Committee for Risk Assessment

RAC

Annex 1

Background document

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

pirimicarb (ISO); 5,6-dimethyl-2-dimethylamino-pyrimidin-4-yl N,N-dimethylcarbamate

EC number: 245-430-1
CAS number: 23103-98-2

CLH-O-0000001412-86-39/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

04 December 2014

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Pirimicarb

EC Number: 245-430-1

CAS Number: 23103-98-2

Index Number: 006-035-00-8

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Version number: 1 **Date: September 2013**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Pirimicarb***
EC number:	***245-430-1***
CAS number:	***23103-98-2***
Annex VI Index number:	***006-035-008***
Degree of purity:	***95% ≤ 97.6%***
Impurities:	**Confidential – please refer to the technical dossier**

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 3* ; H301 Aquatic Acute 1; H400 Aquatic Chronic 1; H410
Current proposal for consideration by RAC	Acute Tox. 3; H301 Acute Tox. 3; H331 Skin Sens. 1B; H317 Carc. 2; H351 Aquatic Acute 1; H400 M= 10 (Acute) Aquatic Chronic 1; H410 M= 100 (Chronic)
Resulting harmonised classification (future entry in Annex VI, CLP	Acute Tox. 3; H301 Acute Tox. 3; H331

Regulation)	Skin Sens. 1B; H317 Carc. 2; H351 Aquatic Acute 1; H400 M= 10 (Acute) Aquatic Chronic 1; H410 M= 100 (Chronic)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.1.	Acute toxicity - oral	**Acute Tox. 3; H301**	Not applicable	**Acute Tox. 3* ; H301**	Not applicable
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	Conclusive but not sufficient
	Acute toxicity - inhalation	**Acute Tox. 3; H331**	Not applicable	Not classified	Not applicable
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	**Skin Sens. 1B; H317**	Not applicable	Not classified	Not applicable
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.6.	Carcinogenicity	**Carc. 2; H351**	Not applicable	Not classified	Not applicable

3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400** **Aquatic Chronic 1; H410**	**M= 10 (Acute)** **M= 100 (chronic)**	**Aquatic Acute 1; H400** **Aquatic Chronic 1; H410**	Not applicable
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger

Pictograms: GHS06, GHS08, GHS09
Hazard statements: H301, H331, H317, H351, H410
Precautionary statements: Precautionary statements are not listed on Annex VI of CLP.

Proposed notes assigned to an entry: None

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: T; N
R-phrases: R23/25, 40, 43, 50-53
S-phrases: (1/2), S36/37-45-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Pirimicarb is a pesticidal active substance that has been reviewed under Directive 91/414/EEC with the UK as the Rapporteur Member State. It is currently listed in Annex VI of Regulation 1272/2008 (CLP Regulation) with Acute Tox. 3*; H301, Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. A proposal to add Acute Tox. 3; H331 and Skin Sens. 1; H317 was discussed and agreed by the Technical Committee on Classification and Labelling (Directive 67/548/EEC) ('TCC&L') in May 2007. However, these agreed additional classifications were not formally adopted by the Commission for inclusion into Annex I of Directive 67/548/EEC before the introduction of the CLP Regulation. A proposal is therefore required in line with Articles 36 and 37 of the CLP Regulation, for amendment of the current harmonised classification of this substance.

2.2 Short summary of the scientific justification for the CLH proposal

Pirimicarb is a pesticidal active substance that has been reviewed under Directive 91/414/EEC with the UK as the Rapporteur Member State.

In 2005 EFSA concluded that the following classification (in accordance with DSD) should be considered T; R23/25, R43, R48/22 (based on haematological effects) and raised a question regarding the application of Carc Cat 3; R40 due to the observation of lung tumours. Classification with N; R50/53 (no specific concentration limits) was also included.

The available data support the existing classification, Acute Tox 3; H301, Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. A proposal to add Acute Tox 3; H331 and Skin Sens 1; H317 was discussed and agreed by the Technical Committee on Classification and Labelling (Directive 67/548/EEC) ('TC C&L') in May 2007. Following reanalysis of available data and the generation of this CLH report, classification with Carc 2; H351 is also proposed. STOT-RE is note proposed as the haematological effects are considered to be inconsistent.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Classification: Acute Tox 3*; H301

Aquatic Acute 1; H400

Aquatic Chronic 1; H410

Labelling: Signal word: Danger

Hazard statements: H301, H410

Pictogram: GHS06, GHS09

Supplemental hazard statement code: None

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The applicant currently classifies as follows

Classification: Acute Tox. 3 ; H301

Acute Tox. 3 ; H331

Skin Sens. 1 ; H317

Aquatic Acute 1; H400

Aquatic Chronic 1; H410

Labelling: Signal word: Danger

Hazard statements: H301, H331, H317, H410

Pictogram: GHS06, GHS09

The majority of notifiers to the C&L Inventory classify as above. The remainder classify in line with the harmonised entry.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pirimicarb is a pesticidal active substance in the meaning of Directive 91/414/EEC. In accordance with Article 36(2) of the CLP Regulation, Pirimicarb should be subject to harmonised classification and labelling.

Pirimicarb already has an entry in Annex VI to CLP; therefore, the present CLH report proposes to update/amend the existing Annex VI entry and does not address all hazard classes.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

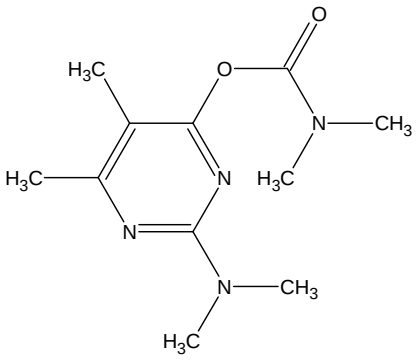
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	245-430-1
EC name:	Pirimicarb 5,6-dimethyl-2-dimethylamino-pyrimidin-4-yl *N*,*N*-dimethylcarbamate
CAS number (EC inventory):	23103-98-2
CAS number:	23103-98-2
CAS name:	Carbamic acid, N,N-dimethyl-, 2-(dimethylamino)-5,6-dimethyl-4-pyrimidinyl ester
IUPAC name:	2-(dimethylamino)-5,6-dimethylpyrimidin-4-yl dimethylcarbamate
CLP Annex VI Index number:	006-035-00-8
Molecular formula:	$C_{11}H_{18}N_4O_2$
Molecular weight range:	238.3

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Pirimicarb	95%	95-97.6%	

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are a number of impurities in Pirimicarb, all of which are present at concentrations of ≤1.6%. These impurities have been taken into account in the proposed classification and labelling and are not considered to be of additional concern. The impurities are considered confidential, so are not listed in this report. Further information can be found in the technical dossier.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

The minimum purity of Pirimicarb is 95%. The studies detailed in this report used Pirimicarb with a purity of 94 - 98.8%. After careful and detailed review by the UK CLP CA and those authorities responsible for the assessment under Directive 91/414/EEC, the technical specification of the current technical material and the material used in the studies are considered to be comparable.

1.3 Physico-chemical properties

Table 8: Summary of physico-chemical properties

The physiochemical properties of Pirimicarb are summarised below.

Property	**Value**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Powdery solid, White-cream, No odour	
Melting/freezing point	Pure Material (98.8%) = 91.6^{o}C (365 K) Technical Material (97.3%) = 87.3-90.7^{o}C	Measured, EEC A1 (Capillary Method) Wollerton C, Husband R 1994
Boiling point	No data	No data
Relative density	Pure Material (98.8%) =1.18 g cm^{-3} at 25^{o}C Technical Material (97.3%) = 1.21 g cm^{-3} at 25^{o}C	Measured, EEC A3 (Pycnometer Method) Wollerton C, Husband R 1994
Vapour pressure	Pure Material (98.8%) = 4.3 x 10^{-7} kPa at 20^{o}C (interpolation)	Measured, EEC A4 (Gas Saturation Method) Wollerton C, Husband R 1994
Surface tension	Technical Material (97.3%) = 58.2 mN/m (0.999g/l solution) at 20.0^{o}C	Measured, EEC A5 (Ring Method) Evans AJ, Mullee DM 2001
Water solubility	Pure Material (98.8%) = 3000 mg/l (purified water) 3600 mg/l (pH 5.2) 3100 mg/l (pH 7.4) 3100 mg/l (pH 9.3) all at 20^{o}C	Measured, EEC A6 (Flask Method) Wollerton C, Husband R 1994
Partition coefficient n-octanol/water	Pure Material (98.8%) Log Pow= 1.7 (purified water) Log Pow = 1.7 (pH 7.1) Log Pow =1.1 (pH 3.9)	Measured, EEC A8 (Shake Flask Method) Wollerton C, Husband R 1994
Flash point	N/A	N/A
Flammability	Technical Material (97.3%) = is not flammable and did not propagate combustion. In addition, experience in handling and use indicates that it is not flammable in contact with air or water.	Measured, EEC A10 Wollerton C, Husband R 1994
Explosive properties	Technical Material (97.3%) = Pirimicarb is determined to be non-explosive	Measured, EEC A14 Tremain SP 2001
Self-ignition temperature	Technical Material (97.3%) = No ignition was detected before the sample melted, as indicated by an exotherm at ~92^{o}C	Measured, EEC A16 Wollerton C, Husband R 1994
Oxidising properties	Technical Material (97.3%) = Pirimicarb is considered not to be an oxidising agent.	Measured, EEC A17 Jackson WA 2002
Dissociation constant	pKa= 4.44 (pure material (98.8%)) at 20^{o}C in water containing 1.23% of co-solvent pH 6.4 (1% dispersion of 20^{o}C) t½ <1 hr.	Measured, OECD 112/pH meter Wollerton C, Husband R 1994

2 MANUFACTURE AND USES

2.1 Manufacture

Pirimicarb is not manufactured in the EU.

2.2 Identified uses

Pirimicarb is used as a selective aphicide in the EU.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

See Table 8

3.1 PHYSIO-CHEMICAL PROPERTIES

The data presented in Section 1.3 (Table 8) show that Pirimicarb does not meet the criteria for classification for physico-chemical hazards and this is not considered further in this proposal.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The following summary is derived from the Draft Assessment Report (DAR) made under Directive 91/414/EEC.

In rats, Pirimicarb was well absorbed (> 80%) and rapidly excreted (60-70% primarily in urine) within 24 hours of oral administration. Distribution after oral exposure was widespread, with the highest concentrations occurring in the liver. There was no evidence of accumulation.

Absorbed Pirimicarb was extensively metabolised. The major pathway involved the loss of the carbamate moiety to produce a range of substituted hydroxypyrimidines. A sex difference was evident in the metabolic profile, with males showing a more extensive range of 4-hydroxypyrimidines than females, although there is no information to suggest that this impacts on the classification.

The toxicokinetic profile of Pirimicarb after inhalation and dermal exposure has not been investigated.

4.1.2 Human information

No data available

4.1.3 Summary and discussion on toxicokinetics

Pirimicarb is well absorbed, extensively metabolised and rapidly eliminated after oral exposure.

RAC general comment
pirimicarb, a pesticidal active substance, has been reviewed under Directive 91/414/EEC with the UK as the Rapporteur Member State. In addition to the existing classification (Acute Tox 3 *; H301, Aquatic Acute 1; H400 and Aquatic Chronic 1; H410), a proposal to add Acute Tox 3; H331 and Skin Sens 1; H317 was discussed and agreed by the Technical Committee on Classification and Labelling (Directive 67/548/EEC) in May 2007. Following reanalysis of available data during the preparation of the CLH report, the dossier submitter (DS) also proposed to remove the minimum classification for acute toxicity by the oral route and to add classification for carcinogenicity as Carc 2; H351.

4.2 Acute toxicity

Three acute toxicity studies on Pirimicarb are available.

4.2.1 Non-human information

Table 9: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and Remarks**
Rat/Wistar 5/sex/dose 100, 150, 200 mg/kg bw in corn oil OECD 401 (1992), GLP Purity: 97.6% (Lees & Connolly, 1995a)	M: 152 mg/kg F:142 mg/kg	Clinical signs of toxicity were observed at all dose levels and included; salivation, wet fur, decreased activity, fasciculations, pinched sides, irregular breathing, diarrhoea and upward curvature of the spine. Surviving animals recovered within 8 days. Gross necropsy for those animals that died during the study revealed red/dark or mottled areas of the lung, gas/fluid filled stomach and dark areas on the liver.
Acute Inhalation		

Method	LD_{50}	Observations and Remarks
Rat/Wistar 5/sex/dose 0, 0.414, 0.747, 1.065 mg/L/4 hr (aerosol) OECD 403 (nose only), GLP Purity: 97.4% (Parr-Dobrzanski, 1994)	M: 0.948 mg/L/4 hr F: 0.858 mg/L/4 hr	Clinical signs of toxicity were observed at all dose levels and included; initial body weight loss (days 2 and 3 in males), decreased activity, shaking, wet fur, hunched posture, chromodacryorrhea, piloerection and stains around the nose. Slow deep/irregular breathing and salivation were seen in most animals exposed to 747 and 1065 µg/l. All surviving animals recovered within 6 days. No abnormal findings were observed at necropsy.

Acute Dermal		
Method	**LD_{50}**	**Observations and Remarks**
Rat/Wistar 5/sex 2000 mg/kg bw (24 hour exposure) OECD 402 (1987), GLP Purity: 97.6% (Lees & Connolly, 1995)	M&F: > 2000 mg/kg	No animals died and there were no signs of systemic toxicity. Local skin irritation (desquamation and some small scabs) was observed at the application sites of one male and two female rats.

4.2.1.1 Acute toxicity: oral

Oral LD_{50} values of 152 and 142 mg/kg bw were derived for male and female rats, respectively.

4.2.1.2 Acute toxicity: inhalation

In an acute inhalation study, the LC_{50} was measured at 0.948 and 0.858 mg/L/4 hr for male and female rats, respectively.

4.2.1.3 Acute toxicity: dermal

A dermal LD_{50} of >2000 mg/kg bw was derived in males and females from a study conducted with rats.

4.2.1.4 Acute toxicity: other routes

No data available

4.2.2 Human information

No data available

4.2.3 Summary and discussion of acute toxicity

Refer to section 4.2.1

4.2.4 Comparison with criteria

The oral LD_{50} values of 152 and 142 mg/kg for male and female rats, respectively, are within the range of $50 < LD_{50} \leq 300$ for classification as Acute Tox. 3; H301.

In an acute inhalation study, the LC_{50} was measured at 0.948 and 0.858 mg/L/4 hr for male and female rats, respectively. These values fall within the criteria for classification as Acute Tox, 3; H331 ($0.5< LC_{50} \leq 1$ mg/L/4 hr).

An LD_{50} of >2000 mg/kg for rats exposed to pirimicarb via the dermal route is above the cut off for classification (2000 mg/kg) under CLP, therefore, no classification is proposed.

4.2.5 Conclusions on classification and labelling

Acute Tox. 3; H301 and Acute Tox. 3; H331

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal Three acute toxicity studies were presented in the CLH report. Studies were conducted by oral, inhalatory and dermal routes in the Wistar-derived SD rat strain. In an acute oral toxicity study in rats in accordance with OECD Test Guideline (TG) 401, LD_{50} values were calculated to be: • 152 mg/kg bw in males and • 142 mg/kg bw in females. Clinical signs of toxicity were observed at all doses from the first day of treatment, and were characteristic of poisoning with an acetyl cholinesterase inhibitor (including salivation, irregular breathing, diarrhoea, fasciculation, upward curvature). The DS proposed that according to the CLP criteria, the current classification for pirimicarb in the acute oral toxicity hazard category 3 ($50 < ATE \leq 300$) and with the hazard statement H301: Toxic if swallowed should remain, but with the removal of the minimum classification (*) for acute toxicity. In an acute dermal toxicity study in rats (in accordance with OECD TG 402), no animals died and there were no signs of systemic toxicity with the applied dose of 2000 mg/kg bw (limit test, 24-hr exposure). Local skin irritation (desquamation and some small scabs) was observed at the application sites of one out of 5 males and two out of 5 female rats.

No classification for acute dermal toxicity was proposed by the DS (LD_{50} > 2000 mg/kg bw).

In an acute inhalation toxicity study (in accordance with OECD TG 403), rats were exposed for 4 hours nose-only, to pirimicarb aerosol with particle size distribution within the recommended range (the mass median aerodynamic diameter (MMAD) ranged from 3.02 µm to 3.46 µm, with a geometric standard deviation from 1.89 µm to 2.04 µm). LC_{50} values were calculated to be:

- 0.948 mg/L in males and
- 0.858 mg/L in females.

Clinical signs of toxicity, characteristic for poisoning with an acetyl cholinesterase inhibitor, were observed at all doses during or immediately after exposure. The DS proposed that according to the CLP criteria, pirimicarb should be classified in the acute inhalation toxicity hazard category 3 (0.5 < ATE ≤ 1.0, for dusts and mists), with the hazard statement H331: Toxic if inhaled.

Comments received during public consultation

Four MSCA supported the proposed classification during public consultation.

Assessment and comparison with the classification criteria

Following a comparison of the available acute oral LD_{50} and inhalation LC_{50} values with the classification criteria, RAC supports the conclusion of the DS that according to the CLP Regulation, pirimicarb should be classified as **Acute Tox. 3 - H301** (Toxic if swallowed) with the removal of the minimum classification (*) and as **Acute Tox. 3 - H331** (Toxic if inhaled).

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

See Table 9.

4.3.2 Comparison with criteria

Substances that have produced significant non-lethal toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following single exposure, are classified as STOT-SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a constant and identifiable toxic effect.

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

The signs that were apparent after single oral and inhalation exposure to Pirimicarb were indicative of acute neurotoxicity resulting from anticholinesterase inhibition. These same effects caused deaths at higher dose levels, for which classification for acute toxicity is proposed. As there was no clear evidence of specific toxic effects on a target organ or tissue that were independent of mortalities, classification as STOT-SE 1 or STOT-SE 2 is not justified.

Since no definitive signs of respiratory tract irritation (irregular breathing is likely to be a consequence of anticholinesterase inhibition) or narcotic effects were observed, no classification for STOT-SE 3 under CLP is required.

4.3.3 Conclusions on classification and labelling

No classification

4.4 Irritation

Not considered in this proposal.

4.5 Corrosivity

Not considered in this proposal.

4.6 Sensitisation

4.6.1 Skin sensitisation

One skin sensitisation study for Pirimicarb is available, which is summarised below.

4.6.1.1 Non-human information

Table 10: Summary table of relevant skin sensitisation studies

Method	**Doses**	**Results**
Guinea pig/Dunkin-Hartley/Female 20 test and 10 vehicle control Purity: 97.3% OECD 406 (1992)- Magnusson and Kligman maximisation study, GLP (Rattray and Leah, 1990)	*Induction* Intradermal = 3% w/v in corn oil Epidermal= 75% w/v in corn oil *Challenge* Left flank= 75% w/v in corn oil Right flank= 30% w/v in corn oil	**Positive** 30% Challenge= 6/19* (32%) and 9/19* (47%) responded at 24 and 48 hours, respectively. 75% Challenge= 4/19* (21%) and 6/19* (32%) responded at 24 and 48 hours, respectively. See table 10(a) below for individual animal scores Positive control (formaldehyde) = 94% response. Negative control (corn oil) = 0% response. *one animal excluded because the bandage slipped.

4.6.1.2 Human information

No data available

4.6.1.3 Summary and discussion of skin sensitisation

In an adjuvant-type, guinea pig maximisation study, Pirimicarb induced skin sensitisation reactions in 47% and 32% of animals challenged with 30% and 75% Pirimicarb, respectively. It should be noted that one animal was excluded because the bandage slipped.

Table 10(a): Individual animal data

	75% challenge		30% challenge	
Animal	24 Hours	48 Hours	24 Hours	48 Hours
1	0	0	3	3
2	0	0	0	0
3	1	1	0	0
4	0	0	3	2
5	1	1	0	0
6	0	0	3	2
7	0	0	0	1
8	0	1	0	0
9	1	1	2	2
10	0	2	0	1
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	1
16	0	0	0	0
17*	0*	0*	0*	0*
18	0	0	1	1
19	1	2	2	2
20	0	0	0	0

0 = no reaction
1 = scattered mild rendess
2 = moderate and diffuse redness
3 = intense redness and swelling
* bandage slipped

4.6.1.4 Comparison with criteria

The criteria for classification (positive in ≥30% of animals) was fulfilled and Pirimicarb should be classified as Skin Sens. 1; H317. In addition, since the intradermal dose used in the skin sensitisation study was >1%, classification in sub-category 1A is not applicable. However, Pirimicarb did not produce a high sensitising response (i.e. the response was not ≥60%) at a 3% induction dose, and therefore should be assigned to sub-category 1B. It should also be noted that

the skin sensitisation rate did not appear to be concentration dependent (i.e. 47% and 32% response with 30% and 75% respectively).

4.6.1.5 Conclusions on classification and labelling

Skin Sens. 1B; H317

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

One skin sensitisation study in accordance with OECD TG 406 (Magnusson and Kligman maximisation test) was conducted using female Guinea pigs (Dunkin-Hartley strain, 20 exposed and 10 control animals). Intradermal induction was performed with 3% dilution and epidermal induction with 75% dilution of pirimicarb in corn oil. Challenge was performed by applying 30% and 75% pirimicarb dilution on the right and left flank, respectively. In animals challenged with 30% pirimicarb dilution, positive reaction (scattered mild redness, intense redness and swelling) was found in 32% of animals (6 out of 19; one animal with slipped bandage was excluded) at the 24 hr reading, and in 47% of animals (9 out of 19) at the 48 hr reading. In animals challenged with 75% pirimicarb dilution, positive reaction (scattered mild or moderate diffuse redness) was found in 21% of animals (4 out of 19) at the 24 hr reading, and in 32% of animals (6 out of 19) at the 48 hr reading (see the table below).

Time after challenge	Pirimicarb dilution at challenge		Negative control
	30%	75%	
24 hours	6/19* (32%)	4/19 (21%)	0/10
48 hours	9/19 (47%)	6/19 (32%)	0/10

*One animal exposed to pirimicarb was excluded due to slipped bandage.

A positive control study, performed 21 months before this study, showed positive reaction to formaldehyde in 94% of animals. No positive result was observed in negative controls. The DS concluded that since the induction dose of 3% pirimicarb dilution caused a positive response in more than 30% of animals, pirimicarb should be classified as Skin Sens. Cat. 1; H317. The DS further proposed sub-category 1B, with the justification that pirimicarb did not produce a sensitising response ≥ 60% at the induction dose of 3%.

Comments received during public consultation

Four MSCAs supported the proposed classification (Skin Sens. 1B; H317), and one of them requested additional clarification on the way of choosing test substance concentrations for the induction and challenge exposure and whether any skin irritation was observed during the induction phase.

Assessment and comparison with the classification criteria

The available skin sensitisation study was conducted in accordance with OECD TG 406, with the possible deviation that during topical induction mild-to-moderate skin irritation was probably not produced after topical induction but this was not reported either in the CLH report or in the DAR. Namely, pirimicarb was not irritant to the skin (according to skin irritancy test), the same dilution was applied for topical induction and challenge (75% dilution; challenge dose should be the highest non-irritant dose), and application of a skin irritant (sodium lauryl sulphate) prior to topical induction was not reported either

in the CLH report or in the DAR.

Nevertheless, the study is regarded as acceptable and the proposed classification, Skin Sens. Cat. 1; H317, is supported by RAC since a positive skin reaction was observed in more than 30% of exposed animals. RAC, however, does not support the proposed sub-categorisation (1B) since the intradermal induction dose was above 1% and the decision regarding sub-categorisation in 1A could not be made. The classification criteria for sub-category 1B are met according to the criteria laid down in the 2nd Adaptation to Technical Progress (ATP) to the CLP Regulation, since ≥ 30% animals responded at > 1% intradermal induction dose. The positive response rate at 3% induction dose is lower than 60% (21% to 47%) and thus does not suggest that it can be ≥ 60% at ≥ 0.1% to <1% induction dose, or ≥ 30% at ≤ 0.1% induction dose. However, the possibility of a response rate compliant with sub-category 1A cannot be completely excluded since there are no experimental data with an induction dose below 1%. RAC concludes that pirimicarb should be classified as Skin Sens. 1 (H317) without sub-categorisation.

4.6.2 Respiratory sensitisation

Not considered in this proposal.

4.7 Repeated dose toxicity

The data on repeated dose toxicity is provided for information to support the classification for carcinogenicity. It is not proposed to classify pirimicarb for this hazard class as agreed at the TCC&L meeting in May 2007. Eight repeated dose toxicity studies for Pirimicarb are available.

4.7.1 Non-human information

Table 11: Summary table of relevant repeated dose toxicity studies

Oral Studies

Dose schedule	Dose levels	Results (effects of major toxicological significance)
Oral short term studies in rats		
90-day study Oral (diet or gavage) Rat/Wistar 25/sex/dose [of which 5/sex/dose were sacrificed after an additional 28-day recovery period] Purity: not specified Non-guideline or GLP Conducted prior to introduction of OECD 408, but has been checked for compliance with this guideline and deviations do not compromise the validity. Conducted prior to adopted of GLP compliance. (Griffiths & Conning, 1968; Hodge, 1995)	Diet: 250 or 750 ppm corresponding to 12.9 or 38.8 mg/kg bw/day in males and 15.3 or 47.1 mg/kg bw/day in females Gavage: 0 and 25 mg/kg bw/day (administered as a 25% dispersion in dispersol OG) for both males and females	**<u>Diet</u>** <u>38.8 (males)/47.1 (females) mg/kg bw/day</u> – *Clinical*: reduced food utilisation (during weeks 1-4). *Haematology*: reduced reticulocytes (M:↓20%-32.8% during weeks 6-13). *Cholinesterase Activity*: reduced plasma cholinesterase activity (M:↓5.8%-14.8%, F:↓5.6%-22.1% during weeks 2-12; no time response trend observed). <u>12.9 (males)/15.3 (females) mg/kg bw/day-</u> *Haematology*: reduced haemoglobin (M:↓4.5%-5.7% during weeks 6-13), reduced lymphocyte counts (F:↓31.8%-40.9% during weeks 6-13). *Cholinesterase Activity*: Reduced plasma cholinesterase activity (M:↓0%-13.2%, F:↓6.5%-17.2% during weeks 2-12, no time response trend observed). **<u>Gavage</u>** <u>25 (males and females) mg/kg bw/day</u> – *Clinical*: 7/25 (M) and 5/25 (F) died (due to trauma from repeated cannulation). *Haematology*: increased haemoglobin (F:↑5.4%-7.8% during weeks 6-13); reduced haemoglobin (M:↓2.6%-5.1% during weeks 6-13), reduced packed cell volume (M:↓5.7%-6.8% during weeks 6-13), increased packed cell volume (F:↑7.5%-8.7% during weeks 6-13), increased lymphocyte counts (M:↑7.8%-57.5% during weeks 6-13). *Cholinesterase Activity*: reduced plasma cholinesterase activity (M:↓29.7%-39.3%, F:↓30.1%-56.6% during weeks 2-12; no time response trend observed and reduced to levels not considered to be adverse during the recovery period), reduced erythrocyte cholinesterase activity (F: >20% inhibition at weeks 1 and 4 only; no time response trend observed and reduced to levels not considered to be adverse during recovery period). *None of the observed changes were considered to be adverse, therefore, the NOAEL was concluded to be 750 ppm (corresponding to 38.8 and 47.1 mg/kg bw/day for male and female rats, respectively).
8 week paired feeding study Oral (diet) Rat/ Wistar 12 females/dose/ feeding group All animals: 8 week recovery period Purity: 97.7% Non-guideline or GLP (There are no	Ad libitum feeding group: 0, 250 and 750 ppm corresponding to 0, 27.5 and 81.9 mg/kg bw/day Restricted feeding group: 0 and 250 ppm corresponding to 0 and 25.7 mg/kg bw/day (food restricted based on consumption in	**<u>Ad libitum feeding group</u>** *<u>81.9 mg/kg bw/day</u>* – *Clinical*: reduced body weight gain (↓12.3%-15.9% during weeks 1-8); reduced food consumption (↓5%), increased food utilisation (g consumed/g weight gained, ↑8.9%). All clinical changes reversed within the recovery period. *<u>27.5 mg/kg bw/day</u>*- *Clinical*: slight reduction in body weight gain (↓1.5%-4.6% during weeks 1-8 reversed within recovery period). **<u>Restricted feeding group</u>** *<u>75 mg/kg bw/day-</u>* *Clinical:* reduced body weight gain (↓2.2-15.4% during weeks 1-8),

comparable guidelines as this was an investigative study only) Conducted prior to the adoption of GLP compliance. (Richards *et al*, 1978)	250 ppm ad libitum feeding group) 0 and 750 ppm corresponding to 0 and 75 mg/kg bw/day (food restricted based on 750 ppm ad libitum feeding group).	increased food utilisation (g consumed/g weight gained). All clinical observations reversed within recovery period. <u>*25.7 mg/kg bw/day*</u> – *Clinical*: reduced body weight gain (↓3.9%-14.7% during weeks 1-8), increased food utilisation. All clinical observations reversed within the recovery period. Haematological and biochemical parameters were not measured in this study. *A NOEL or NOAEL could not be determined for this study due to a reduction in body weight gain observed at the lowest dose (27.5/25.7 mg/kg bw/day).
8 week study Oral (diet) Rats/Wistar 20 females/dose Purity: 97.7% Non-guideline or GLP (There are no comparable guidelines as this was an investigative study only) Conducted prior to the adoption of GLP compliance. (Paul *el al*, 1978)	0, 100, 175, 250 or 750 ppm corresponding to 0, 12.2, 20.4, 29.2 or 84.8 mg/kg bw/day	<u>*84.8 mg/kg bw/day*</u> – reduced bodyweight gain (↓9.7%-12.3% during weeks 4-8); reduced food consumption (↓5.4% during weeks 1-8); increase in food wastage (↑41.5% during weeks 1-8). <u>*29.2 mg/kg bw/day-*</u> slight reduction in body weight gain (↓1.1%-4.2% during weeks 1-8). <u>*12.2 and 20.4 mg/kg bw/day-*</u> no significant treatment related effects. Haematological and biochemical parameters were not measured in this study. *A NOEL of 175 ppm (20.4 mg/kg bw/day) was derived, based on a reduction in body weight gain in the female rats at the next highest dose (29.2 mg/kg bw/day).
Oral short term studies in dogs		
90-day/180-day study Oral (diet) Dog/Beagle Purity: 94% Non-guideline or GLP Conducted prior to the adopted of OECD 409, but checked for compliance. There are some deviations (e.g. the 90 and 180 days studies were inter-related). Conducted prior to the adoption of GLP compliance.	**<u>Part 1</u>** 0, 4, 10 or 25 mg/kg bw/day for 90 days with 28 days recovery **<u>Part 2</u>** 0, 0.4, 1.8 mg/kg bw/day for 90 days or 4 mg/kg bw/day for 180 days For both parts, Pirimicarb was suspended with Dispersol OG and mixed in food	**<u>Part 1</u>** <u>*25 mg/kg bw/day*</u>- *Clinical*: reduced body weight (M:↓1.7%-6.7% during weeks 5-12), 1 male died at day 70 (exhibiting rapid weight loss, urinary incontinence, lethargy and severe anaemia, upon post mortem examination this individual revealed abdominal ascites, a heavy nematode infestation of the ileum and congestion of the thymus, spleen and liver). *Haematology*: anaemia (M: 1/4 (↓66.3% Hb – N.B. this male died day 70), F:1/4 (↓43.2% Hb- reversed within 28 day recovery period)), increased erythrocyte diameter (M:↑3.7%, F:↑5.1%), markedly increased circulating erythroblasts (M&F) in individual dogs. *Cholinesterase Activity*: reduction in plasma cholinesterase activity (M:↓16.5%-37.1%, F:↓7.4%-29.8%), reduction in erythrocyte cholinesterase activity (M: ↓16.8%-27.2% during weeks 6-12; F:↓21.1%-24.8% during weeks 10-12). *Bone marrow*: post-mortem examination of the bone marrow of the male that died showed marked erythropoietic hyperplasia with delayed maturation of the red cell series and the presence of numerous megaloblasts. Examination of the bone marrow for all animals showed an increase in the number of early normoblasts in males during the recovery

