiphos-methyl, one each in rats and mice.

In order to be classified with category 1A, pirimiphos-methyl must be a known human carcinogen, but there is no evidence to support this.

Classification with category 1B must be carried out for substances that are presumed to have carcinogenic potential in humans, largely based on animal evidence. There are no clearly significant tumour findings in the studies presented above to support classification with category 1B.

Substances are placed in category 2 on the basis of evidence of a carcinogenic effect in animals studies that is not sufficiently convincing to place the substance in category 1A or 1B. In order for pirimiphos-methyl to be classified in category 2, there must be evidence of a treatment-related increase in tumours in the available animal studies.

There were no treatment-related neoplastic findings reported in the mouse study.

In rats, there was an increased incidence of pancreatic islet cell adenoma in males, and one male of the top dose group was found to have pancreatic islet cell carcinoma. Both findings were within historical control data provided and are considered a natural occurrence in aged rats, unrelated to treatment. In addition, there were no pre-neoplastic lesions that might suggest a progression to cancer and no other signs of toxicity to the tissues. No increase in pancreatic tumours were observed in female rats or mice treated with pirimiphos-methyl. Therefore, these tumours are not considered to be treatment-related.

There were minor increases in brain tumours observed in rats. Two male rats of the mid and high dose groups were found to have benign meningioma (versus 1 in controls and the low dose groups). The tumour incidences were only marginally above the concurrent control and were within the historical control data range and therefore are not considered sufficient evidence of a carcinogenic response.

In females, 1 rat in the top dose group was found to have a brain ependymoma (not seen in concurrent controls or other treated animals). However, this was within the historical control data range and therefore not considered treatment-related.

A second, rare tumour type (a brain ganglioneuroma) was also observed in one female of the top dose group. This finding was not seen in male rats or in mice treated with pirimiphos-methyl. The tumour was benign and there was no dose response, however this might not be expected for a rare tumour type occurring only at the top dose. Contemporary historical control data from the testing laboratory taken from a 20 year period from 1984-2004 showed no background incidence of this finding in females. However, the HCD did show a spontaneous occurrence of ganglioneuroma in males (1/52 in the adrenals and the thyroid in two studies from 1990 and 1994 respectively).

The dossier submitter concludes that the tumours observed in the pancreas and brains of rats occurred spontaneously and were not related to treatment with pirimiphos-methyl. There were no pre-neoplastic lesions or any other toxicological findings that indicated these tissues were a target organ and no mechanistic basis for tumour formation, raising into question the biological plausibility of the findings. Furthermore, pirimiphos-methyl was found to be non-genotoxic in a battery of *in vitro* and *in vivo* tests and in a robust carcinogenicity study in mice, using higher doses, no tumours were observed.

Therefore, on the basis of the available evidence, pirimiphos-methyl should not be classified for carcinogenicity.

9.12.4 Conclusion on classification and labelling for carcinogenicity

Not classified – data conclusive but not sufficient for classification.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two studies are available to inform on the carcinogenic potential of pirimiphos-methyl, a 2-year study in rats and a 78-week study in mice. The summaries below only relate to the neoplastic findings in these studies; the non-neoplastic findings have been summarized in the section on STOT RE. It is to be noted that as part of the pesticide renewal process, some new historical control data (HCD) were provided by the applicant to aid in the interpretation of the rat study.

Mice

In a GLP and OECD TG 451 compliant study (Anon. *et al.*, 1996), exposure of CD1-mice (50/sex/group) to 0, 50, 200 or 300 ppm pirimiphos-methyl in the diet (equivalent to 0, 9, 36 and 57 mg/kg bw/day) for 78 weeks did not result in treatment-related increases in incidences of neoplastic lesions. Pirimiphos-methyl was therefore concluded to be not carcinogenic in mice.

Rats

In a study pre-dating OECD TG and GLP, groups of 48 Wistar-derived rats/sex were fed diets containing 0, 10, 50 or 300 ppm pirimiphos-methyl (corresponding to mean intakes of 0, 0.4, 2.1 and 12.6 mg/kg bw/d) for 104 weeks (Anon., 1974). At the end of this period 8 rats/sex/dose were maintained on control diet for 4-8 weeks to assess recovery. Towards the end of the study, animals became ill with a respiratory infection and some died. All surviving animals were treated with oxytetracycline during week 86.

The DS noted that the level of detail in reporting was not to current standards, that no statistical analysis was conducted and that there are some inconsistencies within the report, but the overall level of information and investigation was concluded to be adequate.

Aside from some death due to respiratory infection (at its peak, 7, 3, 7 and 6 animals from the control, low, mid and high dose group, respectively, in one week), survival was not affected.

Marginally increased incidences of pancreatic and brain tumours were observed (see Table 1 below). The increases were compared to two sets of HCD. The first set was provided in the original DAR and covers 6 studies of varying terminology between 1965 and 1973. The second set was provided for the renewal process and covers 23 studies in the same strain of rat as in the main study, from the same laboratory, over a period of 20 years (1984-2004).

The DS remarked that uncertainty lies in why the numbers of animals investigated for carcinogenic findings is lower than the total number of animals in the study. It is possible that the lower numbers account for the animals that died due to a respiratory infection that occurred earlier in the study; however there is no information in the study report to support this. In the available study reports, tumour data specifically for the 40 male and 40 females sacrificed after 104 weeks was not available.

The DS further remarked that a direct comparison cannot be made between the HCD and the current study as there is no information to indicate whether the studies ended at 104 weeks or whether they had a recovery period as with the current study. Nevertheless, the extended HCD were still considered to provide useful information, e.g. the pattern of findings in the HCD presented did not seem to change over the 20 year period.

***Table 1**. Increased tumour incidences in rats fed pirimiphos-methyl*

	Males						Females					
Dose (ppm)	0	10	50	300	HCR[#] (%)	HCD* (%) [$]	0	10	50	300	HCR[#] (%)	HCD* (%) [$]
Total number of animals	48	48	48	48			48	48	48	48		
Animals investigated	42	43	45	42			43	45	46	47		
PANCREAS												
Islet cell adenoma	0	0	0	4 (9.5 %)	0-6	0-9.6 [15/23]	1 (2.3 %)	0	0	0	0-4	0-3.8 [9/23]
Islet cell carcinoma	0	0	0	1 (2.4 %)	0-7	0-3.8[§] [5/23]	0	0	0	0	0-2	0-1.9[§] [1/23]
BRAIN												
Meningioma (B)	1 (2.4 %)	1 (2.3 %)	2 (4.4 %)	2 (4.8 %)	0-4	0-3.8 [8/23]	0	0	1 (2.2 %)	0	0	0-1.9 [6/23]
Ependymoma (B/M)	0	0	0	0	0-5	0-1.9 [2/23]	0	0	0	1 (2.1 %)	0	0-1.9 [1/23]
Ganglioneuroma (B)	0	0	0	0	0	0	0	0	0	1 (2.1 %)	0	0

B – benign; M - malignant
[#] HCR - Historic control range from 6 studies with varying terminology from 1965-1973
[*] HCD – Historical control data for Alpk:APfsd Wistar BABU rats from the applicant's laboratory over a 20 year period from 1984-2004
[§] reported as adenocarcinoma
[$] number of studies with tumour findings

Pancreatic tumours

Compared to concurrent controls, there was an increased incidence of pancreatic islet cell adenoma in top dose males (4/42, 9.5 %). In addition, one top dose male had multiple tumours, including a pancreatic islet cell carcinoma. Both findings were however within the HCD provided, showing that up to 5/52 (9.6 %) adenoma and up to 2/52 carcinoma (3.8 %) can occur spontaneously in a study. Given that there were no pre-neoplastic lesions that might suggest a progression to cancer and no other signs of toxicity to the tissues, and that there was no increase in pancreatic tumours in female rats and in mice treated with pirimiphos-methyl, the DS considered the findings in male rats a natural occurrence in aged rats, unrelated to treatment.

Brain tumours

Some minor increases in brain tumours were observed. In male rats, there was an increase in benign meningioma in the mid and high dose group, both presenting 2 animals with this tumour versus 1 animal each in the control and low dose group. Whilst on a percentage basis this finding was above the HCD, historical control incidences of 1 to 2 animals per

study are not uncommon. The DS therefore considered it insufficient evidence of a carcinogenic response.

In female rats, 1 top dose animal (2.1 %) was found to have an ependymoma and another top dose animal (2.1 %) a benign ganglioneuroma, associated with the pituitary gland. No ependymoma or ganglioneuroma were observed in the other treatment or control groups. As to HCD, no ependymoma were observed in the old set, whereas in the new set there was 1/23 studies with 1/52 animals spontaneously developing this tumour type (1.9 %). Hence, the finding of a single untreated animal with an ependymoma was not considered treatment-related by the DS. Neither the old nor the new HCD showed any incidence of females presenting a ganglioneuroma in the brain. However, the DS noted that the new HCD did show a spontaneous occurrence of tumours with the same aetiology in untreated male rats (1/52 (1.9 %) in the adrenals and 1/104 (1.0 %) in the thyroid gland).

Conclusion

The DS concluded that the tumours observed in the pancreas and brains of rats occurred spontaneously and were not related to treatment with pirimiphos-methyl. There were no pre-neoplastic lesions or any other toxicological findings that indicated these tissues were a target organ and no mechanistic basis for tumour formation, raising into question the biological plausibility of the findings. Furthermore, pirimiphos-methyl was found to be non-genotoxic in a battery of *in vitro* and *in vivo* tests and in a robust carcinogenicity study in mice, using higher doses, no tumours were observed. On the basis of the available evidence, pirimiphos-methyl should therefore not be classified for carcinogenicity.

Comments received during public consultation

One MSCA and IND supported the proposal for no classification for carcinogenicity. A second MSCA suggested that the HCD studies in the period 1984-2004 were not compliant and that the pancreatic tumours and brain meningioma should be discussed in more detail considering the weight of evidence. A third MSCA suggested classification in category 2, based on less than 50 animals/sex having been tested, the limited relevance of the HCD values from the period 1984-2004, and the observation that pirimiphos-methyl is a molecule with neurological tropism, raising concern as to the different types of brain tumours found.

In response, the DS agreed that the HCD from 1984-2004 are not contemporary to the 2-year rat study from 1974. Due to the rare nature of some of the findings, however, the DS found these HCD to provide some reassurance that these types of tumours can occur spontaneously in rats. Although the DS acknowledged that the number of animals investigated leaves some uncertainties, this is not believed to lead to a significant problem in interpreting the results as the number of animals were comparable across all dose groups (including the controls). The DS further stood by their arguments and conclusion presented in the CLH report that the pancreas and brain tumours are not related to treatment with pirimiphos-methyl.

Assessment and comparison with the classification criteria

In view of the absence of treatment-related increases in neoplastic effects in the mouse 78-week carcinogenicity study, RAC considers there is no evidence for carcinogenicity of pirimiphos-methyl in mice.

In contrast to mice, marginally increased incidences of pancreatic and brain tumours were observed in a 2-year rat study. RAC notes this study has some limitations in its design (somewhat lower number of animals tested than currently required (but still > 40 per group available for investigation), dose selection, no statistics performed), in its conduct (occurrence of respiratory infections), and in reporting (limited details). Regarding dose selection, RAC notes this may not have been appropriate, given that the rather low top dose of 12.6 mg/kg bw/day (4.5× lower than in mouse 78-week study) was devoid of toxicity (e.g. no effect on mortality, clinical signs, body weight and food consumption), aside from acetylcholinesterase inhibition.

In male rats an increase in islet cell adenoma of the pancreas was observed at the highest dose (4/42, 9.5 %) whereas no such tumours were observed in the concurrent controls or the other exposure groups, nor in the exposed females. Additionally, there was 1 top dose male with an islet cell carcinoma, but this animal had multiple tumours and it was not clear whether this carcinoma was a primary tumour or not. On a percentage basis, the incidence of adenoma was above the highest incidence observed in male rats in the old set of HCD (6 %, in 6 studies over 10 years prior to the 2-year rat study with pirimiphos-methyl from 1974), and just below the highest incidence observed in the new set of HCD (5/52 or 9.6 %, in 23 studies over a 20-year period between 1984 and 2004). The single occurrence of a carcinoma was within the HCD of both periods. RAC considers the comparison with both sets of HCD of limited value, the old set because a part was outside the window of 5 years before/after the performance of the 1974 rat study and no details were available to narrow it down, the new set because these are even less contemporary to the study under consideration. Furthermore, it is unclear whether the HCD concerned 104-week studies, or included a recovery period. The HCD do however indicate that over the approximately 30 years studied there is a consistent low level of spontaneous pancreatic tumour findings, with no evidence of a shift over time. Whereas for the increase in islet cell adenoma a relation with pirimiphos-methyl treatment cannot be totally excluded due to the study limitations and limited value of the HCD, RAC concludes on the basis of a weight of evidence assessment that it does not warrant classification because:

- no such increase was observed in female rats;
- no such increase was observed in mice;
- no pre-neoplastic lesions of the pancreas were observed in the study, nor in any of the other repeated dose toxicity studies with rats;
- pirimiphos-methyl was found to be non-genotoxic *in vivo*;
- it concerns benign tumours, whereas no increase in malignant tumours was observed;
- pancreatic islet cell adenoma and carcinoma seem to occur with low frequency in untreated male rats.

In male rats also a small increase in benign brain meningioma was observed at the two highest dose groups (1 extra case as compared to the concurrent control and low dose groups). Female rats did not show an increase in meningioma (single occurrence at the mid dose), but in the high dose group there was a single female with an ependymoma of

the brain and a single female with a ganglioneuroma. The latter tumour is not a primary tumour of the brain, but arises from the sympathetic ganglia of the autonomic nervous system. Ganglioneuromas are rare, benign tumours that may appear in organs such as thyroid gland, adrenals and pituitary. According to the DAR, the female with the ganglioneuroma was having a pituitary growth, and the ganglioneuroma was not seen in several brain sections but was stated to have been associated with the pituitary. It might therefore have been a pituitary tumour, a common finding in aged rats. The available HCD of two periods show that brain meningioma and ependymoma, but not ganglioneuroma, occur spontaneously at low incidence in rats. Ganglioneuroma do however occur at low incidence in other organ types. As noted for the pancreatic tumours, RAC considers the comparison with the HCD of limited value because they were not contemporary. Whilst noting the study limitations, RAC concludes on the basis of a weight of evidence assessment that the brain tumours do not warrant classification because:

- the increases were very small;
- each tumour type only occurred in one sex;
- no increases in brain tumours were observed in mice;
- no pre-neoplastic lesions of the brain were observed in the study, nor in any of the other repeated dose toxicity studies with rats, including a sub-chronic neurotoxicity study with a slightly higher top dose;
- pirimiphos-methyl was found to be non-genotoxic *in vivo*;
- meningioma and ependymoma seem to occur with low frequency in untreated rats;
- the ganglioneuroma may have been misdiagnosed.

Overall, RAC therefore concurs with the DS that on the basis of the available data in mice and rats, **classification of pirimiphos-methyl for carcinogenicity is not warranted**.

9.13 Reproductive toxicity

9.13.1 Adverse effects on sexual function and fertility

Hazard class not assessed in this dossier.

9.13.2 Adverse effects on development

Hazard class not assessed in this dossier.

9.14 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

9.15 Specific target organ toxicity-repeated exposure

The repeated-dose oral toxicity of pirimiphos-methyl has been investigated in a number of studies in rats, mice and dogs; including a 28-day, 90-day and 2-year study in the rat, a 90-day and 18-month study in the mouse and a 2-year study in the dog. A 21-day study via the dermal route is also available in the rabbit.