Study conducted in 2 parts: **<u>Part 1:</u>** 90 days 4/sex/dose [of which 2/sex/sode were sacrificed after an additional 28-day recovery period] **<u>Part 2:</u>** 90 or 180 days 4/sex/dose (Conning, Garner & Griffiths, 1968)		phase. In females, there was an increase in megaloblasts before and after recovery. *Spleen:* haemopoiesis in the anaemic male dog that died at day 70. *Lymph Nodes*: haemopoiesis in the anaemic male dog that died at day 70; slight increase in reactive changes in the lymph nodes. *Liver*: slight increase in the incidence of focal inflammatory lesions. *<u>10 mg/kg bw/day</u>*- *Haematology*: anaemia (F:1/4 females (↓63.1% Hb)), markedly increased circulating erythroblasts (M&F) in individual dogs. *Bone marrow:* bone marrow examination of all the recovery phase animals revealed increases of myeloblasts and lymphocytes in males. In females, there was an increase in megaloblasts before and after recovery. *Cholinesterase Activity*: reduced plasma cholinesterase activity (M:↓4.8%-16.7%, F:↓3.5%-24.5%), reduced erythrocyte cholinesterase activity (M:↓11.5%-16.4% during weeks 10-12; F:↓9.6%-18.6% during weeks 6 and 10-12). *Spleen:* haemopoiesis in the anaemic female dog. *Lymph Nodes*: haemopoiesis in the anaemic female dog, slight increase in reactive changes in the lymph nodes. *Liver*: slight increase in the incidence of focal inflammatory lesions. *<u>4 mg/kg bw/day</u>- Haematology*: In females, there was an increase in megaloblasts before and after recovery. *Lymph nodes*: slight increase in reactive changes in the lymph nodes. *Liver*: slight increase in the incidence of focal inflammatory lesions. *Since, bone marrow effects were observed at all dose levels, an overall NOEL was not determined for this study. A NOAEL of 10 mg/kg bw/day was determined for erythrocyte cholinesterase inhibition in male and female dogs (<20% inhibition at this dose). **<u>Part 2</u>** *<u>4 mg/kg bw/day (180 days)</u>*- *Clinical:* 1M and 1F developed similar illnesses at weeks 4 and 13, respectively (illness characterised by: lethargy, loss of appetite, weight loss, pyrexia, slight dehydration and passage of a bloody stool) both animals recovered within a few days, however, the male exhibited reoccurrence of symptoms on two occasions. Post mortem examination of both animals revealed haemorrhagic cystitis (polypoid in the female), congested abdominal lymph nodes (M&F) and congested mammary tissue (F). The study report concluded that this illness was not treatment related and is often observed in this strain of dog. *Haematology: F:* reduced serum iron levels at all time points. *Bone marrow:* increased megaloblasts (at 90 and 180 days in both M & F), increased myelocytes-neutophills (at 90 and 180 days in M) and decreased neutrophills (at 90 and 180 days M)), which did not develop into anaemia. *<u>0.4 and 1.8 mg/kg bw/day (90-days)-</u>* no significant treatment related effects. *A NOEL of 1.8 mg/kg bw/day was derived based on observed bone marrow changes in male and females observed at the next highest dose (4

		mg/kg bw/day). *The overall NOEL for both parts of this study was concluded to be 1.8 mg/kg bw/day.
1 year study Oral (diet-capsules) Dog/Beagle 4/sex/dose Purity: 97.5% OECD 452 (1981), GLP. (Horner, 1998)	0, 3.5, 10 or 25/35 mg/kg bw/day (Top dose group- 1 week 35 mg/kg bw/day followed by 2 week recovery period and 25 mg/kg bw/day from week 4 onwards)	<u>*35 mg/kg bw/day-*</u> *Clinical*: tremor, salivation, thin appearance, sides pinched in, unsteady gait, subdued behaviour, irregular breathing and/or occasional coughing, slightly increased incidence of vomiting and regurgitation. Due to toxicity, the top dose of 35 mg/kg bw/day was reduced to 25 mg/kg bw/day after 1 week. <u>*25 mg/kg bw/day*</u>- *Clinical*: tremor; thin appearance, irregular breathing, coughing, marginally increased incidence of fluid faeces, reduced body weight (M:↓4.9-7.1%, F:↓6.1%-9.2% during weeks 2-53), reduced food consumption (F:↓0.6%-23.5% during weeks 2-52), pallor of the mucus membranes (M:2/4), one female was sacrificed following significant bodyweight reduction, pallor (during weeks 34-36) and moderately severe anaemia. *Haematology*: one female exhibiting moderately severe anaemia (decreased RBC count (↓52.1%), haemoglobin (↓53.7%), haematocrit (↓42.0%) and mean cell haemoglobin concentration; increased mean cell volume (↑19.4%), circulating reticulocytes and slight to moderate macrocytosis and hypochromasia). *Cholinesterase Activity*: reduced erythrocyte cholinesterase activity (F: ↓20.7% at week 52), reduced brain cholinesterase activity (F: ↓22% at week 53); reduced plasma albumin (M:↓8.3%-8.6%, F:↓4.4%-11.9% during weeks 13-53), reduced total protein (M:↓6.4%-8.0% during weeks 13-53, F:↓7.1%-9.7% during weeks 26-53). *Bone marrow*: hyperplasia (sacrificed female only), significantly decreased myeloid:erythroid ratio (0.1:1, sacrificed female only), minimal/slight extramedullary haemopoiesis. *Liver*: haemosiderin deposition in the anaemic female sacrificed at week 36, of those animals that survived until the end of the study a minimal/slight increase in haemosiderin deposition was observed (M:3/4). *Spleen*: extramedullary haemopoiesis was observed in the anaemic female sacrificed at week 36, of those animals that survived until the end of the study minimal/slight extramedullary haemopoiesis was observed (M:1/4, F:1/3). <u>*10 mg/kg bw/day-*</u> *Clinical*: slight tremor (F: 1/4). *Cholinesterase Activity*: reduced erythrocyte cholinesterase activity (F:↓ 7.3%), reduced brain cholinesterase activity (F:↓10%). *Spleen*: slight extramedullary haemopoiesis (F1/4) reduced total protein (F:↓9% at week 26). <u>*3.5 mg/kg bw/day-*</u> *Cholinesterase Activity*: reduced erythrocyte cholinesterase activity (F:↓9.6%), reduced brain cholinesterase activity (F:↓2.8%). *A NOEL of 10 mg/kg bw/day was determined for male dogs based on a reduction in body weight, food consumption, plasma albumin and total proteins and increased haemosiderin deposition in the liver and spleen at

		the next highest dose. A NOEL of 3.5 mg/kg bw/day was determined for female dogs based on increased haemosiderin deposition in the spleen, tremors (1 female) and a possible dose related decrease in brain cholinesterase activity at the next highest dose. NB: there was a period of approx 22 hours between dosing and the collection of blood samples for the determination of plasma and erythrocyte cholinesterase activity (i.e. considerable reactivation could have occurred).
2 year feeding study. Oral (diet) Dog/beagle 4/sex/dose. Purity not specified. Non-guideline or GLP Conducted prior to the introduction of OECD 452 but has been checked against it for compliance. There are some deviations in the conduct and reporting of the study but overall it is considered to be acceptable. Conducted prior to adoption of GLP compliance (Garner, Litchfield & Watson, 1971)	0, 0.4, 1.8, or 4 mg/kg bw/day (diluted in maize oil, suspended in Dispersol OG and mixed with food)	*4 mg/kg bw/day-* Slight increase in erthroid:myeloid ratio (F:2/4). *0, 0.4 and 1.8mg/kg bw/day-* No significant treatment related effects. *A NOEL of 4 and 1.8 mg/kg bw/day was derived for males and females, respectively, based on the slight and equivocal effects on erythroid:myeloid ratio in some females at 4 mg/kg bw/day.
16 week study (with 6 week recovery period) Oral (diet) Dog/Foxhound 5M and 5F Purity: 98% Non-guideline or GLP Conducted before the introduction of OECD 409 but checked for compliance with this guideline. There are a number of deviations from the guideline in both the conduct and reporting of the study (e.g. the dosing regime) which make it of	0, 2, 25/50 mg/kg/day Non-standard dosing regime for animals in the 25/50 mg/kg/day dose group M1 25 mg/kg/day (wk 1-4), 50 mg/kg/day (wk 5-6) (sacrificed on day 43) M2 25 mg/kg/day (wk 1-7), 50 mg/kg/day (wk 8-11) M3 25 mg/kg/day (wk 1-7), 50 mg/kg/day (wk 8-11) and 25	One male (M1) died after 43 days (exposed to 25 mg/kg bw/day for 28 days and 50 mg/kg bw/day for 10 days and not dosed for 5 days) with enlargement of the gall bladder, dark bile, areas of diffuse haemorrhage in the small intestine and intussusception of the ileum (it is not clear whether this was treatment related). 5-15% reduction in body weight when exposed to 50 mg/kg bw/day. Haematological parameters were inconsistent with some animals exhibiting reduced haemoglobin (M2 ↓ 72% and F2 ↓ 30%), packed cell volume and erythrocyte count and increased reticulocytes. Other animals showed slight or no changes (e.g. ↓Hb of 6% in M3, 4% in F1 and 13% in F3). Bone marrow changes included ↑ in normoblasts and a tendency to suppress bone marrow activity when dosed with 50 mg/kg/day. This reversed when dose reduced to 25 mg/kg bw/day and all marrow samples were normal one week after dosing. A >6 fold increase in spleen weight of females. However there was a

questionable relevance. It has been included for completeness. Conducted prior to adoption of GLP compliance (Fox, 1978)	mg/kg/day (wk (11-16) F1 25 mg/kg/day (wk 1-4), 50 mg/kg/day (wk 5-6), 25 mg/kg/day (wk 7), 50 mg/kg/day (wk 8-11) and 25 mg/kg/day (wk 11-16) F2 and F3 25 mg/kg/day (wk 1-7), 50 mg/kg/day (wk 8-11) and 25 mg/kg/day (wk (11-16) The substance was added to the diet in a suspension with Tween 80	comparatively low spleen weight in controls. Up to >85% reduction in plasma cholinesterase was observed in all treated animals. Erythrocyte cholinesterase activity was not affected. *A NOEL was not derived for this study due to deficiencies in the methodology.

*All NOEL and NOAEL values provided in the present CLH Report have been derived from the Draft Assessment Report (DAR) produced in accordance with Directive 91/414/EEC.

Dermal Studies

Dose schedule	Dose levels	Results
21-day study Dermal Rat/Alpk:APfSD 5/sex/dose Purity= 97.6% OECD 410 (1981), GLP (Lees & Leah, 1995)	0, 40, 200 or 1000 mg/kg bw/day (5 days per week/ 6 hours per day) Topical application: (paste in deionised water; occlusive dressing)	*1000 mg/kg bw/day-* reduction in brain cholinesterase activity (M:↓26.0%, F:↓22.5%); reduction in plasma cholinesterase activity (M:↓19.7%, F:↓39.7%). *200 mg/kg bw/day-* reduction in brain cholinesterase activity (M:↓10.5%, F:↓11.3%), reduction in plasma cholinesterase activity (F:↓23.4%). *40 mg/kg bw/day-* No significant treatment related effects. *A NOAEL of 200 mg/kg bw/day was determined for both male and female rats based on reduced brain cholinesterase activity observed at the next highest dose.

*All NOEL and NOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

4.7.1.1 Repeated dose toxicity: oral

There are two 8-week studies and one 90-day study available in the rat. A 90/180-day study, 16-week study, 1-year study and 2-year study is available in the dog. No studies are available for the mouse.

Rat studies

90-day study:

In the 90-day study (Griffiths & Conning, 1968; Hodge, 1995), rats were dosed with Pirimicarb either via the diet (M:12.9 or 38.8 mg/kg bw/day, F:15.3 or 47.1 mg/kg bw/day) or via gavage (25 mg/kg bw/day). For those animals that were administered Pirimicarb via the diet, no adverse compound related changes were observed. However, Pirimicarb exposure did induce some small haematological changes, but these did not exhibit a dose or time related response and were inconsistent between the sexes. A slight decrease in plasma cholinesterase activity was observed at 750 ppm (47.1 mg/kg bw/day); however, at most time points this decrease was not considered to be adverse (i.e. less than 20% inhibition). No effects were observed on brain and erythrocyte cholinesterase activity levels.

In the group administered Pirimicarb via gavage, 7/25 males and 5/25 females died or were sacrificed due to trauma from repeated cannulation. These deaths were not considered to be compound related. In those animals that survived, a reduction in plasma cholinesterase activity was observed (M:↓29.7%-↓39.3%, F:↓30.1%-↓56.6% during weeks 2-12). However, the extent of plasma cholinesterase inhibition did not increase with time and preliminary investigations showed that it was only detectable if the examination was conducted within 4 hours of dosing (all cholinesterase examinations in this study were conducted 1 hour after dosing). During the 28-day recovery period, the extent of plasma cholinesterase inhibition reduced to levels not considered to be adverse (M:↓5.3-6.3%, F:↓6.8%-12.1%).

Although, there was no clear dose or time related trends in erythrocyte cholinesterase inhibition, there were indications of an inhibitory effect in males and females at a number of time points. However, at most time points the amount of inhibition was <20% and is, therefore, not considered to be adverse. Brain cholinesterase activity was measured, but no effects were observed.

The differing degree of cholinesterase inhibition observed in rats dosed via gavage or via the diet, is likely to be due to the differences in peak plasma concentration of Pirimicarb attained by these modes of administration.

8-week studies:

The two 8-week rat studies (Richards *et al*, 1987; Paul *et al*, 1978) were conducted to very limited protocols (only clinical observations, body weights, food and water consumption were recorded) and were designed to investigate growth retardation. In both studies, Pirimicarb exposure did not affect clinical condition and water consumption, however, a dose related decrease in body weight and food consumption was observed. In the second study (Paul *et al*, 1978), these effects were not accompanied by a change in food utilisation, suggesting that the body weight reduction may be a result of the substance's un-palatability, rather than a direct sign of substance-related toxicity.

Dog Studies

90/180-day study:

In a study composed of 2-parts (Conning, Garner & Griffiths, 1968), Beagle dogs were treated with Pirimicarb via the diet for either 90 or 180-days. In part 1, dogs (4/sex/dose) were exposed to 0, 4, 10 or 25 mg/kg bw/day Pirimicarb for 90 days and were monitored for a further 28-day recovery period (2/sex/dose). Whereas, part 2 animals (4/sex/dose) were treated with either 0, 0.4 or 1.8 mg/kg bw/day Pirimicarb for 90 days or 4 mg/kg bw/day Pirimicarb for 180 days, with the aim of establishing a no observed effect level for the haematological changes observed in Part 1. The key toxicological effects observed were haematological changes and a reduction in cholinesterase activity. Although, this study was conducted before the introduction of GLP and guidelines, it was broadly compliant with modern guidelines.

PART 1: One male of the top dose group died (week 10, 25 mg/kg bw/day) exhibiting rapid weight loss, lethargy, urinary incontinence and what was described as 'macrocytic anaemia' (↓66.3% Hb). Post-mortem examination of this individual revealed abdominal ascites, a heavy nematode infestation of the ileum and congestion of the thymus, spleen and liver. In the bone marrow, marked erythropoietic hyperplasia, delayed maturation of the red cell series and an increase in the number of megaloblasts was observed. All other dogs survived until the end of the study and showed no clinical signs of abnormality, apart from a slight reduction in body weight (M:↓1.7%-6.7% 25 mg/kg bw/day).

In addition to the male that died at week 10, anaemia was observed in 1/4 females in the 25 mg/kg bw/day group (↓43.2% Hb) and 1/4 females in the 10 mg/kg bw/day group (↓63.1% Hb), however, for the top dose female, this reversed within the 28-day recovery period. Anaemia was not observed in any of the other dogs, but examination of terminal blood films revealed an increased erythrocyte diameter (M:↓3.7% F:↑5.1%, 25 mg/kg bw/day) and an increase in the number of circulating erythroblasts (at ≥10 mg/kg bw/day in individual dogs). Investigation of the bone marrow in all animals revealed an increase in megaloblasts (Males at 10 mg/kg bw/day and females in all treatment groups), lymphocytes (males at 10 mg/kg bw/day) and early normoblasts (males at 25 mg/kg bw/day)). These changes were originally described as megaloblastic. However, subsequent investigations have shown that they are characterised by a reduction in the myeloid-erythroid ratio (an indication of myeloid hypoplasia and/or erythroid hyperplasia) and should be described as a compound dependent haemolytic anaemia of the 'penicillin type' (see Section 4.7.1.6.). Effects on the bone marrow improved during the 28-day recovery period, but did not return to normal during this time.

Gross and microscopic pathological examination revealed haemopoiesis of the spleen and lymph nodes in the male of the 25 mg/kg bw/day and female of the 10 mg/kg bw/day dose group who exhibited severe anaemia upon autopsy. Haemopoiesis was not observed in any of the other dogs; including the third anaemic dog (female of the 25 mg/kg bw/day group) that was maintained for the 28-day recovery period. Treated dogs tended to show a higher incidence of focal inflammatory lesions in the liver and reactive changes in the liver, however, the differences were not marked.

Plasma, erythrocyte and brain cholinesterase activity levels were all examined in this study, but the brain cholinesterase activity measurements were difficult to interpret due to the low number of animals examined (2/sex/group) and a high individual variation. Plasma cholinesterase activity was significantly reduced at 25 mg/kg bw/day (M:↓16.5-37.1%, F:↓7.4-29.8%), when compared with controls. Dose related reductions in erythrocyte cholinesterase activity were also observed in the 10 (M:↓11.5-16.4%, F:↓9.6-18.6%) and 25 mg/kg bw/day groups between weeks 6-12 in males and weeks 10-12 in females. However, these reductions reversed within the 28-day recovery period and were not accompanied by adverse neurological and clinical signs of toxicity.

PART 2: No evidence of any compound-related effect on clinical condition or bodyweights were observed, but one male and one female of the 4 mg/kg bw/day group developed similar illnesses, which were deemed by the study author to be non-treatment related. The haematological parameters measured at 0.4 and 1.8 mg/kg bw/day were not affected by treatment; however, serum iron levels were reduced in females receiving 4 mg/kg bw/day for 180 days.

Changes in the bone marrow were evident in the 4.0 mg/kg bw/day group at 90 and/or 180 days. These included, increased myelocytes (males), decreased neutrophils (males and females) and an increase in megaloblasts (males and females), however, these animals did not develop anaemia throughout the duration of the study.

No effects were observed on plasma/brain cholinesterase activity, organ weights, gross necropsy or microscopic findings.

1-year study:

In the most recent dog study (Horner, 1998), groups of Beagle dogs (4/sex/dose) were administered oral doses (capsules) of 0, 3.5, 10 or 25/35 mg/kg bw/day Pirimicarb (purity: 97.5%) for a period of at least 1 year. The initial high dose was 35 mg/kg bw/day, which resulted in significant treatment-related toxicity including tremors, salivation, thin appearance, sides pinched in, unsteady gait, subdued behaviour, irregular breathing and/or occasional coughing. Treatment at this dose level was suspended after 1 week and the dogs were allowed a 2-week recovery period, before treatment with 25 mg/kg bw/day. Following this reduction in dose, clinical signs of toxicity (tremor, thin appearance, irregular breathing, coughing, marginally increased incidence of fluid faeces, reduced body weight, reduced food consumption and pallor of the mucus membranes) were observed. A single incidence of slight tremors was also observed in one female of the 10 mg/kg bw/day dose group.

One female of the 25 mg/kg bw/day dose group was sacrificed (week 36) due to significant body weight loss, pallor and anaemia. The anaemia observed in this female was first reported at week 13 and was characterised by a reduction in red blood cell count (↓52.1%), haemoglobin (↓53.7%), haematocrit (↓42.0%) and slight to moderate macrocytosis and hypochromasia. These effects were enhanced with time and by week 36 this female was described as having moderately severe anaemia with a marked increase in circulating reticulocytes. Histopathological and bone marrow changes for this female were consistent with an increase in red blood cell breakdown and included an increase in haemosiderin deposition, increased bone marrow cellularity and a significant decrease in the myeloid:erythroid ratio (indicative of increased erythropoietic activity). No signs of anaemia were observed in the haematological and bone marrow investigations for the other treated animals. However, an increase in extramedullary haemopoiesis of the spleen (25 mg/kg bw/day: 1/4 M & 1/3 F and 10 mg/kg bw/day: 1/4 F) and an increase in haemosiderin deposition of the liver (25 mg/kg bw/day: 3/4 M) were observed, which may indicate that the ability of these individuals to cope with the level of red blood cell destruction was being challenged.

There were no changes in plasma, erythrocyte or brain cholinesterase activities that could be attributed to treatment in the female sacrificed at week 36 in the 25 mg/kg bw/day dose group. In surviving animals, there was no evidence of any adverse effects on plasma cholinesterase activity throughout the study. At 25 mg/kg bw/day erythrocyte cholinesterase activity was significantly reduced (>20% reduction in activity) in females at week 52. However, it should be noted that the time interval between dosing and blood sampling appears to be approximately 22 hours, which could have allowed considerable cholinesterase reactivation to occur. At termination, mean brain cholinesterase activity for females receiving 25 mg/kg bw/day was statistically significantly lower than the control values (22% reduced activity).

2-year study:

In the third (2-year) study conducted with beagle dogs, no effects on haematology, cholinesterase activity or clinical condition were observed. However, the dose selection in this study was questionable (top dose of 4 mg/kg bw/day). At the top dose there was a slight increase in the erythroid:myeloid ratio in the bone marrow, which could be an adaptive response to increased red blood cell haemolysis.

16-week study:

The haematological changes observed in the 16-week foxhound study (Fox, 1978) appear to be similar to those observed in the beagle dog. Anaemia and reticulocytosis were associated with treatment at 50 mg/kg/day, with a reduction in haemoglobin, packed cell volume and erythrocyte count and an increase in reticulocytes. Anaemia reversed when the dose level was reduced to 25 mg/kg/day (after week 12) or when treatment stopped. Changes in bone marrow were associated with treatment at 50 mg/kg/bw with an increase in the number of normoblasts from week 9 and a tendency towards suppression of bone marrow activity (hypoplasia). These effects were reversed when the dose was reduced to 25 mg/kg/day (after week 12) and all bone marrow samples were considered normal, one week after cessation of treatment. A marked increase in absolute and relative spleen weight was observed in all treated females when compared with the control female.

Inhibition of plasma cholinesterase was associated with treatment at 25 and 50 mg/kg/day. Inhibition compared with control levels reached up to 85% at 25 mg/kg bw/day and over 90% at 50 mg/kg bw/day. This effect reversed within seven days of the cession of treatment. Erythrocyte cholinesterase activity was not affected. After 43 days of treatment (28 days at 25 mg/kg/day, 10 days at 50 mg/kg/day and not dosed for 5 days), one dog was scarified in extremis following clinical deterioration observed over the preceding 14 days. Gross necropsy of this dog revealed ‘massive’ enlargement of the gall bladder with dark bile, areas of diffuse haemorrhage in the small intestine and intusussception (i.e. inversion/folding back) of the ileum.

However, given the low number of control animals (1 male and 1 female), the lack of historical control data for foxhounds and an unusual dosing regime (including periods of exposure to 25 mg/kg bw/day and periods of exposure to 50 mg/kg bw/day pirimicarb), it is not possible to determine if the changes observed in this study were associated with treatment. Therefore, it is considered that the results of this study are not sufficient for classification and labelling purposes.

4.7.1.2 Repeated dose toxicity: inhalation

No studies available.

4.7.1.3 Repeated dose toxicity: dermal

One 21-day dermal repeated dose study is available in rats (5/sex/dose) in which 0, 40, 200 or 1000 mg/kg bw/day Pirimicarb was applied to the skin under an occlusive dressing. In this study, no signs of clinical toxicity, skin irritation, haematology or macroscopic/microscopic changes were observed. There was a statistically significant reduction in brain cholinesterase activity in both sexes at 1000 mg/kg bw/day, however, this was not accompanied by clinical signs of toxicity and the sampling time was delayed to 18-24 hours after the last topical administration. Statistically significant reductions in plasma cholinesterase activity was also observed in males and females at 200 mg/kg and above, but this is thought to provide evidence of absorption rather than a toxicological effect. Blood samples for cholinesterase measurements were also taken 18-24 hrs after the last topical application.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

Table 12: Summary table of additional repeated dose toxicity studies

Dose schedule	Dose levels	Results
Mechanistic study- to characterise the anaemia observed in pure-bread beagle dogs Dog/Beagle 1/sex/dose Purity= 95% Not GLP or guideline There are no comparable guidelines and the study was conducted prior to adoption of GLP compliance. (Garner *et al*, 1972)	Dosing with Pirimicarb: 25 or 50 mg/kg bw/day (suspended in Dispersol OG and sprayed onto the morning food ration for each dog) Dosing with Haemantinics (vitamin B12, vitamin B6, iron and/or folic acid): No details on the dose and method of administration.	**Effect of dosing with pirimicarb**: *50 mg/kg bw/day*- The female dog did not develop anaemia following 110 weeks of dosing (no treatment related biochemical, haematological and clinical effects observed). The male dog exhibited signs of anaemia (falling haemoglobin levels accompanied by changes in red blood cell morphology, evidence of a few nucleated red blood cells, reticulocytosis and marked erthyroid hyperplasia) after 10 weeks of dosing. Differential cell counts showed a change in the pattern of developing red cells giving the appearance of transitional megaloblastosis. Heamolysis was a prominent feature. *25/mg/kg bw/day*- The male dog did not develop anaemia following 110 weeks of dosing with pirimicarb (no treatment related biochemical, haematological and clinical effects observed). The female dog exhibited signs of anaemia (falling haemoglobin levels accompanied by changes red blood cell morphology, evidence of a few nucleated red blood cells, reticulocytosis and marked erthyroid hyperplasia) after 10 weeks of dosing. Differential cell counts showed a change in the pattern of developing red cells giving the appearance of transitional megaloblastosis. Haemolysis was a prominent feature. **Effect of dosing with pirimicarb and haemantinics**: For those dogs showing signs of developing anaemia following treatment with Pirimicarb additional dosing with haematinics was initiated once haemoglobin levels had fallen to 50% of the pre-experimental value (from week 18 for the male dosed with 50 mg/kg bw/day Pirimicarb and from week 24 for the female dosed with 25 mg/kg bw/day).

In an additional study reported in 1972, unrelated young adult dogs (1/sex/group) were dosed with either 25 or 50 mg/kg bw/day Pirimicarb (purity: 95%; suspended in 'dispersol' OG and sprayed on to the morning food ration in approximate amounts), with the aim of reproducing and characterising the anaemic reaction observed in previous repeated dose dog studies. Blood, urine and bone marrow samples were obtained pre-experimentally and at two weekly intervals for the first 12 weeks of the study. Thereafter, the sampling frequency was determined by the clinical and laboratory findings, or by therapeutic regime.

The female in the 50 mg/kg/day group and the male in the 25 mg/kg/day group were dosed continually for more than 110 weeks, in which time neither dog developed clinical, haematological or histopathological signs of anaemia.

The other two dogs (the male in the 50 mg/kg/day group and female in the 25 mg/kg/day group) exhibited evidence of anaemia (falling haemoglobin levels, accompanied by reticulocytosis and marked erythroid hyperplasia) following 10 weeks of dosing. Peripheral blood films taken from these dogs revealed changes in the size and shape of the red cells and the occurrence of nucleated red cells were increased. No abnormalities were observed in either the white cells or platelets. Once the haemoglobin levels had reduced by 50% (week 18 for the male dosed with 50 mg/kg bw/day and week 24 for the female dose with 25 mg/kg bw/day) these two dogs were selected for additional dosing with haematinics (vitamin B12, vitamin B6, iron and/or folic acid) for up to 32 weeks. The

high doses (exact dose not specified) of heamatinics failed to correct the anaemia, suggesting that haemolysis may be the cause of the observed anaemia. Following the withdrawal of dosing with both Pirimicarb and the haematics, a complete haematological recovery was observed.

Subsequent genealogical and antibody reaction investigations were conducted. The results of the genealogical investigation suggested that sensitivity to Pirimicarb might be genetically determined because the anaemic male in the 50 mg/kg bw/day dose group was found to be the offspring of a female Beagle dog, which was also the mother of an affected dog used in a previous study. Analysis of serum proteins showed that exposure to Pirimicarb produced an antibody reaction in the sera of the two anaemic dogs (accompanied by the absence of a reaction in the 2 unaffected dogs and 20 untreated dogs). Washed red cells from each of the two anaemic dogs were strongly agglutinated by specific antigamma globulin serum, but those of the other two dogs were not. Twenty untreated dogs also gave negative results in this test. Free antibody was demonstrated in the sera of both anaemic dogs but not in the two unaffected dogs nor in sera from 25 untreated dogs. A checkerboard analysis, using sera and cells from 20 untreated dogs did not reveal any cross-section reaction between them. It was shown that the antibody and the antigenicity of the red cells were related in time to the administration of Pirimicarb. The antibody was not present in the pre-experimental serum samples and the red cell possessed the appropriate antigen only when the dogs were receiving Pirimicarb. Withdrawal of Pirimicarb was followed by a marked decline in antibody titre and circulating red cells did not react with specific antiglobulin serum. Characterisation of the antibody showed it to be an immune type (probably IgG).

In an additional follow-up investigation, the female dog previously dosed with 25 mg/kg/day was dosed with 2 mg/kg bw/day for a 14 week period without evidence of a haematological effect. No antibody was detectable in the serum of this animal at the end of this period. The male dog previously dosed with 50 mg/kg/day was dosed with 1 mg/kg/day for a 12-week period without evidence of a haematological effect. The antibody had not been completely absent from the serum of this animal but the titre did not increase, being less than 1 in 2. It was reported that the dose level was then increased 2 mg/kg bw/day although the length of the dosing period was not stated.

4.7.1.7 Summary and discussion of repeated dose toxicity

The key toxicological effects observed in the available repeated dose studies were, haemolytic anaemia and inhibition of cholinesterase activity. These are discussed below:

Anaemia

Signs of haemolytic anaemia or related bone marrow changes were observed in some dogs from 4 mg/kg bw/day. However, individuals displayed a continuum of effects, which ranged from no haematological changes to sub-clinical adaptive changes and anaemia. Anaemia was not observed in rats or mice. Whilst some effects on haematological parameters were observed in rats and mice, these were inconstant between the sexes and did not generally exhibit a dose response. The effects of relevance are summarised in tables 13, 14 and 15 below.

Table 13: Summary of haematological and related effects in rats

Species	Study	Results	Reference
Rat 25/sex/dose	90 day Diet 0, 12.9/15.3 or 38.8/47.1 mg/kg in M/F respectively	*38.8 (M) or 47.1 (F) mg/kg/day* M: ↓ reticulocytes 32.8% (wk 6) and 20% (wk 13) *12.9 (M) or 15.3 (F) mg/kg/day* M:↓Hb 4.5%-5.7%	Griffiths & Conning 1968
Rat 25/sex/dose	90 day Gavage 0 and 25 mg/kg/day	*25 mg/kg/day* M: ↓Hb 2.6-5.1%, ↓PCV 5.7-6.8% F: ↑Hb 5.4-7.8%, ↑PCV 7.5-8.7%	Hodge, 1995
Rat 64/sex/dose	2 year Diet 0, 75, 250 or 750 ppm	*750 ppm (changes from control weeks 13-104)* F; ↑ Hb (0.6-5.3%), ↑Hct 0.7-5% M; ↑MCV 2.1-3.3%, ↑Mean cell Haemoglobin (2.1-4%)	Tinston 1992

Table 14: Summary of haematological effects in mice

Species	Study	Effects	Reference
Mice 55/sex/dose	80 week Diet 0, 50, 200, 700 ppm	*700 ppm* M ↑RBC (6.4%), ↓MCV (8%), ↓Mean cell haemoglobin (5.8%) F; ↑Mean cell haemoglobin concentration (3%), ↑ platelet count (30.1%), ↓ MCV (9.1%), ↓ Mean cell haemoglobin (6.7%)	Rattray 1998

Table 15: Summary of haematological effects in dogs

Species	Study	*Results*	Reference
Dog (Beagle) 4/sex/dose	90 day (diet) 0, 4, 10 and 25 mg/kg/day	*25 mg/kg/day* **M**: 1/4 (died on day 70) ↓Hb (66.3%), ↑erythrocyte diameter (3.7%), ↑ circulating erythroblasts, marked erythropoietic hyperplasia in the bone marrow with delayed maturation of red blood cell series and the presence of numerous megaloblasts. Haemopoiesis in the spleen. 3/4 remaining M: ↑ circulating erythroblasts. ↑ number of early normobalsts during recovery period. **F**: 1/4 ↓Hb (43.2%) (reversed with the 28 day recovery period), ↑ erythrocyte diameter (5.1%), ↑ circulating erythroblasts	Conning, Garner & Griffiths, 1968

		F: 3/4 ↑ circulating erythroblasts. ↑ in megaloblasts before and after recovery. *10 mg/kg bw/day* **M**: ↑ circulating erythroblasts, ↑ myeloblasts and lymphocytes in bone marrow in recovery period. **F:** 1/4 ↓Hb (63.1%), ↑ circulating erythroblasts, ↑ increase in megaloblasts before and after recovery. Haemopoiesis in the spleen and lymph nodes **F**:3/4 ↑ circulating erythroblasts ↑ increase in megaloblasts before and after recovery. *4 mg/kg bw/day* F; ↑ megaloblasts before and after recovery	
Dog (Beagle) 4/sex/dose	180 days (diet) 4 mg/kg/day	*4 mg/kg/day* M: Bone marrow: ↑ megaloblasts, ↑ myleocytes-neutrophils and ↓ neutrophils F: ↓ serum iron levels. Bone marrow: ↑ megaloblasts	Conning, Garner & Griffiths, 1968
Dog (Beagle) 4/sex/dose	1 year (diet capsule) 0, 3.5, 10 and 25/35 mg/kg/day	*25 mg/kg/day (reduced from 35 mg/kg/day)* M: Minimal/slight haemosiderin deposition liver (3/4), minimal/slight extramedullary haemopoiesis spleen (1/4). F: 1/4 (sacrificed during week 36): ↓ RBC (52.1%), ↓Hb (53.7%), ↓ Hct (42%) and mean cell haemoglobin concentration; ↑MCV (19.4%), circulating erythrocytes and slight to moderate macrocytosis and hypochromasia. Bone marrow hyperplasia, significantly decreased myeloid:erythroid ratio (0.1:1). Minimal haemosiderin deposition in the liver. Extramedullary haemopoiesis in the spleen. For females that survived to termination there were no treatment related haematological changes. However, in 1female, minimal/slight extramedullary haemopoiesis was observed in the spleen. *10 mg/kg bw/day* F: 1/4 slight extramedullary haemopoiesis in the spleen. The study report noted that it could not be conclusively attributed to treatment with pirimicarb.	Horner, 1998
Dog (Beagle)	2 years Diet	*4 mg/kg/day* F: 2/4 ↑ erythroid:myeloid ratio	Garner, Litchfield & Watson,

4/sex/dose	0, 0.4, 1.8 or 4 mg/kg/day		1971
Dog (Foxhound) 5M and 5F (3M and 3F treated with 25/50 mg/kg/day	16 week Diet 0, 2, 26 and 50 mg/kg/day Note that there was a non-standard dosing regime with exposure to 25 mg/kg/day and periods of exposure to 50 mg/kg/day. Study included for completeness.	<u>50 mg/kg/day/25 mg/kg/day</u> Haematological parameters were inconsistent with some animals exhibiting reduced haemoglobin (M2 ↓ 72% and F2 ↓ 30%), packed cell volume and erythrocyte count and increased reticulocytes. Other animals showed slight or no changes (e.g. ↓Hb of 6% in M3, 4% in F1 and 13% in F3). Bone marrow changes included ↑ in normoblasts and a tendency to suppress bone marrow activity when dosed with 50 mg/kg/day. This reversed when dose reduced to 25 mg/kg bw/day and all marrow samples were normal one week after dosing. A >6 fold increase in spleen weight of females. However there was a comparatively low spleen weight in controls.	Fox, 1978
Dog Beagle 1/sex/dose 50 and 25 mg/kg/day	110 weeks	*<u>50 mg/kg/day</u>* M: ↓ Hb (50%), changes in RBC, reticulocytosis, marked erythroid hyperplasia, change in appearance of developing cells, appearance of transitional megaloblasts, haemolysis. F: No anaemic effects <u>25 mg/kg/day</u> M; No anaemic effects F; ↓ Hb (50%), changes in RBC, reticulocytosis, marked erythroid hyperplasia, change in appearance of developing cells, appearance of transitional megaloblasts, haemolysis. Note that when Hb had ↓ by 50% the animals were treated with haematinics.	Garner et al, 1972

Table 16: Summary of anaemia in dogs

Dose (mg/kg/d)	90 DAY	16 WEEK (NB: non-standard dosing, summary included for completeness)	180 DAYS	110 WEEKS	1 YEAR	2 YEAR
50	-	***1/2 M; anaemia*** ***1/3 F ; anaemia*** Bone marrow changes F; Increased spleen weight	-	***1/1 M; anaemia*** 1/1 F; no anaemic effects	-	-
25	***1/4 M: anaemia (died))*** 3/4 M: ↑ circulating erythroblasts. ↑ number of early normobalsts during recovery period. ***1/4 F: anaemia*** 3/4 F: ↑ circulating erythroblasts. ↑ in megaloblasts before and after recovery.	Effects reversed or reduced when dosing reduced	-	1/1 M; No anaemic effects ***1/1 F; anaemia***	(NB – dose reduced from 35 mg/kg/day) M: slight haemosiderin deposition liver (3/4), slight extramedullary haemopoiesis spleen (1/4). ***1/4 F; anaemia (sacrificed)***, minimal/slight extramedullary haemopoiesis (spleen) 1/3 others.	-
10	M: ↑ myeloblasts and lymphocytes in bone marrow in recovery period. ***1/4 F; anaemia*** F:3/4 ↑ increase in megaloblasts before and after recovery.	-	-	-	*F: 1/4* slight extramedullary haemopoiesis in the spleen.	-
4	F; ↑ megaloblasts before and after recovery	-	M: Effects in bone marrow and delayed RBC maturation. F: ↓serum iron levels. Effects in bone marrow and delayed RBC maturation.	-	-	F 2/4: ↑ erythroid: myeloid ratio

No effects on haematological parameters below 4 mg/kg/day and therefore lower doses not included in the table.

Cholinesterase inhibition

The mechanism of action of pirimicarb is inhibition of acetylcholinesterase. 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 JMPR guidance,

> 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. 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.
>
> iii) 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.

Reductions in cholinesterase activity were observed in both rats and dogs, but in a number of studies (Horner, 1988 and Lees &Leah, 1995) the sampling time was >4 hours after dosing, allowing time for enzyme reactivation.

Erythrocyte and brain cholinesterase activity were reduced in some studies to levels considered to be adverse (>20%). However, these effects were transient (enzyme reactivation occurs within 4 hours of dosing) and did not increase in severity during the course of the study. Therefore, we consider that this effect is better characterised as an acute or single dose effect.

In the majority of studies, a reduction in erythrocyte and brain cholinesterase activity was not accompanied by adverse clinical or neurological effects. However, in the most recent dog study (Horner, 1988), individuals of the top dose group (35/25 mg/kg bw/day) exhibited tremors, salivation, irregular breathing, coughing and a reduction in body weight. Similar effects were also observed in the acute oral and acute inhalation studies (see section 4.2); in these studies, the adverse neurological and clinical effects lead to mortalities, for which classification as Acute Tox. 3 (H301) and Acute Tox. 3 (H331) has been proposed.

A reduction in plasma cholinesterase activity is not considered adverse and is generally used as an indication of absorption rather than toxicity.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

A classification for STOT-RE is indicated when significant adverse effects occur at or below 100 mg/kg/d. It should be noted that this value is based on a 90 day study in rats and should be amended accordingly for studies of a longer or shorter duration in line with the guidance on the application of the CLP criteria (e.g. ≤ 25/mg/kg/day for a 1 year study, ≤ 12 mg/kg/day for a 2 year study). However, the effects of concern were observed in dogs and the guidance values may need further consideration to account for the species differences. Therefore expert judgement is required.

Isolated deaths/sacrifices were observed in two anaemic dogs (1/4 males at 25 mg/kg bw/day in the 90/180-day study and 1/4 females in the 35/25 mg/kg bw/day dose group in the 1-year study). However, for each of these animals it was not clear whether anaemia was the cause of death. Gross necropsy of the dog that died at 25 mg/kg bw/day in the 90/180 day study revealed abdominal acites and a heavy nematode infestation of the ileum, in addition to anaemia. Whilst significant toxicity (tremor, thin appearance, irregular breathing, reduced body weight and reduced food consumption) was observed in the 35/25 mg/kg bw/day dose group in which the one anaemic female was sacrificed.

Effects on haematological parameters or related bone marrow changes were observed in some dogs from 4 mg/kg bw/day in 90/180 day and 2 year studies, which is below the general guidance value (in the rat) for classification with STOT-RE. However, at this does level, the effects were limited to delayed red blood cell maturation and effects in the bone marrow (refer to tables 15 and 16). At higher dose levels, individuals displayed a continuum of effects, which ranged from no haematological changes to sub-clinical adaptive changes and anaemia. This wide spectrum of responses occurred between individuals within dose groups and across study durations. For example, in the 90/180 day study (conning, Garner & Griffiths, 1968), most dogs did not develop anaemia; only 1/4 males and 1/4 females of the 25 mg/kg bw/day dose group and 1/4 females in the 10 mg/kg bw/day dose group developed anaemia. In the 1 year guideline study, which utilised comparable doses, again most dogs did not develop anaemia; only 1/4 females exhibited anaemia at 25 mg/kg/day. Of those dogs that did not develop anaemia at 25 mg/kg/day, 3/4 males did show a minimal/slight increase in haemosiderin deposition in the liver and 1/4 males and 1/3 surviving females showed haemopoiesis in the spleen. None of the animals dosed with 10 mg/kg/day developed anaemia and there were no treatment related effects on haematological parameters; 1/4 females at this does level did show slight haemopoiesis in the spleen, although the study report noted that this could not conclusively be attributed to treatment with pirimicarb.

In a mechanistic study in which male and females dogs (1/sex/dose) received 25 or 50 mg/kg/day pirmicarb for 110 weeks, inconsistent results were again obtained. At 50 mg/kg/day the female did not develop anaemia (with no treatment related haematological effects observed) but the male did exhibit signs of anaemia. At 25 mg/kg/day the male did not develop anaemia (with no treatment related haematological effects observed) but the female did exhibit signs of anaemia.

Anaemeia was not observed in rats or mice. Small changes in the haematological parameters were observed, but these findings did not generally exhibit a dose response and were inconsistent between the sexes.

According to the review paper 'Hazard Classification of Chemicals Inducing Haemolytic Anaemia: An EU Regulatory Perspective' by Muller *et al* (2006) and the CLP guidance document, chronic haemolytic anaemia is a serious effect that warrants classification if the observations are consistent and constitute a toxicologically adverse response to treatment, rather than an adaptive response. Study findings should be interpreted in totality. Since, no signs of anaemia were observed in rats and mice (in repeated dose and/or carcinogenicity studies) and most individuals in the dog studies exhibited either no signs of anaemia or adaptive changes only, it is considered that the effects observed in individual dogs are inconsistent and are not sufficiently severe to justify classification of Pirimicarb with STOT-RE.

4.8.1 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No Classification

4.9 Germ cell mutagenicity (Mutagenicity)

Seven mutagenicity studies on Pirimicarb are available. ***These are summarised below as supportive information***.

4.9.1 Non-human information

Table 17: Summary table of *in vitro* mutagenicity studies

Method	Organism/Strain	Concentrations Tested	Result
Bacterial Mutation Assay (Ames) Purity: 97.6% OECD 471 (1997), GLP (Callander, 1995)	*S. tyhpimurium* (TA1535, TA1537, TA98 and TA100) and *E. coli* (WP2P and WP2PuvrA)	100-500 µg/plate (dissolved in DMSO)	**Negative** ± S9 metabolic activation
Bacterial Mutation Assay (Ames) Purity:98% OECD 471 (1997), GLP (Truman, 1980)	*S. typhimurium* (TA1535, TA1537, TA1538, TA100 and TA98)	4, 20, 100, 500 or 2500 µg/plate (dissolved in DMSO)	**Negative** ± S9 metabolic activation
Mammalian cell gene mutation test Purity: 97.5% OECD 476, GLP (Clay, 1996)	L5178 TK +/- mouse lymphoma cells	≤1400 µg/ml (-S9) ≤237 µg/ml (+S9)	**Negative** – S9 metabolic activation In the absence of S9, Pirimicarb exposure induced isolated increases in mutant frequency; however, these were not reproducible or dose-related. **Positive** + S9 metabolic activation In the presence of S9, Pirimicarb gave a reproducible, dose-related increase in mutant frequency in two out of 3 experiments. The observed increases in mutant frequency were primarily due to increases in mutants with a small colony size, possibly indicative of a clastogenic effect.
Mammalian Chromosome Aberration Test Purity: 98.2% Not guideline, GLP (Wildgoose *et al*, 1987)	Human Lymphocytes	50, 250, 500 µg/ml	**Negative** ± S9 metabolic activation Concentration related reductions in mitotic activity were observed in cultures from both donors, demonstrating that Pirimicarb is biologically active in this test system.

Table 18: Summary table of *in vivo* mutagenicity studies

Method	Organism/Strain	Concentrations Tested	Result
Mouse Micronucleus Test Purity:97.3% Not guideline, GLP (Jones and Howard, 1989)	C57BL/6JFCD-1/Alpk mice/5/sex/group	43.3 or 69.3 mg/kg bw	**Negative** The study report states that Pirimicarb was tested at the maximum tolerated dose in both males and females.
Unscheduled DNA Synthesis Assay Purity: 98.3% Not guideline, GLP (Kennelly, 1990)	Alpk:APfSD rat hepatocytes	50, 100, 200 mg/kg	**Negative** Signs of toxicity were observed at ≥100 mg/kg (4/10 animals died at 200 mg/kg and at 100 mg/kg bw/day signs of cholinergic toxicity were observed).
Dominant Lethal Study Purity: Not guideline or GLP (McGregor, 1974)	CD-1 mice/15/dose (males)	0, 10, 20 mg/kg bw/day	**Negative**

4.9.1.1 In vitro data

The genotoxic potential of Pirimicarb has been assessed in four *in vitro* studies including; two Ames tests, a TK +/- mouse lymphoma assay and a chromosome aberration assay. In all studies, the negative and positive controls behaved appropriately. Pirimicarb was not mutagenic in either the Ames tests or the chromosome aberration assay. However, in some replicates of the TK+/- mouse lymphoma assay, Pirimicarb induced a positive, reproducible and dose-related response, when in the presence of metabolic activation. The increased mutant frequency was reported to be primarily due to small colony size mutants, indicative of a clastogenic effect.

4.9.1.2 In vivo data

The genotoxic potential of Pirimicarb has been investigated in two somatic cell *in vivo* studies (mouse micronucleus assay and an unscheduled DNA synthesis assay) and one germ cell *in vivo* study (dominant lethal study) all of which were negative. In all studies, the negative and positive controls behaved appropriately.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

The potential genotoxicity of Pirimicarb has been well investigated. The only evidence of genotoxic activity was found in some replicates of the TK+/- mouse lymphoma assay in the presence of S9 metabolic activation. The increased mutant frequency was reported to be primarily due to small colony size mutants, indicative of a clastogenic effect. However, there is no other evidence to support this finding as clastogenicity was not detected in the *in vitro* cytogenic test or the *in vivo* mouse micronucleus assays.

4.9.5 Comparison with criteria

The mutagenicity data presented in Tables 17 and 18 is given as supportive information only. No classification is proposed for mutagenicity.

4.9.6 Conclusions on classification and labelling

No Classification

4.10 Carcinogenicity

There are two carcinogenicity studies available in rats and three carcinogenicity studies available in mice. However, due to an outbreak of disease, followed by drug treatment and high levels of mortality, the findings of one rat study and one mouse study have been rendered unreliable. These studies have not been considered in the classification and labelling evaluation, but are presented in Table 20 for completeness.

4.10.1 Non-human information

Table 19: Summary table of carcinogenicity studies considered adequate for classification and labelling purposes

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Studies in Rats		
2 years Oral (diet) Rat/Alderley Park (Sprague Dawley derived) 64/sex/dose of which 11 or 12/sex/dose were sacrificed early (week 52) An additional satellite experiment was conducted to determine the effect on plasma, erythrocyte and brain cholinesterase activity (36/sex/dose - with 8/sex/dose being sacrificed at weeks 27, 53, 79 and 105). Purity= 97.3-97.6 % OECD 453, GLP (Tinston, 1992)	Rats from both the main study and the satellite experiment were dosed with: 0, 75, 250 or 750 ppm, corresponding to 0, 3.7, 12.3 or 37.3 mg/kg bw/day in males and 0, 4.7, 15.6 or 47.4 mg/kg bw/day in females	**Non-neoplastic findings:** High mortality rates were observed after approximately 1 year of treatment. However, this occurred across all treatment groups in similar numbers and in a similar time response pattern. There was no evidence of disease or infection that might have compromised the interpretation of this study. *750 ppm (37.3(M)/47.4(F) mg/kg bw/day)–* *Clinical:* 24 (M) and 36 (F) survived to termination, reduced body weight gain (M:↓2.5%-13.4%; F:↓9.6%-18.7% during weeks 1-105), reduced food consumption (M:↓1.8%-12.4%; F:↓5.1%-7.7% during weeks 6-104), reduced food utilisation (M:↓5.5%, F:↓8.7% during weeks 1-4). *Haematology*: small increase in haemoglobin (F:↑0.6%-5.3% during weeks 13-104), increased haematocrit (F:↑0.7%-5.0% during weeks 13-104), increased mean cell volume (M:↑2.1%-3.3% during weeks 13-104), increased mean cell haemoglobin (M:↑2.1%-4.0% during weeks 13-104). *Biochemical:* increased levels of plasma cholesterol (M:↑16.6%-57.6%%, F:↑21.6%-36.6% during weeks 13-104), increased triglycerides (M:↑11.7%-55.3%, F:↑8.3%-47.0% during weeks 13-104). *Cholinesterase Activity:* consistently reduced plasma cholinesterase activity in females (F:≤↓28%), occasional reduced plasma cholinesterase activity in males (M: up to 27% at week 78). *Brain:* increased incidence of slight/moderate necrosis (M:6 of which 5 died before the end of the study). *Adrenal Cortex:* increased incidence of minimal/slight vacuolation (M:48 compared to 39 in the control, F:6 compared to 3 in the control). *Kidney*: increased incidence of minimal/slight pelvic transitional cell hyperplasia (M:44, F:48), increased incidence of minimal/slight pelvic ectasia (M:8, F:5). *Liver:* increased incidence of minimal hepatocyte hypertrophy (M:5 compared to M:0 in the control) and minimal/slight altered hepatocytes (clear cell) (M:5 compared to M:0 in the control), increase in relative liver weight (M:↑9.2% F:↑11.7%). *Nervous system*: increased severity of static nerve demyelination, increased severity and incidence (M:25 compared to M:20 in the control and F:15 compared to F:2 in the control) of voluntary muscle degeneration *250 ppm (12.3(M)/15.6(F) mg/kg bw/day) –* *Clinical:* 25 (M) and 33 (F) survived to termination, reduced body weight gain (F:↓3.2%-11.4% during weeks 1-105). *Biochemical:* increased plasma cholesterol (F:↑17.8%), increased triglycerides (F:33.2%). *Cholinesterase Activity:* reduced plasma cholinesterase activity (≤↓28%). *Brian:* brain necrosis (M:1 which died before the end of the study). *Kidney:* increased incidence of pelvic transitional cell hyperplasia (M:42, F:41), increased incidence of pelvic ectasia (M:5, F:5). *75 ppm (3.7(M)/4.7(F) mg/kg/bw/day) – Clinical:* 18 (M) and 32 (F) survived to termination. *Brain*: brain necrosis (M:2 both died before the end of the study). *Kidney:* pelvic transitional cell hyperplasia (M: 34, F:35), pelvic vascular ectasia

(M:1, F:2) .

<u>*0 ppm (0 mg/kg bw/day) –*</u> 22 (M) and 37 (F) survived to termination. *Brain:* brain necrosis (M:1 which died before the end of the study). *Kidney:* transitional epithelial hyperplasia (M:33, F:26), pelvic vascular ectasia (M:2, F:3).

<u>Neoplastic findings (decedents and terminal):</u>

No statistically significant increase in the incidence of any particular tumour type.

Brain astrocytoma was observed in 1 male at 75 ppm in the interim kill group. Interim kill data is not included in the table below.

Findings (number of animals with tumours)	Males (ppm)			
	0	**75**	**250**	**750**
Brain- astrocytoma (malignant)				
Decedents	0[a]/ 30[b]	0/ 35	2/ 27	2/ 28
Terminal kill	0[a]/22[b]	2/18	0/25	1/24
Total	0[a]/52[b] (0%)[c]	2/53 (3.8%)	2/52 (3.8%)	3/52 (5.8%)
Brain – meningioma (benign)				
Decedents	1/30	0/35	3/27	1/28
Terminal kill	1/22	1/18	1/25	0/24
Total	2/52 (3.8%)	1/53 (1.9%)	4/52 (7.7%)	1/52 (1.9%)
Findings (number of animals with tumours)	**Females (ppm)**			
	0	**75**	**250**	**750**
Brian- astrocytoma (malignant)				
Decedents	0/16	1/20	0/19	2/16
Terminal kill	0/37	0/32	0/33	0/36
Total	0/53 (0%)	1/52 (1.9%)	0/52 (0%)	2/52 (3.8%)
Brain – meningioma (benign)				
Decedents	0/16	0/20	0/19	0/16
Terminal kill	0/37	0/32	0/33	2/36
Total	0/53 (0%)	0/52 (0%)	0/52 (0%)	2/52 (3.8%)
Uterus-stromal cell sarcoma (malignant)				
Decedents	0/15	0/20	0/19	1/16
Terminal kill	0/37	0/32	0/33	1/36
Total	0/52 (0%)	0/52 (0%)	0/52 (0%)	2/52 (3.8%)

		Uterus- stromal cell polyp (benign) Decedents 2/15 1/20 2/19 2/16 Terminal kill 3/37 5/32 5/33 8/36 Total 5/52 (9.6 %) 6/52 (11.5%) 7/52 (13.5%) 10/52 (19.2%) Mammary gland- fibroadenoma (benign) Decedents 0/15 3/20 1/19 1/16 Terminal kill 3/36 2/31 1/33 5/35 Total 3/51 (5.9%) 5/51 (9.8%) 2/52 (3.8%) 6/51 (11.8%) Mammary gland- adenoma (benign) Decedents 1/15 1/20 0/19 1/16 Terminal kill 0/36 0/31 1/33 1/35 Total 1/51 (2%) 1/51 (2%) 1/52 (1.9%) 2/51 (3.9%) a- number of animals with tumour, b- total number of animals examined, c- incidence
Studies in Mice		
80 week study Oral (dietary) Mice C57BL/10J$_f$CD-1 Alpk/55/sex/ dose Purity= 97.5 % OECD 451, GLP (Rattray, 1998)	0, 50, 200 or 700 ppm corresponding to 0, 6.7, 26.6 or 93.7 mg/kg bw/day in males and 0, 9.0, 37.1 or 130.3 mg/kg bw/day in females.	**<u>Non-neoplastic findings</u>** Survival was >85% across all dose groups. <u>*700 ppm (M:93.7 mg.kg bw/day, F:130.3 mg/kg bw/day)*</u>- *Clinical:* reduced body weight (M:↓1.3%-5.5%, F:↓0.5%-8.0% during weeks 2-81); reduced food consumption (M:↓2.5%-9.5% during weeks 2-80); reduced food utilisation (M:↓21.0%, F:↓13.3% during weeks 1-12), slight increase in the incidence of eye discharge (F), increase in the incidence of subcutaneous masses (F). *Haematological:* increased red blood cell count (M:↑6.4%, F:↑7.9%, week 81), increased mean cell haemoglobin concentration (F:↑3%, week 81), increased platelet count (F:↑30.1%, week 81); reduced mean cell volume (M:↓8.0%, F:↓9.1%, week 81), reduced mean cell haemoglobin (M:↓5.8%; F:↓6.7%, week 81). *Lung:* lymphoid proliferation (F:23); squamous cyst (F:1) *Eye*; increased incidence of discharge. *Kidney:* increased incidence of urine distension (M), mononuclear cell infiltration/interstitial (M:10, F:8), mononuclear cell infiltration/pelvic (M:15, F:14). *Liver:* increase in relative liver weight (M:↑20.5%; F:↑18.3%). <u>*200 ppm (M:26.6 mg/kg bw/day, F:37.1 mg/kg bw/day)-*</u> *Lung:* lymphoid proliferation (F:18). *Kidney:* mononuclear cell infiltration/interstitial (M:6, F:6), mononuclear cell infiltration/pelvic (M:13, F:21). *Spleen:* pigmentation (F:1). <u>*50 ppm (M:6.7 mg/kg bw/day, F:9.0 mg/kg bw/day)*</u>- *Clinical:* reduced body weight (M:2.6%-3.6% during weeks 8-49). *Lung:* lymphoid proliferation (F:18). *Kidney:* mononuclear cell infiltration/interstitial (M:2, F:5), mononuclear cell infiltration/pelvic (M:9, F:12).