Table 9: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**
ORAL STUDIES		
28 Day oral study in rats Rats, Wistar (12/sex/dose) Dietary Pre-dating OECD 407 and GLP (Anon., 1975) STOT-RE 1: ≤ 30 mg/kg bw/day STOT-RE 2: > 30, ≤ 300 mg/kg bw/day	Pirimiphos-methyl (purity 97 %) Oral exposure via the diet Doses: 0, 5, 8, 10 and 50 ppm [Equivalent to: 0.6, 1, 1.2 and 6 mg/kg bw/day (calculated using standard conversion factor)]	**≤ 50 ppm (6 mg/kg bw/day):** There were no toxicologically relevant effects at any dose. NOAEL: 50 ppm (5 mg/kg bw/day)
90 Day oral study in rats Rats, Alderley Park SPF (20/sex/dose) Dietary Predating OECD 408 and GLP (Anon., 1970) STOT-RE 1: ≤ 10 mg/kg bw/day STOT-RE 2: > 10, ≤ 100 mg/kg bw/day	Pirimiphos-methyl (purity 93.1 %) Oral exposure via the diet Doses: 0, 8, 80 and 360 ppm [equivalent to 0, 0.7, 7 and 32 mg/kg bw/day(calculated using standard conversion factor)]	**360 ppm (32 mg/kg bw/day):** ↓ Body weight gain in females (~ 20 %) Inhibition of brain cholinesterase activity in females (42 %) in week 12 - not reversible in 4 week recovery time. Inhibition of erythrocyte cholinesterase activity in males (34 – 60 %) and females (48 – 60 %) in weeks 2 – 12 – fully reversible in males only after 4 weeks **80 ppm (7 mg/kg bw/day):** ↓ Body weight gain in females (~ 20 %) Inhibition of brain cholinesterase activity in females (20 %) in week 12 – not reversible in 4 week recovery time Inhibition of erythrocyte cholinesterase activity in females (22 - 24 %, weeks 6 - 12) and in males (21 %, week 6 only) – fully reversible in males in 1 week and in females after 4 weeks **8 ppm (0.7 mg/kg bw/day):** There were no toxicologically relevant effects at this dose.

<table>
<tr>
<td></td>
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<td>
Table to show inhibition of cholinesterase activities [% inhibition compared to controls (absolute values given)] in Alderley Park SPF rats fed diets containing pirimiphos-methyl for 90 days.
<table>
<tr><td>Week</td><td>1 pre-</td><td>1</td><td>2</td><td>6</td><td>12</td><td>1 recovery</td><td>4 recovery</td><td>12</td><td>4 recovery</td></tr>
<tr><td>Dose (ppm)</td><td colspan="7">Erythrocyte</td><td colspan="2">Brain</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Males 0</td><td>1.0[£]</td><td>1.0</td><td>0.9</td><td>1.4</td><td>1.3</td><td>0.9</td><td>1.1</td><td>30.6[#]</td><td>30.5</td></tr>
<tr><td>8[$]</td><td>+27</td><td>+2</td><td>+11</td><td>-5</td><td>+8</td><td>+31</td><td>+20</td><td>+3</td><td>+2</td></tr>
<tr><td>80[$]</td><td>+31</td><td>+6</td><td>+10</td><td>-21</td><td>+10</td><td>+13</td><td>+5</td><td>1</td><td>+9</td></tr>
<tr><td>360[$]</td><td>+17</td><td>+8</td><td>-34</td><td>-60</td><td>-11</td><td>-34</td><td>+16</td><td>-12</td><td>-14</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Females 0</td><td>1.2[£]</td><td>1.0</td><td>1.2</td><td>1.4</td><td>1.2</td><td>1.0</td><td>1.1</td><td>31.8[#]</td><td>31.4</td></tr>
<tr><td>8[$]</td><td>-1</td><td>+28</td><td>-3</td><td>-5</td><td>-14</td><td>+11</td><td>+4</td><td>-6</td><td>+1</td></tr>
<tr><td>80[$]</td><td>-14</td><td>+35</td><td>-1</td><td>-24</td><td>-22</td><td>-6</td><td>+3</td><td>-20</td><td>-21</td></tr>
<tr><td>360[$]</td><td>-18</td><td>-5</td><td>-48</td><td>-51</td><td>-60</td><td>-29</td><td>-22</td><td>-42</td><td>-35</td></tr>
</table>
[£] - μmoles/ml/min
[#] - ΔpH/g/h
[$] - % inhibition compared to controls

NOAEL: 8 ppm (0.7 mg/kg bw/day)
</td>
</tr>
<tr>
<td>Two-generation reproduction study
Oral (dietary)
Rat (Sprague Dawley)
28/sex/dose (F_0)
24/sex/dose (F_1)
OECD 416
GLP

(Anon., 1995)

STOT-RE 1: ≤ 10 mg/kg bw/day
STOT-RE 2: > 10, ≤ 100 mg/kg bw/day</td>
<td>Pirimiphos-methyl (86.7 % pure)
Dose: 0, 10, 40 oe 160 ppm
[Equivalent to 0, 1, 3 or 12 mg/kg bw/day (F_0) and 0, 1, 4 or 15 mg/kg bw/day (F_1)]

Exposure: 10 weeks pre-mating and then during gestation and lactation phases (21 days)</td>
<td>
Reproductive effects are not included in this table as the endpoint “reproductive toxicity” is not assessed in this dossier.

<u>160 ppm (12/15 mg/kg bw/day, F_0/F_1 respectively):</u>

↓ Body weight gain in females during gestation and lactation (~ 10 %)

Inhibition of brain cholinesterase activity in F_0 females (53 %) and F_1 females (44 %) at sacrifice.

Inhibition of erythrocyte cholinesterase activity in F_0 males (33 – 36 %) and females (46 – 48 %) during pre-mating and sacrifice and F_1 males (37 %) at sacrifice and F_1 females during pre-mating (47 %).

<u>40 ppm (3/4 mg/kg bw/day F_0/F_1 respectively):</u>

Inhibition of brain cholinesterase activity in F_0 females (26 %) at sacrifice.

Inhibition of erythrocyte cholinesterase activity in F_0 males (22 %) at sacrifice and females (21 – 27 %) during pre-mating and sacrifice

<u>10 ppm (1 mg/kg bw/day):</u>

Inhibition of erythrocyte cholinesterase activity in F_1 males (21 %) during pre-mating.

(see table below for more details)
</td>
</tr>
</table>

Table to show cholinesterase inhibition [% inhibition when compared to controls (absolute values given)] in Sprague Dawley rats fed diets containing pirimiphos-methyl

	F0 to F1 generation			F1 to F2 generation	
Timing	**pre-dosing**	**pre-mating**	**sacrifice**	**pre-mating**	**sacrifice**
Dose (ppm)					
Erythrocyte					
Male 0[#]	989	1250	1476	945	1272
10 (%)	+23	-4	-10	**-21**	- 8
40 (%)	+2	**-22***	- 17**	-1	-17**
160 (%)	+44	**- 36****	**-33*****	**-36**	**-37****
Females 0[#]	1575	1513	1234	1085	439
10 (%)	+113	-9*	-6	+8	+12+
40 (%)	+10	**-21*****	**27*****	-17	-3 [+]
160 (%)	+5	**-46*****	**48*****	**47****	-7 [+]
Brain					
Males 0[£]	NP	NP	16113	NP	12558
10 (%)	NP	NP	-4	NP	+3
40 (%)	NP	NP	-4	NP	-4
160 (%)	NP	NP	-13***	NP	-18
Females 0[£]	NP	NP	14735	NP	14674
10 (%)	NP	NP	-10* [$]	NP	-6
40 (%)	NP	NP	**-26*****	NP	-16*
160 (%)	NP	NP	**-53*****	NP	**-44*****

NP - not performed
[$] - only 2/14 animals below control range
[+] - Control value low
[£] - iU/mg [#] - iU/l
* P<0.05 ** P<0.01 *** P<0.001

Sub-chronic neurotoxicity study Oral (dietary) Rat (Sprague Dawley) 10/sex/group OECD 424 GLP (Anon., 1995a)	Pirimiphos-methyl (89.8 % pure) Dose: 0, 2, 30 or 300 ppm (Equivalent to: 0, 0.2, 2.1/2.4 or 21.1/24.7 mg/kg bw/day males/females) Exposure: 92 days	**<u>300 ppm (21.1/24.7 mg/kg bw/day:</u>** Inhibition of brain cholinesterase activity in males: 20 – 30 % and females: 32 – 51 % (determined at study termination) Inhibition of erythrocyte cholinesterase activity in males (37 – 45 %, weeks 3 - 13) and females (38 - 46 %, weeks 3 - 13) **<u>30 ppm (2.1/2.4 mg/kg bw/day):</u>** No statistically significant or biologically significant adverse effects on brain or erythrocyte cholinesterase activities. No other toxicologically relevent findings. There was no evidence of neuropathy in this study.

<table>
<tr>
<td>STOT-RE 1: ≤ 10 mg/kg bw/day
STOT-RE 2: > 10, ≤ 100 mg/kg bw/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-year combined chronic toxicity / carcinogenicity
Oral (dietary)
Rat (Wistar derived)
48/sex/dose in main treatment group
Satellite group 24/sex/dose
Pre-dates OECD and GLP guidelines
(Anon, et al., 1974)
STOT-RE 1: ≤ 1.25 mg/kg bw/day
STOT-RE 2: > 1.25 ≤ 12.5 mg/kg bw/day</td>
<td>Pirimiphos-methyl (86.8% pure)
Dose: 0, 10, 50 & 300 ppm
Equivalent to: 0, 0.4, 2.1 and 12.6 mg/kg bw/day (mean value across both sexes)
Exposure: 104 weeks for the main carcinogenicity cohort, 52 weeks for the satellite group - cholinesterase testing at 12, 26 and 52 weeks</td>
<td><u>300 ppm (12.6 mg/kg bw/day):</u>
Inhibition of brain cholinesterase activity in males (31 – 38 %, weeks 26 and 104) and females (30 – 36 %, weeks 12-52) – reversible to levels not considered adverse (< 20 %) (both males and females)
Inhibition of erythrocyte cholinesterase activity in males (24 – 37 %, weeks 12 - 104) and females (27 - 43 %, weeks 2 - 104) – fully reversible within 4 weeks (both males and females)
(See table below for more details)
Organs:
Liver:
↑ Severe fatty vacuolation in females only (17 % females affected versus 2.3 % in controls)
Testis:
↑ Cystic vacuolation of the epididymis (4/42 males versus 0 in controls)
<u>50 ppm (2.1 mg/kg bw/day):</u>
Inhibition of brain cholinesterase activity (22 – 29 %, weeks 26 and 104) in males - fully reversible within 4 weeks
(See table below for details)
Table to show cholinesterase inhibition [% inhibition when compared to controls (absolute values given)] in Wistar rats fed diets containing pirimiphos-methyl
<table>
<tr><th>Week</th><th>pre-</th><th>2</th><th>12</th><th>26</th><th>52</th><th>104</th><th>1 recovery</th><th>4 recovery</th></tr>
<tr><td>Dose (ppm)
Erythrocyte</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Males 0[£]</td><td>0.9</td><td>1</td><td>1.3</td><td>1.4</td><td>1.2</td><td>1</td><td>0.9</td><td>1.2</td></tr>
<tr><td>10[$]</td><td>-2</td><td>+7</td><td>+27</td><td>+9</td><td>-10</td><td>-2</td><td>+19</td><td>+24</td></tr>
<tr><td>50[$]</td><td>+7</td><td>0</td><td>0</td><td>-9</td><td>-15</td><td>-9</td><td>+1</td><td>+2</td></tr>
<tr><td>300[$]</td><td>0</td><td>-16</td><td>-24</td><td>-37</td><td>-25</td><td>-31</td><td>-15</td><td>+3</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Females 0[£]</td><td>1</td><td>1.2</td><td>1.3</td><td>1.7</td><td>1.1</td><td>1.4</td><td>1.1</td><td>1.2</td></tr>
<tr><td>10[$]</td><td>+15</td><td>+6</td><td>+8</td><td>-11</td><td>+3</td><td>+13</td><td>0</td><td>-6</td></tr>
<tr><td>50[$]</td><td>+5</td><td>-7</td><td>+2</td><td>-13</td><td>-10</td><td>-4</td><td>-12</td><td>+10</td></tr>
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		300$ +14 **-37** **-30** **-43** **-38** **-27** **-32** 0 **Brain** Males 0# NP NP 32.2 34 28 30.2 NP 25.5 10$ NP NP -3 -2 -5 -9 NP +7 50$ NP NP -3 **-22** -4 **-29** NP +2 300$ NP NP -17 **-31** -9 **-38** NP -6 Females 0# NP NP 32.8 26.2 24.3 26.1 NP 26.9 10$ NP NP -5 +2 +5 +3 NP -1 50$ NP NP -14 0 0 -6 NP -4 300$ NP NP **-34** **-36** **-30** -19 NP -14 £ - µmoles/ml/min # - ΔpH/g/h $ - % inhibition compared to controls NP –not performed **10 ppm (0.4 mg/kg bw/day):** No treatment-related findings. NOAEL: 10 ppm (0.4 mg/kg bw/day)
90 Day oral study in mice Mice, CD-1 (10/sex/dose) Dietary OECD 408 (1981) GLP (Anon., et al., 1996) STOT-RE 1: ≤ 10 mg/kg bw/day STOT-RE 2: > 10, ≤ 100 mg/kg bw/day	Pirimiphos-methyl (purity 86.7 %) Oral exposure via the diet Doses: 0, 10, 30, 90, 270 and 810 ppm (equivalent to: ♂ 0, 2, 6, 20, 63 or 178 mg/kg bw/day and ♀ 0, 3, 9, 26, 80 or 284 mg/kg bw/day)	**810 ppm (178/284 mg/kg bw):** Dose terminated after week 1 due to severe toxicity **270 ppm (63/80 mg/kg bw):** Inhibition of brain cholinesterase activity at week 13 in males (81 %) and in females (75 %) Inhibition of erythrocyte cholinesterase activity at weeks 1-13 in males (68 – 75 %) and in females (68 – 78 %) **90 ppm (20/26 mg/kg bw):** Inhibition of brain cholinesterase activity at week 13 in males (44 %) and in females (35 %) Inhibition of erythrocyte cholinesterase activity at weeks 1-13 in males (57 - 71 %) and in females (51 - 70 %) **30 ppm (6/9 mg/kg bw):** Inhibition of erythrocyte cholinesterase activity in males (58 - 59 %) at weeks 3-13 and in females (26 - 52 %) at weeks 1-13 **10 ppm (2/3 mg/kg bw):** Inhibition of erythrocyte cholinesterase activity at weeks 3 and 13 in males (42 - 45 %), and in females (26 - 34 %)

Table to show inhibition of cholinesterase activities [% inhibition compared to controls (absolute values given)] in CD-1 mice fed diets containing pirimiphos-methyl for 90 days.

Week	**1**	**3**	**13**	**13**
Dose (ppm)	**Erythrocytes**			**Brain**
Males 0 (iU/litre)	1775	1753	1990	23614
10 (%)	-38	**-42***	-45	-6
30 (%)	-42	**-59*****	**-58****	-10**
90 (%)	**-57***	**-70*****	**-71****	**-44*****
270 (%)	**-68****	**-74*****	**-75****	**-81*****
Females 0 (iU/litre)	2036	1979	1446	23131
10 (%)	-8	**-34***	-26	-7
30 (%)	**-26***	**-52*****	**-38***	-3
90 (%)	**-51*****	**-70*****	**-55****	**-35*****
270 (%)	**-73*****	**-78*****	**-68*****	**-75*****

* - p<0.05 ** - p<0.01 *** - p<0.001

NOAEL: Could not be determined due to inhibition of erthycocyte cholinesterase activity observed at all dose levels.

78 week carcinogenicity

Oral (dietary)

Mouse (CD-1)

50/sex/dose

Satellite group (sacrificed at 52 weeks): 10/sex/dose

OECD 451

GLP

(Anon., et al., 1996)

STOT-RE 1: ≤ 1.65 mg/kg bw/day

STOT-RE 2: > 1.65 ≤ 16.5 mg/kg bw/day

Pirimiphos-methyl (purity 86.7 or 89.8% (2 batches)

Dose: 0, 50, 200 & 300 ppm

Equivalent to: 0, 9, 36 and 57 mg/kg bw/d (mean value across both sexes)

Exposure: 78 weeks with satellite group killed at 52 weeks

<u>300 ppm (57 mg/kg bw/day):</u>

Inhibition of brain cholinesterase activity in males (79 – 80 %) and in females (58 – 66 %)

Inhibition of erythrocyte cholinesterase activity in males (75 - 88 %) and females (79 – 84 %)

<u>200 ppm (36 mg/kg bw/day):</u>

Inhibition of brain cholinesterase activity in males (55 – 70 %) and in females (44 – 62 %)

Inhibition of erythrocyte cholinesterase activity in males (55 - 83 %) and females (62 – 78 %)

<u>50 ppm (9 mg/kg bw/day):</u>

Inhibition of brain cholinesterase activity in males only (21%, week 52 only)

Inhibition of erythrocyte cholinesterase activity in males (21 - 57 %) and females (48 – 65 %)

(See table below for more details)

Table to show cholinesterase activities [% inhibition when compared to controls (absolute values given)] in CD-1 mice fed diets containing pirimiphos-methyl

		Erythrocyte (%)		**Brain (%)**	
Week		**52**	**78**	**52**	**78**
Dose (ppm)					
Males	0	2408[#]	3327[#]	15282[£]	20882[£]

		50	**-57***	**-47***	**-21***	-13
		200	**-76***	**-83***	**-55***	**-70***
		300	**-75***	**-88***	**-79***	**-80***
	Females	0	2886[#]	3240[#]	14698[£]	11630[£]
		50	**-48***	**-65***	-8	+6
		200	**-77***	**-78***	**-62***	**-44***
		300	**-79***	**-84***	**-66***	**-58***

***** = p<0.05
[#] - iU/l
[£] - iU/mg

NOAEL: Could not be determined due to inhibition of erythocyte cholinesterase activity observed at all dose levels.