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<td></td>
<td>
<u>0 ppm (M&F:0 mg/kg bw/day)-</u> Lung: lymphoid proliferation (F:13). Kidney: mononuclear cell infiltration/interstitial (M:6, F:5), mononuclear cell infiltration/pelvic (M:4, F:12).

<u>Neoplastic finding</u>
No overall increase in tumour incidence.
<table>
<tr><th rowspan="2">Finding</th><th colspan="4">Males (ppm)</th></tr>
<tr><th>0</th><th>50</th><th>200</th><th>700</th></tr>
<tr><td>Lung- Pulmonary Adenoma</td><td>1[a]/55[b] (1.8%)[c]</td><td>1/55 (1.8%)</td><td>1/55 (1.8%)</td><td>3/55 (5.5%)</td></tr>
<tr><th rowspan="2">Finding</th><th colspan="4">Females (ppm)</th></tr>
<tr><th>0</th><th>50</th><th>200</th><th>700</th></tr>
<tr><td>Lung- Pulmonary Adenoma</td><td>0/55 (0.0%)</td><td>0/55 (0.0%)</td><td>0/55 (0.0%)</td><td>6/55 (10.9 %)</td></tr>
<tr><td>Lung- Keratinising Squamous Epithelioma</td><td>0/55 (0.0%)</td><td>0/55 (0.0%)</td><td>0/55 (0.0%)</td><td>1/55 (1.8%)</td></tr>
</table>
a- number of animals with tumour, b- total number of animals examined, c- incidence
</td>
</tr>
<tr>
<td>Life time feeding study - 94-96 weeks
Oral (diet)

Mice
Alderley Park swiss-derived
60/sex/dose

Terminal kills were made when mortality approached 80% (week 94 for females and week 96 for males)

Purity= 97.7-98.2 %

OECD 451, not GLP

(Sotheran et al, 1980)</td>
<td>0 (two groups), 200, 400 or 1600 ppm</td>
<td>
NB: Terminal kills were made when mortality approached 80% (week 94 for females and week 96 for males)

<u>Non-neoplastic findings:</u>
<u>1600 ppm-</u> 78.3% (M) and 88.6% mortality by week 94. Increased mortality rates during weeks 30-60 and shortly before termination. Clinical: reduced body weight gain (M:↓13.0%-36.5% , F:↓21.1%-41.2% during weeks 1-92); reduced food consumption (M:↓22.3%), reduced food utilization (M:↓16.1%, F:↓20.6% during weeks 1-12).
<u>400 ppm</u>- 71.2% (M) and 76.4% (F) mortality by week 94. Clinical: reduced body weight gain (F:↓10%); reduced food utilization (M:↓6.2%, F:↓7.9% during weeks 1-12)
<u>200 ppm-</u> 76.7% (M) and 70% (F) mortality by week 94. No treatment related signs of toxicity.
<u>0 ppm-</u> 66.7% (M) and 69.6% mortality by week 94 in control group 1. 73.3% (M) and 78.1 % (F) mortality at week 94 in control group 2.

<u>Neoplastic findings (descendants and terminal):</u>
No overall increase in tumour incidence.
<table>
<tr><th rowspan="2">Finding</th><th colspan="5">Males (ppm)</th></tr>
<tr><th>0</th><th>0</th><th>200</th><th>400</th><th>1600</th></tr>
<tr><td>Tumour bearing animals</td><td>42</td><td>45</td><td>41</td><td>42</td><td>46</td></tr>
<tr><td>Lung –pulmonary adenoma</td><td>9[a]/59[b] (15.3 %[c])</td><td>8/60 (13.3 %)</td><td>9/59 (15.3 %)</td><td>8/59 (13.6 %)</td><td>17/58 (29.3 %)</td></tr>
<tr><td>Liver- hyperplasic nodules and benign tumours</td><td>3/58 (5.2 %)</td><td>9/59 (15.3 %)</td><td>5/59 (8.5%)</td><td>9/58 (15.5 %)</td><td>15/57 (26.3 %)</td></tr>
<tr><td>Liver- nodules with morphological signs of malignancy</td><td>4/58 (6.9</td><td>6/59 (10.2 %)</td><td>13/59 (22.0 %)</td><td>8/58 (13.8 %)</td><td>17/57 (29.8 %)</td></tr>
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			%)				
		Immune system (lymphocytes) – Lymphosarcoma	13*	15*	13*	13*	14*
		Finding	**Females (ppm)**				
			0	**0**	**200**	**400**	**1600**
		Tumour bearing animals	42	43	42	45	46
		Lung –pulmonary adenoma	9/59 (15.3 %)	4/59 (6.8 %)	9/59(15.3%)	11/59 (18.6 %)	18/59 (30.5 %)
		Liver- hyperplasic nodules and benign tumours	1/58 (1.7 %)	2/59 (3.4 %)	3/57 (5.3%)	6/58 (10.3 %)	4/59 (6.8%)
		Liver- nodules with morphological signs of malignancy	2/58 (3.5 %)	0/59 (0.0 %)	3/57 (5.3%)	3/58 (5.2%)	5/59 (8.5%)
		Immune system (lymphocytes) – Lymphosarcoma	11*	25*	18*	18*	24*
		Ovary- papillary cystadenoma	0/55 (0%)	0/55 (0%)	1/58 (1.7%)	3/55 (5.5%)	3/56 (5.4%)
		Mammary gland- adenocarcinoma	0/56 (0%)	0/58 (0%)	1/57 (1.8%)	1/57 (1.8%)	4/57 (7%)
		a- number of animals with tumour, b- total number of animals examined, c- incidence, * number examined not specified					

Table 20: Summary table of carcinogenicity studies judged inadequate for classification and labelling purposes

Due to the poor health of the animals and the high rates of mortality (not related to treatment with Pirimicarb) reported in the following studies, we do not consider this data to be sufficient for classification and labelling purposes. However, for completeness the studies are presented below.

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Studies in Rats		
2 year study Oral (diet) Rat Sprague-Dawley or Wistar 48/dose Purity= 97.3 % Not guideline or GLP (Samuels *et al*, 1975)	*Experiment 1:* 0, 750 or 2500 ppm male Sprague-Dawley Rats *Experiment 2:* 0, 750 or 2500 ppm to male Wistar Rats *Experiment 3:* 0 or 750 ppm to Wistar rat offspring, dosed from conception (24/sex/dose).	**An outbreak of respiratory disease, followed by (unspecified) drug treatment and high levels of mortality, render these findings unreliable.** *Experiment 1- Male Sprague Dawley:* **Non-neoplastic findings-** Prior to respiratory infection (week 40), mortality and clinical signs of infection were acceptable. *2500 ppm* – 34/48 animals died by week 104; *Clinical:* reduced body weight gain, reduced food consumption, increased food conversion; *Gross Pathology:* non-treatment related lung effects associated with respiratory infection only. *750 ppm* – 33/48 animals died by week 104; *Gross Pathology:* non-treatment related lung effects associated with respiratory infection only. *0 ppm* – 41/48 animals died by week 104; *Gross Pathology:* non- treatment related lung effects associated with respiratory infection. **Neoplastic findings-** *2500 ppm* – reticulum cell lymphoma of the lung observed in 6/48 animals. *750 ppm* – reticulum cell lymphoma of the lung observed in 4/48 animals. *0 ppm* – reticulum cell lymphoma of the lung observed in 1/47 animals. *Experiment 2- male Wistar:* **Non- neoplastic findings-** In all treatment groups respiratory disease occurred by week 43 which was reportedly only partly controlled by treatment with an unspecified drug. *2500 ppm* –37/48 animals died by week 104; *Clinical:* consistently reduced body weight gain, reduced food consumption, increased food conversion. *750 ppm* – 37/48 animals died by week 104. *0 ppm* – 28/48 animals died by week 104. **Neoplastic findings-** No treatment-related increases in absolute or specific tumour incidence were observed. *Experiment 3- Wistar rat offspring (dosed from conception):* **Non-neoplastic findings-** In all treatment groups respiratory disease occurred from week 43, which was treated with oxytetracycline (weeks 43-93) or sulphadimidine (week 89). *750 ppm* – 15/24 (M) and 13/24 (F) died by week 104; reduced body weight *0 ppm* – 15/24 (M) and 12/24 (F) died by week 104. **Neoplastic findings-** 750 ppm- mammary gland fibroadenoma (benign) observed in 7/24 (F) 0 ppm – mammary gland fibroadenoma (benign) observed in 4/23 (F)
Studies in Mice		
80 week study Oral (diet)	0, 300 or 1500 ppm	**An outbreak of respiratory disease, followed by treatment with oxytetracycline or sulphadimidine and high levels of mortality, render these findings unreliable.**

Mice 'Alderley Park'/50/sex/dose

Purity= 97.3 %

Not guideline or GLP

(Palmer and Samuels, 1974)

Non-neoplastic findings:

1500 ppm- 34/50 (M) and 36/50 (F) died by week 80; reduced body weight.

Gross pathology revealed congested, consolidated and/or oedematous lungs in all groups.

300 ppm- 35/50 (M) and 39/50 (F) died by week 80.

0 ppm- 34/50 (M) and 30/50 (F) died by week 80.

Neoplastic findings (descendants and terminal):

Finding	Males (ppm)		
	0	**300**	**1500**
Pulmonary adenoma	0[a]/46[b] (0%)[c]	6/49 (12.2%)	6/46 (13%)
Pulmonary carcinoma/adenocarcinoma	1/49 (2.2%)	2/49 (4.1%)	0/47 (0%)
Finding	**Females (ppm)**		
	0	**300**	**1500**
Pulmonary adenoma	3/46 (6.1%)	4/49 (8.3%)	5/46 (10.6%)
Pulmonary carcinoma/adenocarcinoma	0/49 (0%)	2/48 (4.2%)	0/47 (0%)

a- number of animals with tumour, b- total number of animals examined, c- incidence

4.10.1.1 Carcinogenicity: oral

As shown in Table 19, there is one rat study (Tinson, 1992) and two mouse studies (Rattray, 1998; Southeran *et al*, 1980), which are considered to be of sufficient quality for inclusion in the present classification and labelling analysis. The key effects observed in these studies are described below.

Rat

In a 2-year study (Tinston, 1992), treatment of male and female Alderley Park (Sprague Dawley derived) rats (64/sex/dose of which 11 or 12 were scarified early) with Pirimicarb (0, 75, 250 and 750 ppm corresponding to 0, 3.7, 12.3 and 37.3 mg/kg bw/day in males and 0, 4.7, 15.6 and 47.4 mg/kg bw/day in females) resulted in an overall increase in the total number of tumours. This was the result of an increase in a variety of different neoplasms occurring in different tissues. Affected organs included the brain, uterus and mammary gland, for which the increased tumour incidence followed a statistically significant trend. A detailed analysis and discussion of these tumour findings is presented below.

A large number of deaths were observed in this study, due to an increased mortality rate after approximately one year of treatment (22, 18, 25, and 24 males survived to termination at 0, 3.7, 12.3 and 37.3 mg/kg bw/day and 37, 32, 33 and 36 females survived to termination at 0, 4.7, 15.6 and 47.4 mg/kg bw/day). However, this was observed in all groups (in similar numbers and following a similar time response pattern), indicating that it was not a treatment related effect. There was no evidence of disease or infection that might have compromised the findings of this study.

Generalised toxicity including a reduction in bodyweight gain (up to 13.4% (M) and 18.7% (F) during the course of the study), reduced food consumption and changes in haematological and biochemical parameters was observed at the top dose (750 ppm). Plasma cholinesterase activity was consistently reduced in females (>28% reduction) and occasionally reduced in males (> 27% at week 78 at 250 ppm) at 250 and 750 ppm. Non-neoplastic findings observed upon necropsy included a small increase in the incidence of necrosis of the brain (M: 1, 2, 1, 6 at 0, 75, 250 and 750 ppm), vacuolation of the adrenal cortex (M: 48 at 750 ppm compared to 39 in controls), pelvic vascular ectasia (M: 2, 1, 5 and 8, F: 3, 2, 5 and 5 at 0, 75, 250 and 750 ppm), kidney transitional cell hyperplasia (M: 33, 34, 42 and 44, F: 26, 35, 41 and 48 at 0, 75, 250 and 750 ppm), increased severity of sciatic nerve demyelination and an increased incidence and severity of voluntary muscle degeneration. However, the study report concluded that changes in the nerve, voluntary muscle, adrenal cortex and kidney represent a treatment-related exacerbation of spontaneous age related changes. Changes in the liver, including increased relative weight (M: 9.2%, F:11.7%), hepatocyte hypertrophy (M: 5 compared to 0 in control) and altered hepatocytes (clear cell) (M: 5 compared to 0 in the control) were also observed at 750 ppm.

Three two-year rat dietary studies conducted in the mid-1970s are also available (Samuels *et al*, 1975). These studies are deemed inadequate due to an outbreak of respiratory disease, high mortality rates and antibiotic treatment, however, they contain several findings that were seen in later studies (lung and mammary gland tumours) and may provide additional supporting information. In the first two studies, male Sprague-Dawley or male Alderley Park (Wistar-derived) rats were dosed with 750 and 2500 ppm for 2 years. In the third study, both male and female Alderley Park rats received 750 ppm for 2 years.

Brain

Astrocytoma

Exposure to Pirimicarb at 75-750 ppm (equivalent to 3.7-37.3 mg/kg bw/day and 4.7-47.4 mg/kg bw/day in males and females, respectively) resulted in an increase in the incidence of brain astrocytomas (malignant) in Alderley Park (Sprague Dawley derived) rats (Tinston, 1992). In males, the increase followed a dose response pattern (0, 3.8, 3.8 and 5.8% incidence at 0, 3.7, 12.3 and 37.3 mg/kg bw/day), which rose to the top of the historical control range at the highest dose (historical control taken from 15 studies conducted between 1982-1992: range: 0-5.8%). In females, the incidence did not follow a dose response pattern (0, 1.9, 0 and 3.9% incidence at 0, 4.7, 15.6 and 47.4 mg/kg bw/day), but reached the top of the historical control range at the highest dose (historical control taken from 15 studies conducted between 1982-1992: range: 0-3.9%). Although, brain astrocytomas are considered to be a rare tumour, the historical control data for this strain of rat show that brain astrocytomas were observed at low levels in many of the two year studies conduced at the same laboratory (Table 21). This suggests that the incidence of brain astrocytomas observed in this study may be a reflection of the normal background rate of this tumour type, rather than a treatment related effect.

Table 21- Historical control incidence of brain astrocytomas in 15 2-year carcinogenicity studies conducted with Alderley Park (Sprague Dawley derived) rats at the Central Toxicology Laboratory, Zeneca.

Study code	Start date	Incidence excluding interim kill	
		Male	Female
PR0542	1982	1/52 (1.9%)	2/52 (3.8%)
PR0575	1983	0/64 (0.0%)	1/64 (1.6%)
PR0590	1984	5/104 (4.8%)	1/104 (1.0%)
PR0609	1984	0/52 (0.0%)	0/52 (0.0%)
PR0626	1985	1/52 (1.9%)	1/52 (1.9%)
PR0648	1985	2/52 (3.8%)	1/52 (1.9%)
PR0679	1986	2/52 (3.8%)	1/52 (1.9%)
PR0683	1987	1/52 (1.9%)	1/52 (1.9%)
PR0719	1988	0/52 (0.0%)	0/52 (0.0%)
PR0492	1989	0/56 (0.0%)	1/56 (1.8%)
PR0789	1990	3/52 (5.8%)	0/52 (0.0%)
PR0810	1990	3/52 (5.8%)	0/52 (0.0%)
PR0890	1992	0/52 (0.0%)	2/52 (3.8%)
PR0892	1992	2/52 (3.8%)	1/52 (3.8%)
PR0936	1992	1/52 (1.9%)	0/52 (0.0%)

Meningioma

An increase in the incidence of brain meningioma (benign) was also observed in the mid dose group for males (3.8, 1.9, 7.7 and 1.9% incidence at 0, 3.7, 12.3 and 37.3 mg/kg bw/day) and the top dose group for females (0, 0, 0, and 3.8% at 0, 4.7, 15.6 and 47.4 mg/kg bw/day) (Tinston, 1992). Although, the increases exceeded the historical control range (historical control taken from 14 studies conducted between 1983-1992: range: M:0-1.9% F:0-1.9%, Table 22), they did not follow a dose response pattern in males and the incidence observed in females was the same or below that observed in the male concurrent control group. This suggests that the incidence of brain meningioma observed in this study may reflect the normal background rate in this strain of rats.

Table 22- Historical control incidence of brain meningioma in 14 2-year carcinogenicity studies conducted with Alderley Park (Sprague Dawley derived) rats at the Central Toxicology Laboratory, Zeneca.

Study code	Start date	Incidence excluding interim kill	
		Male	Female
PR0575	1983	1/64 (1.7%)	0/64 (0.0%)
PR0590	1984	1/104 (1.0%)	1/104 (1.0%)
PR0609	1984	1/52 (1.9%)	1/52 (1.9%)
PR0626	1985	0/52 (0.0%)	0/52 (0.0%)
PR0648	1985	0/52 (0.0%)	1/52 (1.9%)
PR0679	1986	1/52 (1.9%)	0/52 (0.0%)
PR0683	1987	0/52 (0.0%)	0/52 (0.0%)
PR0719	1988	1/52 (1.9%)	0/52 (0.0%)
PR0492	1989	0/56 (0.0%)	0/56 (0.0%)
PR0789	1990	1/52 (1.9%)	1/52 (1.9%)
PR0810	1990	0/52 (0.0%)	0/52 (0.0%)
PR0890	1992	0/52 (0.0%)	0/52 (0.0%)
PR0892	1992	0/52 (0.0%)	0/52 (0.0%)
PR0936	1992	0/52 (0.0%)	0/52 (0.0%)

Mammary gland

Fibroadenoma

A small increase in the incidence of mammary gland fibroadenoma (benign) (5.9, 9.8, 3.8 and 11.8% at 0, 75, 250 and 750 ppm) was observed in the low and top dose treated female rats (Tinston, 1992). However, this increase did not follow a dose response pattern and was within the historical control range (historical control taken from 14 studies conducted between 1983-1992: range: 3.8-19.2%).

An increase in the incidence of benign mammary gland fibroadenomas was also observed in female Wistar rats (Samuels *et al*, 1975) (29.2% at 750 ppm compared to 17.4% in the control); a strain known to possess a high spontaneity rate for this tumour type (historical control 36.1% in female Wistar rats) (Poteracki and Kathleen, 1998). However, these findings are not considered to provide reliable evidence of the carcinogenic potential of pirimicarb, due to the poor study design (small group sizes), high mortality rate and the presence of a chronic respiratory infection, followed by unspecified drug treatment.

Uterus

Stromal Cell Sarcoma

An increase in the incidence of the malignant tumour type, stromal cell sarcoma was observed at the top dose (0, 0, 0, and 3.8% incidence at 0, 3.7, 12.3 and 37.3 mg/kg bw/day) in Alderley Park (Sprague Dawley derived) rats. However, this incidence was within the historical control range (historical control taken from 14 studies conducted between 1983-1992: range: 0-3.8%), suggesting that it may be a reflection of the normal background rate in this strain of rats.

Stromal Cell polyp

A dose dependent increase (9.6, 11.5, 13.5 and 19.2% at 0, 3.7, 12.3, and 37.3 mg/kg bw/day) in the incidence of stromal cell polyp (benign) was also observed in the uterus of Alderley Park (Sprague Dawley derived) rats. However, this incidence was within the historical control range (historical control taken from 14 studies conducted between 1983-1992: range: 0-23.1%), suggesting that it may be a reflection of the normal background rate in this strain of rats.

Lung

Reticulum cell lymphoma

In the older rat study (Samules *et al*, 1975), a dose-related increase in the incidence of reticulum cell lymphoma was observed in the lungs of male Alderley Park (Sprague Dawley derived) rats at the 750 and 2500 ppm dose levels (2.1%, 8.3% and 12.5% incidence at 0, 750 and 2500 ppm). The study authors suggest that this may have been caused by chronic respiratory infection, rather than administration of the test substance and that the increased incidence is made to appear more prominent by an increased mortality rate in the control group. In addition, group sizes were smaller than recommended in current guidelines, there was limited reporting detail and a high mortality was observed across all groups. Consequently, these findings alone are not considered to provide reliable information on the carcinogenic potential of Pirimicarb; however, lung tumours were also observed in the available mouse studies.

Mouse

As shown in Table 19, there are two studies in the mouse (Rattray, 1988 and Sotheran *et al*, 1980), which are considered to be of sufficient quality for classification and labelling purposes. In both studies, Pirimicarb did not induce an overall increase in the total number of tumours. However, increased tumour incidences that followed a statistically significant trend, were observed in the lung, liver, immune system, ovaries and mammary gland. A detailed analysis and discussion of these tumour types is presented below.

In a lifetime feeding study (Southeran *et al*, 1980), groups of Alderley Park Swiss-derived mice (60/sex/dose) were administered Pirimicarb at 0 (2 groups), 200, 400 and 1600 ppm until mortality approached 80% (M: week 96, F: week 94); when the remaining animals were sacrificed. General signs of toxicity were observed at 1600 ppm; including a reduction in body weight gain (ranging from M:↓13.0%-36.5% , F:↓21.1%-41.2% during weeks 1-92), food consumption (M:22.3%) and food utilisation (M:16.1%, F:20.6% during weeks 1-12). However, microscopic examinations did not reveal any treatment-related non-neoplastic findings. Due to an unusual study design, the number of historical control studies for comparison with this study are limited (3 studies, 6 control groups).

In another study (Rattay, 1998), C57 black mice (50/sex/dose) were dosed with 0, 50, 200 or 700 ppm Pirimicarb for 80 weeks. This strain of mouse was selected due to its lower and less variable normal background incidence of both lung and liver tumours, compared to Alderley Park Swiss-derived mice. Some small signs of toxicity were observed at the top dose, which included a small decrease in body weight (ranging from M:1.3%-5.5%, F:0.5%-8.0% during weeks 2-81), reduced food consumption (M:2.5%-9.5% during weeks 2-80), eye discharge (females) and an increase in the incidence of subcutaneous masses (females). At necropsy the non-neoplastic changes observed included an increase in the incidence of lung lymphoid proliferation (13, 18, 18 and 23 females at 0, 50, 200 and 700 ppm), renal pelvis mononuclear infiltration (6, 9, 13 and 10 males and 5, 5, 21 and 8 females at 0, 50, 200 and 700 ppm), decreased incidence of urine distension (males) and an increase in relative liver weight (M:20.5%, F:18.3%). Although, industry considered 700 ppm to be in exceedance of the MTD, the observed effects are not sufficiently severe to support this conclusion.

An 80-week dietary study conducted in Alderley Park Swiss-derived mice in 1974 is also available (Palmer and Samuels, 1974). However, this study is deemed inadequate due to an outbreak of respiratory disease, high mortality rates and antibiotic treatment.

Lung

Pulmonary adenoma

An increase in the incidence of pulmonary adenomas was observed in both Alderley Park and C57BL mice. In the study conducted with Alderley Park mice (Southeran *et al*, 1980), the incidence of pulmonary adenomas in the top dose males (15.3, 13.3, 15.3, 13.6 and 29.3% at 0, 0, 200, 400 and 1600 ppm) and the top and mid dose females (15.3, 6.8, 5.3, 18.6 and 30.5% at 0, 0, 200, 400 and 1600 ppm) exceeded the historical control range (historical control taken from 3 studies/6 control groups conducted between 1977-1983: range: M: 6.6-18.3% F:0-13.3%, Table 23). However, the top dose level was considered to exceed the MTD (maximum tolerated dose), due to a large reduction in body weight gain (ranging from M:↓13.0%-36.5% , F:↓21.1%-41.2% during weeks 1-92).

In C57 mice, an increase in the incidence of pulmonary adenomas was observed at the top dose level (700 ppm) in males (1.8, 1.8, 1.8 and 5.5% at 0, 6.7, 26.6 and 93.7 mg/kg bw/day) and females (0, 0, 0 and 10.9% at 0, 9.0, 37.1 and 130.3 mg/kg bw/day) (Rattray, 1988). Since the incidence in females was outside the historical control range (historical control taken from 6 studies conducted between 1994-1997: range: F: 0.0-3.6%, Table 24), these tumours are considered to be treatment related. A single incidence of benign keratinising squamous epithelioma was also observed in one female of the 700 ppm dose group and was considered to be supportive of a treatment related effect in the lungs. The increase in pulmonary adenomas observed in males lies within the historical control range (historical control taken from 6 studies conducted between 1994-1997: range: M:1.8-7.3%, Table 24) and therefore, may be a reflection of the normal background rate in this strain of rat.

A dose-related increase in the incidence of pulmonary adenomas was also observed in an older study conducted with Alderley Park mice (0%, 12.2% and 13.0% in males and 6.1%, 8.3% and 10.6% in females at 0, 300 and 1500 ppm) (Palmer and Samuels, 1974). However, these findings are not considered to provide reliable evidence of the carcinogenic potential of Pirimicarb, due poor study design, high mortality rate and the presence of a chronic respiratory infection, followed by treatment with oxytetracycline and sulphadimidine.

Table 23- Historical control incidence of pulmonary adenoma in 3 lifetime carcinogenicity studies (6 control groups) conducted with Alderley Park mice at the Central Toxicology Laboratory, Zeneca.

Study code	Start date	Incidence excluding interim kill	
		Male	Female
PM0006	1978	8/60 (13.3%)	8/60 (13.3%)
PM0006	1978	11/60 (18.3%)	5/60 (8.3%)
PM0366	1979	4/61 (6.6%)	4/61 (6.6%)
PM0366	1979	10/60 (16.7%)	4/60 (6.7%)
PM0574	1983	4/50 (8.0%)	0/50 (0.0%)
PM0574	1983	4/50 (8.0%)	0/50 (0.0%)

Table 24 - Historical control incidence of pulmonary adenoma in 6 80-week carcinogenicity studies conducted with C57BL mice at the Central Toxicology Laboratory, Zeneca.

Study code	Start date	Incidence excluding interim kill	
		Male	Female
PM0979	1994	4/55 (7.3%)	1/55 (1.8%)
PM0983	1995	2/55 (3.6%)	2/55 (3.6%)
PM1000	1995	1/55 (1.8%)	2/55 (3.6%)

PM1000	1995	2/55 (3.6%)	1/55 (1.8%)
PM1040	1996	1/50 (2.0%)	0/55 (0.0%)
PM1080	1997	1/55 (1.8%)	0/55 (0.0%)

Liver

Hyperplasic nodules, benign tumours and nodules with signs of malignancy

In the study conducted with Alderley Park mice (Southeran *et al*, 1980), the incidence of liver tumours described as hyperplastic nodules/benign tumours, exceeded the historical control range (Table 25) in males treated with 1600 ppm (5.2, 15.3, 8.5, 15.5 and 26.3% at 0, 0, 200, 400 and 1600 ppm) and females treated with 200, 400 and 1600 ppm (1.7, 3.4, 5.3, 10.3 and 6.8% at 0, 0, 200, 400 and 1600 ppm). The incidence of liver tumours with signs of malignancy also exceeded the historical control range (Table 26) in male mice treated with 200 and 1600 ppm (6.9, 10.2, 22.0, 13.8 and 29.8% at 0, 0, 200, 400 and 1600 ppm) and female mice treated with 1600 ppm (3.5, 0.0, 5.3, 5.2 and 8.5% at 0, 0, 200, 400 and 1600 ppm). Although, the incidence of liver tumours exceeded the historical control range they did not follow a dose response pattern and no pre-neoplastic lesions were reported. Consequently, the increase in liver tumours observed in this study were considered to provide limited evidence of carcinogenicity. No liver tumours were reported in the study conducted with C57 mice, however, lower doses were used in that study.

Table 25 - Historical control incidence of hyperplastic nodules and benign tumours in the Alderley Park mouse, 1978-1983

		Incidence excluding interim kill	
Study code	**Start date**	**Male**	**Female**
PM0006	1978	7/60 (11.7%)	2/60 (3.3%)
PM0006	1978	10/60 (16.7%)	0/60 (0.0%)
PM0366	1979	11/61 (18.0%)	1/61 (1.6%)
PM0366	1979	13/60 (21.7%)	2/60 (3.3%)
PM0574	1983	4/50 (8.0%)	1/50 (2.0%)
PM0574	1983	6/50 (12.0%)	1/50 (2.0%)

Table 26 - Historical control incidence of malignant liver tumours in the Alderley Park mouse, 1978-1983

		Incidence excluding interim kill	
Study code	**Start date**	**Male**	**Female**
PM0006	1978	6/60 (10.0%)	2/60 (3.3%)
PM0006	1978	5/60 (8.3%)	1/60 (1.7%)
PM0366	1979	11/61 (18.0%)	2/61 (3.3%)
PM0366	1979	9/60 (15.0%)	5/60 (8.3%)

PM0574	1983	8/50 (16.0%)	2/50 (4.0%)
PM0574	1983	7/50 (14.0%)	0/50 (0.0%)

Ovary

Papillary cystadenoma

An increase in the incidence of papillary cystadenoma (a benign epithelial tumour) (0, 0, 1.7 5.5 and 5.4% at 0, 0, 200, 400 and 1600 ppm) was observed in the ovaries of Alderley Park mice. Although, no pre-neoplastic lesions were reported in the ovaries, the tumour incidence exceeded both concurrent and historical controls (historical control taken from 6 studies conducted between 1994-1997: range: F: 0.0-2.1%, Table 27) in the mid and top dose. In the absence of mechanistic data to explain the observed increase of this tumour type in female Alderley Park mice, these tumours are considered to be treatment related.

No ovary tumours were reported in the study conducted with C57 mice; however, lower doses were applied in this study.

Table 27 - Historical control incidence of papillary cystadenoma in 6 80-week carcinogenicity studies conducted with C57BL mice at the Central Toxicology Laboratory, Zeneca.

Study code	**Start date**	**Incidence excluding interim kill**
		Females
PM0006	1978	0/60 (0.0%)
PM0006	1978	0/60 (0.0%)
PM0366	1979	0/61 (0.0%)
PM0366	1979	0/61 (0.0%)
PM0574	1983	1/48 (2.1%)
PM0574	1983	1/50 (2.0%)

Mammary gland

Adenocarcinomas

A dose related increase in the incidence of adenocarcinoma was observed in the mammary glands of female Alderley Park mice (0, 0, 1.8, 1.8, 7% incidence at 0, 0, 200, 400 and 1600 ppm). At the top dose, the incidence exceeded the historical control range (histrorical control taken from 3 studies, 6 control groups conducted between 1978-1983: range: 0-2.1%). However, this dose was considered to exceed the MTD (large reduction in body weight gain (ranging from M:↓13.0%-36.5% , F:↓21.1%-41.2% during weeks 1-92) and consequently, the increase in mammary gland tumours was considered to provide limited evidence of carcinogenicity.

No mammary gland tumours were reported in the study conducted with C57 mice, however, the doses were used in this study were lower.

Immune system

Lymphosarcoma

A high incidence of lymphosacrcoma was observed in the study conducted with Alderley Park mice (Southeran *et al*, 1980). However, for both males (13, 15, 13, 13 and 14 animals at 0, 0, 200, 400 and 1600 ppm) and females (11, 25, 18, 18 and 24 animals at 0, 0, 200, 400 and 1600 ppm) the incidence observed in treatment groups did not differ significantly from the concurrent controls. Therefore, the occurrence of this tumour type is considered incidental and not related to treatment.

4.10.1.2 Carcinogenicity: inhalation

No data available

4.10.1.3 Carcinogenicity: dermal

No data available

4.10.2 Human information

No data available

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

The carcinogenic potential of Pirimicarb has been investigated in rats and mice.

In the rat, an increase in the incidence of tumours in the brain (astrocytoma and meningioma), mammary gland (fibroadenoma) and uterus (stromal cell sacrcoma and stromal cell polyp) were observed. However, it was considered that these tumours are unlikely to be related to treatment because they did not follow a dose response pattern and/or were within the historical control range.

In the mouse, an increase in the incidence of pulmonary adenomas was reported in both Alderley Park and C57BL strains. Whilst the incidence in the Alderley Park mice exceeded historical controls at the top dose, this was also considered to exceed the MTD. In C57BL mice, which have a lower spontaneous background incidence for this tumour type, the incidence exceeded the historical

control range in top dose females and is therefore considered treatment related. A single incidence of benign keratinising squamous epithelioma and pre-neoplastic squamous cyst was also observed in top dose female C57 mice and is considered to be supportive of a treatment related effect in the mouse lung. Although, no lung tumours were observed in the study conducted with Alderley Park (Sprague Dawley derived) rats, high numbers of mortalities were observed in this study after 1 year of treatment (occurred in all groups at similar numbers).

In the ovary, an increase in papillary cystadenoma was observed in the mid and high dose groups of Alderley Park mice. Although, no pre-neoplastic lesions were reported in the ovaries the incidence exceeded the historical control range and is considered to be treatment related.

In the liver, an increased incidence of both benign and malignant tumours was observed in Alderley Park mice. However, this did not follow a dose response pattern and no pre-neoplastic lesions were reported. The occurrence of these tumours is considered to provide limited evidence of carcinogenicity.

At the top dose level, the incidence of mammary gland adenocarcinoma exceeded the historical control range in Alderley Park mice, however, this dose was considered to be in exceedance of the MTD. The increased incidence of mammary gland adenocarcinoma is considered to provide limited evidence of carcinogenicity.

Although, the ovary, liver and mammary gland tumours were not reported in the study conduced with C57 mice, lower doses were used in that study.

4.10.5 Comparison with criteria

The carcinogenicity of Pirimicarb has been investigated in rats (Alderley Park (Sprague-Dawley derived)) and mice (C57BL and Alderley Park). In the rat, the observed increased tumour incidences were not considered to be attributable to treatment. However, high numbers of mortalities were reported in this study after 1-year of treatment (observed in similar numbers across all groups), which may have effected its quality. In the mouse, Pirimicarb appeared to have a carcinogenic effect in the lung (observed in two strains), liver, ovaries and mammary gland. Based on these findings, classification for carcinogenicity is justified.

In accordance with the criteria in the CLP Regulation, classification in Category 1A is reserved for substances known to have a carcinogenic effect in humans. Since there is not evidence of Pirimicarb having cause cancer in humans, classification in Category 1A is not justified.

Where no human evidence is available, classification in Categories 1B or 2 is based on the strength of evidence in animals. Classification in Category 1B is for substances for which there is sufficient evidence to demonstrate carcinogenicity in animals, and Category 2 where there is limited evidence. On consideration of all the available data, Pirimicarb is considered to demonstrate limited evidence of carcinogenicity in animals. This is based on several factors, which weaken the available evidence. For example, treatment related tumours were only reported in one species (mice), many tumour incidences only exceeded the historical control range at doses above the MTD (mammary gland adenocarcinoma and pulmonary adenomas in Alderley Park mice)/in one sex (pulmonary adenomas in female C57 mice), pre-neoplatic lesions were not reported (ovary tumours in Alderley Park mice) or a clear dose response pattern was not observed (liver tumours in Alderley Park mice). However, in the absence of mechanistic data to dismiss the relevance of these tumours for humans, they are considered to be treatment related and provide limited evidence of carcinogenicity.

In view of these considerations, the available evidence is deemed to match the criteria for classification as a Category 2 carcinogen. There are no grounds to draw attention to a particular route of exposure on the label.

4.10.6 Conclusions on classification and labelling

Carc. 2 (H351)

RAC evaluation of carcinogenicity
Summary of the Dossier submitter's proposal Out of two carcinogenicity studies available in rats and three carcinogenicity studies available in mice, only one rat study and two mice studies were considered reliable by the DS. The findings of one rat study and one mouse study have been rendered unreliable due to poor study designs and an outbreak of respiratory disease with high mortality and antibiotic treatment. The reliable carcinogenicity studies provided the basis for the classification proposal by the DS. *Two-year oral study in rats (Tinton, 1992)* The study was performed in accordance with OECD TG 453 with Alderley Park (Sprague Dawley derived) rats, 64 per sex/dose (0, 75, 250 and 750 ppm pirimicarb in the diet, corresponding to 0, 3.7, 12.3 and 37.3 mg/kg bw/d in males and 0, 4.7, 15.6 and 47.4 mg/kg bw/d in females), of which 11 or 12/sex/dose were sacrificed at week 52. In an additional satellite group (36/sex/dose - with 8/sex/dose being sacrificed at weeks 27, 53, 79 and 105), plasma, erythrocyte and brain cholinesterase activity was determined. *Mortality* After approximately one year of treatment, high mortality was observed in males in all treatment groups (52% to 65%) and in the controls (58%), and somewhat lower in females (29% in controls, 31% to 38% in exposed groups). Mortality rates were not dose-related and followed a similar time pattern. Therefore, they were not considered treatment-related. No evidence of disease or infection that might have compromised the findings of this study was observed. *General toxicity* During the course of the study, at 250 and 750 ppm a reduction in body weight gain (up to 13% in males and 19% in females), reduced food consumption (at 750 ppm) and changes in haematological parameters (increased mean cell volume and mean cell haemoglobin in males; increased haemoglobin, haematocrit and mean cell haemoglobin in females) and biochemical parameters (increased plasma cholesterol and triglycerides, decreased alkaline phosphatase, in both sexes) were observed. At 750 ppm, increased relative liver weights (9.2% in males, 11.7% in females) were measured. There were no treatment-related clinical signs. *Acetyl cholinesterase activity* Plasma cholinesterase activity was consistently reduced (up to 28%) in females and occasionally in males (up to 27%) at 250 and 750 ppm. Brain and erythrocyte cholinesterase activities were not affected, but the time between sampling and cholinesterase activity analysis was not precisely reported. *Non-neoplastic effects* An increase in the incidence of necrosis of the brain, vacuolation of the adrenal cortex,

pelvic vascular ectasia, kidney transitional cell hyperplasia, voluntary muscle degeneration, minimal hepatocyte hypertrophy and minimal to slightly altered hepatocytes (clear cell), were observed in males (mainly at the top dose), as well as an increase in severity of sciatic nerve demyelination (at the top dose). There was no association between astrocytoma and brain necrosis.
In female rats, there was an increased incidence of kidney transitional epithelial hyperplasia and pelvic vascular ectasia (at the top and mid dose), and increased incidence of voluntary muscle degeneration and severity of sciatic nerve demyelination (at the top dose).
Sciatic nerve, voluntary muscle, adrenal cortex and kidney changes were interpreted as a treatment-related exacerbation of spontaneous age related changes.

Regarding neoplastic effects, a dose-related increase in the incidence of astrocytoma in males and uterine stromal cell polyp in females was observed. In addition, increased incidence of meningioma in the mid dose males and top dose females, and uterine stromal cell sarcoma and mammary gland fibroadenoma in top dose females, was found (Table 1 in the Section "Supplemental information" in the background document). Nevertheless, the DS reported that these tumours are unlikely to be treatment-related because they were within the historical control range (Table 5 in "Supplemental information") and/or did not follow a dose-response pattern:

- Astrocytoma: Incidence in male and female rats was at the upper limit of the historical control range (0-5.8% for the same strain, years 1982-1992), and in the females it did not follow a dose-related pattern.
- Meningioma: The increases exceeded the male and female historical control range (0-1.9%), but did not follow a dose response pattern in males and the incidence observed in females at the top dose (3.8%) was the same as for male controls (which was also above the historical control range).
- Uterine stromal cell polyp: The incidences were within the historical control range (0-23.1%).
- Uterine stromal cell sarcoma: An increased incidence was observed only at the top dose and was within the historical control range (0-3.8%).
- Mammary gland fibroadenoma: The incidences did not follow a dose-related pattern and were within the historical control range (3.8-19.2%).

Two-year oral study in rats (Samuels, Hodge and Palmer, 1975)
The results of this study are provided for information as they are considered unreliable. The study design comprised three experiments:
1) male Sprague-Dawley rats, 48 animals/dose, dosed at 0, 750 or 2500 ppm;
2) male Wistar-derived rats, 48 animals/dose, dosed at 0, 750 or 2500 ppm;
3) Wistar-derived pregnant rats fed at 0 or 750 ppm of pirimicarb, their offspring (24/sex/dose) dosed at 0 or 750 ppm for 2 years.

In this study, increased incidences of reticulum cell lymphoma of the lung in Sprague-Dawley rats (2.1%, 8.3% and 13% at 0, 750 and 2500 ppm, respectively) and mammary gland fibroadenoma in Wistar rats (17% and 29% at 0 and 750 ppm, respectively) were observed. The findings from this study are not considered reliable due to high mortality rate (up to 85%) caused by a respiratory disease (started after 40 to 50 weeks of exposure, unsuccessfully treated with antibiotics) and poor study design (small group sizes, only two doses tested in the third experiment, unspecified antibiotic treatment). Reticulum cell lymphoma of the lung was suggested (by the study authors) to arise in peribronchial lymphoid tissue due to chronic respiratory infection, and mammary gland fibroadenoma incidences were within the historical control range for females of this strain (18-45%, total incidence 36.1%).

Heighty-week oral study in mice (Rattray, 1998)
The study was performed in accordance with OECD TG 451 in C57BL/10JfCD-1 Alpk (C57

black) mice, 55/sex/dose (0, 50, 200 or 700 ppm corresponding to 0, 6.7, 26.6 or 93.7 mg/kg bw/d in males and 0, 9.0, 37.1 or 130.3 mg/kg bw/d in females). This study was conducted because the older 80-day oral study in Alderley Park mice (Palmer and Samuels, 1974) was considered unreliable since it was a non-guideline and non-GLP study and an outbreak of respiratory disease with high mortality occurred. In the new study, a C57 black mouse strain was used because it has lower and less variable spontaneous incidence of lung and liver tumours compared to Alderley Park Swiss derived strains.

Mortality
Survival was > 85% across all dose groups. There were no treatment-related effects on mortality.

General toxicity
During the course of the study (weeks 2-81), reduced body weight (up to 5.5% in males and up to 8% in females) was observed at the top dose (700 ppm) (Table 2 in "Supplemental information"). Reduced food consumption (up to 9.5%) in males, reduced food utilisation in both sexes (by 21% in males and 13.3% in females, weeks 1-12), increased incidence of subcutaneous masses and eye discharge in females, were also found at this dose level. Subcutaneous masses were not related to adverse histophatological changes. Regarding haematological parameters (see Table 2 in "Supplemental information"), a dose–related increase in red blood cell (RBC) count and decrease in mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) was observed in both sexes. Haemoglobin levels, however, remained unchanged across study groups. At the top dose, an increase in platelet count was found in females. Absolute and relative liver weights were increased at 200 and 700 ppm in both sexes, but there were no associated pathological microscopic changes.

Non-neoplastic effects
A slight increase in the incidence of interstitial mononuclear cell infiltration in the kidney was observed in both sexes at the top dose. An increase in the incidence of pelvic mononuclear cell infiltration in the kidney was dose-related in males, and in the females was observed at the mid dose (200 ppm). Lymphoid proliferation in the lungs increased in a dose-related pattern in females. An increase in spleen pigmentation incidence, observed in top dose females, was in the study report described as melanin deposition, spontaneously occurring in this strain of mice.

The incidence of lung adenoma was increased in males and females at the top dose (5.5% *vs.* max. 1.8% in all other male groups; 10.9% *vs.* 0% max. in all other female groups; see also Table 2 in "Supplemental information" in Annex 2). In females at the top dose, one case of keratinising squamous lung epithelioma was recorded.

Lung adenoma in males was within the historical control range for this strain (1.8-7.3%, median 2.8%). However, in the females the incidence of 10.9% at the top dose was markedly higher than the upper value of the historical control range for female mice of the same strain (0-3.6%, median 1.8%), and was therefore considered as treatment-related by the DS. A single incidence of benign keratinising squamous epithelioma at the top dose was considered to be supportive of a treatment-related effect in the lungs. The DS mentioned that the dose level of 700 ppm does not exceed the maximum tolerated dose (MTD), since the observed effects are not sufficiently severe.

Lifetime feeding study in mice (Sotheran et al., 1980)
A life time feeding study (94-96 weeks) in mice (Sotheran *et al*., 1980) was performed in accordance with OECD TG 451 (non-GLP study), in 60 Alderley Park Swiss-derived mice/sex/dose (0, 200, 400 or 1600 ppm; food consumption was difficult to measure due

to large amounts of food wastage that was often damp when weighed).

Mortality
During weeks 30 to 60 and shortly before termination, the mortality rate increased in females at 1600 ppm (up to 89%). Other groups did not differ in mortality rates compared to controls (Figure 2 in Supplemental information). Terminal kills were made when mortality approached 80%, i.e. in week 94 for females and week 96 for males.

General toxicity
There were no specific clinical signs related to treatment, but the top dose (1600 ppm) animals of both sexes showed reduced body weight gain during the experiment (Figure 3 in "Supplemental information"). Reduced body weight gain was also observed at 400 ppm, but only during the first 8 weeks of the study.
Food consumption and food utilisation was reduced in males and females at 1600 ppm.

Non-neoplastic effects were not observed at microscopic tissue examination.

Increased incidences of lung adenomas were observed in males at 1600 ppm (29.2%) and females at 400 and 1600 ppm (18.6% and 30.5%, respectively). These values were also above the historical control range (males: 6.6-18.3%, median 10.7%; females: 0-13.3%. median 6.7%, years 1978-1983). Nevertheless, the highest dose, 1600 ppm, exceeded the MTD (markedly reduced body weight gain during the study).

Liver hyperplastic nodules and benign tumours increased in incidence which exceeded the historical control range (males: 8-21.7%, median 14.4%; females: 0-3.3%, median 2.0% in females) in males treated with 1600 ppm (26.3%) and in all exposed females (3.5% to 10.3%), but without a dose response relationship. There was also an increased in the incidence of liver tumours with signs of malignancy. The incidence exceeded the historical control range (8.3-18%, median 14.5%) in males at 200 and 1600 ppm (22.0% and 29.8%, respectively) and in females dosed at 1600 ppm (8.5%; historical control range 0-8.3%, median 3%). Although, the increase in both types of tumours was above the historical control range, it did not follow a dose-response pattern and no pre-neoplastic lesions were reported. The DS, therefore, concluded that the increase in liver tumours observed in this study provides limited evidence of carcinogenicity.

The incidence of lymphosarcoma was rather high, but equally distributed across study groups in both sexes. Therefore, it is not considered substance-related. The incidence of mammary gland adenocarcinoma was above the historical control range only at the highest dose, considered to exceed the MTD. Therefore, this tumour is considered to provide limited evidence of carcinogenicity.

In addition, ovary tumours and papillary cystadenoma were also increased. Papillary cystadenomas (a benign epithelial tumour) were observed at doses of 400 and 1600 ppm. The incidences exceeded both concurrent (0%) and historical controls (0-2.1%, median 0). The DS considered that in the absence of mechanistic data to explain the observed increase of this tumour type in female Alderley Park mice, these tumours are considered to be treatment related.

Heighty-week oral study in mice (Palmer and Samuels, 1974)
In an older, unreliable 80-week oral study in mice (Palmer and Samuels, 1974), Alderley Park mice (50/sex/dose) were dosed at 0, 300 or 1500 ppm pirimicarb in diet. In males and females, a dose-dependent increase in the incidence of pulmonary adenoma was observed (Table 4 in "Supplemental information"). However, since this is not a guideline or GLP study, and an outbreak of respiratory disease with high mortality (despite the treatment with tetracycline or sulphadimidine) occurred, these findings are taken with caution by the DS.

Supportive information from repeated dose toxicity and genotoxicity studies
Pre-neoplastic lesions were not reported in a 90-day oral study in rats. In other rat studies it was not stated that histopathology examination was performed. In dog studies, histopathological changes were related to haemolytic anaemia not observed in rodent studies. Since these studies are presented only as supportive information for carcinogenic toxicity evaluation, no classification is proposed for repeated dose toxicity.
The DS concluded that the potential genotoxicity of pirimicarb has been well investigated in *in vitro* and *in vivo* assays. The only positive result, indicative of a clastogenic effect, was found in some replicates of the TK+/- mouse lymphoma assay in the presence of S9 metabolic activation. Genotoxic effect, however, was not confirmed in other *in vitro* tests (bacterial reverse mutation tests, mammalian chromosome aberration test), or in *in vivo* studies (mouse micronucleus test, UDS in rat liver, dominant lethal assay) (for a more detailed description, please see "Supplemental information"). Since these data are given as supportive information only, no classification is proposed for mutagenicity.

The DS concluded that considering all available data, pirimicarb demonstrated limited evidence of carcinogenicity in animals. Tumours that are considered treatment-related are:

- lung adenoma in females C57 black mice at the top dose (markedly higher than the upper value of historical control range);
- ovary tumours - papillary cystadenoma in Alderley Park mice observed at mid and top dose (exceeded both concurrent and historical controls).

The increase in liver tumours (in low and top dose males and top dose females) and mammary gland adenocarcinoma (in top dose females) observed in a life-time feeding study in Alderley Park mice, provides only limited evidence of carcinogenicity.

Several factors weaken the available evidence: the treatment related tumours were reported only in one species (mice) and one sex (females); many tumour incidences exceeded the historical control range only at doses above the MTD (mammary gland adenocarcinoma and pulmonary adenomas in Alderley Park mice); pre-neoplastic lesions were not reported (ovary tumours in Alderley Park mice); a clear dose-response pattern was not observed (liver tumours in Alderley Park mice).

According to the DS, mechanistic data that could dismiss the relevance of these tumours for humans are not available. Therefore, these tumours are considered to be treatment-related and provide limited evidence of carcinogenicity. Classification as a Category 2 carcinogen was therefore proposed by the DS, without specifying a particular route of exposure.

Comments received during public consultation

Three MSCAs supported the classification proposal from the DS. One industry representative however opposed to the proposed classification as Carc. 2 with the following arguments:

- the effects are observed at doses exceeding the MTD (more than 10% reduction in body weight gain) in two mice strains and as a consequence, they should not be considered relevant for classification;
- there is no evidence of pre-neoplastic lesions, all tumours were benign, with no progression to carcinoma;
- type II bronchio-alveolar adenomas seen in mice are not generally seen in man;
- pirimicarb was demonstrated to be non-genotoxic;
- there are limited control data for ovarian tumour type occurring in the study in Alderley Park mice;
- the type of ovarian tumour that occurred in the mouse study is not commonly observed as a treatment-associated ovarian lesion in rodent carcinogenicity

studies.

Assessment and comparison with the classification criteria

Three carcinogenicity studies, a one 90-day oral study in Alderley Park (Sprague Dawley-derived) rats (Tinston 1992), and two mouse studies – an 80-week oral study in C57 black mice (Rattray, 1998) and a lifetime feeding study in Alderley Park Swiss-derived mice (Sotheran *et al*., 1980), considered by the DS to be of sufficient quality for inclusion in the classification and labelling analysis, were considered by RAC. The findings of the other three studies in rodents are briefly presented for completeness, but they are deemed unreliable due to poor study design and an outbreak of respiratory disease that led to increased mortality and treatment with antibiotics.

In the rat study (see Tables 1 and Table 4 in "Supplemental information"), a dose-related increase in the incidence of astrocytoma in males and uterine stromal cell polyp in females was observed. Also, an increased incidence of meningioma in the mid dose males and top dose females, and uterine stromal cell sarcoma and mammary gland fibroadenoma in top dose females, was found. RAC supports the DS's and DAR rapporteur's conclusion that these tumours are not clearly treatment-related because they were within the historical control range (e.g. brain astrocytoma, uterine stromal cell polyp, uterine stromal cell sarcoma, mammary gland fibroadenoma, Table 5 in "Supplemental information") or did not follow a clear dose-response pattern (e.g. brain meningioma, mammary gland fibroadenoma). In addition, when interim kill data were included (Tables 1 and 4 in "Supplemental information"), only data for terminal kill and intercurrent deaths are presented, to be comparable with the historical control data that did not include an interim kill) no dose-response pattern was observed for astrocytoma incidence in males (0% in controls, 3% in low dose, 2% in mid dose and 3% in top dose males). Other tumours presented in Table 1 ("Supplemental information"), namely benign thymoma and adrenal gland adenoma, did not follow a dose-related pattern either or were present only at the top dose (adrenal gland adenoma in females).

In the reliable 80-day study in C57 black mice (Tables 2 and 4 in "Supplemental information") an increased incidence of lung adenomas in top dose females (700 ppm) was found, exceeding both concurrent and historical control values (3 fold higher than the upper value of the historical control range). The relevance of this finding was challenged by industry during public consultation.

1. Lung adenoma occurring at the dose level potentially exceeding the MTD

During public consultation, Industry argued that 700 ppm in mice is above the MTD due to a 23% decrease in total body weight gain compared to controls. The guidance on the application of the CLP criteria (version 4.0, Novembre 2013) states that in lifetime bioassays "...*the highest dose needs to induce minimal toxicity, such as characterised by an approximately 10% reduction in body weight gain (maximal tolerated dose, MTD dose)*". The MTD is the highest dose of the test agent during the bioassay that can be predicted "*not to alter the animal's normal longevity from effects other than carcinogenicity* (CLP guidance, version 4.0, November 2013)."

The following survival data and non-neoplastic effects were recorded in females dosed at 700 ppm:

- treatment did not affect mortality rate in any exposed group at the survival rates greater than 85% across all dose groups;
- a slight increase in the incidence of eye discharge and subcutaneous masses that was not related to adverse histopathological changes;
- an increase in relative liver weight (18%) not associated with pathological microscopic changes;
- changes in red blood cells parameters (<10% difference from control values in RBC count, MCV and MCHC) that were not indicative of anaemia (no effect on

haemoglobin level);
- increased platelet count by 30% compared to controls; however, platelet count has a wide range of values in healthy animals (e.g. in adult male C57BL/6 mice it ranged from 620-1200 x 10^3/µL; Barrios *et al*., 2009), and adverse effects related to thrombocytosis (i.e. thrombotic events) were not reported;
- increased incidence of lymphoid proliferation in the lungs and of spleen pigmentation; however, pigmentation of the spleen (and of some other organs) and lymphoid accumulation in lungs, liver and lacrimal glands, are common non-neoplastic findings in the black mouse (Brayton, 2009).

The RAC does not consider the changes listed above as indicators of severe toxicity. Nevertheless, in the event that the top dose would be considered to be above the MTD, classification remains an option according to the CLP guidance: "*If a test compound is only found to be carcinogenic at the highest dose(s) used in a lifetime bioassay, and the characteristics associated with doses exceeding the MTD as outlined above are present, this could be an indication of a confounding effect of excessive toxicity. This may support a classification of the test compound in Category 2 or no classification*."

2. The relevance of type II pneumocyte-arising tumours for humans
Although rare, benign and malignant tumours that arise from type II pneumocytes occur in humans: alveolar adenoma, a very rare benign tumour (WHO histological classification of tumours of the lung), and bronchioloalveolar carcinoma represent less than 4% of all lung tumours (Read *et al*., 2004). Bronchioloalveolar carcinoma is a type of lung adenocarcinoma, predominantly of non-mucinous form composed primarily of type II pneumocytes or Clara cells (Lonardo, 2013). Type II pneumocytes are crucial for repair of the injured alveolus since they differentiate into alveolar epithelial type I cells (Wang *et al*., 2007). Type II cells possess proliferative potential so they could accumulate mutations that initiate tumour development (Lin *et al*., 2012). In the open literature it is stated that "*Spontaneous lung tumours in mice are similar in morphology, histopathology, and molecular characteristics to human adenocarcinomas. Mouse models for lung cancer can thus serve as a valuable tool not only for understanding the basic lung tumor biology but also for the development and validation of new tumour intervention strategies as well as for the identification of markers for early diagnosis*" (Meuwissen and Berns, 2005). Therefore, in the opinion of RAC, also in agreement with the DS proposal, human relevance of lung adenomas in mice, which are considered to arise from type II pneumocytes, could not be excluded. In addition, no mechanistic data were presented that could dismiss the relevance of these tumours for humans.

3. Increased incidence of lung adenomas observed only in females
In the Rattray study conducted in C57BL mice, top dose females received an almost 40% higher dose than top dose males (130.3 mg/kg bw/d *vs.* 93.7 mg/kg bw/d), although it is questionable whether this difference could explain the two times higher lung adenoma incidence in females compared to males. Nevertheless, according to the CLP guidance: "*There may be cases where tumours are only observed in one sex... A default position is that such tumours are still evidence of carcinogenicity and should be evaluated in light of the total tumorigenic response...*". In humans, alveolar adenoma and bronchioloalveolar carcinoma has a slight female predominance (WHO histological classification of tumours of the lung; Zell *et al.*, 2005), and the incidence for all adenocarcinomas was recorded to be more than two times higher in women than in men (Radzikowska *et al*., 2004).

Based on these arguments, RAC concludes that lung adenoma in female mice are treatment-related and relevant for pirimicarb classification. In addition, a single incidence of benign keratinising squamous epithelioma in top dose females is considered to be supportive of a treatment-related effect in the lungs, since this type of lung tumour rarely occurs spontaneously in mice or rats (Dixon *et al.,* 2008). In the lifetime study conducted in Alderley Park Swiss derived mice (Tables 3 and 4 in "Supplemental information") an

increased incidence of lung adenoma, liver tumours, mammary gland tumour and ovary tumour was observed in exposed animals.