2 Year oral study in dogs

Dogs, Beagle

(4/sex/dose)

Capsule

Pre-dates OECD and GLP

(Anon., 1973)

Pirimiphos-methyl (supplied in 6 batches, 4 of unspecified purity and 2 of 97 and 99 % purity)

Capsule administration

Doses: 0, 0.5, 2 and 10 mg/kg bw/day

(No dose was administered in week 24)

<u>10 mg/kg bw/day:</u>

Inhibition of brain cholinesterase activity in males and females (54 %)

Inhibition of erythrocyte cholinesterase activity throughout study in males and females (37 – 77 %)

<u>2mg/kg bw/day:</u>

Inhibition of erythrocyte cholinesterase activity throughout study in males and females (29 - 38 %)

<u>0.5 mg/kg bw/day:</u>

There were no toxicologically relevant effects at this dose.

Table to show inhibition of cholinesterase activities in Beagle dogs orally administered capsules of pirimiphos-methyl for 2 years.

Week	**Pre**	**1**	**2**	**4**	**12**	**25/26**	**51/52**	**103/104**
Dose (mg/kg bw/d)								
Erythrocyte cholinesterase [#] (%)								
Males + females 0	0	-8	-2	-2	0	0	-18	-9
0.5	0	-4	-7	0	-2	-0	-12	-10
2	0	-5	-9	-11	**-14****	**-29****	**-38****	**-29****
10	0	**-37****	**-54****	**-62****	**-68****	**-60****	**-77****	**-72****
Brain cholinesterase [£] (%)								
Males + females 0.5	NP	NP	NP	NP	NP	NP	NP	-15*
2	NP	NP	NP	NP	NP	NP	NP	-18*
10	NP	NP	NP	NP	NP	NP	NP	**-54***

		NP = not performed # = % inhibition using pre-dose mean £ = % inhibition using control value, excluding outliers * = p<0.05 **= p<0.01 NOAEL: 0.5 mg/kg bw/day
DERMAL STUDIES		
21 Day dermal study in rabbits Rabbits, New Zealand White (5/sex/dose) Pre-dates OECD and GLP (Anon., 1980) STOT-RE 1: ≤ 85 mg/kg bw/day STOT-RE 2: > 85 ≤ 857 mg/kg bw/day	Pirimiphos-methyl (Purity 90.6 %) Occlusive, 6h/day Doses: 0, 4, 40 and 400 mg/kg bw/day in propylene glycol	**400 mg/kg bw/day:** Inhibition of erthyrocte cholinesterase activity in males (21 %) and females (79 %) **40 mg/kg bw/day:** Inhibition of erthyrocte cholinesterase activity in females (31 %) **4 mg/kg bw/day:** There were no toxicologically relevant effects at this dose. NOAEL: 4 mg/kg bw/day

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

Pirimiphos-methyl has been studied in repeat dose oral studies in rats, dogs and mice and in a repeated dose dermal study in rabbits. Information is also available from a carcinogenicity study in rats and one in mice (2 years), a two-generation study in rats and two neurotoxicity studies, also carried out in rats.

One of the most significant effects observed throughout all of the following studies was cholinesterase inhibition.

Cholinesterase inhibition

Most of the toxicity studies measured cholinesterase activity in plasma, erythrocytes (RBC) and brain as a surrogate for disruption of cholinergic neurotransmission. Assessment of the adversity of cholinesterase inhibition at any particular dose level has been performed in a hierarchical manner with consideration of the Joint Meeting on Pesticide Residues (JMPR) guidance (WHO - JMPR, 1999):

i. Clinical signs: - evidence of altered cholinergic neurotransmission is considered adverse. If there are no clinical signs, consider cholinesterase inhibition.

ii. Brain acetylcholinesterase: - inhibition by > 20 % which is statistically significant ($p < 0.05$) is considered adverse if it fits a dose- or time-related trend. Inhibition of < 20 % is not considered adverse.

iii. Erythrocyte acetylcholinesterase: - inhibition of > 20 % which is statistically significant ($p < 0.05$) is considered adverse if it fits a dose or time related trend. Inhibition of < 20 % is not considered adverse.

iv. Plasma butyrylcholinesterase: - Is considered only as a marker of exposure, unless no other cholinesterase measurements have been performed. Inhibition of > 20 % which is statistically significant ($p < 0.05$) would then be considered as an indication of adversity if it fits a dose or time related trend. Inhibition of < 20% is not considered adverse.

However, in dealing with biological systems, it is not always meaningful to adhere to rigid criteria, and other issues have been considered, case-by-case, in reaching conclusions regarding particular sets of data. For example, the range of values within a group has been addressed when mean values are close to the 'cut-offs'; for studies using small numbers of animals the relevance of statistical testing is taken into account.

9.15.1.1 Oral studies

The following section provides a study-by-study summary of the effects observed following repeated dosing of pirimiphos-methyl. This is followed by a discussion of the data and a weight of evidence summary table comparing all studies with the classification criteria.

Rats

A 28-day, 90-day and 2-year study are available to investigate the effect of repeated doses of pirimiphos-methyl *via* the oral route in rats.

28-day study

In a non-GLP study from 1975, pirimiphos-methyl (purity 97 %) was administered to groups of young Wistar-derived rats (12/sex) at dose levels of 0, 5, 8, 10 and 50 ppm in the diet for a period of 28 days [equivalent to 0.6, 1, 1.2 and 6 mg/kg bw/day (as calculated using a standard conversion factor for subacute studies taken from EFSA Journal 2012)]. There were no clinical signs of toxicity and no significant effects on bodyweight gain or food consumption.

Plasma and erythrocyte cholinesterase assays were carried out on tail vein blood samples from all animals twice pre-experimentally and on 5 animals/sex/dose group at days 1, 7 & 21 or days 3, 14 & 28. Brain cholinesterase determinations were carried out on 5 males and 5 females from each group at 28 days. Plasma cholinesterase activity was consistently inhibited in top dose group animals (about 30 % in males and 50 % in females) throughout the period of dosing. Erythrocyte cholinesterase activity was not affected at any dose throughout the experimental period. Brain cholinesterase activity showed a statistically significant ($p < 0.05$; 10 – 15 %) depression at the top dose level in both sexes, but not at lower dosages. There were no notable macroscopic findings at autopsy and no histopathological investigations were performed.

90-day study

In a non-GLP study from 1970, Alderley Park SPF rats (20/sex/dose group) were administered dietary levels of 0, 8, 80 or 360 ppm of pirimiphos-methyl (purity 93.1 %) for a period of 90 days [equivalent to 0.7, 7 and 32 mg/kg bw/day (as calculated using a standard conversion factor for subchronic studies taken from EFSA Journal (EFSA Scientific Committee, 2012))]. Recovery groups of 5/sex/group received treated diet for 90 days followed by 28 days of control diet. Plasma and erythrocyte cholinesterase activities were determined pre-dosing, at weeks 1, 2, 4, 6, 8, 10 and 12 and at weeks 1 and 4 of the recovery period. Brain cholinesterase measurements were performed at terminal sacrifice. Data were not analysed statistically. No clinical signs of toxicity were reported.

Reductions in body weight gain (approximately 20 %) and food utilisation were present in females receiving 80 and 360 ppm. Plasma cholinesterase activity was depressed (> 30 %) in the top two dose groups from week

two, returning to normal within one week after cessation of dosing. Erythrocyte cholinesterase activity was reduced in the top dose groups (34 – 60 % in males and 48 – 60 % in females) and to a lesser extent in mid-dose males and females (21 - 24 %), returning to normal during the four-week recovery period. Brain cholinesterase activity was inhibited in 80 and 360 ppm females (20 % and 42 % respectively) and to a lesser extent in top dose males (12 %), and was still depressed at the end of the recovery period.

At the end of the study, organ bodyweight ratios were found to be unaffected by treatment and there were no notable macro- or histopathological findings.

Two-generation study

Pirimiphos-methyl (purity 86.7%) was investigated over two generations in Sprague Dawley rats. Groups (28/sex F_0 and 24/sex F_1) received diets containing 0, 10, 40 or 160 ppm ≥10 weeks prior to mating (F0) throughout mating, gestation, lactation and post-weaning. Blood samples for plasma and erythrocyte cholinesterase determinations were taken early in the morning from 14/sex/group pre-treatment (F_0), pre-mating (F_0 and F_1) and at sacrifice (F_0 and F_1). Brain acetylcholinesterase determinations were performed on frontal cortex samples obtained at sacrifice from the animals used for blood sampling.

There were no effects on mating, fertility, litter size, pup weight or pup survival in either generation. Body weight gain was reduced (~ 10%) in top dosed females during gestation and lactation. Erythrocyte cholinesterase activities were found to be inhibited by > 20 % in F_0 males and females treated with 160 ppm during premating (36 % and 46 % respectively) and at sacrifice (33 and 48 % respecitvely). At 40 ppm, inhibition was 22 % in males (pre-mating) and 21 and 27 % in females (pre-mating and sacrifice respectively). In females, brain cholinesterase was also to be inhibited at sacrifice (26 and 53 % at 40 and 160 ppm respectively). No effects to brain or erythrocyte cholinesterase activity was noted at 10 ppm in the F_0 generation males and females.

In top-dosed males and females of the F_1 generation, erythrocyte cholinesterase activities were inhibited by 36 and 37 % in males (pre-mating and sacrifice respectively) and 47 % in females (pre-mating). No inhibition of erythrocyte cholinesterase was noted at 40 ppm in either sex of this generation. Brain cholinesterase was inhibited in F_1 females at the top dose only at sacrifice (44 %).

Two-year (carcinogenicity) study

In a 1974 carcinogenicity study in Wistar-derived rats, a satellite group of animals (24/sex/dose) received a dose of 0, 10, 50 or 300 ppm pirimiphos-methyl (equivalent to 0, 0.4, 2.1 or 12.6 mg/kg bw/day in both males and females) in their diet for up to 52 weeks. Groups of 8 males and females/dose were killed at 12, 26 and 52 weeks and changes to cholinesterase activities were noted.

Erythrocyte cholinesterase activity was inhibited in top dose (12 mg/kg bw/day) males (24 – 37 %) and females (27 – 43 %), but was found to be fully reversible by the end of the 4 week recovery period. Brain cholinesterase activity was also inhibited in males of the mid and top dose groups and females of the top dose group. In top dose animals, this inhibition ranged from 31-38 % in males and 30-36 % in females, but was reversible to levels below 20 % by the end of the 4 week recovery period. In mid dose males, inhibition of brain cholinesterase inhibition ranged from 22-29 %, depending on the week of study, and was fully reversible within the 4 week recovery period.

Mice

A 90-day study is available to investigate the effect of repeated doses of pirimiphos-methyl *via* the oral route in mice. Further information is available from an 18-month carcinogenicity study in mice.

90-day study

In this GLP-compliant study, carried out in 1993, groups of CD-1 mice (10/sex/dose) were fed diets containing 0, 10, 30, 90, 270 or 810 ppm pirimiphos-methyl (purity 86.7 %) (doses equivalent to 0, 2, 6, 20, 63 or 178 mg/kg bw/day in males and 0, 3, 9, 26, 80 or 284 mg/kg bw/day in females) for 13 weeks.

The top dose level of 810 ppm (178/284 mg/kg bw/day) was terminated after 2 weeks due to severe toxicity (cyanosis, pilo-erection and hunched posture).

Plasma cholinesterase activity was reduced by > 35 % in all pirimiphos-methyl treated groups after 1 week and by > 90 % at termination in groups receiving 30 ppm (6-9 mg/kg bw/day in m/f) and above. A dose-related inhibition of erythrocyte cholinesterase activity (> 8 %) was seen at all dose levels from week 1 onwards. Brain cholinesterase activity was clearly reduced at 90 ppm (20/26 mg/kg bw/day) and 270 ppm 63/80 mg/kg bw/day) in both males and females (males: 44 % and 81 % respectively and females: 35 % and 75 % respectively). In most cases the inhibition observed was statistically significant.

18-month (carcinogenicity) study

In a 1996 study, the chronic toxicity and carcinogenicity of pirimiphos-methyl was tested in CD-1 mice. Groups of mice (50/sex/dose) were used in the main study, fed diets containing 0, 50, 200 or 300 ppm (equivalent to 0, 9, 36 and 57 mg/kg bw/day) pirimiphos-methyl (≥ 86.7 % purity) for 78 weeks and a satellite group of 10/sex/dose was killed after 52 weeks. The top dose level was initially 400 ppm, but this was reduced after the first week due to body weight loss.

Survival to week 78 was > 60 % in all groups, and was similar in all female groups over most of the study, but there was an increase in mortality at week 60 (percentage not specified). An initial increased level of early deaths was evident at 200 ppm and 300 ppm in males; the likely causes were anticholinesterase effects, nephropathy or urinary bladder obstruction.

Clinical signs showed a dose-related pattern of severity and incidence at 200 and 300 ppm, including pilo-erection, dark eyes, hunched posture, cyanosis and agitation; tremors were noted at 300 ppm. After an initial reduction in body weight gain in the top- and mid- dose groups, values were similar in all groups for the remainder of the study (from approximately week 6 onwards).

In mice dosed with 200 or 300 ppm, there was an increased incidence of thymic lymphoid atrophy, a finding which appeared to be associated with poor general condition. This observation was seen only in mice dying or sacrificed during the study (but not in animals surviving to 78 weeks).

Cholinesterase activities in erythrocytes were inhibited by > 20 % in all treatment groups in males and females at both 52 weeks (57 – 75 % in males and 48 – 79 % in females) and 78 weeks (47 – 88 % in males and 65 – 84 % in females). Brain cholinesterase activity was inhibited by > 20 % in males in all treatment groups (21 – 79 %) and in females of the mid and top dose groups (62 – 66 %) at 52 weeks. At 78 weeks, brain cholinesterase activity was inhibited in the mid and top dose groups in males and females (70 -80 % in males and 44 – 58 % in females). Plasma cholinesterase activities were also reduced, dose-relatedly, by > 80 % in all treated groups.

Dogs

In a study initiated in 1970, beagle dogs (4/sex/dose level) received pirimiphos-methyl (in 0.1 ml corn oil *via* gelatine capsules) at 0, 0.5, 2 or 10 mg/kg bw/day for 2 years [it is not certain if controls received a capsule]. The test material was supplied in 6 batches, 4 of unspecified purity with the remainder of 97% and 99% purity. Dosing was suspended on week 24 due to solidification of the test material.

Clinical signs of toxicity were mainly confined to the top dose group and included loose faeces and vomiting (within half an hour of dosing). Loss of appetite and body condition occurred from week 3 in 2 males at the top dose level and resulted in a loss of weight. One of the dogs showed improved bodily condition and rapid

weight gain from week 7 onwards. The other dog showed no improvement until week 10, when appetite improved and there was subsequent gain of bodyweight.

Red blood cell cholinesterase activity was consistently depressed in animals receiving 10 mg/kg bw/day and in the latter half of the study at 2 mg/kg bw/day. There was a marked reduction in brain cholinesterase activity (54 %) in dogs receiving 10 mg/kg bw/day together with dose-related, statistically significant decreases at lower doses (15 and 18 % at 0.5 and 2 mg/kg bw/day respectively).

9.15.1.2 Dermal studies

In a study initiated in 1979, groups of NZ White rabbits (5/sex/group) received pirimiphos-methyl (90.6% pure) dermally, 5d/week for 3 weeks. Application was at 0, 4, 40 or 400 mg/kg bw/d in propylene glycol (2 ml/kg bw/d) under occlusive conditions for 6 hours, when sites were wiped. The study was not performed according to GLP, but the study was subject to Quality Assurance evaluation. The study pre-dates the requirement for formal GLP compliance.

Erythrocyte cholinesterase activity looked to be inhibited in top dose and mid dose females; with an indication of a dose-response relationship (79 % and 31 % inhibition at the top and mid dose levels respectively). However, whilst activity was inhibited in males at the top dose (66 %), activity across the dose groups showed no dose-response. Brain cholinesterase activity varied greatly between animals and groups. The small group sizes, discontinuous dosing pattern and wide inter-animal variations significantly compromised the value of this study.

9.15.1.3 Discussion of repeated dose toxicity

The most significant toxicological effects in the repeated dose studies available are associated with inhibition of acetylcholinesterase, measured in plasma, erythrocytes and brain. Inhibition of activity was observed in all 90-day oral studies in rats and mice and in the two year feeding studies in rats, mice and dogs. There was also some evidence of acetylcholinesterase inhibition in a dermal study in rabbits. In all of the studies, there were no consistent evidence of effects on clinical chemistry, haematology or at gross or microscopic examinations, other than those related to acetylcholinesterase inhibition.

In a 90-day repeated dose study and a reproductive toxicity study (approximately 90 days in duration) in rats, adverse effects to cholinesterase activities were observed from doses of 4-7 mg/kg bw/day. Erythrocyte activity was observed to be reduced by up to 27 % in both males and females, with full recovery noted following one week of cessation of dosing. Brain cholinesterase activity was reduced to a similar extent but there was no evidence of recovery in females following cessation of dosing for up to four weeks.

Inhibitory effects to brain cholinesterase in males only were observed from a dose of 2.1 mg/kg bw/day in a 2 year study in rats with recovery noted by week 4, following cessation of dosing.

Similarly, in mice, effects to cholinesterase activities were noted from a dose of 2/3 mg/kg bw/day in a 90-day repeated dose study. In particular, erythrocyte cholinesterase activity was inhibited in males and females to by 26 – 59 % from week 1. The 78-week carcinogenicity study showed cholinesterase inhibition effects in erythrocytes from a dose of 9 mg/kg bw/day in males and females (21 – 65 %) and in the brain of males only at week 52 (21 %). There was no data on recovery in this study.

In dogs, effects to cholinesterase activity were noted from a dose of 2 mg/kg bw/day in a 2-year study. These were inhibition of erythrocyte activity in males and females from week 25 (29 – 38 %).

In all cases, the level of inhibition increased with increasing dose. However, rarely did the severity of inhibition increase with time, with the exception of the 2-year dog study where inhibition of cholinesterase levels in erythrocytes did appear to increase in the first year only. Effects were seen throughout each of the

studies, at low doses and where measured, complete recovery was not always observed by the end of the 4-week recovery period.

Clinical signs of acetylcholinesterase inhibition were not seen in rats at doses equivalent to 32 mg/kg bw/day, but were reported in dogs at 10 mg/kg bw/d. In mice clinical signs were recorded at doses equal to 63 mg/kg bw/d which produced > 70 % inhibition of brain acetylcholinesterase, but not at 20 mg/kg bw/d which produced 50 % inhibition of erythrocyte acetylcholinesterase and 35 % inhibition of brain acetylcholinesterase.

In the acute toxicity study, carried out *via* the oral route, clinical signs of neurotoxicity were observed at doses of 500, 1000 and 2000 mg/kg bw (Section 9.3). These included salivation, tip toe gait and an upward curvature of the spine. It could be assumed that the cause of death in this study was acetylcholinesterase inhibition, although this was not measured, based on the high levels of acetylcholinesterase inhibition observed throughout the repeated dose studies which were carried out at much lower doses. If this were so, consideration that the effects observed in the repeated dose studies would be better characterised as an acute or single dose effect, covered by classification for acute toxicity by the oral route. According to the guidance on the application of the CLP criteria (Version 4.1 – June 2015, Section 3.9.1),

"where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure."

However, the effects observed in the repeated dosing study occurred at doses much lower than those used in the acute toxicity study (> 100-fold less), therefore it cannot be said that the toxicity occurred at a "similar dose" and to classify only with acute toxicity, category 4, would not appear to be sufficient.

To conclude, erythrocyte and brain cholinesterase activity were reduced in all studies of > 90 days in length to levels considered to be adverse (> 20 %). Plasma cholinesterase activity was also reduced by more than 20 % in some studies; however a reduction in plasma cholinesterase activity alone is not considered adverse and is generally used as an indication of absorption rather than toxicity. In the majority of studies, the reduction in erythrocyte and brain cholinesterase activity was not accompanied by adverse clinical effects and there was no reported evidence of neurological effects in any study. However, a greater than 20 % reduction of brain cholinesterase levels alone is deemed relevant enough for classification purposes.

9.15.2 Comparison with the CLP criteria

In a number of repeated dose studies in rats, mice and dogs, pirimiphos-methyl has been found to cause marked inhibition of acetylcholinesterase in both brain and erythrocytes to levels considered adverse, according to the recommendations of the WHO JMPR. According to the recommendations, the inhibition of brain cholinesterase activity and clinical signs are considered to be the primary endpoints of concern in toxicological studies on compounds that inhibit acetylcholinesterases. Inhibition of erythrocyte acetylcholinesterase is also considered to be an adverse effect, insofar as it is used as a surrogate for brain and peripheral nerve acetylcholinesterase inhibition, when data on the brain enzyme are not available. Significant inhibition of brain and erythrocyte acetylcholinesterase by 20 % or more represents a clear toxicological effect.

In both rats and mice dosed orally for 90 days and in rabbits treated dermally for 21 days, effects occurred at doses relevant for classification with STOT-RE 1 that were not always found to be reversible (≤ 10 mg/kg bw/day for a 90 day oral study and ≤ 85 mg/kg bw/day for a 21 day dermal study) (See table below).

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PIRIMIPHOS-METHYL (ISO); O-[2-(DIETHYLAMINO)-6-METHYLPYRIMIDIN-4-YL] O,O-DIMETHYL PHOSPHOROTHIOATE

Table to show the weight of evidence analysis of effects observed at doses relevant for classification with STOT-RE 1 and STOT-RE 2 in rats, mice and dogs.

Study	**Doses tested (mg/kg bw/day)**	**Criteria for STOT-RE 1 (based on those for a 90-day oral study in rats and extrapolated using Haber's rule)**	**Effects Observed**	**Criteria for STOT-RE 2 (based on those for a 90-day oral study in rats and extrapolated using Haber's rule)**	**Effects Observed**
90-Day oral study in rats (diet)	0, 0.7, 7 and 32	≤ 10 mg/kg bw/day	**7 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in females (**non-reversible**) Inhibition of erythrocyte acetylcholinesterase activity in males and females (fully reversible by 4 weeks post-recovery) ↓Body weight gain in females (~ 20 %)	> 10 mg/kg bw/day, ≤ 100 mg/kg bw/day	**32 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in females (**non-reversible**) Inhibition of erythrocyte acetylcholinesterase activity in males and females (fully reversible in males by 4 weeks post-recovery)
Two-generation study in rats (diet) (~90 days)	0, 1, 3/4 and 12/15 (F_0/F_1)	≤ 10 mg/kg bw/day	**1 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in F_1 males (pre-mating) **3/4 mg/kg bw/day**: Inhibition of brain acetylcholinesterase activity in F_0 females Inhibition of erythrocyte acetylcholinesterase activity in F_0 males	> 10 mg/kg bw/day, ≤ 100 mg/kg bw/day	**12/15 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in F_0 and F_1 females Inhibition of erythrocyte acetylcholinesterase activity in F_0 and F_1 males and females ↓Body weight gain in females (~ 10 %)
Sub-chronic Neurotoxicity study in rats (diet) (~90 days)	0, 0.2, 2.1/2.4 and 21.2/24.7 (males/females)	≤ 10 mg/kg bw/day	No treatment-related findings at < 2.4 mg/kg bw/day	> 10 mg/kg bw/day, ≤ 100 mg/kg bw/day	**21.1/24.7 mg/kg bw/day:** Significant inhibition of brain and erythrocyte acetylcholinesterase activities.

2-Year carcinogenicity study in rats (diet)	0, 0.4, 2.1 and 12.6	≤ 1.25 mg/kg bw/day	No treatment-related findings at 0.4 mg/kg bw/day	> 1.25 mg/kg bw/day, ≤ 12.5 mg/kg bw/day	**2.1 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in males (reversible 4 weeks post-dosing) **12.6 mg/kg bw/day:** Inhibition of brain and erythrocyte acetylcholinesterase activity in males and females
90-Day oral study in mice (diet)	0, 2/3, 6/9, 20/26, 63/80 and 178/284 (males/females)	≤ 10 mg/kg bw/day	**2/3 mg/kg bw/day**: Inhibition of erythrocyte acetylcholinesterase activity in males and females **6/9 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in males and females	> 10 mg/kg bw/day, ≤ 100 mg/kg bw/day	**20/26 mg/kg bw/day:** Inhibition of brain and erythrocyte acetylcholinesterase activities in males and females **63/80 mg/kg bw/day:** Inhibition of brain and erythrocyte acetylcholinesterase activities in males and females.
78-Week carcinogenicity study in mice (diet)	0, 9, 36 and 57 (mean value across both sexes)	≤ 1.65 mg/kg bw/day	Not tested to doses relevant for classification with STOT-RE 1	> 1.65 mg/kg bw/day, ≤ 16.5 mg/kg bw/day	**9 mg/kg bw/day**: Inhibition of brain cholinesterase activity in males only. Inhibition of erythrocyte acetylcholinesterase activity in both males and females
2-Year oral study in dogs (capsule)	0, 0.5, 2 and 10	≤ 1.25 mg/kg bw/day	No treatment-related findings at 0.5 mg/kg bw/day	> 1.25 mg/kg bw/day, ≤ 12.5 mg/kg bw/day	**2 mg/kg bw/day:** Inhibition of erythrocyte activity in males and females. **10 mg/kg bw/day:** Inhibition of brain and erythrocyte activities in males and females.
21-Day dermal study in rabbits	0, 4, 40 and 400	≤ 85 mg/kg bw/day	**40 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in females.	> 85 mg/kg bw/day, ≤ 857 mg/kg bw/day	**400 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in males and females.

STOT-RE 1 is assigned on the basis of findings of significant or severe toxicity. In this context "significant" means changes which are clearly indicative of functional disturbance or morphological changes which are

toxicologically relevant. Inhibition of acetylcholinesterase leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission. Therefore, on the basis of the finding of inhibition of brain and erythrocyte acetylcholinesterase activity (> 20 %) following oral administration of doses relevant for classification with STOT-RE 1 in a number of studies in rats and mice, pirimiphos-methyl should be classified with specific target organ toxicity following repeated dosing by the oral route.

9.15.3 Conclusion on classification and labelling for STOT RE

STOT-RE 1 - H372: Causes damage to organs (inhibition of acetylcholinesterase activity) through prolonged or repeated exposure. Data conclusive and sufficient for classification.

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

Summary of the Dossier Submitter's proposal

The repeated dose oral toxicity of pirimiphos-methyl has been investigated in a number of studies in rats, mice and dogs. These include a 28-day, 90-day and 2-year study in the rat, a 90-day and 78-week study in the mouse and a 2-year study in the dog. Information is also available from an oral 2-generation and an oral sub-chronic neurotoxicity study in rats. For the dermal route a 3-week study in the rabbit is available.

The most significant effect observed throughout all these studies was acetylcholinesterase inhibition, measured in plasma, erythrocytes and brain (see short study summaries below, with more details presented in Table S1 under Supplemental information). In all of the studies, there were no consistent evidence of effects on clinical chemistry, haematology or at gross or microscopic examinations, other than those related to acetylcholinesterase inhibition.

Rat, 28-day (Anon., 1975; pre-dating OECD TG and GLP)

In a 28-day dietary study in rats, no adverse effects were observed up to and including the highest dose of 6 mg/kg bw/day (brain cholinesterase activity was significantly reduced at the high dose (11-13 %) but not to levels > 20 %).

Rat, 90-day (Anon., 1970; pre-dating OECD TG and GLP)

In a 90-day dietary study in rats, a reduction in female body weight gain of approximately 20 % was observed at 7 and 32 mg/kg bw/day. At these doses, females also showed a reduction in brain cholinesterase activity of 20 % or more which did not recover within 4 weeks. Erythrocyte cholinesterase activity was reduced more than 20 % in males and females at 32 mg/kg bw/day from week 2 and in females at 7 mg/kg bw/day from week 6. Only in females at 32 mg/kg bw/day the reduction did not fully recover within 4 weeks.

Rat, 92-day neurotoxicity study (Anon., 1995a; according to OECD TG 424 and GLP)

In a 92-day dietary neurotoxicity study in rats, a reduction in erythrocyte cholinesterase activity of more than 20 % was observed from week 3 at the highest dose of 21.1 (males) or 24.7 (females) mg/kg bw/day. The same dose also induced a reduction in brain cholinesterase

activity of more than 20 % in both sexes. No effects were observed at the next lower dose level of approximately 2 mg/kg bw/day and below.

Rat, 2-generation study (Anon., 1995; according to OECD TG 416 and GLP)

In a dietary 2-generation study in rats, a reduction in female body weight gain of approximately 10 % was observed at the highest dose of 12 (F_0) and 15 (F_1) mg/kg bw/day. Erythrocyte cholinesterase activity was reduced at some time points at 1 (males only), 3/4 (F_0/F_1) and 12/15 (F_0/F_1) mg/kg bw/day. Brain cholinesterase activity was reduced by more than 20 % in females at 3 (F_0) and 4 (F_1) mg/kg bw/day and above.

Rat, combined chronic and carcinogenicity study (Anon, et al., 1974; pre-dating OECD TG and GLP)

In a dietary chronic (2-year) study in rats, a small increase in severe fatty vacuolation of the liver was observed in females at the highest dose of 12.6 mg/kg bw/day (17 % versus 7 % in controls). At the same dose, also a small increase in cystic vacuolation of the epididymis was observed (4/42 males versus 0 in controls). Erythrocyte cholinesterase activity was reduced by more than 20 % at the highest dose in both males (from week 12) and females (from week 2), but was reversible within 4 weeks. Brain cholinesterase activity was reduced by more than 20 % at 2.1 (males only) and 12.6 mg/kg bw/day. At the highest dose this reduction was not fully reversible within 4 weeks, although the level of inhibition diminished to < 20 %.

Mice, 90-day (Anon., 1996; according to OECD TG 408 and GLP)

In a dietary 90-day study in mice, the highest dose group (178/284 mg/kg bw/day m/f) was terminated after week 2 due to severe toxicity. Clinical signs (cyanosis, piloerection and hunched posture), slight reductions in body weight and reduced food consumption were observed at the next lower dose of 63/80 mg/kg bw/day (m/f) but not at lower doses. Erythrocyte cholinesterase activity was reduced by more than 20 % from week 1 at 2 (males) and 9 (females) mg/kg bw/day and above. In females at 3 mg/kg bw/day such a reduction was observed from week 3 onwards. Brain cholinesterase activity was reduced in all groups, but by more than 20 % only from 20/26 mg/kg bw/day (m/f).

Mice, combined chronic and carcinogenicity study (Anon. et al., 1996; according to OECD TG 451 and GLP)

In a dietary chronic (78-week) study in mice, the highest dose was reduced from 400 ppm (72 mg/kg bw/day) to 300 ppm (57 mg/kg bw/day) after week 1 due to body weight loss. An initial increased level of early deaths was evident at 36 and 57 mg/kg bw/day in males; likely causes of the deaths were anticholinesterase effects, nephropathy or urinary bladder obstruction. Clinical signs showed a dose-related pattern of severity and incidence at 36 and 57 mg/kg bw/day and included pilo-erection, dark eyes, hunched posture, cyanosis and agitation, with tremors additionally noted at 57 mg/kg bw/day. In mice from the 36 and 57 mg/kg bw/day groups, dying or sacrificed during the study (but not in animals surviving to 78 weeks), there was an increased incidence of thymic lymphoid atrophy, a finding which appeared to be associated with poor general condition. A reduction in cholinesterase activity of more than 20 % was observed in erythrocytes from the lowest tested dose of 9 mg/kg bw/day. In brain this reduction was mainly seen from 36 mg/kg bw/day, but males at 9 mg/kg bw/day also showed a reduction of more than 20 % at the interim sacrifice after 52 weeks.