Pirimicarb treatment in males did not affect survival rate in exposed groups, which was rather low at all dose levels (21.7-28.8% in exposed groups, 33.3% and 26.6% in controls). In the top dose males (1600 ppm), besides decreased body weight gain (by 24%), non-neoplastic adverse effects were not reported. On the other hand, the highest dose significantly decreased survival rate in females (11.4% compared to 30.4% and 21.9% in two control groups), as well as body weight gain (for 41%). Therefore, this dose is considered to exceed the MTD in female mice.

Lung adenoma incidence was increased in males at the highest dose and in females at the highest and mid dose. However, spontaneous lung adenoma incidence in this mouse strain is high and variable (up to 18.3% in males and 13.3% in females according to historical control range; 15% and 6.8% in two contemporary female control groups).

The increased incidence of benign liver tumours in females and malignant liver tumours in males was above the historical control range, but did not follow a dose-related pattern. An increased incidence of benign liver tumours in males (26.3%) occurred at the top dose, and was not markedly above the historical control range (8-21.7%, median 14.4%). An increased incidence of malignant liver tumours in females occurred only at the highest dose that exceeded the MTD, and was slightly above (for 0.2%) the upper limit of the historical control range (0-8.3%, median 3.3%).

Mammary gland adenocarcinoma occurred at an incidence exceeding the historical control range only at the top dose, that was above the MTD.

Ovarian papillary cystadenoma (benign epithelial tumour) occurred at an incidence above the historical control range in mid and top dose females, without a clear dose-response pattern (5.5% and 5.4% at mid and high dose, respectively).

This study was however not considered reliable by RAC (in agreement with the DS) due to very low survival rates in all dose groups, including a less than 25% survival rate in control females, and 26.7% and 23.3% survival rates in control and low dose males, respectively. In addition, tumour incidence data were not adjusted for survival (namely, since animals that died early are expected to have a lower risk of tumour than animals that died later, the absence of dose-response cannot be reliably confirmed) and relevant historical control data were rather limited (3 studies with 2 control groups per study). In light of these issues, together with high and variable spontaneous lung adenoma incidence in this mouse strain, RAC considers the increased incidence of tumours in this study only as a supportive evidence for carcinogenic potential of pirimicarb.

According to the CLP criteria, a substance should be classified in Category 1B if "*a causal relationship has been established between the agent and an increased incidence of malignant neoplasms, or of a combination of benign and malignant neoplasms in at least two species or in two independent studies in one species*". Substances may also be classified in Category 1B according to CLP if they produce an "*increased incidence of tumours in both sexes of a single species in a well-conducted study or if the substance leads to an unusual degree of malignant neoplasms in one species and sex*". For pirimicarb the carcinogenicity findings are not considered to fulfil these conditions; RAC is of the opinion that the pirimicarb data do not justify a classification in CLP Category 1B.

If there is limited evidence of carcinogenicity in animal studies, classification as a Category 2 carcinogen or even no classification is possible. Following the weight of evidence approach, classification in Category 2 for pirimicarb is proposed by RAC based on limited evidence of carcinogenicity in animal studies according to CLP criteria:

- "*the evidence of carcinogenicity is restricted to a single experiment*": only the C57 black mouse study is considered to substantially indicate treatment-related tumourigenesis;
- "*the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential*": increase in benign lung tumour incidence is considered as relevant for classification.

Therefore, RAC agrees with the DS proposal to classify pirimicarb as a carcinogen of Category 2 (Carc. 2), with the hazard statement H351: Suspected of causing cancer (without specifying a particular route of exposure), based on the increased incidence of lung adenomas in female C57 black mice and the absence of mechanistic data that could dismiss the relevance of these lung adenomas for humans.

RAC considers that a single incidence of benign keratinising squamous epithelioma in C57 black mice females at the highest dose is considered to be supportive of a treatment-related effect in the lungs. Also, an increased incidence of lung adenomas, liver tumours and ovarian papillary cystadenoma in Alderley Park Swiss derived mice in a study with low survival rates without survival-adjustment for tumour incidence and in a strain with higher and more variable spontaneous incidence of lung tumours than C57 black mouse strain, are considered as supportive evidence for the carcinogenic potential of pirimicarb.

Supplemental information - In depth analyses by RAC

Repeated dose toxicity studies
Eight repeated dose toxicity studies conducted with pirimicarb were summarised by the DS as supportive information. The main substance-related toxicological effects observed were haemolytic anaemia and inhibition of cholinesterase activity.
Haemolytic anaemia was observed in dogs and not in rodents (rats or mice). In dogs, anaemic effects ranged from no haematological changes to sub-clinical adaptive changes and anaemia. It is described as the 'penicillin type' haemolytic anaemia, probably IgG-mediated, with a possibly genetically determined sensitivity to pirimicarb.
Reductions in cholinesterase activity were observed in rats and dogs. In some studies, erythrocyte and brain cholinesterase activities were reduced to potentially adverse levels (by >20%), but in the majority of cases this was not accompanied by adverse clinical or neurological effects. A reduction in plasma cholinesterase activity is not considered as an adverse effect, but is instead used as an exposure indicator.
In the repeated dose toxicity study in rats, in which organs were histopathologically examined (90-day oral study in rats, Griffiths and Conning, 1968), dose levels of 250 and 750 ppm did not affect body weights, although food utilisation was reduced in females dosed at 750 ppm during weeks 1-4. No substance-related macroscopic or microscopic findings or effects on organ weights were observed.

Genotoxicity of pirimicarb
Pirimicarb was not genotoxic in bacterial reverse mutation tests (Callander, 1995; Truman, 1980), and no clastogenic response was observed in an *in vitro* study in human lymphocytes (Wildgoose *et al.*, 1987).
In the ***in vitro*** **mouse lymphoma mutation assay**, pirimicarb was **mutagenic** in the presence of S9 metabolic activation (Clay, 1996). Mutant frequency increase was largely related to an increase in the small colony size of mutants, "considered to be associated with large-scale chromosome deletions".
Three *in vivo* genotoxic assays, namely a mouse micronucleus test (Jones and Howard, 1989), UDS in rat liver (Kennelly, 1990) and dominant lethal assay (McGregor, 1974), were negative.

Carcinogenicity studies

Table 1. Summary table for the 2-year rat carcinogenicity study (Tinston, 1992)

		Pirimicarb dose (ppm)			
	HC (%)	**0**	**75**	**250**	**750**
Males					
Survival [N (%)]		22 (42)	18 (35)	25 (48)	24 (46)
Total bw gain, g (% of Controls)		559	585 (105)	553 (99)	523 (94)
Non-neoplastic changes [N (%)][a]					
N of examined animals		64	64	64	64
Adrenal cortical fat vacuolation		39 (61)	36 (56)	37 (58)	48 (75)
Brain necrosis		1 (1.6)	2 (3.1)	1 (1.6)	6 (9.4)
Transitional epithelial hyperplasia – kidney		33 (52)	34 (53)	42 (66)	44 (69)*
Pelvic vascular ectasia – kidney		2 (3.1)	1 (1.6)	5 (7.8)	8 (13)
Liver hypertrophy		0	0	0	5 (8)
Altered hepatocytes (clear cell)		0	1 (2)	0	5 (8)
Sciatic nerve demyelination		55 (86)	51 (80)	52 (81)	50 (78)
Voluntary muscle degeneration		20 (31)	17 (27)	23 (36)	25 (39)
Neoplastic changes [N (%)]					
N of examined animals		52	53	52	52
Brain – astrocytoma	0-5.8	0	2 (3.8)	2 (3.8)	3 (5.8)
Brain – meningioma	0-1.9	2 (3.8)	1 (1.9)	4 (7.7)	1 (1.9)
Thymoma		0	0	1 (1.9)	0
Adrenal gland – cortical adenoma		0	0	1 (1.9)	0
Females					
Survival [N (%)]		37 (70)	32 (62)	33 (63)	36 (69)
Total bw gain (% of Controls)		395	381 (96)	371 (94)	345 (87)
Non-neoplastic changes [N (%)]					
N of examined animals		64	64	64	64
Adrenal cortical fat vacuolation		3 (4.7)	7 (11)	4 (6.3)	6 (9.4)
Brain necrosis		0	0	0	0
Transitional epithelial hyperplasia – kidney		26 (41)	35 (55)	41 (64)	48 (75)*
Pelvic vascular ectasia – kidney		3 (4.7)	2 (3.1)	5 (7.8)	5 (7.8)
Liver hypertrophy		0	0	1 (1.6)	0
Altered hepatocytes (clear cell)		0	1 (1.6)	0	0
Sciatic nerve demyelination		54 (84)	53 (83)	53 (83)	54 (84)
Voluntary muscle degeneration		0	0	0	2 (13)
Neoplastic changes [N (%)]					
N of examined animals		53	52	52	52
Brain – astrocytoma	0-3.8	0	1 (1.9)	0	2 (3.8)
Brain – meningioma	0-1.9	0	0	0	2 (3.8)
Thymoma		0	1 (1.9)	0	3 (5.8)
Adrenal gland – cortical adenoma		0	0	0	2 (3.8)
Uterus – stromal cell sarcoma	0-3.8	0	0	0	2 (3.8)
Uterus – stromal cell polyp	0-23.1	5 out of 52 (9.6)	6 (12)	7 (14)	10 (19)
Mammary gland fibroadenoma	3.8-19	3 out of 51 (5.9)	5 out of 51 (9.8)	2 (3.8)	6 out of 51 (12)
Mammary gland adenoma		1 out of 51 (2)	1 out of 51 (2)	1 (1.9)	2 out of 51 (3.9)

Data are taken from the CLH report and from the DAR. HC – historical control. *Significantly different from the Control (Pearson's chi-square test or Fisher's exact test, $P<0.05$). [a]For neoplastic changes interim kill data were not included.

Table 2. Summary table for the 80-week C57 black mouse carcinogenicity study (Rattray, 1998)

		Pirimicarb dose (ppm)			
	HC (%)	**0**	**50**	**200**	**700**
Males					
Total bw gain, g (% of Controls)		11.7	11.1 (95)	11.7 (100)	10.3 (88)
Relative liver weight				↑	↑20.5%
Haematology (% of Control)					
Red blood cell count (RBC)		-	101.7	103.5*	106.4*
Mean cell volume		-	98.3	97.5*	92.0*
Mean cell haemoglobin (MCH)		-	98.1*	98.1*	94.2*
Haemoglobin[a]		-	99.7	101.5	100.3
Mean cell haemoglobin conc.		-	100	100.7	102.7*
Platelet count		-	94.0	98.7	104.2
Non-neoplastic changes (N)					
Kidney – interstitial mononuclear cell infiltration		6	2	6	10
Kidney – pelvic mononuclear cell infiltration		4	9	13	15
Lung – lymphoid proliferation		4	8	8	6
Spleen – increased pigmentation		3	1	4	2
Neoplastic changes [N (%)]					
N of examined animals		55	55	55	55
Lung adenoma	1.8-7.3	1 (1.8)	1 (1.8)	1 (1.8)	3 (5.5)
Lung - keratinising squamous epithelioma		0	0	0	0
Females					
Total bw gain, g (% of Controls)		10.9	10.8 (99)	10.6 (97)	8.4 (77)
Relative liver weight				↑	↑18.3%
Subcutaneous masses					↑
Eye discharge					↑
Haematology (% of Control)					
Red blood cell count (RBC)		-	102.4	102.8	107.9*
Mean cell volume		-	98.8	97.4*	90.9*
Mean cell haemoglobin (MCH)		-	99.3	98.7	93.3*
Haemoglobin[a]		-	101.7	101.4	100.6
Mean cell haemoglobin conc.		-	100.7	102.0*	103.0*
Platelet count		-	93.7	95.7	130.1*
Non-neoplastic changes (N)					
Kidney – interstitial mononuclear cell infiltration		5	5	6	8
Kidney – pelvic mononuclear cell infiltration		12	12	21	14
Lung – lymphoid proliferation		13	18	18	23
Spleen – increased pigmentation		1	1	1	7
Neoplastic changes [N (%)]					
N of examined animals		55	55	55	55
Lung adenoma	1.8-3.6	0	0	0	6 (10.9)
Lung - keratinising squamous epithelioma		0	0	0	1 (1.8)

[a]Haemoglobin values were calculated from group averages of RBC and MCH values (RBC x MCH). * Statistically significant difference, $p \leq 0.05$ (Student's t-test).

Table 3. Summary table for the lifetime Alderley Park mouse carcinogenicity study (Sotheran *et al*., 1980)

	Pirimicarb dose (ppm)				
HC (%)	**0**	**0**	**200**	**400**	**1600**
Males					

Survival (%)		33.3	26.7	23.3	28.8	21.7
Total bw gain, g (% of Controls)		20.8	26.0	19.3 (93)	23.8 (114)	15.8 (76)
Tumour bearing animals		42	45	51	42	46
Neoplastic changes [N with tumour/N examined (%)]						
Lung adenoma	6.6-18.3	9/59 (15.3)	8/60 (13.3)	9/59 (15.3)	8/59 (13.6)	17/58 (29.3)
Lung carcinoma		0/59	1/60 (1.7)	0/59	0/59	1/58 (1.7)
Hyperplastic nodules and benign tumours in liver	8-21.7	3/58 (5.2)	9/59 (15.3)	5/59 (8.5)	9/58 (15.5)	15/57 (26.3)
Liver nodules with morphological signs of malignancy	8.3-18	4/58 (6.9)	6/59 (10.2)	13/59 (22.0)	8/58 (13.8)	17/57 (29.8)
Lymphosarcoma[b]		13	15	13	13	14
Females						
Survival (%)		30.4	21.9	30.0	23.6	11.4*
Total bw gain, g (% of Controls)		22.9	22.6	24.0 (105)	20.6 (90)	13.4 (59)
Tumour bearing animals		42	43	42	45	46
Neoplastic changes [N with tumour/N examined (%)]						
Lung adenoma	0-13.3	9/59 (15.3)	4/59 (6.8)	9/59 (15.3)	11/59 (18.6)	18/59 (30.5)
Lung carcinoma		0/59	1/59 (1.7)	0/59	1/59 (1.7)	0/59
Hyperplastic nodules and benign tumours in liver	0-3.3	1/58 (1.7)	2/59 (3.4)	3/57 (5.3)	6/58 (10.3)	4/59 (6.8)
Liver nodules with morphological signs of malignancy	0-8.3	2/58 (3.5)	0/59	3/57 (5.3)	3/58 (5.2)	5/59 (8.5)
Lymphosarcoma[b]		11	25	18	18	24
Ovary – papillary cystadenoma	0-2.1	0/55	0/55	1/58 (1.7)	3/55 (5.5)	3/56 (5.4)
Mammary gland adenocarcinoma	0-2.1	0/56	0/58	1/57 (1.8)	1/57 (1.8)	4/57 (7)

[a]Body weight gain expressed as % of control values was calculated with the average value from two Control groups. [b]Number examined was not specified. *Significantly different from the Control (Pearson's chi-square test or Fisher's exact test, P<0.05)

Figure 2a. Mortality rates in male mice in the Sotheran *et al*. (1980) study (corrected for accidental deaths using the Kaplan-Meier formula)

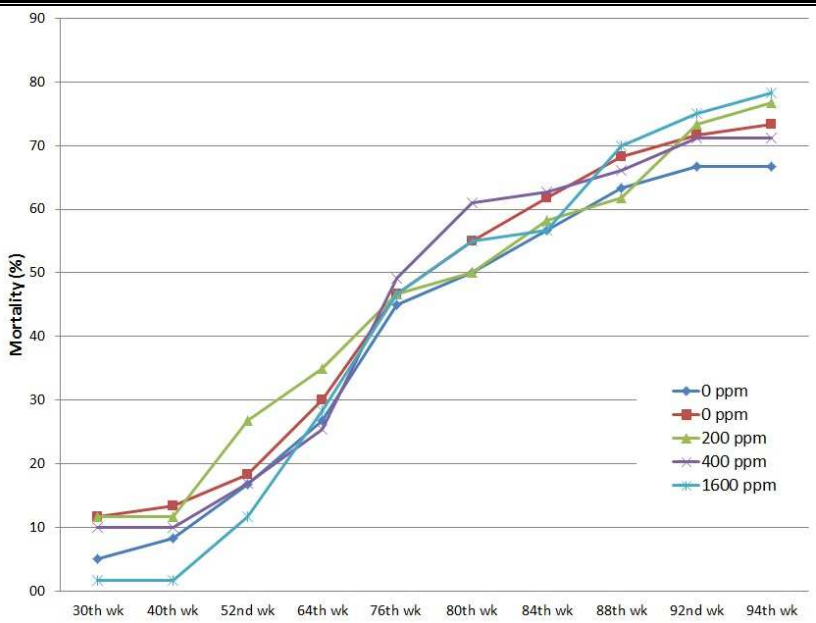


Figure 2b. Mortality rates in female mice in the Sotheran *et al*. (1980) study (corrected for accidental deaths using the Kaplan-Meier formula)

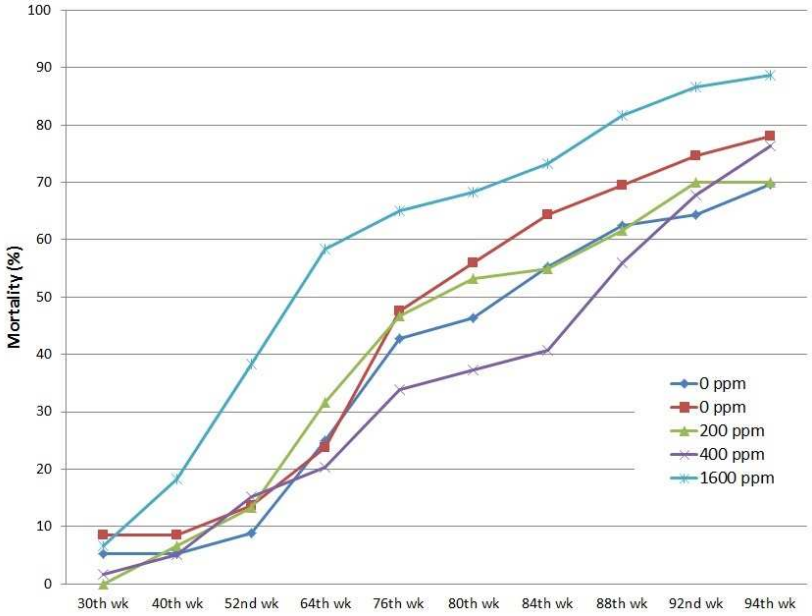


Figure 3a. Male mice body weight gain in the Sotheran *et al*. (1980) study

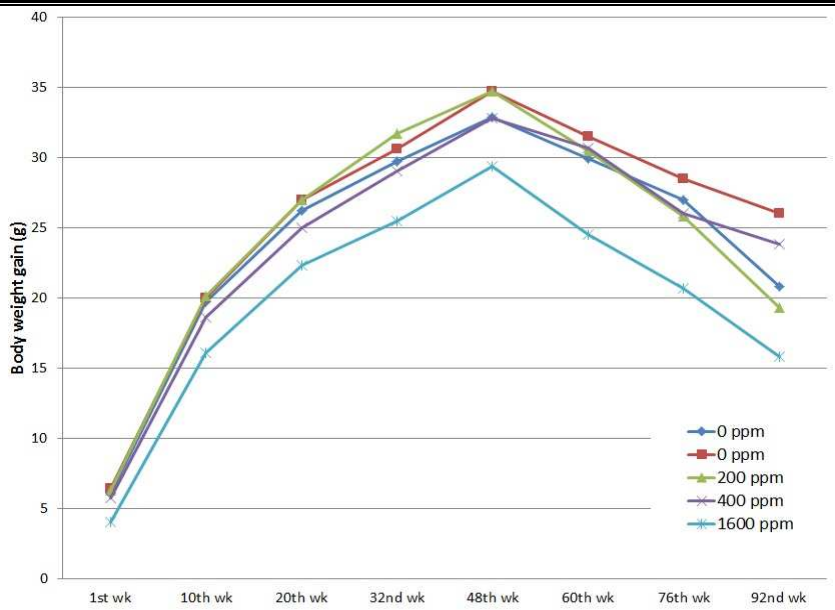


Figure 3b. Female mice body weight gain in the Sotheran *et al*. (1980) study

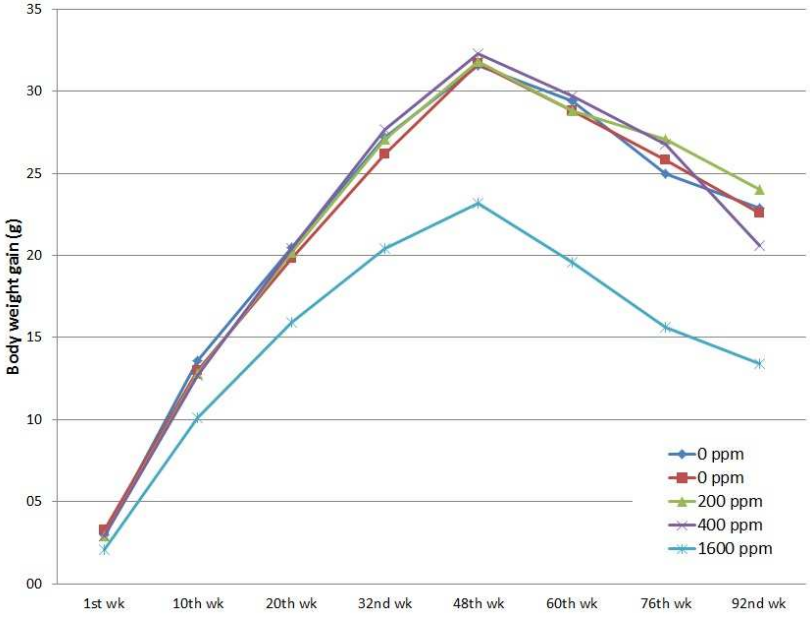


Table 4. Comparative table of relevant neoplastic changes (%) in the rodent carcinogenicity studies

Study		**Pirimicarb dose** (ppm)			
2-year rat study (Tinston, 1992)	**HC**	**0**	**75**	**250**	**750**
Males					
Survival (%)		*42*	*35*	*48*	*46*
% of Control bw gain		*(ref)*	*105*	*99*	*94*
Brain – astrocytoma	1.9 (0-5.8)	0	3.8	3.8	5.8
Brain – meningeoma	0 (0-1.9)	**3.8**	1.9	**7.7**	1.9
Females					

Survival (%)		*70*	*62*	*63*	*69*	
% of Control bw gain		*(ref)*	*96*	*94*	***87***	
Brain – astrocytoma	1.8 (0-3.8)	0	1.9	0	3.8	
Brain – meningioma	0 (0-1.9)	0	0	0	**3.8**	
Uterus – stromal cell sarcoma	0-3.8	0	0	0	3.8	
Uterus – stromal cell polyp	0-23.1	9.6	12	14	19	
Mammary gland fibroadenoma	3.8-19	5.9	9.8	3.8	12	
Mammary gland adenoma		2	2	1.9	3.9	
2-year oral in rats (Samuels *et al.*,1975)	**HC**	**0**			**750**	**2500**
Survival in male SD rats (%)		***15***			***31***	***29***
Lung - Reticulum cell lymphoma (SD rats)		2.1			8.3	13
Survival in female Wistar rats (%)		50			46	-
Mammary gland fibroadenoma (Wistar)	36.1 (18-45)	17			29	-
80-week, C57 black mouse (Rattray 1998)	**HC**	**0**	**50**	**200**	**700**	
Males						
Survival (%)				*>85%*		
% of Control bw gain		*(ref)*	*95*	*100*	***88***	
Lung adenoma	2.8 (1.8-7.3)	1.8	1.8	1.8	5.5	
Females						
Survival (%)				*>85%*		
% of Control bw gain		*(ref)*	99	97	***77***	
Lung adenoma	1.8 (0-3.6)	0	0	0	**10.9**	
Lung - keratinising squamous epithelioma		0	0	0	1.8	
Life-time, Alderley Park mice (Sotheran *et al.*, 1980)	**HC**	**0**	**0**	**200**	**400**	**1600**
Males						
Survival (%)		*33.3*	*26.7*	*23.3*	*28.8*	*21.7*
% of Control bw gain		-	-	*93*	*114*	***76***
Lung adenoma	10.7 (6.6-18.3)	15.3	13.3	15.3	13.6	**29.3**
Lung carcinoma		0	1.7	0	0	1.7
Hyperplastic nodules and benign tumours in liver	14.4 (8-21.7)	5.2	15.3	8.5	15.5	**26.3**
Liver nodules with morphological signs of malignancy	14.5 (8.3-18)	6.9	10.2	**22.0**	13.8	**29.8**
Lymphosarcoma (N)		13	15	13	13	14
Females						
Survival (%)		*30.4*	*21.9*	*30.0*	*23.6*	*11.4*
% of Control bw gain		-	-	*105*	*90*	***59***
Lung adenoma	6.7 (0-13.3)	15.3	6.8	15.3	**18.6**	**30.5**
Lung carcinoma		0	1.7	0	1.7	0

Hyperplastic nodules and benign tumours in liver	2.0 (0-3.3)	1.7	**3.4**	**5.3**	**10.3**	**6.8**
Liver nodules with morphological signs of malignancy	3.3 (0-8.3)	3.5	0	5.3	5.2	**8.5**
Lymphosarcoma (N)		11	25	18	18	24
Ovary – papillary cystadenoma	0 (0-2.1)	0	0	1.7	**5.5**	**5.4**
Mammary gland adenocarcinoma	0-2.1	0	0	1.8	1.8	**7**
80-week mice (Palmer & Samuels, 1974)		**0**		**300**		**1500**
Survival (%) males		*32*		*30*		*32*
Lung adenoma - males		0		12.2		13.0
Survival (%) females		*40*		*22*		*28*
Lung adenoma-females		6.1		8.3		10.6

HC – historical control range incidence (%), presented as median (range) or as a range (if individual study incidence data were not available). Incidences that are above HC range are in **bold**.

Table 5. Historical control range (from CLH dossier) for tumours in rat and mouse strains used in described carcinogenicity studies

Rodent strain & tumour type	**Time period**	**N of studies**	**Total N**		**N (%) with tumour**		**Incidence (%)**	
			M	F	M	F	M	F
Alderley Park SD-derived rats:								
Astrocytoma	1982 - 1992	15	848	848	21 (2.5)	12 (1.4)	1.9 (0-3.8) [0-5.8]	1.8 (0-1.9) [0-3.8]
Meningeoma	1983 - 1992	14	796	796	6 (0.8)	4 (0.5)	0 (0-1.9) [0-1.9]	0 (0-1) [0-1.9]
Mammary gland fibroadenoma*	1983 - 1992	14	-	N/A	-	N/A	-	3.8-19.2
Uterine stromal cell sarcoma*	1983 - 1992	14	-	N/A	-	N/A	-	0-3.8
Uterine stromal cell polyp*	1983 - 1992	14	-	N/A	-	N/A	-	0-23.1
Alderley Park Wistar-derived rats:								
Mammary gland fibroadenoma[†]	1990 - 1995	5	-	465	-	168 (36)	-	18-45
Alderley Park Swiss-derived mice:								
Pulmonary adenoma	1978 - 1983	3	341	341	41 (12)	21 (6.2)	10.7 (8-16.7) [6.6-18.3]	6.7 (0-8.3) [0-13.3]
Hyperplastic nodules and benign tumours in liver	1978 - 1983	3	341	341	51 (15)	7 (2.1)	14.4 (11.7-18) [8-21.7]	2.0 (1.6-3.3) [0-3.3]
Malignant liver nodules	1978 - 1983	3	341	341	46 (14)	12 (3.5)	14.5 (10-16) [8.3-18]	3.3 (1.7-4) [0-8.3]

Ovary- papillary cystadenoma	1978 - 1983	3	-	341	-	2 (0.6)	-	0 (0-2.0) [0-2.1]
Mammary gland* adenocarcinoma	1978 - 1983	3	-	N/A	-	N/A	-	0-2.1
C57BL mice:								
Pulmonary adenoma	1994 - 1997	6	325	330	11 (3.4)	6 (1.8)	2.8 (1.8-3.6) [1.8-7.3]	1.8 (0-3.6) [0-3.6]

Historical control incidence (%) data are from the laboratory performing carcinogenicity studies, unless stated otherwise. The data are presented as median (interquartile range) [range] or as a range (minimal-maximal value) where individual study incidence data were not available. N – number; M – males; F – females, N/A – not available; *only the number of studies and incidence ranges were available from the CLH dossier; [†]Charles River Laboratory in Kingston, NY, or Portage, MI (from the reference stated in the CLH report, Poteracki and Walsh, 1998).

4.11 Toxicity for reproduction

Not considered in this proposal.

4.12 Other effects

Not considered in this proposal.

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Not considered in this proposal.

4.12.1.2 Immunotoxicity

Not considered in this proposal.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Pirimicarb photodegrades in the environment – where available, data on the metabolites have also been discussed.