Dogs, 2-year (Anon., 1973; pre-dating OECD TG and GLP)

In a 2-year study in dogs (4/sex/dose) using oral capsules, brain cholinesterase activity was significantly reduced at 0.5, 2 and 10 mg/kg bw/day, but by more than 20 % only at the highest dose. Erythrocyte cholinesterase activity was reduced by more than 20 % at 2 (from week 25/26) and 10 mg/kg bw/day (from week 1). Clinical signs of toxicity were mainly observed in the top dose group and included loose faeces and vomiting (within half an hour of dosing). Loss of appetite and body condition occurred from week 3 in 2 males at the top dose level and resulted in a loss of weight. However, body weight gain improved in these two animals from week 7 or 10.

Dermal study in rabbits (Anon., 1980; pre-dating OECD TG and GLP)

In a 3-week dermal study in rabbits (5/sex/dose, treatment on 5 days/week), erythrocyte cholinesterase activity was reduced above 20 % in females at all dose levels (4, 40 and 400 mg/kg bw/day) and in males at 400 mg/kg bw/day. Brain cholinesterase activity varied greatly between animals and groups. Mortalities (cause unknown) were seen in 3 top dose animals, 1 mid-dose animal and 1 low dose animal but not in controls.

In evaluating the effects on acetylcholinesterase inhibition in the available studies, the DS referred to the recommendations of the WHO JMPR, stating that the inhibition of brain cholinesterase activity and clinical signs are considered to be the primary endpoints of concern in toxicological studies on compounds that inhibit acetylcholinesterases. Inhibition of erythrocyte acetylcholinesterase is also considered to be an adverse effect, insofar as it is used as a surrogate for brain and peripheral nerve acetylcholinesterase inhibition, when data on the brain enzyme are not available. In line with these recommendations, the DS considered a significant inhibition of brain and erythrocyte acetylcholinesterase by 20 % or more to represent a clear toxicological adverse effect, even when not accompanied by clinical signs. As inhibition of acetylcholinesterase leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors and disrupted neurotransmission, the DS further considered the adverse effects to warrant classification for STOT RE, if occurring at levels at or below the cut-off for classification.

When looking at the oral data available on pirimiphos-methyl, cholinesterase activity inhibition in the brain or in erythrocytes by more than 20 % was observed in all studies with rats, mice and dogs (at almost all dose levels tested), with the exception of the 28-day rat study. There was also some evidence of acetylcholinesterase inhibition in the dermal rabbit study. The inhibition was dose dependent but rarely increased with time. It was not always found to be (fully) reversible. Clinical signs were only observed in dogs (at 10 mg/kg bw/day) and mice (at dose levels from 36 mg/kg bw/day), at the higher dose levels tested. No clinical signs were observed in rats tested up to 32 mg/kg bw/day in the repeated dose studies. In contrast, in acute oral studies in rats, clinical effects were observed at doses ranging from 500 to 2000 mg/kg bw. The DS assumed that these effects as well as the observed mortality at 2000 mg/kg bw were caused by cholinesterase activity inhibition. Whilst recognizing that the effects in the repeated dose studies could also be an acute or single dose effect, the DS noted that the effects on cholinesterase activity occurred at much lower dose levels (> 100 fold less) in the repeated dose studies as compared to the acute studies. Given this difference, the DS concluded that the toxicity did not occur at a "similar dose" and that classification only with acute toxicity, category 4, would not appear to be sufficient.

According to the DS, the lowest levels at which cholinesterase inhibition above 20 % occurred was approximately 3-7 mg/kg bw/day in rats (as observed in oral 90-day studies), 2/3 mg/kg bw/day in mice (in oral 90-day study), 2 mg/kg bw/day in dogs (in oral 2-year study) and 40 mg/kg bw/day in rabbits (in dermal 3-week study). Since these levels in rats, mice and rabbits are below the cut-off for classification with STOT RE 1 (10 mg/kg bw/day for an oral 90-day study, 85 mg/kg bw/day for a dermal 3-week study), classification with STOT RE 1 was proposed. No specification of the route was suggested; the suggested target organ was "inhibition of acetylcholinesterase activity".

Comments received during public consultation

One MSCA agreed to the proposed classification in general and another MSCA specifically agreed that an inhibition of brain or erythrocyte of more than 20 % is deemed relevant enough for classification purposes and agreed with classification with STOT RE 1. With reference to a previous RAC opinion on phosmet, also an inducer of acetylcholinesterase inhibition, IND proposed classification with STOT SE 1 because the cholinesterase activity is rarely associated with any clinical effect and inhibition is observed both after single and repeated exposure without evidence that the effect increases with increased duration of exposure. Therefore, it is unclear whether the observed inhibition is an acute or a repeated dose effect. In their response the DS pointed to the very much lower doses at which inhibition occurred following repeated dosing as compared to single dosing, and remained of the opinion that classification with STOT RE is justified.

Assessment and comparison with the classification criteria

An overview of the effects observed on cholinesterase inhibition and/or clinical signs in studies with repeated dosing of pyrimiphos-methyl is presented in Table S1 in the Background Document. This table includes some studies that were not mentioned in the CLH report but were present in the DAR.

It can be seen that the level of cholinesterase inhibition is dependent on dose but not so much on study duration. It was not always found to be (fully) reversible, with the activity of acetylcholinesterase in brain more slowly recovering than that in erythrocytes.

It can further be seen that in most studies clinical signs typical for organophosphates were not observed (nor were microscopical lesions in the nervous system), despite considerable inhibition of brain and erythrocyte acetylcholinesterase. In rats, clinical effects indicating neurotoxicity were not observed in any of the repeated dose studies described in the CLH report. RAC however notes that in these studies pirimiphos-methyl was tested at relatively low dose levels of 0.2-32 mg/kg bw/day, and that these levels hardly, if at all, induced general toxicity. Higher doses could therefore have been tested, which could possibly have resulted in clinical signs. This is supported by the findings at higher doses in a rat oral developmental toxicity study, which was not mentioned in the CLH report but was present in the DAR. In this (10-day) study, clear toxicity was observed at 150 mg/kg bw/day, but not at 1.5 and 15 mg/kg bw/day. The toxicity included clinical signs (abnormal gait, urinary incontinence, piloerection and body tremors), death (1/24 dams), and reduced maternal body weight gain (~ 50 %) and food consumption (~ 15 %). In mice, the doses tested were higher than in most rat studies, indeed resulting in general toxicity and signs of neurotoxicity. Severe toxicity at 178/284 mg/kg bw/day (m/f) (90-day study) and body weight loss at 72 mg/kg bw/day

(chronic study) was evident within the first 1-2 weeks of treatment. Clinical signs indicating effects on the nervous system were observed from 36 mg/kg bw/day in the chronic study, and at 63/80 mg/kg bw/day (m/f) in the 90-day study. For dogs only a 2-year study was available, showing at the highest tested dose of 10 mg/kg bw/day loose faeces, vomiting and transient body weight effects. According to the study authors these effects may be secondary to the capsule dosing in a small volume (0.1 mL) to which the dogs adapted for the latter 80 % of the study. Given that the effects were already observed within the first weeks of the study, more severe effects might be expected in dogs in studies of shorter duration with higher dose levels.

RAC notes that in the available study summaries the time of onset of the clinical effects in rats, mice and dogs is not described. Hence, it is unclear whether the signs related to cholinergic inhibition appeared following repeated exposure or already in response to the first exposure(s). Comparison of the occurrence of clinical effects, after acute and repeated exposure, is only possible for rats (for mice and dogs there are no acute studies). Even for rats it can only be done on the basis of the developmental toxicity study, where gavage doses of 150 mg/kg bw/day resulted in abnormal gait, urinary incontinence, piloerection and body tremors, and one out of 24 dams dying. This observation contrasts with the absence of such effects in an acute oral neurotoxicity study with rats (also not mentioned in the CLH report but present in the DAR; see Table S2 under Supplemental information) at the same dose also by gavage. When further comparing the level of brain and erythrocyte acetylcholinesterase inhibition after acute and repeated exposure (see Table S3 under Supplemental information), it can be observed that comparable levels of inhibition after single exposure to 150 mg/kg bw/day can be achieved by much lower repeated exposures.

The above indicates that repeated exposure to a certain pirimiphos-methyl dose results in more severe effects than a single dose. Although there is limited evidence of accumulation of cholinesterase inhibition with repeated dosing, the slow recovery of brain and erythrocyte cholinesterase activity observed in some rat studies could be evidence of accumulation of the substance or its metabolites or slow reversibility of the binding to cholinesterase.

Based on the above comparisons (including the slow recovery of the inhibition), and assuming that also in mice and dogs the effects are not a response to the first exposure(s), RAC considers classification for STOT RE (as proposed by the DS) more appropriate than classification for STOT SE (as suggested during public consultation). Whereas there are indeed some similarities in the toxicity profile between the two acetylcholinesterase inhibitors, pirimiphos-methyl and phosmet, as remarked during public consultation, RAC notes also some differences. For phosmet, the level of acetylcholinesterase inhibition induced by a single dose of 22.5 mg/kg bw was at the same level (in erythrocyte) or higher (in brain) as that of a similar dose level in studies of longer duration. For pirimiphos-methyl that was not the case (see comparison above). Moreover, in an acute neurotoxicity study, clinical signs typical for organophosphate exposure were observed at an oral phosmet dose of 36 mg/kg bw, i.e. a dose lower than those triggering the acute oral toxicity classification for phosmet (category 3, 50 < ATE ≤ 300 mg/kg bw). For pirimiphos-methyl, such clinical signs were observed in acute studies within the dose-range triggering its acute toxicity category 4 classification (300-2 000 mg/kg bw, ATE = 1 414 mg/kg bw).

According to the criteria, classification for STOT RE based on evidence from studies in animals requires significant and/or severe toxic effects of relevance to humans at low (category 1) or moderate (category 2) exposure. 'Significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant, whereas for 'severe'

the effects are generally more profound or serious and significantly impact on health. Effects qualifying for classification include among others 'significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell)' (CLP Annex I 3.9.2.7.3(b)) and 'any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters' (CLP Annex I 3.9.2.7.3(c)).

The DS considered a statistically significant and greater than 20 % inhibition of brain and erythrocyte acetylcholinesterase sufficient to fulfil the criteria, without the need to have adverse clinical effects indicative of neurotoxicity present. RAC notes that the cut-off of 20 % inhibition is used for risk assessment of acetylcholinesterase inhibitors, and that in general much higher reduction is needed for clinical effects to become manifest. From that perspective, placing the evidence/presence of clinical signs first in the hierarchy of adversity assessment of cholinesterase inhibition (conform the WHO JMPR guidance), might possibly meet the CLP requirement for adversity more than solely a ≥ 20 % inhibition of brain or erythrocyte acetylcholinesterase. Yet, also the inhibition seems to qualify, given criterion (c) above. Recognising the adversity, in particular of brain acetylcholinesterase inhibition, and that the degree of acetylcholinesterase inhibition that can be tolerated without clinical symptoms can vary between individuals and substances, RAC supports the approach of the DS. RAC subsequently supports the proposal for STOT RE 1; H372, because in most studies in rats, mice and dogs, a ≥ 20 % inhibition of brain or erythrocyte acetylcholinesterase was observed at dose levels around or below 10 mg/kg bw/day, the guidance value for STOT RE 1 for a 90-day oral study. RAC notes that the DS has extrapolated this guidance value for study durations other than 90 days, according to Haber's rule. It is however doubtful whether that is appropriate in this particular case, given that a maximum level of inhibition of erythrocyte acetylcholinesterase was already achieved after a couple of weeks of treatment, which did not further increase with longer study duration/treatment.

In the 3-week dermal toxicity study in rabbits, inhibition of erythrocyte cholinesterase activity was observed at all dose levels (4-400 mg/kg bw/day, on 5 days/week), some of which (or all, depending on whether extrapolation is appropriate) would warrant classification for STOT RE 1/2 (guidance values are 20 and 200 mg/kg bw/day, respectively, for a 90-day dermal study).

No repeated dose inhalation study was available. In an acute inhalation study (not mentioned in the CLH report but present in the DAR), inhibition of plasma and erythrocyte cholinesterase activity was observed, showing systemic bioavailability following inhalation exposure. Therefore, classification for STOT RE via the inhalation route cannot be excluded.

Overall, RAC supports classification of pirimiphos-methyl with **STOT RE 1; H372**, without specification of the route and with the **nervous system** as target organ.

Supplemental information - In depth analyses by RAC

In Table S1 an overview is presented of effects observed at doses relevant for classification with STOT RE. Although criteria for STOT RE 1/2 are given based on extrapolation from the guidance values for a 90-day study (in line with DS proposal), RAC doubts the appropriateness of the extrapolation in this case, given that the level of inhibition of acetylcholinesterase activity did not increase with study duration.

Table S1. *Overview of effects observed in studies with repeated dosing.*

Study	**Doses tested (mg/kg bw/day)**	**GV in mg/kg bw/day for STOT RE 1**	**Effects Observed**	**Criteria for STOT RE 2 (GV in mg/kg bw/day)**	**Effects Observed**
28-day oral study in rats (diet)	0, 0.6, 1, 1.2 and 6	≤ 30	No treatment-related findings at ≤ 6 mg/kg bw/day	> 30, ≤ 300	No treatment-related findings at ≤ 6 mg/kg bw/day
90-day oral study in rats (diet), plus 4-week recovery period	0, 0.7, 7 and 32	≤ 10	**7 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in females (20 %; **non- reversible**) Inhibition of erythrocyte acetylcholinesterase activity in males (21 %; week 6 only) and females (22-24 % in weeks 6-12) (fully reversible by 4 weeks post-recovery) ↓Body weight gain in females (~ 20 %) [No clinical signs]	> 10, ≤ 100	**32 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in females (42 %; **non-reversible**) Inhibition of erythrocyte acetylcholinesterase activity in males (34-60 % in weeks 2-6; fully reversible by 4 weeks post-recovery) and females (48-60 % in weeks 2-12; **non-reversible**) ↓Body weight gain in females (~ 20 %) [No clinical signs]
Two- generation study in rats (diet) (~90 days)	0, 1/1, 3/4 and 12/15 (F_0/F_1)	≤ 10	**1/1 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in F_1 males (21 %; premating only) **3/4 mg/kg bw/day**: Inhibition of brain acetylcholinesterase activity in F_0 females (26 %) Inhibition of erythrocyte acetylcholinesterase activity in F0 males (22 %; premating only) and females (21-27 %) [No clinical signs]	> 10, ≤ 100	**12/15 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in F_0 and F_1 females (53 and 44 %, resp.) Inhibition of erythrocyte acetylcholinesterase activity in F_0 males (33-36 %) and females (46-48 %) and in F_1 males (36-37 %) and females (47 %; premating only) ↓Body weight gain in females (~ 10 %) [No clinical signs]

Sub-chronic Neurotoxicity study in rats (diet) (~90 days)	0, 0.2/02, 2.1/2.4 and 21.2/24.7 (males/ females)	≤ 10	No treatment-related findings at ≤ 2.1/2.4 mg/kg bw/day	> 10, ≤ 100	**21.1/24.7 mg/kg bw/day:** Significant inhibition of brain (males 20-30 %, females 32-51 %) and erythrocyte acetylcholinesterase activities (males 37-45 % and females 38-46 % in weeks 3-13) [No clinical signs, no neuropathy, no effects on FOB]
2-Year carcinogenicity study in rats (diet), plus 4-8 week recovery period	0, 0.4, 2.1 and 12.6	≤ 1.25	No treatment-related findings at 0.4 mg/kg bw/day	> 1.25, ≤ 12.5	**2.1 mg/kg bw/day:** Inhibition of brain acetylcholinesterase activity in males (22-29 %; fully reversible 4 weeks post-dosing) **12.6 mg/kg bw/day:** Inhibition of brain (males 31-38 %, females 30-36 %; reversible to inhibition levels < 20 % within 4 weeks post-dosing) and erythrocyte acetylcholinesterase activity (males 24-37 % in weeks 12-104, females 27-43 % in weeks 2-104; fully reversible 4 weeks post-dosing) [No clinical signs]
90-day oral study in mice (diet)	0, 2/3, 6/9, 20/26, 63/80 and 178/284 (males/ fem ales) [Top dose terminated after 2 weeks due to severe toxicity]	≤ 10	**2/3 mg/kg bw/day**: Inhibition of erythrocyte acetylcholinesterase activity in males (38-45 % in weeks 1-13) and females (26-34 % in weeks 3-13) **6/9 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in males (42-59 %) and females (26-52 %) in weeks 1-13	> 10, ≤ 100	**20/26 mg/kg bw/day:** Inhibition of brain (males 44 %, females 35 %) and erythrocyte acetylcholinesterase activities (males 57-71 % and females 51-70 % in weeks 1-13) **63/80 mg/kg bw/day:** Inhibition of brain (males 81 %, females 75 %) and erythrocyte acetylcholinesterase activities (males 68-75 % and females 68-78 % in weeks 1-13) Clinical signs (cyanosis, piloerection and hunched posture)