5.1 Degradation

Table 28: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis Guideline subdivision N 161-1	Pirimicarb is hydrolytically stable at environmentally relevant pH and temperature		Huynh & Mathis, 1996
Aquatic photolysis SETAC guidelines and EPA Guideline subdivision N 161-2	DT_{50} = 2.6 hours at pH 5 DT_{50} = 1.9 hours at pH 7		Hamlet, 1997
Aquatic photolysis SETAC guidelines, Frank and Klöffer model	DT_{50} = 12 hours DT_{50} = 264 hours	summer for top 0-30cm water column in winter for top 0-30cm water column	Giese & Müller, 1992
Aquatic photolysis SETAC guidelines, Frank and Klöffer model and US EPA Guideline 161-2	DT_{50} = 16 hours DT_{50} = 290 hours	summer for top 0-30cm water column winter for top 0-30cm water column	Moffatt, 1994
Water/sediment simulation SETAC and BBA guidelines	DT_{50} total system 156 - 185 days		Kirkpatrick & Kellett, 1992

5.1.1 Stability

Hydrolysis (Huynh & Mathis, 1996)

A hydrolysis study following EPA guidelines using ^{14}C-pirimidinyl radiolabelled pirimicarb (purity 97.9 %) is available. The test was run at pH 5, 7 and 9 at 25^{o}C over 32 days in the dark. Less than 5 % hydrolysis was observed under all pH conditions and pirimicarb is considered hydrolytically stable under environmentally relevant pH and temperature conditions.

Aqueous photolysis

Study 1 (Hamlet, 1997)

An aqueous photolysis study following SETAC guidelines (and EPA Guideline subdivision N 161-2) using ^{14}C-pirimidinyl radiolabelled pirimicarb (purity 99.2 %) showed that pirimicarb undergoes photodegradation. The study involved subjecting buffered (pH 5 and 7) sterile pure water samples with 1.04 µg/ml test substance to continuous irradiation (calculated to be the equivalent to 31 hours of summer sunlight at 30°N) at 25°C and wavelength 300-400 nm. Pirimicarb was observed to photodegrade extensively.

For the DAR process the experimental first order half-lives at 25°C were calculated as 2.6 hours at pH 5 and 1.9 hours at pH 7.

Various degradants were identified including three major degradants at ≥ 10 % applied radioactivity (AR) (R034885 [maximum 17.9 % AR], R031805 [maximum 27.8 % AR], R016210 [maximum 26.9 % AR][1]) and two minor degradants (R034836, R035140[2]). Half-life values for degradants were not calculated due to insufficient data.

The study DT_{50} values were 3.2 hours at pH 5 and 2.28 hours at pH 7 in Florida summer sunlight (30°N). This is considered to equate to DT_{50} values of 3.3 hours at pH 5 and 2.38 hours at pH 7 in European summer sunlight (50°N).

Study 2 (Giese & Müller, 1992)

The quantum yield and phototransformation of pirimicarb (purity 98.8 %) in water was evaluated following SETAC guidelines. Test samples were irradiated at 315 nm for 8 hours at 20°C and unknown pH. Quantum yield for direct phototransformation was 4.4 x 10^{-3}. Using the Frank and Klöpffer simulation model, estimated half-lives for the top 0-30cm of a pure water column in central Europe were 12 hours in June and 264 hours (approximately 11 days) in December.

Study 3 (Moffatt, 1994)

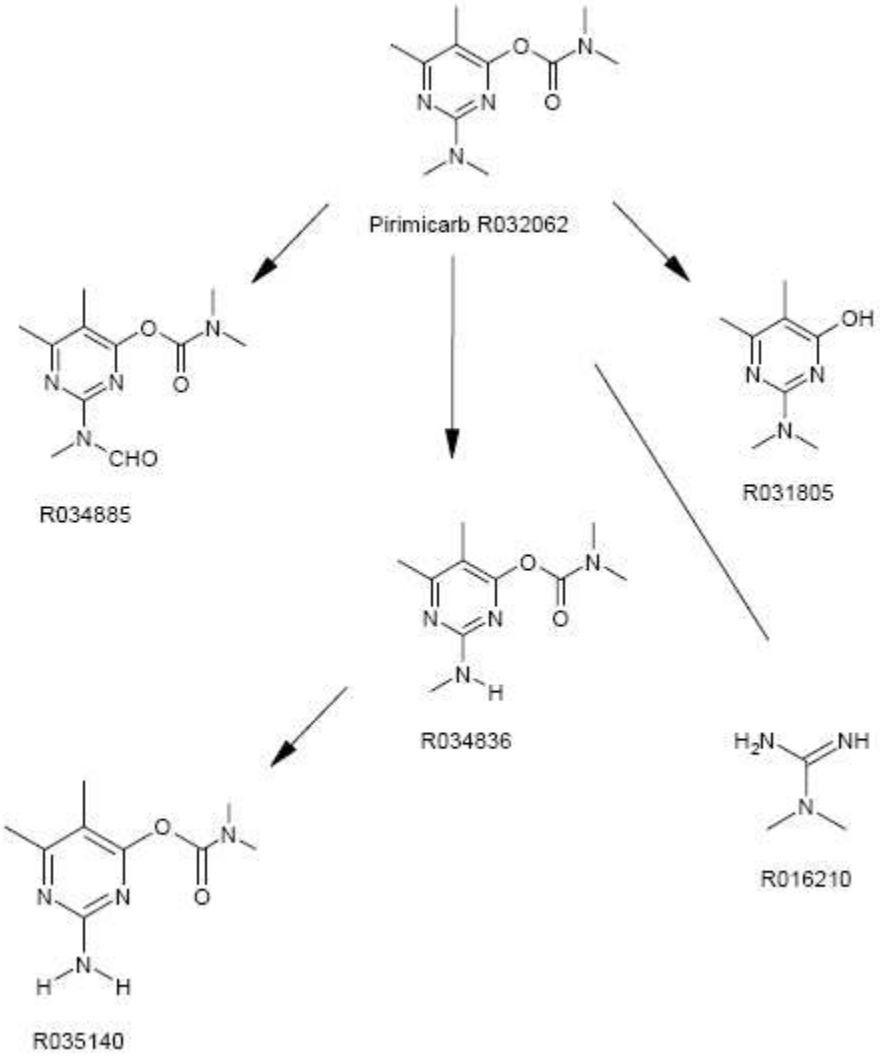
The quantum yield and phototransformation of pirimicarb (purity 98.4 %) in water was evaluated following SETAC and US EPA 161-2 guidelines. Test samples were irradiated at 313nm at 20°C and unknown pH until 2-12% of the pirimicarb was degraded. Quantum yield for direct phototransformation was 9.5 x 10^{-3}. Using the Frank and Klöpffer simulation model, estimated half-lives for the top 0-30cm of a pure water column in central Europe were 16 hours in summer and 290 hours (approximately 12 days) in winter in mid-European conditions.

On the basis of three studies, pirimicarb is considered to photodegrade in aquatic environment. A proposed aquatic photodegradation pathway for pirimicarb is presented in figure 1.

[1] R34885: 5,6-dimethyl-2-(methylformamido) pyrimidin-4-yl dimethylcarbamate
R31805: 2-dimethylamino-5,6-dimethylpyrimidin-4-ol
R16210: 1,1-dimethylguanidine

[2] R34836 : 5,6-dimethyl-2-(methylamino) pyrimidin-4-yl dimethylcarbamate
R35140: 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate

Figure 1 - Proposed aquatic photodegradation pathway for pirimicarb



5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

A ready biodegradation study is not available for pirimicarb.

5.1.2.3 Simulation tests

Study 1 (Kirkpatrick & Kellet, 1992)

Following SETAC and BBA guidelines, the aerobic water/sediment degradation of pirimicarb was assessed using water/sediment from the Brown Carrick Hill and Auchingilsie water bodies. The study using ^{14}C-pirimidinyl radiolabelled pirimicarb (purity > 97.4 %) involved incubating test flasks without plants over 100 days in the dark at 20 ± 2 ^{o}C. The study guidelines specify a sediment/water ratio of 1:4 to 1:10. For the pirimicarb study, the sediment/water ratio was 1:3.6 meaning slightly more sediment was present than the guideline range.

In the Brown Carrick Hill system, the water pH was 7.2 and sediment pH was 6.9. The sediment phase comprised 8 % clay, 22 % silt, and 70 % sand. The organic carbon content was 1.1 %.

In the Auchingilsie system, the water pH was 7.2 and sediment pH was 7.2. The sediment phase comprised 16 % clay, 33 % silt, and 51 % sand. The organic carbon content was 1.8 %.

In both systems aquatic pirimicarb concentrations decreased during the experiment with an increased in pirimicarb concentrations in sediment and pirimicarb degradants R034836, R034885 and R031805. However, no degradant was observed at ≥ 10 % AR. The pirimicarb DT_{50} in water at 20 ^{o}C in the dark for the Brown Carrick Hill system was 55 days and for the Auchingilsie system was 36 days. As the concentration of pirimicarb in sediment was still increasing at study termination in both systems, and limited decline was observed, reliable DT_{50} values for the sediment phase could not be calculated. Minimal mineralization was observed with a maximum of 1.5 % carbon dioxide after 100 days in the Auchingilsie system.

Total pirimicarb in the Brown Carrick Hill system (sediment and water) declined from an initial 94.3% to 62.3% AR after 100 days. A semi-logarithmic plot of total pirimicarb against time was bi-phasic in nature but a half-life of 194 days was calculated by the study authors from the data. For the Auchingilsie system total primicarb fell from 93.7% to 62.4% AR at 100 days, this decline was also bi-phasic in nature and a whole system half-life of 166 days was calculated from the data. Different whole system first order DT_{50} values are included in the EFSA pesticide peer review conclusion on pirimicarb (10th August 2005) but their derivation is unclear, nevertheless they also indicate DT_{50}ss >156 days and therefore confirm a lack of rapid degradation.

The distribution of AR in each system is presented in Tables 29 and 30. A proposed aquatic degradation pathway for pirimicarb is presented in figure 2.

Table 29 – Applied Radioactivity (AR) distribution in Brown Carrick Hill system

Component Detected	Phase	Distribution of Radioactivity (% Applied)[a]								
		Days Incubation								
		0	0.25	1	2	7	14	30	60	100
Pirimicarb	water	88.5	88.3	64.8	68.6	57.8	48.5	36.7	27.6	22.6
	sediment	5.9	6.2	22.2	14.7	20.3	27.3	35.7	42.7	40.0
R034836	water	2.2	2.1	1.6	1.4	1.2	1.4	2.9	2.0	1.6
	sediment	0.2	0.1	0.5	0.3	0.6	0.9	1.5	2.1	1.9
R034885	water	2.2	2.5	2.3	2.0	1.2	1.3	2.0	1.2	1.2
	sediment	0.2	0.2	0.8	0.4	0.8	1.1	1.8	1.4	1.7
R031805	water	0.7	0.7	0.5	0.5	0.7	0.9	0.8	0.6	0.6
	sediment	0.2	0.2	0.4	0.2	0.6	0.4	0.6	1.4	2.4
Polars	water	1.8	2.3	1.3	1.1	0.8	2.0	2.4	1.3	1.6
	sediment	0.8	1.0	2.1	2.7	5.3	3.5	6.8	4.3	4.3
Others[b]	water	1.1	1.3	0.8	0.9	1.0	0.8	0.4	0.3	0.6
	sediment	0.3	0.3	0.9	0.7	1.7	1.3	1.4	1.5	1.7
Unextracted		0.9	0.6	2.4	1.1	4.3	3.4	6.2	7.9	13.4
Volatiles		nd	nd	nd	nd	0.0	0.1	0.3	0.5	0.8
Total Recovery		104.7	105.5	100.3	94.3	96.1	92.6	99.2	94.5	94.2

nd Not detected

[a] Any summation differences in values within tables result from rounding of numbers within individual calculations

[b] Others - radioactivity distributed through regions of the chromatogram other than those specified and which did not contain any discrete radioactive peaks

Table 30 – Applied Radioactivity (AR) distribution in Auchingilsie system

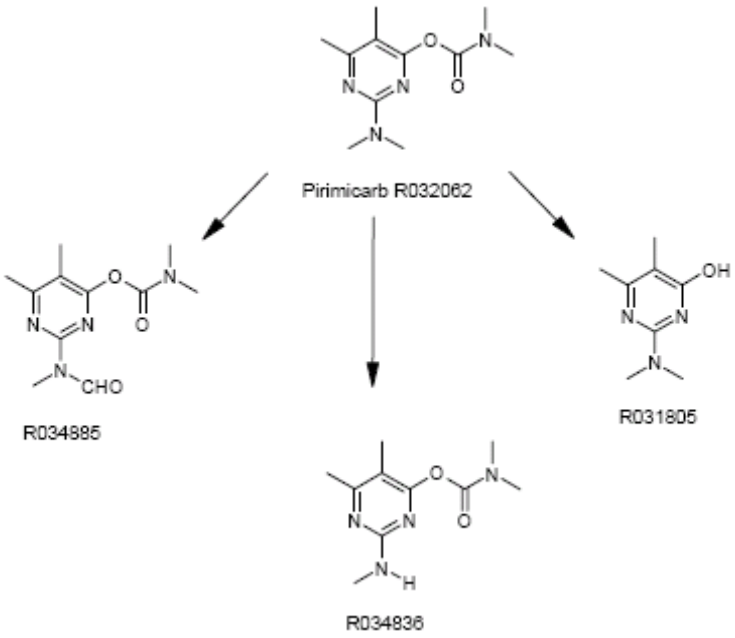
Component Detected	Phase	Distribution of Radioactivity (% Applied)[a]								
		Days Incubation								
		0	0.25	1	2	7	14	30	60	100
Pirimicarb	water	92.9	91.9	83.0	86.8	72.6	58.2	33.4	20.8	13.3
	sediment	0.9	0.8	7.9	1.9	8.2	22.2	40.9	45.5	48.0
R034836	water	2.7	3.4	2.2	2.1	2.0	1.8	1.8	2.2	1.7
	sediment	0.1	0.1	0.3	0.2	0.3	0.9	1.7	3.3	3.2
R034885	water	2.1	2.4	1.8	1.8	1.8	1.2	0.8	0.9	0.8
	sediment	0.1	0.1	0.3	0.2	0.4	0.7	1.4	3.0	2.3
R031805	water	0.6	0.8	0.6	0.7	0.9	0.8	1.6	0.8	0.5
	sediment	0.1	0.1	0.2	0.3	0.3	0.9	0.8	1.0	2.6
Polars	water	1.9	2.4	1.4	1.6	1.5	1.4	1.4	1.6	1.0
	sediment	0.8	0.7	2.9	2.3	2.2	1.7	8.7	8.4	3.9
Others[b]	water	1.1	1.3	1.3	1.3	1.4	1.0	0.3	0.5	0.4
	sediment	0.3	0.3	0.8	0.6	0.9	0.9	3.2	3.0	1.5
Unextracted		0.1	0.2	1.9	0.6	3.3	8.8	3.8	8.5	9.6
Volatiles		nd	nd	nd	0.0	0.0	0.0	0.1	0.8	1.5
Total Recovery		103.6	104.2	104.2	100.1	95.5	100.4	99.7	100.0	90.1

nd Not detected

[a] Any summation differences in values within tables result from rounding of numbers within individual calculations

[b] Others - radioactivity distributed through regions of the chromatogram other than those specified and which did not contain any discrete radioactive peaks

Figure 2 - Proposed degradation pathway for pirimicarb based on aquatic water /sediment simulation study



5.1.2.4 Additional information

Soil degradation (Miles & Hamlet, 1998)

Studies of aerobic and anaerobic degradation in soil studies at 20°C in the dark were conducted to USA EPA (Environmental Fate Section N Part 162-1, 1982) guidelines (deviations from SETAC (March 1995) guidelines were: Soil moisture content was maintained at 71 - 75% 1/3 bar, not 40-50% MWHC). The soils were treated with ^{14}C pyrimidinyl-labelled pirimicarb at a rate equivalent to 1.49kg a.s./ha. Treated samples were removed at intervals (up to 372 days) during the incubation and analysed for parent and major metabolites. $^{14}CO_2$ and any other volatilised radioactivity from the incubation systems were trapped and quantified. Limited mineralisation with a maximum of 3 % Applied Radioactivity after 103 days was observed.

5.1.3 Summary and discussion of degradation

Pirimicarb is considered hydrolytically stable at environmentally relevant pH and temperature conditions.

Pirimicarb undergoes aquatic photolysis. Two studies estimated winter half-lives for the top 0-30 cm of a pure water column as ~11-12 days. A third study calculated DT_{50} values of 3.2 hours at pH 5 and 2.28 hours at pH 7 in Florida summer sunlight (30°N), which is considered to equate to DT_{50} values of 3.3 hours at pH 5 and 2.38 hours at pH 7 in European summer sunlight (50°N).Three major photodegradants were observed: R34885, R31805 and R16210. Although this suggests a primary environmental half-life of less than 16 days, the actual degree of photodegradation in the environment (where local conditions will vary and affect degradation rates) is uncertain. Photolysis is therefore considered to be not relevant for classification purposes, but it is relevant for the interpretation of algal toxicity studies.

A ready biodegradation study is not available.

In the water/sediment simulation study conducted in two systems in the absence of light, pirimicarb did not significantly mineralise (up to 1.5 % AR after 100 days). Pirimicarb was observed to slowly partition from the water phase to sediment and the water DT_{50} was 36 to 55 days. The whole system first order DT_{50} for pirimicarb was reported to be 166 and 194 days in each system.

Overall, pirimicarb is unlikely to undergo greater than 70 % ultimate degradation in the aquatic environment within 28 days, and so is not considered rapidly degradable for the purposes of classification.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Pirimicarb (Lane and Rowe, 1991)

Following OECD Guideline 106 and using ^{14}C-pirimidinyl labelled pirimicarb the adsorption/desorption of pirimicarb (purity 96.1 %) was assessed using four soils (sand, sandy loam, clay loam and sandy loam). The adsorption K_{foc} ranged from 45 to 730 ml/g. The desorption K_{doc} ranged from 75.2 to 1380 ml/g. On this basis pirimicarb is considered slightly mobile.

Degradants (Millais & Kaur, 2000a; Millais & Kaur, 2000b; Vaughn & Lane, 1994; and O'Hara, 2000)

Three studies following OECD Guideline 106 and using radiolablled ^{14}C-pirimidinyl degradants R034836 (purity >98 %), R034885 (purity >99 %), R031805 (purity >97 %) and R034865 (purity >97 %) are available.

The adsorption K_{foc} for R034836 ranged from 34 (sandy loam) to 4320 (silty clay) ml/g. The adsorption K_{foc} for R034885 ranged from 57 (sandy loam) to 867 (silty clay) ml/g. The adsorption K_{foc} for R031805 ranged from 130 (sandy loam) to 80000 (silty clay loam) ml/g. The adsorption K_{foc} for R034865 ranged from 179 (sandy loam) to 9650 (silty clay) ml/g.

5.2.2 Volatilisation

Pirmicarb has a low vapour pressure of 4.3 x 10^{-7} kPa at 20 °C (Wollerton & Husband, 1994a) and a calculated low Henry's Law Constant of 2.9 x 10^{-5} Pa m^3mol^{-1} at pH 5.2, and 3.3 x 10^{-5} Pa m^3mol^{-1} at pH 7.4 and pH 9.3 based on measured data. On this basis pirimicarb is considered unlikely to partition the air.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Pirimicarb has a log K_{ow} of 1.7 at 20 ^{o}C pH 7.1 (Wollerton & Husband, 1994a). As this is below 3, indicating a low bioaccumulation potential, a fish aquatic bioaccumulation study has not been conducted.

5.3.1.2 Measured bioaccumulation data

5.3.2 Summary and discussion of aquatic bioaccumulation

5.4 Aquatic toxicity

Key data for classification are presented below in Tables 31, 32 and 33, with additional information presented in the relevant sections below. Relevant information pertaining to aquatic degradants is also included.

This information is obtained mainly from the October 2003 DAR for pirimicarb under Dir. 91/414/EEC and also the August 2004 Addendum 1 to the DAR and August 2005 EFSA Conclusion (EFSA Scientific Report (2005) 43) - which contains the peer reviewed and agreed endpoints for pirimicarb. The key studies used for classification are all conducted to GLP and without significant deviation from the relevant guidelines. All endpoints are derived using appropriate methods and measured test concentrations where appropriate. Although studies were

checked to provide some additional information, they are considered to be reliable for use in hazard classification therefore (Klimisch 1) and they have not been evaluated again in detail.

Table 31: Summary of relevant information on aquatic toxicity

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Pirimicarb 98.9 %	*Oncorhynchus mykiss*	US EPA 72-1 and OECD 203	96-h LC_{50}	79 mg/l	Static Mean measured conc^{n}s 100-103%, therefore based on nominals	Kent *et al*, 1998a and 1998b
Pirimicarb 98.9 %	*Pimephales promelas*	US EPA 72-1	96-h LC_{50}	>100 mg/l	Static Based on highest nominal test conc^n	Magor *et al*, 1998
R34865 100 %	*Oncorhynchus mykiss*	OECD 203	96-h LC_{50}	>120 mg/l	Static Nominal conc^n	Daniel *et al*, 2001a
Pirimicarb 96-98 %	*Oncorhynchus mykiss*	OECD 204	28-d NOEC	<18 mg/l	Semi-static Based on mean measured test conc^n	Tapp *et al*, 1989
Pirimicarb 97.5 %	*Pimephales promelas*	EPA 72-4	36-d NOEC based on growth	10 mg/l	Flow-through Based on mean measured test conc^n	Kent *et al*, 1996

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Pirimicarb
Two GLP static 96-hour acute toxicity to fish studies are available.

Study 1 (Kent *et al*, 1998a and 1998b): The study followed US EPA guideline 71-1 and OECD guideline 203 and used *Oncorhynchus mykiss* (rainbow trout). The purity of pirimicarb was 98.9 %. Based on nominal concentrations the 96-h LC_{50} was 79 mg/l with 65-100 mg/l 95 % confidence intervals and the NOEC was 18 mg/l.

Study 2 (Magor *et al*, 1998): The study followed US EPA guideline 71-1 and used *Pimephales promelas* (fathead minnow). The 96-h LC_{50} was considered >100 mg/l, the highest nominal concentration tested.

A further prolonged toxicity to fish study (Tapp *et al*, 1989) is available using pirimicarb. Following GLP and OECD Guideline 204, a semi-static method and using *Oncorhynchus mykiss* (rainbow trout), the 28 day NOEC based on measured concentrations was below the lowest exposure concentration tested of 18 mg/l. However, for classification purposes, this prolonged endpoint is very unlikely to be as low as the chronic NOEC for *Daphnia* (see below).

Degradant R34865 (Daniel *et al*, 2001a)
A static 96-hour acute toxicity to fish study following GLP and OECD Guideline 203 using *Oncorhynchus mykiss* (rainbow trout) is available. Based on nominal concentrations in a limit test the 96-h LC_{50} was considered >120 mg/l.

5.4.1.2 Long-term toxicity to fish

Pirimicarb (Kent *et al*, 1996)

Following US EPA guideline 72-4[3], a flow-through test system and using *Pimephales promelas* (fathead minnow), the 36-day mean measured NOEC was 10 mg/l based on reduced growth.

5.4.2 Aquatic invertebrates

Table 32: Summary of relevant information on aquatic toxicity to aquatic invertebrates

[3] The study was performed to an in-house procedure based on US EPA Guideline 540/9-86-138 (1986) which also satisfies the requirements of EPA Office of Pesticides Program 72-4 Fish early life stage and aquatic invertebrate life cycle studies (Pesticide Assessment Guidelines subdivision E – Hazard Evaluation. 1982).

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Pirimicarb 97.5 %	*Daphnia magna*	US EPA 72-2 and OECD 202	48-h EC_{50}	0.017 mg/l	Static Mean measured 100-109%, therefore based on nNominal conc^{n}	Kent & Shillabeer, 1996a and 1996b
Pirimicarb 99 %	*Daphnia magna*	Not specified (see below discussion)	48-h EC_{50}	0.011-0.033 mg/l	Static Nominal conc^{n}	Hamer, 1995
R35140 90 %	*Daphnia magna*	Not specified	48-h EC_{50}	0.09 mg/l	Static Nominal conc^{n}	Hamer, 1998a
R34836 98 %	*Daphnia magna*	OECD 202	48-h EC_{50}	0.056 mg/l	Static Nominal conc^{n}	Kent *et al*, 1995a
R34885 96 %	*Daphnia magna*	OECD 202	48-h EC_{50}	0.018mg/l	Static Nominal conc^{n}	Kent *et al*, 1995b
R31805 100 %	*Daphnia magna*	Not specified	48-h EC_{50}	>100 mg/l	Static Nominal conc^{n}	Hamer, 1998b
R34865 100 %	*Daphnia magna*	OECD 202	48-h EC_{50}	>120 mg/l	Static Nominal conc^{n}	Daniel *et al*, 2001b
R16210 97 %	*Daphnia magna*	OECD 202	48-h EC_{50}	28 mg/l	Static Nominal conc^{n}	Hamer, 1998c
Pirimicarb 96.0 %	*Daphnia magna*	OECD 202	21-d NOEC Length	0.0009 mg/l	Semi-static Based on mean measured test Nominal conc^{n}	Thompson *et al*, 1989

5.4.2.1 Short-term toxicity to aquatic invertebrates

Pirimicarb
Two static 48-hour acute toxicity to *Daphnia magna* studies are available.

Study 1 (Kent & Shillabeer, 1996a and 1996b): This study was conducted to Following GLP and according to EPA Guideline 540/9-85-005 (1986) which satisfies the requirements of EPA/OPP Pesticide Assessment Guidelines subdivision E - 72-2 (Acute Toxicity Test for Freshwater Invertebrates, 1982). It was also stated to comply with , EPA guideline 72-2 and OECD guideline 202 (and EU method C2,). The 48 hour EC_{50} based on nominal concentrations was 0.017 mg/l with 95 % confidence levels 0.014 to 0.02 mg/l. Results were reported as nominal as since mean measured concentrations were 100 to 109 % of nominal values.

Study 2 (Hamer, 1995): The study was not run to GLP and the guideline was not detailed but the study is considered to broadly follow standard guidelines and is presented as supporting

information. The 48 hour EC_{50} based on nominal concentrations was 0.011 to 0.033 mg/l reflecting 0 % immobilisation at 0.011 mg/l and 100 % immobilisation at 0.033 mg/l.

Twenty-three further acute toxicity to freshwater invertebrate studies are available and presented in the DAR (Reference 4). These were mainly screening studies used in the pesticide aquatic risk assessment to highlight the particular sensitivity of *Daphnia* to pirimicarb. None of the $L(E)C_{50}$ values are lower than the data presented above so they are not summarised further.

Degradants
Acute toxicity to *Daphnia magna* studies are available for the degradants R35140 (Hamer, 1998a), R34836 (Kent *et al*, 1995a), R34885 (Kent *et al*, 1995b), R31805 (Hamer, 1998b), R34865 (Daniel *et al*, 2001b) and R16210 (Hamer, 1998c).

The lowest EC_{50} value is 0.018 mg/l for R34885 based on nominal concentrations from a 48-h static, GLP study following OECD 202.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A study on pirimicarb was conducted to OECD 202 Part II, *Daphnia* sp Reproduction Test (adopted (dated 1984) and according to GLP, . This was a semi-static test system and using *Daphnia magna*, ; the 21-d mean measured NOEC was 0.0009 mg/l based on length and 0.0017 mg/l based on reproduction.

5.4.3 Algae and aquatic plants

Table 33: Summary of relevant information on aquatic toxicity to algae and aquatic plants

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Pirimicarb 98.5 %	*Pseudokirchneriella subcapitata*	OECD 201	24-96-h E_rC50 24-96-h NOE_rC	180 mg/l 50 mg/l	Static Based on mean measured test Measured $conc^n$	Thompson, 1985
R34865 100 %	*Pseudokirchneriella subcapitata*	OECD 201	72-h E_rC50 72-h NOE_rC	>120 mg/l 56 mg/l	Static Nominal $conc^n$	Magor & Shillabeer, 2001
R31805 100 %	*Pseudokirchneriella subcapitata*	OECD 201	72-h E_rC50 72-h NOE_rC	>120 mg/l 120 mg/l	Static Nominal $conc^n$	Daniel & Shillabeer, 2000

Pirimicarb

Study 1 (Thompson 1985): A 96-hour, GLP, static algal growth inhibition study is available using *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) and following OECD Guideline 201 (1983). The 0 to 24 hours growth rate was slower than the remaining test period suggesting a lag phase of non-exponential growth. To ensure the E_rC_{50} reflected exponential growth, it was based on measurements from 24 to 96 hours. Based on mean measured concentrations, the 24-96h E_rC_{50} was 180 mg/l and the NOE_rC was 50 mg/l. Potential degradation due to exposure to light in this test was therefore taken into account.

Degradants

Following GLP and OECD 201, two algal growth inhibition studies are available for R34865 and R31805 (Magor & Shillabeer, 2001; Magor & Shillabeer, 2000). Based on nominal test concentrations, the 72-h E_rC_{50} was >120 mg/l and the 72-h NOE_rC was 120 mg/l for biomass in both studies. The 72-hour NOErC for growth rate was 56 mg/l for R34865.

5.4.4 Other aquatic organisms (including sediment)

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Pirimicarb is considered hydrolytically stable at environmentally relevant pH and temperature conditions. It undergoes aquatic photolysis in the laboratory, but this is considered irrelevant for classification purposes. A ready biodegradation study is not available. In the absence of light in the water/sediment simulation study, pirimicarb did not significantly mineralise (up to 1.5 % AR over 100 days). Pirimicarb was observed to slowly partition from the water phase to sediment and the water DT_{50} was 36 to 55 days. The total system first order DT_{50} was reported to be 166 and 194 days in each of the two systems studied (>156 days in EFSA peer review conclusion).