78-week carcinogenicity study in mice (diet)	0, 9, 36 and 57/72 (mean value across both sexes) [Top dose reduced after 1 week due to bw loss]	≤ 1.65	Not tested to doses relevant for classification with STOT RE 1	> 1.65, ≤ 16.5	**9 mg/kg bw/day**: Inhibition of brain cholinesterase activity in males (21 %) at 52 weeks only Inhibition of erythrocyte acetylcholinesterase activity in both males (47-57 %) and females (48-65 %) **36 and 57 mg/kg bw/day:** Inhibition of brain (males 55-80 %, females 44-66 %) and erythrocyte acetylcholinesterase activities (males 75-88 %, females 77-84 %) Clinical signs (pilo-erection, dark eyes, hunched posture, cyanosis and agitation; + tremors at 57 mg/kg bw/day)
2-year oral study in dogs (capsule)	0, 0.5, 2 and 10	≤ 1.25	No treatment-related findings at 0.5 mg/kg bw/day	> 1.25, ≤ 12.5	**2 mg/kg bw/day:** Inhibition of erythrocyte activity in males and females from week 25/26 (29-38 %) **10 mg/kg bw/day:** Inhibition of brain (54 %) and erythrocyte acetylcholinesterase activities (37-77 %, from week 1) in males and females Clinical signs (loose faeces and vomiting)
3-week dermal study in rabbits, with treatment on 5d/week	0, 4, 40 and 400	≤ 120 [DS had 85, but 15 and not 21 days of treatment]	**4 and 40 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in females (21 and 26 %, resp.)	> 120, ≤ 1200 [DS > 85, ≤ 857]	**400 mg/kg bw/day:** Inhibition of erythrocyte acetylcholinesterase activity in males (48 %) and females (78 %)
Oral developmental toxicity study in rats (gavage) GD7-16 [study only reported in DAR, not in CLH report]	0, 1.5, 15 and 150	≤ 90	No treatment-related findings at ≤15 mg/kg bw/day	> 90, ≤ 900	**150 mg/kg bw/day:** Clinical signs (abnormal gait, urinary incontinence, piloerection, body tremors); 1/24 died [No cholinesterase determinations performed]

Oral developmental toxicity study in rabbits (gavage) GD6-18 [study only reported in DAR, not in CLH report]	0, 12, 24 and 48	≤ 70	**12, 24 and 48 mg/kg bw/day:** Inhibition of erythrocyte cholinesterase activity on GD19 (21-62 %), but reversible to inhibition levels < 20 % on GD29 Inhibition of brain cholinesterase activity at 48 (38 %) on GD29 [No clinical signs]	> 70, ≤ 700	
Sub-chronic delayed neurotoxicity study hens (gavage) (90 days) [study only reported in DAR, not in CLH report]	0, 0.5, 1.0, 2.5, 5.0 and 10	≤ 10	**2.5 and 10 mg/kg bw/day:** Mortality, clinical signs (weakness, ruffled feathers, leg stiffness, exaggerated leg movements, unsteadiness or wing drooping), but no clinical signs of ataxia or histopathological changes ➔ No delayed neurotoxicity [No cholinesterase and NTE determinations performed]	> 10, ≤ 100	

Table S2 presents the effects observed in the acute oral studies available, in order to compare the doses and effects after acute and repeated exposure. This is possible only for rats (see Table S3).

Table S2*. Overview of effects observed in acute oral studies*

Study	**Doses tested (mg/kg bw/day)**	**Effects Observed**
Acute oral study in rats (gavage)	500, 1000 and 2000	**500, 1000 and 2000 mg/kg bw**: Clinical signs indicative of neurotoxicity at all dose levels, with dose relationship increase in severity Mortality at 2000
Acute oral neurotoxicity study in rats (gavage) [study only reported in DAR, not in CLH report]	0, 15, 150 and 1500	**1500 mg/kg bw**: Clinical signs, including convulsions, up to day 5; effect on FOB on day 1; inhibition of brain (males 67 %, females 59 %; average over 6 regions) and erythrocyte cholinesterase activity (males 71 %, females 63 %) **150 mg/kg bw**: Inhibition of brain (males 9-29 % with average 17 %, females 6-28 % with average 16 %) and erythrocyte cholinesterase activity (males 39 %, females 21 %) **15 mg/kg bw**: Inhibition of erythrocyte cholinesterase activity (males only, 26 %)
Acute delayed neurotoxicity	100	**100 mg/kg bw**: Mortality; transient clinical signs up to 10 days post-dosing in surviving animals (subdued appearance, unsteadiness, leg paralysis and inability to

study in hens [study only reported in DAR, not in CLH report]		stand); inhibition of brain cholinesterase activity (> 75 %); no clinical signs of delayed neurotoxicity and no effect on neuropathy target esterase (NTE) or on histopathology ➔ no acute delayed neurotoxicity

Table S3. Comparison of doses and effects in acute and repeated dose studies with rats

	ACUTE STUDIES	REPEATED DOSE STUDIES	DEVELOPMENTAL TOXICITY STUDY
Doses tested (in mg/kg bw (/day))	15-2000	0.2-32	1.5-150
Clinical signs	From 500	No	At 150
Mortality	At 2000	No	1/24 at 150
Inhibition of brain cholinesterase activity > 20 %	From 150	At 2.1 (2-year males), 3 (2-gen F0 females), 7 (90-day females), 12.6 (2-year), 12/15 (2-gen F0/F1 females), 21.1/24.7 (90-d neurotox) and 32 (90-day females)	n/a
inhibition of erythrocyte cholinesterase activity > 20 %	From 15 (males), from 150 (females)	At 1 (2-gen F1 males), 3 (2-gen F0), 7 (90-day), 12.6 (2-year), 12/15 (2-gen F0/F1), 21.1/24.7 (90-day neurotox) and 32 (90-day)	n/a

9.16 Aspiration hazard

Hazard class not assessed in this dossier.

10 EVALUATION OF ENVIRONMENTAL HAZARDS

Pirimiphos-methyl is a broad-spectrum organophosphate insecticide with restricted pesticidal use only within grain stores and related industrial storage sites for post-harvest treatment of cereal grain, or as a fabric hygiene treatment of storage facilities or grain handling equipment. Available environmental fate and hazard studies have been considered under Regulation 1107/2009, refer to RAR Volume 3 Annex B Section B8: Environmental Fate and Behaviour (2016) and Volume 3 Annex B Section B9: Ecotoxicology (2016). The key information pertinent to determining a hazard classification is presented.

10.1 Rapid degradability of organic substances

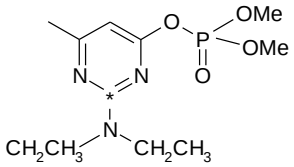
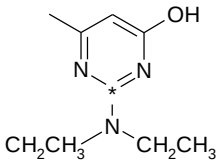
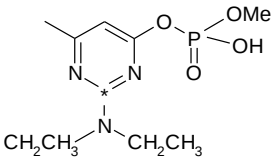
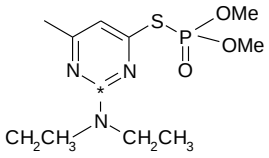
Table 10: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Aqueous hydrolysis in sterile solutions.	DT_{50} water: 2 days at pH 4, 7 days at pH 5, 117 days at pH 7, 75 days at pH 9 (25 ^{0}C). Degradants:	Study is considered valid.	Hand, 1996, (RAR Vol 3CA B8 Section CA.B.8.2.2)

Method	Results	Remarks	Reference
EPA, Subdivision N, Section 161-1 and EEC Method C7 guidelines. GLP.	R046382 max 99.1% AR R402186 max 12.8% AR Mineralisation not directly reported.		
Aqueous photolysis. EPA 161-2 and 161-3 guidelines. GLP.	DT_{50} in water in presence of light: 0.46 hours at pH 5, 0.47 hours at pH 7 (at 25 °C). Photodegradates: R046382 max 63% AR R290438 max 14.5% AR Mineralisation not directly reported.	Study is considered valid.	Powel, 1999, (RAR Vol 3CA B8 Section CA.B.8.2.3)
Water / sediment system DT_{50}. BBA Guidelines Part IV, Section 5-1, 1990 GLP.	Whole system DT_{50}: Arithmetic mean 9.4 days (at 20 °C) Mineralisation: Total volatiles reached a maximum of 35% AR at 59 days and had a maximum value at 100 days of 31.2% AR	Study is considered valid.	Kirkpatrick, 1994, (RAR Vol 3CA B8 Section CA.B.8.2.5)

DT_{50} values have not been recalculated to 12 °C since it is evident from the mineralization data generated at 20 - 25 °C that pirimiphos-methyl is not rapidly degradable. Re-calculation of DT_{50}s to 12 °C would result in longer values but does not change the outcome of the classification.

Table 11: Structure of parent and aquatic degradants

Parent	R046382	R402186	R290438
 * position of radiolabel	 * position of radiolabel	 * position of radiolabel	 * position of radiolabel
Pirimiphos-methyl	O-2-diethylamino-6-methylpyrimidin-4-ol	O-2-diethylamino-6-methylpyrimidin-4-yl-O-methylphosphorothioate	S-2-diethylamino-6-methylpyrimidin-4-yl-O,O-dimethylphosphorothioate

10.1.1 Ready biodegradability

No studies on ready biodegradability are available.

10.1.2 BOD_5/COD

No BOD_5/ COD reported for pirimiphos-methyl.

10.1.3 Hydrolysis

An aqueous hydrolysis study (Hand, 1996) is available following GLP and EPA, Subdivision N, Section 161-1. Pirimiphos-methyl was applied to sterile buffer solutions of pH 4, 5, 7 and 9 and stored at 25 °C for 30 days in the dark. It was demonstrated that pirimiphos-methyl underwent hydrolytic degradation dependant on the

pH, with the shortest DT_{50} values in acidic pH (DT_{50} 2 days at pH 4, 7 days at pH 5, 117 days at pH 7 and 75 days at pH 9). Two degradation products were determined as O-2-diethyl amino-6-methylpyrimidin-4-yl O-methyl phosphorothioate (R402186) and 2-diethylamino-6-methylpyrimidin-4-ol (R046382) at maxima of 12.8% AR and 99.1% AR respectively. Mineralisation was not directly reported in the study however based on the levels of metabolite remaining at the end of the study it was clear that mineralisation was insignificant.

10.1.4 Other convincing scientific evidence

10.1.4.1 Water, water-sediment data (including simulation studies)

A water sediment study conducted to Biological Research Centre for Agriculture and Forestry (BBA), Germany (Guidelines Part IV, Section 5-1, December 1990) and GLP (Kirkpatrick 1994). The rate and route of degradation of pirimiphos-methyl was investigated in two different water sediment systems. The test substance was applied to the water at 15 µg/cm^2. The systems were incubated under aerobic conditions in the laboratory and maintained in dark conditions at 20 °C ± 2 °C for up to 100 days. The total mean recovery of radioactivity from each test system at each sampling time was greater than 93 % of the applied radioactivity in all cases. Pirimiphos-methyl dissipated rapidly from the water phase ($DissT_{50}$ less than 1day), partitioning to sediment was rapid. Degradation in the whole water/sediment systems was also fairly rapid (10.3 days and 8.51 days). Pirimiphos-methyl was degraded by hydrolysis to form a major metabolite up to approximately 60 % applied radioactivity (2-diethylamino-4-hydroxy-6-methyl pyrimidine; R46382). A further metabolite reached a maximum of approximately 20 % of applied radioactivity after 30 days and declined to 2-4 % after 100 days (*O*-2-diethylamino-6-methylpyrimidin-4-yl *O*-methyl phosphorothioate: R402186). This study also demonstrated that volatilisation from water reached a maximum of 31.2 % AR. The total amount of volatiles reached a maximum of 35 % throughout the duration of the study. Therefore the level of mineralisation is below 70 % and the study demonstrated that pirimiphos-methyl was not rapidly degraded according to CLP criteria.

10.1.4.2 Photochemical degradation

An aqueous photolysis study (Powel, 1999) following GLP and EPA 161-2 and 161-3 guidelines is available. Test solutions were incubated at pH 5 and 7 at 25 °C under constant irradiation. Pirimiphos-methyl was calculated as having a photolytic first order DT_{50} of 0.46 and 0.47 hours of Florida Summer Sunlight at pH 5 and 7 respectively. Photolysis resulted in the major degradate 2-diethylamino-6-methylpyrimidin-4-ol (R046382), reaching maximum 63 % AR at the end of the study at both pH values, and S-2-diethylamino-6-methylpyrimidin-4-yl-O,O-dimethylphosphorothioate (R290438) being formed up to 14.5 %, but degrading rapidly to final levels of 2.8 % and 3.3 % AR at pH 5 and pH 7 respectively. Mineralisation was not directly reported in the study but based on the levels of metabolites it was clear that levels of mineralisation were insufficient to classify the active substance as rapidly degraded.

10.2 Summary and discussion of degradation

Pirimiphos-methyl demonstrates pH dependence with regard to hydrolytic stability, with most rapid degradation occurring at pH 4. The level of mineralisation was not reported within the hydrolysis study, however based on the levels of metabolite remaining at the end of the study it was clear that mineralisation was insignificant.

No ready biodegradability studies are available.

No aerobic mineralisation study was submitted, and no study was requested since the proposed use precluded significant exposure to larger water bodies.

Pirimiphos-methyl was observed to undergo rapid primary degradation in the aqueous photolysis study. A DT_{50} value of 0.47 hours was calculated. Photolysis is of uncertain relevance as a route of degradation in

typical European aquatic environments and, given the available data, there is insufficient information in this case to evaluate photodegradation in terms of mineralisation. Based on the levels of metabolites it was clear that levels of mineralisation were insufficient to classify the active substance as rapidly degraded. Therefore, aquatic photolysis is not considered further in relation meeting the criteria for rapid degradation.

In an aerobic water/sediment study, pirimiphos-methyl a maximum of 35 % mineralisation to CO_2 was observed over the course of 100 days. Total system $DegT_{50}$ values generated an arithmetic mean value of 9.4 days (at 20 °C).

Overall, the degradation information does not provide sufficient data to show pirimiphos-methyl is ultimately degraded, to >70 % degradation within 28 days (equivalent to a half-life < 16 days), or transformed to non-classifiable products as there is no available ecotoxicity data on the degradation products. Consequently, pirimiphos-methyl is considered 'not rapidly degradable' for the purpose of classification and labelling.

10.3 Environmental fate and other relevant information

Adsorption/ desorption in 6 soils was assessed in Hartfree *et al*, 1993. The study was not carried out to a specific guideline but is considered to meet the requirements included in SETAC 1995 Section 4 guidance and was GLP compliant. The Kf_{oc} was determined to be 950-8500 ml/g (geometric mean 2204 ml/g) which indicates that pirimiphos-methyl is likely to partition to sediment in aquatic systems.

Volatilisation from soil and leaf structures (MacIver et al, 1996), volatilisation was 21.9 % AR and 34.4 % AR from soil and leaf surface respectively after 24 hours. The study was performed according to BBA Guidelines Part IV Method 6.1 and was GLP compliant.

Calculation of half-life with atmospheric hydroxyl radicals (Hayes, 1998), with the DT_{50} air determined to be 0.8 hours.

Literature search: No relevant scientifically peer-reviewed open literature references were identified for pirimiphos-methyl.

Table 12: Endpoints from other environmental fate studies

Method	Results	Remarks	Reference
Adsorption and desorption in soil. GLP.	Geometric mean Koc 2204ml/g, arithmetic mean 3042ml/g	Study is considered valid	Hartfree et al, 1993 (RAR Vol 3 CA B8 section CA.B.8.1.2)
Volatilisation from soil and leaf surface. BBA Guidelines Part IV Method 6.1. GLP.	Vapour pressure $2.0x10^{-3}$ Pa Volatility, Henry's law constant 0.06 Pa m^3 mol^{-1}	Study is considered valid	MacIver and Hand, 1996, (RAR vol3 CA B8 Section CA B.8.3.1)
Calculation of half-life with atmospheric hydroxyl radicals	Air DT_{50}: 0.8 hours	Study is considered valid.	Hayes, 1998, (RAR Vol 3CA B8 Section CA.B.8.3.2)

10.4 Bioaccumulation

Table 13: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water	Log P_{ow} = 4.2 at 20 °C	Accepted in the original DAR.	Husband, 1997
Experimental aquatic BCF OECD Guideline 305 GLP	BCF_{ss} (corrected, 5% lipid content) : 1013.4 K_u: 1008 K_d: 0.84	Flow through, 28 days exposure, 14 days depuration	Anon., 2007

10.4.1 Estimated bioaccumulation

Estimations are not included in this section as relevant experimental data are available.

10.4.2 Measured partition coefficient and bioaccumulation test data

An experimental aquatic study, Anon. (2007), to determine the bioconcentration potential (BCF) of pirimiphos-methyl (purity 99.2%, 99.3% radiolabelled) is available following GLP and OECD Guideline 305. The study used a mixture of radiolabelled ^{14}C- pirimiphos-methyl and unlabelled test substance (ratio 1:1), in a flow-through system with Rainbow Trout (Oncorhynchus mykiss) with exposure to high and low concentrations of test substance at 10 and 1 µg pirimiphos-methyl/L respectively. Additionally, a solvent control was set up. The exposure period ran for 28 days followed by a 14 day depuration period. Test substance concentrations in water and fish as well as wet weight of fish were determined throughout the study.

No mortalities or signs of toxicity were observed in the control and treatment group over the test period. The lipid content of control fish sampled over the test period remained constant considering the variability of individual values and the lowest mean lipid content from the uptake period (6.3 %) was used for lipid normalization calculations.

The steady-state bioconcentration factor in whole fish was calculated as 1251.2 and 1276.9 for the low and high concentrations respectively. The uptake and depuration constants were calculated as 684.041 and 0.570 for the low concentration and 1008.410 and 0.840 for the high concentration respectively. Adjusting for the lowest average lipid content of exposed fish (6.3 %), the lipid adjusted whole fish BCFSS was 1013.4.

10.4.3 Summary and discussion of aquatic bioaccumulation

The log K_{ow} value of 4.20 for pirimiphos-methyl is greater than the CLP log K_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate under CLP. An experimental bioconcentration study in fish is available to consider bioaccumulation further.

In the experimental study, the whole fish BCF value for pirimiphos-methyl was 1013.4, greater than the CLP trigger values of 500. Therefore for classification purposes, pirimiphos-methyl is considered to have to the potential to bioaccumulate.

10.5 Acute aquatic hazard

The ecotoxicological data available for pirimiphos-methyl are composed predominantly of studies submitted as part of the original approval of pirimiphos-methyl, in addition to two new acute aquatic studies performed on fish and aquatic invertebrates. The studies submitted as part of the original approval predated guidelines and GLP classification, and the endpoints were based upon nominal concentrations. Due to the age of original evaluation, full study summaries are not available for these studies, although they have been previously reviewed for the pesticide assessment of pirimiphos and deemed reliable and acceptable for risk assessment purposes under Dir. 91/414/EEC and as part of the original classification and labelling proposal. As a result, these studies are considered valid as part of the weight of evidence in this proposal.

The new studies submitted as part of the renewal evaluation of pirimiphos-methyl under Reg. (EC) 1107/2009 are to current guidelines, have been performed to GLP and the endpoints based upon mean measured concentrations. For all critical endpoints required for classification, more recent and validated GLP studies are available. These data are supportive of the results obtained in the older studies. There are no data available on the degradants of pirimiphos-methyl but these are not considered in relation to the classification of the parent compound.

Table 14: Summary of relevant information on acute aquatic toxicity

Guideline	Species	Endpoint Data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[1]	
Fish							
Non-guideline Predates GLP	*Oncorhynchus mykiss (*formerly *Salmo gairdneri)*	Mortality	Static	96 hr	LC_{50}	0.404 (nom)	Anon. (1978)
Non-guideline Predates GLP	*Oncorhynchus mykiss (*formerly *Salmo gairdneri)*	Mortality	Static	96 hr	LC_{50}	0.200 (nom)	Anon. (1973a)
Non-guideline Predates GLP	*Cyprinus carpio*	Mortality	Static	48 hr	LC_{50}	1.400 (nom)	Anon. (1973a)
OECD 203 (1992) GLP	*Cyprinus carpio*	Mortality	Flow-through	96 hr	LC_{50}	0.76 (mm)	Anon. (2005)
Aquatic invertebrates							
EPA – 660/3-75-009 Predates GLP	*Daphnia magna*	Immobility	Static	48 hr	EC_{50}	0.00021 (nom)	Evered and Doma (1976)
OECD 202 (2004) GLP	*Daphnia magna*	Immobility	Static	48 hr	EC_{50}	0.000314 (mm)	Liedtke (2015)
Algae / aquatic plants							

Non-guideline Predates GLP	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)	Growth rate and morphology	Static	96 hr	E_rC_{50}	4.9 (mm)	Smyth et al. (1989)

[1] nom: endpoint based upon nominal concentrations, mm: endpoint based upon mean measured concentrations

10.5.1 Acute (short-term) toxicity to fish

Four acute fish studies are available for pirimiphos-methyl. Anon. (2005) was performed to OECD 203 (1992) and according to GLP. The other three were not performed to any guidelines or GLP as they pre-date the existence of the guidelines and GLP certification, although the format was broadly in line with OECD 203.

Anon., 2005

The acute toxicity of pirimiphos-methyl (90.5% w/w purity) to Common Carp (*Cyrpinus carpio*) was determined under flow-through conditions in a 96 hour test. Groups of seven fish were exposed to nominal concentrations of 0.22, 0.46, 1.0, 2.2 and 4.6 mg pirimiphos-methyl/L (0.15, 0.36, 0.57, 1.7 and 3.4 mg pirimiphos-methyl/L mean measured concentrations, upon which the results are based), alongside a dilution water control and a solvent control, all in 48 L of water. Observations for mortalities and symptoms of toxicity were made at 3, 24, 48, 72 and 96 hours. Mortalities were observed at mean measured concentrations of 0.15 mg a.s./L and above. Symptoms of toxicity observed included lethargy and were observed at concentrations of 0.15 mg/L and above. No mortality or symptoms of toxicity were observed in the controls. After 96 h, zero or one mortalities occurred at concentrations up to and including 0.57 mg pirimiphos-methyl/L; for 1.7 and 3.4 mg pirimiphos-methyl/L, mortality was 100 %. Sublethal effects were observed at 0.36 and 0.57 mg pirimiphos-methyl/L at 96 hours. Based on mean measured concentrations, the 96 hour LC_{50} was 0.76 mg pirimiphos-methyl/L.

Anon 1978

A study summary is not available. The study was a 96 hour static test, non-GLP, broadly in line with OECD 203. The 96h LC_{50} based upon nominal concentrations is 0.404 mg pirimiphos-methyl/L.

Anon., 1973a

A study summary is not available. The study was a 96 hour static test, non-GLP, broadly in line with OECD 203. The 96h LC_{50} based upon nominal concentrations is 0.200 mg pirimiphos-methyl/L.

Anon., 1973a

A study summary is not available. The study was a 48 hour static test, non-GLP, broadly in line with OECD 203. The 48h LC_{50} based upon nominal concentrations is 1.400 mg pirimiphos-methyl/L.

10.5.2 Acute (short-term) toxicity to aquatic invertebrates

Two aquatic invertebrate studies are available for pirimiphos-methyl; Liedtke (2015), was performed to OECD 202 (2004) and according to GLP, and Evered and Doma (1976) was performed to EPA-660/3-75-009, but pre-dated GLP certification.

Liedtke A., 2015

The acute toxicity of pirimiphos-methyl (90.9 % w/w purity) to *Daphnia magna* was determined under static conditions in a 48 hour test. Four replicates of five daphnids were exposed to a range of nominal concentrations of 0.000046, 0.0001, 0.000220, 0.000460 and 0.001 mg pirimiphos-methyl/L (mean measured 0.000044, 0.000102, 0.000218, 0.000453 and 0.000952 mg pirimiphos-methyl/L, respectively, upon which the results are based), alongside a dilution water control and a solvent control. The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. After 48 hours, no immobility was observed for concentrations up to and including 0.000218 mg pirimiphos-methyl/L and there was no immobility observed in the dilution water or solvent controls. For 0.000453 and 0.000952 mg pirimiphos-

methyl/L, 100% mortality was observed at 48 hours. Based on mean measured concentrations the 48-hour EC_{50} was 0.000314 mg pirimiphos-methyl/L.

Evered and Doma, 1976

The acute toxicity of pirimiphos-methyl (99.5 % w/w purity) to *Daphnia magna* was determined under static conditions in a 48 hour test. Three replicates of ten daphnids were exposed to a range of nominal concentrations of 0.00001, 0.00005, 0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.05 mg pirimiphos-methyl/L alongside a water control and a solvent control. The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. After 48 hours, no immobility was observed for concentrations up to and including 0.00005 mg pirimiphos-methyl/L and there was no immobility observed in the dilution water or solvent controls. For 0.005, 0.01 and 0.05 mg pirimiphos-methyl/L, 100% mortality was observed at 48 hours. The results of GIC analysis showed that the initial concetration was correct. 50 and 90% of the compound had degraded after 24 and 48 hours respectively. The 48h EC_{50} based upon nominal concentrations is 0.00021 mg pirimiphos-methyl/L (confidence limit 0.00015-0.00031 mg/L).

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

A single algae study, Smyth et al. (1989), is available that was performed to OECD 201 and was GLP compliant, although a study summary is not available. The 96 hour study tests the effects of pirimiphos-methyl on the growth and morphology of the green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a static test. The 96 hour E_rC_{50} was 4.9 mg pirimiphos-methyl/L, and the NOE_rC was 0.56 mg pirimiphos-methyl/L based upon mean measured concentrations.

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

No other acute aquatic organism studies are available.

10.6 Long-term aquatic hazard

Table 15: Summary of relevant information on chronic aquatic toxicity

Guideline	Species	Endpoint Data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[1]	
Fish							
OECD 204 (1992) GLP	*Oncorhynchus mykiss (*formerly *Salmo gairdneri)*	Mortality, growth	Flow-through	28 d	$NOEC_{growth}$	<0.023 (mm)	Anon. et al. (1990)
Aquatic invertebrates							
OECD 202 (2004) GLP	*Daphnia magna*	Immobility, growth	Semi-static	21 d	$NOEC_{survival}$ $NOEC_{growth}$ $NOEC_{reproduction}$	0.000050 (nom) 0.000050 (nom) 0.000050 (nom)	Rapley and Hamer (1991)
Algae / aquatic plants							

Non-guideline Predates GLP	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)	Growth rate and morphology	Static	96 hr	NOE_rC	0.56 (mm)	Smyth et al. (1989)

[1] nom: endpoint based upon nominal concentrations, mm: endpoint based upon mean measured concentrations

10.6.1 Chronic toxicity to fish

A single chronic toxicity study to fish performed with pirimiphos-methyl. Anon. et al. (1990) was a prolonged toxicity test based upon, the now deleted test guideline OECD 204, and was in compliance with GLP. Rainbow trout were exposed in groups of 10 to nominal concentrations of 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg pirimiphos-methyl/L (purity 90 %) in a flow-through test system for 28 days. A dilution water control group and a solvent control group were also employed. Actual concentrations of pirimiphos-methyl were determined by chemical analysis on 10 occasions during the 28-day study. Mortality was recorded daily, with symptoms of toxicity recorded on days 4, 7, 10, 14, 21 and 28. Fish weight gain was reduced at all concentrations in comparison with the controls. The general symptoms of toxicity noted in this study were reduced or stopped feeding, quiescence, dark colouration, rapid respiration, sounding, weakness, loss of balance, laboured respiration and coughing. Based on mean measured concentrations the 4, 7, 10, 21 and 28-day median lethal concentrations (LC_{50} values) for rainbow trout were 0.64, 0.61, 0.61, 0.61 and 0.61 mg/L, respectively. The mean measured NOEC for pirimiphos-methyl technical, based on symptoms of toxicity, was <0.023 mg pirimiphos-methyl/L.

10.6.2 Chronic toxicity to aquatic invertebrates

Rapley and Hamer (1991) was conducted to OECD 202 and was GLP compliant. *Daphnia magna* (less than 24 hours old) were exposed to pirimiphos-methyl (purity 89.3%) in water in a static system for 21 days at 19-21ºC. The nominal test concentrations were 0.000025, 0.000050, 0.0001, 0.0002 and 0.0004 mg pirimiphos-methyl/L, equivalent to mean measured concentrations 0.000028, 0.000050, 0.00009, 0.00019, 0.00036. Given that these measured concentrations were within 80-120% of the nominals, the endpoints were based upon nominal concentrations. The controls were exposed to dilution water only and to acetone for the solvent control. The *Daphnia* were transferred to freshly prepared solutions three times a week with chemical analysis with ages test solutions and new solutions upon renewal. Daily assessments were made for mortality and symptoms of toxicity. In each chamber with surviving *Daphnia*, the number of young were counted and removed on each transfer day and on day 21 the lengths of the surviving adult *Daphnia* were measured. There was no significant effect on reduction in length for any of the *Daphnia* groups that survived to the end of the test. There was a significant effect on reproduction at concentrations greater than and including 0.0001 mg pirimiphos-methyl/L. At concentration of 0.000025 and 0.000050 mg pirimiphos-methyl/L there were no significant effects on survival. The 7, 14 and 21 day nominal EC_{50} for pirimiphos-methyl were 0.00024, 0.00008 and 0.00008 mg pirimiphos-methyl/L, whilst the overall nominal NOEC for survival, reproduction and length was 0.000050 mg pirimiphos-methyl/L.

10.6.3 Chronic toxicity to algae or other aquatic plants

As discussed in the acute toxicity to algae and other aquatic plants (Section 10.5.3), a single algal study, Smyth et al. (1989), is available that was performed to OECD 201 and was GLP compliant, although a study summary is not available. The 96 hour study tests the effects of pirimiphos-methyl on the growth and morphology of the green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a static test. The 96 hour mean measured NOE_rC was 0.56 mg pirimiphos-methyl/L.

10.6.4 Chronic toxicity to other aquatic organisms

No other chronic aquatic organism studies are available.

10.7 Comparison with the CLP criteria

10.7.1 Acute aquatic hazard

Acute aquatic toxicity data is available for fish, aquatic invertebrates and algae. The most sensitive acute aquatic endpoint is the nominal 48 hour EC_{50} of 0.00021 mg pirimiphos-methyl/L for *Daphnia magna*, and therefore pirimiphos-methyl should be classified as Aquatic Acute category 1. As this EC_{50} is > 0.0001 but ≤ 0.001 mg/L, it should also attract an Acute M-factor of 1000.

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

Within the classification criteria, pirimiphos-methyl is considered 'not rapidly degradable' (Section 10.3).

Pirimiphos methyl has a log K_{OW} of 4.20, greater than the CLP cut off of ≥ 4, indicating a potential to bioaccumulate. An experimental bioconcentration study in fish resulted in a whole fish BCFss of 1013.4 (corrected for 5 % lipid content). As this is also greater than the CLP BCF trigger of 500, pirimiphos-methyl is considered to have a high potential for bioaccumulation (Section 10.4.1).

Chronic or long-term aquatic toxicity data on pirimiphos-methyl are available for fish, aquatic invertebrates and algae, however the study on fish is conducted according to OECD TG 204 which is not considered sutiable as a long term study. Therefore, the classification for fish will also be considered based on the acute toxicity data. Aquatic invertebrates were considered the most sensitive group based upon a 21 day nominal NOEC for survival, growth and reproduction of 0.000050 mg pirimiphos-methyl/L for Daphnia magna. This is > 0.00001 but ≤ 0.0001 mg/L, and therefore, since pirimiphos-methyl is considered 'not rapidly degradable' and potentially bioaccumulative, pirimiphos-methyl should be classified as Aquatic Chronic category 1 with a Chronic M-factor of 1000.