On this basis pirimicarb is considered not rapidly degradable or readily biodegradable for classification purposes.

A bioaccumulation study is not available, but the pirimicarb $logK_{ow}$ value of 1.7 is lower than the trigger values under CLP.

Acute toxicity to fish, invertebrates and algae data are available for pirimicarb and selected aquatic degradants. Overall, pirimicarb is considered more toxic than its degradants and since it is not rapidly degradable there is likely to be minimal exposure to these degradants, the focus of this classification will therefore be on pirimicarb itself. Pirimicarb is a selective aphicide and invertebrates are the most acutely and chronically sensitive trophic level tested. The acute toxicity to *Daphnia magna* 48-h EC_{50} is in the range >0.01 to <0.1 mg/l. A long-term aquatic ecotoxicity study is available using *Daphnia magna* with a 21-d NOEC of 0.0009 mg/l based on length.

<u>Regulation EC 1272/2008</u>

Based on acute ecotoxicity data for invertebrates with a $L(E)C_{50}$ values < 1 mg/l, Aquatic Acute 1 is applicable with an acute M-factor of 10 based on 0.01< $L(E)C_{50}$ ≤0.1 mg/l. Based on chronic ecotoxicity data for invertebrates with a 21-d NOEC <0.1 mg/l, Aquatic Chronic 1 is applicable with a chronic M-factor of 100 based on a 0.0001< NOEC ≤0.001 mg/l for a not rapidly degradable substance.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Aquatic Acute 1; H400 M=10
Aquatic Chronic 1; H410 M=100

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Pirimicarb is currently classified in annex VI of CLP as Aquatic Acute 1 and Aquatic Chronic 1. The DS proposed to add M-factors and classify the substance accordingly as Aquatic Acute 1 (M=10) and Aquatic Chronic 1 (M=100).

Degradation

A hydrolysis study conducted according to US EPA guideline subdivision N 161-1 was run at pH 5, 7 and 9 at 25°C over a period of 32 days in the dark. The study indicates that pirimicarb is hydrolytically stable since less than 5% hydrolysis was observed under the environmental conditions used in the study.

The studies of aqueous photolysis showed that pirimicarb undergoes photodegradation in water. In the first study, carried out according to SETAC and US EPA 161-2 guidelines at pH 5 and 7 using Xenon lamp at 25°C for periods equivalent to 31 hours of summer sunlight at 30°C, the experimental first order half-lives were calculated as 2.6 hours at pH 5 and 1.9 hours at pH 7. In the second study, following SETAC guidelines, pirimicarb was irradiated at 315 nm in purified water for 8 hours at 20°C and unknown pH. The estimated half-lives, determined by the Frank and Klopffer simulation model at irradation conditions equivalent to central Europe (Frank and Klopffer, 1988, 1989) for the top 0-30 cm of a pure water column were 12 hours in June and 264 hours in December. In the third study, performed according to SETAC and US EPA 161-2 guidelines subdivision at 20 °C and unknown pH, test samples were irradiated at 313 nm until 2-12% of pirimarb was degraded. Using the Frank and Klopffer simulation model, the estimated half-lives for the top 0-30 cm of a pure water column in central Europe were 16 hours in summer and 290 hours in winter.

No data on ready biodegradability are available.

A water/sediment simulation study, carried out according to SETAC and German BBA guidelines, using ^{14}C-pirimidinyl radiolabelled pirimicarb, was run over 100 days in the dark at 20±2 °C using two pond systems. The sediment/water ratio (1:3.6) in the experiment was slightly higher than the range specified in the guidelines (1:4 to 1:10).

In both systems, pirimicarb concentrations in water decreased during the experiment with an increase in pirimicarb concentrations in sediment and pirimicarb degradants (R034836, R034885 and R031805[4]). No degradant was observed at ≥ 10% of applied radioactivity (AR). Up to 1.5% of AR was completely mineralized to carbon dioxide after 100 days.

For pirimicarb, the half-lives in water were 55 and 36 days while reliable DT_{50} in sediment could not be determined as concentrations of pirimicarb were still increasing in sediment at the end of the study. For the whole systems, half-lives of 194 and 166 days were calculated by the study authors from the data. Different values for whole systems half-lives (185 and 156) are included in the EFSA pesticide peer review conclusion on pirimicarb (2005), but their derivation is unclear.

Aerobic and anaerobic degradation in soil studies, according to US EPA 162-1 and 162-3 guidelines, were carried out at 20°C in the dark using soils treated with ^{14}C-pirimidinyl

[4] R034836: 5,6-dimethyl-2-(methylamino) pyrimidin-4yl dimethylcarbamate
R034885: 5,6-dimethyl-2-(methylformamido) pyrimidin-4yl dimethylcarbamate
R031805: 2-dimethylamino-5,6-dimethylpyrimidin-4ol

radiolabelled pirimicarb at a rate equivalent to 1.49 kg a.s./ha. Treated samples were removed at intervals (up to 372 days) during the incubation and analysed for parent and major metabolites, $^{14}CO_2$ and any other volatilised radioactivity were trapped and quantified. Limited mineralization with a maximum of 3% AR was observed at day 112.

The DS concluded that pirimicarb is not considered as rapidly degradable.

Bioaccumulation

Pirimicarb has a log Kow of 1.7 (20°C , pH 7.1). A bioaccumulation study was not conducted because the bioaccumulation potential of pirimicarb is low.

Aquatic toxicity

The DS provided aquatic toxicity data for each trophic level on pirimicarb and on the most relevant metabolites. However, only information on pirimicarb was considered relevant, as it is considered more toxic than its degradants. Further, pirimicarb is not rapidly degradable and exposure to its degradants is likely to be minimal.

Regarding short-term toxicity, two tests with fishes (*Oncorhynchus mykiss* and *Pimephales promelas*), two with aquatic invertebrates *(Daphnia magna)* and one test with algae (*Pseudokirchneriella subcapitata*) were provided. Regarding long-term toxicity, there are three available tests, two with fishes (*Oncorhynchus mykiss* and *Pimephales promelas*) and one with aquatic invertebrates *(Daphnia magna)*.

Invertebrates are known as the most acutely and chronically sensitive trophic level. Between the acute toxicity tests, the study on *Daphnia magna* (according to US EPA 72-2 and OECD TG 202, under GLP) is considered as the decisive study, with a static 48-h EC_{50} =0.017 mg/L (nominal concentration). This result is supported by a second study on *Daphnia magna*, not perfomed according to GLP and without following a specific test guideline, with a 48-h EC_{50} value (nominal concentration) between 0.011 and 0.033 mg/L.

The long-term aquatic key study is the *Daphnia magna* study (according to OECD 202, part II (1984), GLP). The results are reported with a semi-static 21-d NOEC =0.0009 mg/L based on length, and =0.0017 mg/L based on reproduction.

The following table summarises the reported studies on aquatic toxicity for pirimicarb. The key values used for the purpose of classification are shown in bold.

Test Guideline	**Purity**	**Species**	**Remarks**	**Endpoint**	**Toxicity values in mg/L**	**Ref.**
Short-term toxicity to fish						
US EPA 72-1 and OECD TG 203	98.9%	Rainbow trout (*Oncorhynchus mykiss*)	Static Mean measured conc 100-103%, therefore based on nominals	96-h LC_{50}	79	Kent *et al.*, 1998a and 1998b
US EPA 72-1	98.9%	Fathead minnow (*Pimephales promelas*)	Static Based on highest nominal test conc	96-h LC_{50}	>100	Magor *et al*., 1998
Long-term toxicity to fish						
OECD TG	96-98	Rainbow trout (*Oncorhynchus*	Semi-static	28-d NOEC	<18	Tapp *et*

204	%	*mykiss*)	Based on mean measured test conc			*al.*, 1989
EPA 72-4	97.5 %	Fathead minnow (*Pimephales promelas*)	Flow-through Based on mean measured test conc	36-d NOEC based on growth	10	Kent *et al.*, 1996
Short-term toxicity to aquatic invertebrates						
US EPA 72-2 and OECD TG 202	97.5 %	*Daphnia magna*	Static Mean measured 100-109%, therefore based on Nominal conc	48-h EC_{50}	**0.017**	Kent & Shillabeer, 1996a and 1996b
Not specified	99 %	*Daphnia magna*	Static Nominal conc	48-h EC_{50}	0.011-0.033	Hamer, 1995
Long-term toxicity to aquatic invertebrates						
OECD TG 202, part II (1984)	96.0 %	*Daphnia magna*	Semi-static Nominal conc	21-d NOEC Length 21-d NOEC Reproduction	**0.0009** 0.0017	Thompson *et al.*, 1989
Toxicity to Algae						
OECD TG 201	98.5%	Green algae (*Pseudokirchneriella subcapitata*)	Static Based on mean measured test Measured conc	24-96-h E_rC_{50} 24-96-h NOE_rC	180 50	Thompson, 1985

Comments received during public consultation

Four MSCAs submitted comments during public consultation supporting the proposed environmental classification. In particular, two MSCAs underlined the need to describe those key studies on which the classification is based, in a more detailed way. One MSCA suggested just an addition regarding additional data for acute fish toxicity which however does not change the proposed classification. The DS answered these comments in the RCOM stating that there is sufficient information in the CLH report to allow a decision on the environmental classification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal that pirimicarb should be considered as not rapidly degradable, based on the fact that less than 70% of pirimicarb degraded within 28 days in the hydrolysis study, no ready biodegradability study is available and less than 70% of the substance is not ultimately degraded in the water/sediment study. Although the studies of aqueous photolysis suggest that pirimicarb undergoes photodegradation, the actual degree

of photodegradation in the aquatic environment is uncertain and not relevant for classification purposes.

Bioaccumulation
Pirimicarb has a measured log Kow of 1.7 (20 °C, pH 7.1). This log Kow is below the trigger of log kow = 4. Therefore RAC agrees with the conclusion of the DS that pirimicarb has no significant bioaccumulation potential.

Aquatic toxicity

Acute aquatic hazard

Acute toxicity data are available for all three trophic levels. Invertebrates are the most sensitive taxonomic group. The lowest reliable short-term aquatic toxicity result for *Daphnia magna* is 48-h EC_{50} = 0.017 mg/L (nominal concentration).

Chronic aquatic hazard

Long-term aquatic toxicity data are available for all three trophic levels. The lowest value is for invertebrate species *Daphnia magna*, with a 21-d NOEC = 0.0009 mg/L (nominal concentration).

Pirimicarb is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation.

The lowest available result obtained for pirimicarb in a short-term test is EC_{50} value of 0.017 mg/L in *Daphnia magna*. In agreement with the DS proposal, RAC concludes that pirimicarb therefore fulfils the criteria for classification as **Aquatic Acute 1** with an **M-factor=10**, because the value is in the range: 0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L.

In addition, the lowest available result from a long-term test obtained for pirimicarb is a NOEC value of 0.0009 mg/L in *Daphnia magna*. Pirimicarb therefore also fulfils the criteria for classification as **Aquatic Chronic 1** with an **M-factor=100**, because the value is in the range: 0.0001 mg/L < NOEC ⩽ 0.001 mg/L and the substance is not rapidly degradable.

6 OTHER INFORMATION

No other information provided.

7 REFERENCES

1. Draft Assessment Report (DAR) – public version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance pirimicarb of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.1-B.5- December 2004

2. Draft Assessment Report (DAR) – public version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance pirimicarb of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B6, part 1- December 2004

3. Draft Assessment Report (DAR) – public version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance pirimicarb of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B6, part 2- December 2004

4. Draft Assessment Report (DAR) – public version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance pirimicarb of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B7-B9 and Appendicies - December 2004

5. NTP (2005) Historical control report, gavage, corn oil, Sprague-Dawley, females, 2005

Specific References as provided in Draft Assessment Report (DAR) – public version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance pirimicarb of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 2, Annex A,

Physical and Chemical Properties

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Wollerton C, Husband R	1994	Pirimicarb: Physico-chemical Study on Pure Active Ingredient. Jealott's Hill Research Station Zeneca Report No. RJ1612B GLP, Unpublished	Y	SYN
Wollerton C, Husband R	1994	Pirimicarb: Physico-Chemical Study on Technical Grade Active Ingredient.	Y	SYN

Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
		Jealott's Hill Research Station Zeneca Report No. RJ1613B GLP, Unpublished		
Huynh TT, Mathis SMG	1996	Pirimicarb: Aqueous Hydrolysis in pH5, 7 and 9 Solutions at 25 Degrees C. Jealott's Hill Research Station Zeneca Report No. RJ2051B GLP, Unpublished	Y	SYN
Hamlet JM	1997	Pirimicarb: Aqueous Photolysis at pH5 and pH7 at 25 degrees C. Jealott's Hill Research Station Zeneca Report No. RJ2298B GLP, Unpublished	Y	SYN
Moffatt F	1994	Pirimicarb: Quantum Yield and Environmental Half-Life for Direct Phototransformation in Aqueous Solution. Jealott's Hill Research Station Zeneca Report No. RJ1542B GLP, Unpublished	Y	SYN
Wollerton C, Husband R	1994	Pirimicarb: Dissociation Constants of Pirimicarb and its Metabolites R34836, R31805 and R34865. Jealott's Hill Research Station Zeneca Report No. RJ1548B GLP, Unpublished	Y	SYN
Hayes S	2001	Pirimicarb : Calculation of Half-Life by Reaction with Atmospheric Hydroxyl Radicals	N	SYN

Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
		Jealotts Hill Research Station Zeneca report N. RIC7332 GLP, Unpublished		
Tremain SP	2001	Pirimicarb Technical Grade Active Ingredient – Determination of Explosive Properties. SafePharm Laboratories SPL Report No. 1292/011 GLP, Unpublished	Y	SYN
Evans AJ, Mullee DM	2001	Pirimicarb Technical Grade Active Ingredient – Determination of Surface Tension. SafePharm Laboratories SPL Report No. 1292/014 GLP, Unpublished	Y	SYN
Jackson WA	2002	Oxidising Properties – Pirimicarb Syngenta Technology and Projects Process Hazards Section PHS Ref : HT02/186 Non-GLP, Unpublished	Y	SYN

Toxicology and Metabolism

Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Brown A	1997a	Pirimicarb: Whole Body Autoradiography in the Rat. Report No. P/5383 GLP, Unpublished	Y	SYN
Brown A	1997b	Pirimicarb: Excretion and Tissue Distribution of a Single Oral Dose (1 mg/kg) in the Rat. Report No. P/5329 GLP, Unpublished	Y	SYN
Brown A	1997c	Pirimicarb: Excretion and Tissue Distribution of a Single Oral Dose (50 mg/kg) in the Rat. Report No. P/5381 GLP, Unpublished	Y	SYN
Brown A	1997d	Pirimicarb: Excretion and Tissue Distribution of a Single Oral Dose (1 mg/kg) in the Rat Following Repeat Dosing. Report No.P/5382 GLP, Unpublished	Y	SYN
Gledhill AJ	1998	Pirimicarb: Biotransformation in the Rat. Report No. P/5530 GLP, Unpublished	Y	SYN
Lees D, Connolly HJ	1995a	Pirimicarb: Acute Oral Toxicity to the Rat. Report No. P/4802 GLP, Unpublished	Y	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Lees D, Connolly HJ	1995b	Pirimicarb: Acute Dermal Toxicity to the Rat. Report No. P/4855 GLP, Unpublished	Y	SYN
Parr-Dobrzanski RJ	1994	Pirimicarb: 4-Hour Acute Inhalation Toxicity Study in the Rat. Report No. P/4522 GLP, Unpublished	Y	SYN
Lees D, Connolly HJ	1995c	Pirimicarb: Skin Irritation to the Rabbit. Report No. P/4858 GLP, Unpublished	Y	SYN
Lees D, Doyle CE	1995	Pirimicarb: Eye Irritation to the Rabbit. Report No. P/4706 GLP, Unpublished	Y	SYN
Rattray N, Leah AM	1990	Pirimicarb: Skin Sensitisation to the Guinea Pig. Report No. P/3087 GLP, Unpublished	N	SYN
Paul D, Richards D, Banham PB, Weight TM	1995	First Revision to Pirimicarb: Growth Study to Determine a No-Effect Level in the Female Rat. Report No. /P/408 Non GLP, Unpublished	Y	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Richards D, Banham PB, Weight TM	1978	Pirimicarb: Paired Feeding Study in the Female Rat. Report No. /P/407 Non GLP, Unpublished	Y	SYN
Griffiths D, Conning DM	1995	First Revision to Ninety-Day Oral Toxicity of PP062 - Albino Rats. Report No. /R/237 Non GLP, Unpublished	Y	SYN
Griffiths D, Conning DM	1995	First Revision to Ninety-Day Oral Toxicity of PP062 - Albino Rats. Individual Animal Data Report No. /R/237 Non GLP, Unpublished	Y	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Hodge MCE	1995a	Subchronic Studies with Pirimicarb (PP062) in the Beagle Dog: Ninety-day Oral Toxicity of PP062 - Beagle Dogs (Part 1) and Oral Toxicity of PP062 - Beagle Dogs (Part 2). Report No. /P/4593 Incorporating /R/241 and /R/248 (Previously /241 and /248) Non GLP, Unpublished	Y	SYN
Hodge MCE	1995a	Subchronic Studies with Pirimicarb (PP062) in the Beagle Dog: Ninety-day Oral Toxicity of PP062 - Beagle Dogs (Part 1) and Oral Toxicity of PP062 - Beagle Dogs (Part 2). Individual Animal Data Report No. P/4593 Incorporating CTL/R/241 and R/248 (Previously 241 and 248) Non GLP, Unpublished	Y	SYN
Fox T	1978	Pirimicarb: Dietary Toxicity Study in Foxhounds. Report No. 543 plus addendum P/420 Non GLP, Unpublished	N	SYN
Horner SA	1998	Pirimicarb: 1 Year Oral Toxicity Study in Dogs. Report No P/5690 GLP, Unpublished	Y	SYN
Horner SA	1998	Pirimicarb: 1 Year Oral Toxicity Study in Dogs. Individual Animal Data Report No. P/5690 GLP, Unpublished	Y	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Hodge MCE	1995b	First Revision to Pirimicarb (PP062): 2-Year Feeding Study in Beagle Dogs. Report No. R/337 Non GLP, Unpublished	Y	SYN
Garner R, Phillips CE, Slack P	1995	First Revision to Pirimicarb (PP062): Pirimicarb Anaemia (A Chemically-Induced Autoimmune Haemolytic Anaemia). Report No. P/61 Non GLP, Unpublished	Y	SYN
Lees D, Leah AM	1995	Pirimicarb: 21-Day Dermal Toxicity to the Rat. Report No. P/4805 GLP, Unpublished	Y	SYN
Lees D, Leah AM	1995	Pirimicarb: 21-Day Dermal Toxicity to the Rat. Individual Animal Data Report No P/4805 GLP, Unpublished	Y	SYN
Callander RD	1995	Pirimicarb: An Evaluation of the Mutagenic Potential Using *S. typhimurium* and *E. coli.* Report No. P/4798 GLP, Unpublished	Y	SYN
Wildgoose J, Howard CA, Richardson CR, Randall V	1987	Pirimicarb: A cytogenetic study in human lymphocytes *in vitro*. Report No. P/1655 GLP, Unpublished	Y	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Trueman RW	1980	An Examination Of Pirimicarb For Potential Mutagenicity Using The Salmonella/Microsome Reverse Mutation Assay. Report No. P/540 GLP, Unpublished	N	SYN
Clay P	1996	Pirimicarb: L5178Y $TK^{+/-}$ Mouse Lymphoma Mutation Assay. Report No: P/5080 GLP, Unpublished	Y	SYN
Kennelly JC	1990	Pirimicarb: Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes *in vivo.* Report No. P/2824 GLP, Unpublished	N	SYN
Jones K, Howard CA	1989	Pirimicarb (technical): An Evaluation in the Mouse Micronucleus Test. Report No. P/2641 GLP, Unpublished	N	SYN
McGregor DB	1974	Dominant Lethal Study in Mice of ICI PP062. Report No. C/256 Non GLP, Unpublished	N	SYN
Tinston DJ	1992	Pirimicarb: Two Year Feeding Study in Rats. Report No. P/3040 GLP, Unpublished	Y	SYN

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Tinston DJ	1992	Pirimicarb: Two Year Feeding Study in Rats. Individual Animal Data Report No. P/3040 GLP, Unpublished	Y	SYN
Rattray NJ	1998	Pirimicarb: 80 Week Carcinogenicity Study in Mice. Report No. P/5839 GLP, Unpublished	Y	SYN
Rattray NJ	1998	Pirimicarb: 80 Week Carcinogenicity Study in Mice. Individual Animal Data Report No. P/5839 GLP, Unpublished	Y	SYN
Sotheran MF, Banham PB, Jackson DG, Taylor K, Weight TM, Woollen BH	1980	Pirimicarb: Lifetime Feeding Study in the Mouse. Report No. P/0491 Non GLP, Unpublished	N	SYN
Sotheran MF, Banham PB, Jackson DG, Taylor K, Weight TM, Woollen BH	1980	Pirimicarb: Lifetime Feeding Study in the Mouse. Individual Animal Data Report No. P/0491 Non GLP, Unpublished	N	SYN
Moxon ME	1991	Pirimicarb: Multigeneration Study In The Rat Report No. P/2940 GLP, Unpublished	N	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Moxon ME	1991	Pirimicarb: Multigeneration Study In The Rat. Individual Animal Data Report No. P/2940 GLP, Unpublished	N	SYN
Hodge MCE	1989	Pirimicarb: Teratogenicity Study in the Rat. Report No. P/2745 GLP, Unpublished	N	SYN
Hodge MCE	1989	Pirimicarb: Teratogenicity Study in the Rat. Individual Animal Data Report No. P/2745 GLP, Unpublished	N	SYN
Milburn GM	1989	Pirimicarb: Teratogenicity Study in the Rabbit. Report No. /P/2680 GLP, Unpublished	N	SYN
Milburn GM	1989	First Amendment to Pirimicarb: Teratogenicity Study in the Rabbit. Report NoP/2680 GLP, Unpublished	N	SYN
Milburn GM	1989	Pirimicarb: Teratogenicity Study in the Rabbit. Individual Animal Data Report No. /P/2680 GLP, Unpublished	N	SYN

Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Lees D	2001	Pirimicarb Metabolite 062/06 (R031805): 90 Day Dietary Toxicity Study In Rats. Individual Animal Data Syngenta UK Syngenta Report No. CTL/ PR1184/ REGULATORY/REPORT GLP, Unpublished	Y	SYN

Environmental Fate and Behaviour

Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Miles PD, Hamlet J	1998	Pirimicarb: Metabolism in Aerobic and Anaerobic Soils under Laboratory Conditions. Zeneca Agrochemicals, Jealott's Hill Research Station Report No. RJ2682B GLP, Unpublished	Y	SYN

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Lane MCG, Rowe D	1991	Pirimicarb: Adsorption and Desorption Equilibria in Soil. Zeneca Agrochemicals, Jealott's Hill Research Centre Report No. RJ0941B GLP, Unpublished	N	SYN
Millais AJ, Kaur AK	2000a	Pirimicarb: Adsorption and Desorption Properties of R034836, a Soil Degradate, in Six Soils. Huntingdon Life Sciences Ltd Report No. ZCA 046/003356 GLP, Unpublished	Y	SYN
Millais AJ, Kaur AK	2000b	Pirimicarb: Adsorption and Desorption Properties of R034885, a Soil Degradate, in Six Soils. Huntingdon Life Sciences Ltd Report No. ZCA 045/003144 GLP, Unpublished	Y	SYN
Vaughan PC, Lane MCG	1994	Pirimicarb: Adsorption and Desorption Properties in Soil of R31805, a Major Soil Metabolite. Zeneca Agrochemicals, Jealott's Hill Research Station Report No. RJ1587B GLP, Unpublished	Y	SYN
O'Hara CD	2000	Pirimicarb: Adsorption and Desorption Properties of R34865, a Soil Degradate, in Six Soils. Huntingdon Life Sciences Ltd Report No. ZCA 047/002954 GLP, Unpublished	Y	SYN

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Huynh TT, Mathis SMG	1996	Pirimicarb: Aqueous Hydrolysis in pH5, 7 and 9 Solutions at 25 Degrees C. Zeneca Agrochemicals, Jealott's Hill Research Station Report No. RJ2051B GLP, Unpublished	Y	SYN
Giese G, Müller J	1992	Photolysis of Pirimicarb in Water including Quantum Yield. Fraunhofer-Institut für Umweltchemie und Ökotoikologie,Grafschaft, 5948 Schmallenberg. Report No. ICI-002/7-21 GLP, Unpublished	Y	SYN
Moffatt F	1994	Pirimicarb: Quantum Yield and Environmental Half-Life for Direct Phototransformation in Aqueous Solution. Zeneca Agrochemicals, Jealott's Hill Research Station Report No. RJ1542B GLP, Unpublished	Y	SYN
Kirkpatrick D, Kellett DN	1992	^{14}C-Pirimicarb: The Degradation in Water/Sediment Systems. Huntingdon Research Centre Ltd Report No. HRC/ISN 282/921039 GLP, Unpublished	Y	SYN

Ecotoxicology

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Kent SJ, Magor SE, Shillabeer N	1998	Pirimicarb : acute toxicity to rainbow trout (*Oncorhynchus mykiss*). Report No. 15272 (6403/B) GLP, Unpublished	Y	SYN
Kent SJ, Magor SE, Shillabeer N	1998	Addendum to Pirimicarb : acute toxicity to rainbow trout (*Oncorhynchus mykiss*). Report No. 15272 (6403/B) GLP, Unpublished	Y	SYN
Magor SE, Kent SJ, Shillabeer N	1998	Pirimicarb : acute toxicity to fathead minnow (*Pimephales promelas*). Report No. 20036 (6404/B) GLP, Unpublished	Y	SYN
Magor SE, Kent SJ, Shillabeer N	1998	Addendum to Pirimicarb : acute toxicity to fathead minnow (*Pimephales promelas*). Report No. 20036 (6404/B) GLP, Unpublished	Y	SYN
Daniel M, Magor SE, Shillabeer N	2001a	R034865: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*). Report No: 47536 (6900/B) GLP, Unpublished	Y	SYN
Tapp SJ, Sankey SA, Caunter JE, Harland BJ	1989	Pirimicarb : determination of the 28-day LC_{50} to rainbow trout (*Salmo gairdneri*). Report No. B/3573 GLP, Unpublished	N	SYN
Kent SJ, Morris DS, Shillabeer N	1996	Pirimicarb : chronic toxicity to fathead minnow (*Pimephales promelas*) embryos and larvae. Report No. 15280 (5676/B) GLP, Unpublished	Y	SYN

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Kent SJ, Shillabeer N	1996	Pirimicarb : acute toxicity to *Daphnia magna.* Report No. 15271 (5798/B) GLP, Unpublished	Y	SYN
Kent SJ, Shillabeer N	1996	Addendum to Pirimicarb : acute toxicity to *Daphnia magna*. Report No. 15271 (5798/B) GLP, Unpublished	Y	SYN
Hamer MJ	1995	Pirimicarb : toxicity of the technical material to aquatic invertebrates. Jealott's Hill Research Station Report No. TMJ 3423B Not GLP, Unpublished	Y	SYN
Hamer MJ, Gentle WE, Ashwell JA	1999	Pirimicarb : acute toxicity tests with freshwater invertebrates. Jealott's Hill Research Station Report No. TMJ 4076B Not GLP, Unpublished	N	SYN
Hamer MJ	1998a	R35140: acute toxicity of the pirimicarb degradate 062/03 to *Daphnia magna.* Jealott's Hill Research Station Report No. TMJ 3977B Not GLP, Unpublished	Y	SYN
Kent SJ, Sankey SA, Banner AJ, Magor SE	1995a	R34836: acute toxicity to *Daphnia magna.* Report No. 5317/B GLP, Unpublished	Y	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Kent SJ, Sankey SA, Banner AJ, Magor SE	1995b	R34885: acute toxicity to *Daphnia magna.* Report No. 14543 (5335/B) GLP, Unpublished	Y	SYN
Hamer MJ	1998b	R31805: acute toxicity of the pirimicarb degradate 062/06 to *Daphnia magna.* Jealott's Hill Research Station Report No. TMJ 3976B Not GLP, Unpublished	Y	SYN
Daniel M, Magor SE, Shillabeer N	2001b	R034865: Acute Toxicity to *Daphnia magna*. Report No. 47537 (6901/B) GLP, Unpublished	Y	SYN
Hamer MJ	1998c	R16210: acute toxicity of the pirimicarb degradate 062/10 to *Daphnia magna.* Jealott's Hill Research Station Report No. TMJ 3978B Not GLP, Unpublished	Y	SYN
Thompson RS, Williams TD, Tapp JF	1989	Pirimicarb: determination of chronic toxicity to *Daphnia magna*. Report No. B/3509 GLP, Unpublished	N	SYN
Thompson RS	1985	Pirimicarb : Toxicity to the green alga *Selenastrum Capricornutum.* Report No: B/2617 GLP, Unpublished	N	SYN
Magor SE, Shillabeer N	2001	R034865 : Toxicity to the Green Alga *Selenastrum capricornutum.* Report No: 6902/B GLP, Unpublished	Y	SYN

Author	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or Unpublished**	**Data protection claimed Y/N**	**Owner**
Daniel M, Shillabeer N	2000	Pirimicarb : Toxicity of the metabolite R31805 to the green alga *Selenastrum capricornutum*. Report No: 6912/B GLP, Unpublished	Y	SYN

8 ANNEXES

None