One acute GLP study on fish conducted according to OECD 203 is avalaible. The 96 hr LC50 for common carp is 0.76 mg pirimiphos-methyl/L, which is similar to the values obtained in 3 non-GLP, non-guideline studies which ranged from 0.200 to 1.400 mg pirimiphos-methyl/L. Pirimiphos-methyl is considered to be not rapidly degradable and the experimental determining BCF is ≥ 4, so since the 96 hour LC50 is ≤ 1 it should be classified with Aquatic Chronic category 1 with an M Factor of 1.

The most stringent outcome of the two methods of classification should be used, so pirimiphos-methyl should be classified as Aquatic Chronic category 1 with a Chronic M-factor of 1000.

10.8 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

Data conclusive and sufficient for classification.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The pirimiphos-methyl is an active substance used as an insecticide in the meaning of Regulation (EU) No 1107/2009. It has a current entry in Annex VI to the CLP regulation for classification as Aquatic Acute 1 and Aquatic Chronic 1. Based on the available data on aquatic toxicity and fate of pirimiphos-methyl the dossier submitter (DS) proposed to update the environmental classification to Aquatic Acute 1 (M=1 000) and Aquatic Chronic 1 (M=1 000) according to CLP Regulation.

Degradability

A hydrolysis study according to U.S. EPA, Subdivision N, Section 161-1 and EEC Method C.7 guidelines, in compliance with GLP, was run at pH 4, 5, 7 and 9 at 25 °C for 30 days in the dark. Pirimiphos-methyl undergoes hydrolytic degradation, depending on the pH value, with the shortest DT_{50} values at acidic pH. The first order DT_{50} values at 25 °C were determined to be 2, 7, 117 and 75 days at pH 4, 5, 7 and 9 respectively. In neutral and basic conditions two degradation products R402186 and R046382 were formed whilst in acidic conditions only R046382 was observed.

The photodegradation of radio-labelled pirimiphos-methyl was studied according to EPA FIFRA 161-2 and 161-3 guidelines and in compliance with GLP. Pirimiphos-methyl degraded extensively with an estimated first order DT_{50} of 0.46 and 0.47 hours of Florida Summer Sunlight at pH 5 and 7 respectively. Photolysis of pirimiphos-methyl resulted in one major degradate R046382, reaching maximum 63 % AR at the end of the study at both pH values. A further degradate R290438 was formed up to 14.5 % during the study, but degraded rapidly to final levels of 2.8 % and 3.3 % AR at pH 5 and 7 respectively.

No studies on ready biodegradability are available.

A water/sediment simulation study, carried out according to BBA Guidelines Part IV, Section 5-1, 1990 and in compliance with GLP, was run using two different systems in dark conditions at 20°C ± 2 °C for up to 100 days. Under aerobic conditions pirimiphos-methyl dissipated relatively rapidly from the water phase in both test systems ($DissT_{50}$ less than 1day), partitioning to sediment was rapid. A primary degradation of pirimiphos-methyl in the whole water/sediment systems was also fairly rapid (10.3 days and 8.51 days). Pirimiphos-methyl was degraded by hydrolysis to form a major metabolite (R46382) up to approximately 60 % AR. A further metabolite (R402186) reached a maximum of approximately 20 % AR after 30 days and declined to 2-4 % after 100 days. Volatilisation from water reached a maximum of 31.2 % AR. The total amount of volatiles reached a maximum of 35 % throughout the duration of the study.

Based on the information above, the DS concludes that pirimiphos-methyl is not considered to be rapidly degradable for the purposes of environmental classification according to guidance on Regulation (EC) 1272/2008.

Bioaccumulation

The Partition coefficient n-octanol/water of pirimiphos-methyl was > 4 at 20 °C (Husband, 1997).

An experimental aquatic BCF was available following GLP and OECD TG 305. The study used a mixture of radiolabelled and unlabelled test substance in a ratio of 1:1. A flow-through system was used with Killifish (*Oryzias latipes*) and two exposure concentrations: 10 and 1 µg/L. The exposure period ran for 28 days followed by a 14 day depuration period. The steady-state bioconcentration factor in whole fish, adjusted in 5 % lipid content, was 1 013.4.

Based on a BCF greater than 500 and a Log K_{ow} greater then 4, pirimiphos-methyl is considered to have a potential for bioaccumulation.

Ecotoxicity

The ecotoxicological test results from available acute and chronic toxicity studies in the CLH report performed on pirimiphos-methyl are summarized in the following table:

Method	Test organism	Test system	Results			Test conc.	Referenc
			Endpoint	LC_{50}/EC_{50} [mg/L]	NOEC [mg/L]		
Fish							
Non-guideline Predates GLP	*Oncorhynchus mykiss*	Static 96 h	Mortality	0.404		nom	Anon. (1978)
Non-guideline Predates GLP	*Oncorhynchus mykiss*	Static 96 h	Mortality	0.200		nom	Anon. (1973a)
Non-guideline Predates GLP	*Cyprinus carpio*	Static 48 h	Mortality	1.400		nom	Anon. (1973a)
OECD TG 203 (1992) GLP	*Cyprinus carpio*	Flow-through 96 h	Immobility	0.76		mm	Anon. (2005)
OECD TG 204 (1992) GLP	*Oncorhynchus mykiss*	Flow-through 28 d	Growth	0.61	< 0.023	mm	Anon. *et al*. (1990)
Aquatic invertebrates							
EPA – 660/3-75-009 Predates GLP	***Daphnia magna***	**Static 48 h**	**Immobility**	**0.00021**		**nom**	Evered and Doma (1976)
OECD TG 202 (2004) GLP	*Daphnia magna*	Static 48 h	Immobility	0.000314		mm	Liedtke (2015)
OECD TG 202 (1984) GLP	***Daphnia magna***	**Static 21 d**	**Survival, growth, reproduction**		**0.000050**	**nom**	Rapley an Hamer (1991)
Algae							
OECD TG 201 GLP	*Pseudokirchneriella subcapitata*	Static 96 h	Growth rate and morphology	4.9	0.56	mm	Smyth *et al*. (1989)

Acute toxicity

Four acute studies to fish for the pirimiphos-methyl are reported by the DS. Only one of them (*Anon. 2005*) was performed according to OECD TG 203 and to GLP, for the others no guidelines were followed and the studies summary were not available. All of the provided LC_{50} values ranged from 1.4 to 0.2 mg/L.

For aquatic invertebrates two acute toxicity studies are available and included in the CLH Report; *Liedtke 2015*, performed according to OECD TG 202 (2004) and GLP criteria and *Evered and Doma 1976*, conducted following EPA-660/3-75-009 and pre-dated GLP certification.

In the relevant study by *Liedtke 2015*, the acute toxicity of pirimiphos-methyl *to Daphnia magna* was determined in a 48 h static test system following OECD TG 202 (GLP). Based on mean measured concentrations, the 48 h EC_{50} to *Daphnia magna* was 0.000314 mg/L. This study is considered acceptable with fulfilled validity criteria.

The lowest acute endpoint value was obtained in the acute toxicity study by *Evered and Doma 1976,* with a nominal 48 h EC_{50} of 0.00021 mg/L for *Daphnia magna*. However the study cannot be regarded as valid because measured concentrations are available only for two test concentration, showing that the range of 80-120 % of the nominal is not achieved. Therefore, no measured concentrations are available and nominal concentrations are inadequate for deriving the acute end-point.

Therefore, the mean measured 48 h EC_{50} of 0.000314 mg/L is regarded as the most sensitive endpoint used for aquatic acute classification.

A single algal study (*Smith et al. 1989*) for pirimiphos-methyl is provided in the CLH Report. This study is a 96 h static test, performed according to OECD TG 201 and GLP compliant, and provides both acute and long-term endpoints. Based upon mean measured concentrations, a 96 h E_rC_{50} value of 4.9 mg/L and a NOE_rC of 0.56 mg/L have been determined for the effects of pirimiphos-methyl on growth rate of green algae *Pseudokirchneriella subcapitata*.

Chronic toxicity

A single chronic toxicity study to fish is available (*Anon. et al., 1990*). The study was a prolonged toxicity test to *Oncorhynchus mykiss* based on OECD TG 204 guideline and GLP compliant. A 28 day NOEC value of < 0.023 mg/L was determined based on mean measured concentrations. Although the study is considered valid, is not considered suitable as a long term study, being a prolonged acute study with fish mortality as the major endpoint examined.

A single chronic toxicity study on aquatic invertebrates (*Rapley and Hamer, 1991*) is provided as valid key study in the CLH Report. The chronic toxicity of pirimiphos-methyl to *Daphnia magna* was determined in a 21-day static test system, according to OECD TG 202 (1984) and GLP criteria. The mean measured concentrations were within 80-120 % of the nominals. The overall 21 day nominal NOEC for all tested endpoints (survival, growth, reproduction) was 0.000050 mg/L for *Daphnia magna*. This is regarded as the most sensitive endpoint used for aquatic chronic classification.

Comments received during public consultation

Four Member States (MS) and two organisations (ORG) contributed during public consultation stating a general agreement with the proposed environmental classification. Two MS noted some editorial errors, which were clarified by the DS.

Assessment and comparison with the classification criteria

Degradability

RAC agrees with the DS proposal to consider pirimiphos-methyl as not rapidly degradable. The degradation information does not provide sufficient data to show pirimiphos-methyl is ultimately degraded with a half-life < 16 days (equivalent to a degradation > 70 % within 28 days), or transformed to non-hazardous products as there is no available ecotoxicity data on the degradation products.

Bioaccumulation

The experimental whole fish BCF value for pirimiphos-methyl is 1 013.4, greater than the CLP trigger values of 500. The test is valid and usable for the purposes of classification.

The log K_{OW} value of 4.2 is above the CLP trigger value of 4 intended to identify substances with a potential to bioaccumulate.

In conclusion, RAC agrees with the DS that pirimiphos-methyl is considered to have a potential to bioaccumulate under CLP.

Aquatic toxicity

Acute aquatic hazard

Aquatic invertebrates are the most sensitive species, the lowest valid acute end-point is a 48 h EC_{50} of 0.000314 mg/L based on mean measured concentrations. This value is below the classification criterion of ≤ 1 mg/L for the hazard category Aquatic Acute 1. The appropriate M-factor is 1 000, since the toxicity is within the range ($0.0001 < L(E)C_{50} \leq 0.001$).

Chronic aquatic hazard

The most sensitive organisms are aquatic invertebrates with a 21 d NOEC = 0.000050 mg/L based on nominal concentrations. The study is reliable and usable for the classification purposes. This value is lower than the classification criterion for aquatic Chronic Category 1 (0.1 mg/L) for not rapidly degradable substances in the aquatic environment. The appropriate M-factor is 1 000, since the toxicity is within the range of $0.00001 < EC_{10}$ (NOEC) ≤ 0.0001. However, as there aren't valid long-term toxicity data for fish the lowest acute toxicity value must be compared with the CLP criteria following table 4.1.0(b)(iii). The highest reliable acute toxicity for fish is a value of 0.76 mg/L, which results in a classification of aquatic Chronic 1 with an M-factor of 1 for a not rapidly degradable substance ($0.1 < L(E)C_{50} \leq 1$). As the most stringent outcome is used for classification, pirimiphos-methyl should be classified as Aquatic Chronic 1, M-factor = 1 000.

In summary, RAC agrees with the DS that Pirimiphos-methyl should be classified as:

Aquatic Acute 1; H400, M-factor of 1 000;

Aquatic Chronic 1; H410, M-factor of 1 000.

11 EVALUATION OF ADDITIONAL HAZARDS

11.1 Hazardous to the ozone layer

Not assessed in this dossier.

12 ADDITIONAL LABELLING

Not applicable.

13 REFERENCES

Draft Assessment Report – Pirimiphos-methyl – Volume 3 – Annex B2 – Toxicology and metabolism – October 2013

Draft Renewal Assessment Report – Pirimiphos-methyl – Volume 3 – Annex B2 – Physical and chemical properties – 2016

Draft Renewal Assessment Report – Pirimiphos-methyl – Volume 3 – Annex B6 – Toxicology and metabolism – 2016

Draft Renewal Assessment Report – Pirimiphos-methyl – Volume 3 – Annex B8 – Environmental fate and behaviour – 2016

Draft Renewal Assessment Report – Pirimiphos-methyl – Volume 3 – Annex B9 – Ecotoxicology behaviour – 2016

EFSA Scientific Report, 2005. *Conclusion on the peer review of pirimiphos-methyl*, s.l.: s.n.

Toxicology and human health references:

Callendar, R., 1984. Pirimiphos-methyl: An evaluation in the salmonella mutagenicity assay., s.l.: s.n.

Cross, M., 1986. Pirimiphos-methyl (Technical grade): Assessment of mutagenic potential using L5178Y mouse lymphoma cells, s.l.: s.n.

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, scientific panals and units in the absence of actual measured data. EFSA Journal, 10(3), p. 2579.

Hanna & Dyer, 1975. Mutagenicity of organophospahte compoundsin bacteria and drosophila. Mutation research.

Howard, 1986. Sister chromatid exchange assay, s.l.: s.n.

Husband, R. 1997. Pirimiphos-Methyl: Physical and Cehmical properties of pure material, Zeneca Agrochemicals, GLP, Unpublished.

Husband, R. 1998. Pirimiphos-Methyl: Physical and Cehmical properties of technical material, Zeneca Agrochemicals, GLP, Unpublished.

Rajini et al., 1986. Mutagenic properties of pirimiphos-methyl in male mice. Bull. Environ. Contam. And Toxicol. 36, 680-684

Seiller, J., 1972. A survey of the mutagenicity of various pesticides. Experimentia, 29(5), pp. 622-623.

Seiller, J., 1976. Evaluation of some pesticides for mutagenicity. Excerpta Medica – Amsterdam – ICS 376, 398-404. Not GLP. Published

Shirasu, 1984. Mutagenicity of pesticides. In: Mutation, cancer and malformation. New York: s.n., pp. 617 - 624

Sokolowski, A., 2015a. Chromosome aberration test in human lymphocytes in vitro, s.l.: s.n.

Sokolowski, A., 2015. Pirimiphos-methyl - Salmonella Typhimurium and Escherichia Coli reverse mutation assay, s.l.: s.n.

Trueman, 1983. Mammalian cell transformation test, s.l.: s.n.

WHO - JMPR, 1999. Joint meeting on pesticide residues, Rome, Italy: Food and Agricultural Organisation - United Nations.

Wildgoose, 1986. Clastogenicity in vitro, s.l.: s.n.

Environmental fate and ecotoxicology references:

Arnold, DJ et al. 1976. Degradation of the pesticide in soil under lab conditions.
Report number; PP511/0482, Not GLP

Everett, CJ et al., 1984, Pirimiphos-methyl: Toxicity of 3 Actellic Formulations to First Instar *Daphnia magna*. Report No. RJ0360B, GLP

Evered, P. and Doma, S., 1976, Pirimiphos-methyl: toxicity to first instar *Daphnia magna*. Non-GLP, Unpublished

Hartfree, Y et al. 1993. Pirimiphos-methyl: adsorption and desorption in soil, Report number; PP511/0488. GLP

Hand LH. 1996 P-Methyl: aqueous hydrolysis in pH 4, 5, 7 & 9 Solutions at 25°C. Report number; PP511/0494. GLP

Hayes, SE. 1998. P-methyl – Calculation of half-life with Atmospheric Hydroxyl radicals. Report number: PP511/0620. GLP

Kirkpatrick, D. 1994. ^{14}C Pirimiphos-methyl: The Degradation in Water: sediment Systems. Report Number ISN310/931971, (Syngenta File No. PP511/0499). GLP

Liedtke A (2015) "Pirimiphos-methyl – Acute Toxicity to *Daphnia magna* in a 48-hour Immobilisation Test". Report number D40033, GLP

MacIver JM and Hand LH. 1996. Pirimiphos-methyl: Volatilization from soil and leaf surfaces following application as an EC formulation. Report number: 95JH207 / RJ2124B. GLP

Powel SP. 1999. Pirimiphos-methyl: Aqueous photolysis in pH 5 & 7 at 25°C. Report number; PP511/0497. GLP

Rapley J. H. and Hamer M., 1991, Pirimiphos-methyl*:* Chronic toxicity to *Daphnia magna.* Report number RJ0940B, GLP

Smyth DV, Tapp JF and Sankey SA. 1989. Pirimiphos-methyl: Determination of Toxicity to the Green Alga (*Selenastrum capricornutum*), Report No. BL/B/3512, GLP

14 ANNEXES

Annex I – Full historical control data pertaining to the carcinogenicity study in rats (separate document)

Annex II – Confidential reference list (separate document)