CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Penthiopyrad

EC Number: **n/a**

CAS Number: **183675-82-3**

Index Number: **Not yet assigned**

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CONTENTS

Part A.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Penthiopyrad**
EC number:	**n/a**
CAS number:	**183675-82-3**
Annex VI Index number:	**Not yet assigned**
Degree of purity:	**980 g/kg** **Penthiopyrad is a racemic mixture of the R and S enantiomers.**
Impurities:	**None of relevance to the classification and labelling. Full information is provided in 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	Not applicable
Current proposal for consideration by RAC	Aquatic Acute 1; H400: Very toxic to aquatic life Acute M factor = 1 Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects Chronic M factor = 1
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1; H400: Very toxic to aquatic life Acute M factor = 1 Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

	Chronic M factor = 1

1.3 Proposed harmonised classification and labelling

1.4 Table 3: Proposed classification

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 for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1 H400; Very toxic to aquatic life** **Aquatic Chronic 1 H410; Very toxic to aquatic life with long lasting effects**	**Acute M factor = 1** **Chronic M factor = 1**	Not classified	Not relevant
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

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

Labelling:

Pictogram(s):	GHS09
Signal word:	Warning
Hazard statements:	H410; Very toxic to aquatic life with long lasting effects
Precautionary statements:	Not included in Annex VI of CLP

Proposed notes assigned to an entry:

None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Penthiopyrad is an active substance in the scope of Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC). There is no entry on Annex VI of CLP and there have been no previous classification and labelling discussions for this substance. As such, penthiopyrad is subject to harmonised classification and labelling in accordance with Article 36(2) of CLP.

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

2.2 Short summary of the scientific justification for the CLH proposal

The EFSA conclusion on the peer review of the pesticide risk assessment (2013) notes that concern was raised for the carcinogenic potential of penthiopyrad during the experts meeting. This was due to an increased incidence of hepatocellular adenomas in male mice. It should be noted that an increased incidence of thyroid follicular adenomas was also observed (in male rats), but these were not considered to be relevant to humans during the expert meeting. A full assessment of the available data is presented in section 4.10 of this report. The increased incidence of liver adenomas occurred at the top dose in male mice and fell well within the historical control range for this tumour type. Further, the historical control data indicate that the concurrent control value was unusually low. The implications of the increased incidence of thyroid follicular tumours in male rats have been evaluated using the ECHA Guidance on the Application of the CLP Criteria and the Specialised Experts guidance on non-genotoxic thyroid carcinogens (ECBI, 1999). Consequently, no classification for carcinogenicity is proposed.

The EFSA conclusion also considers classification with Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 appropriate. Penthiopyrad is not considered to be bioaccumulative and aquatic acute toxicity data indicate that fish are the most sensitive trophic group with L(E)C_{50} values in the range 0.1 to 1.0 mg/l. The lowest value is 0.290 mg/l for Fathead Minnow (*Pimephales promelas*). On this basis penthiopyrad should be classified as Aquatic Acute 1; H400 with an M factor of 1. Adequate chronic toxicity data are available and the lowest value is a 33-day NOEC for Fathead Minnow (*Pimephales promelas*) of 0.051 mg/l. Given this is in the range 0.01 to 0.1 mg/l and the substance is considered non-rapidly degradable, penthiopyrad should be classified as Aquatic Chronic 1 with an M factor of 1. A full assessment of the data is provided in section 5.

2.3 Current harmonised classification and labelling

Penthiopyrad is not currently listed on Annex VI of CLP.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling

Aquatic Acute 1; H400 – Very toxic to aquatic life

Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects

There are currently no entries on the C&L Inventory for penthiopyrad.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Penthiopyrad is an active substance in the scope of Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC). There is no entry on Annex VI of CLP and there have been no previous classification and labelling discussions for this substance. The substance is therefore subject to the harmonised classification and labelling process in accordance with Article 36(2) of CLP and no further justification is required.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

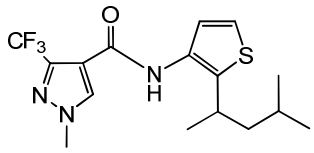
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	Not available
EC name:	Not applicable
CAS number (EC inventory):	Not applicable
CAS number:	183675-82-3
CAS name:	1H-Pyrazole-4-carboxamide,N-[2-(1,3-dimethylbutyl)-3-thienyl]-1-methyl-3-(trifluoromethyl)-
IUPAC name:	1-methyl-*N*-[2-(4-methylpentan-2-yl)-3-thienyl]-3-(trifluoro methyl)-1*H*-pyrazole-4-carboxamide
CLP Annex VI Index number:	Not yet assigned
Molecular formula:	$C_{16}H_{20}F_3N_3OS$
Molecular weight range:	359.4 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
≥ 98%			**Penthiopyrad is a racemic mixture of the R and S enantiomers.**

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are a number of process impurities in the substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided 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

Penthiopyrad was shown to be a racemic mixture of the two optical isomers, R and S penthiopyrad. The batches used in the studies referenced in this report are considered to be representative of the substance as manufactured and identified above. The possible impact of a different enantiomer ratio is not considered.

1.3 Physico-chemical properties

All studies were considered to be reliable in the DAR. Reference can be made to the DAR Volume 3 Section B2 (B.2.1): Physical and Chemical Properties, January 2012 and Addendum 1 to the DAR – Volume 3 Section B2 Physical and Chemical Properties, September 2012

Table 8: Summary of physico – chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid white powder	Tomiya K. 2008a-f	98.6 – 99.8%
Melting/freezing point	108.7 °C ± 0.2 °C (381.9 K ± 0.2K)	Tognucci A. 1999a	OECD 102/EEC A.1(Differential Thermal Calorimetry) 99.9% pure GLP
Boiling point	Boiling point not determined due to decomposition at 314°C.	Tognucci A.1999b	OECD 103/EEC A.2 (DTC and Capillary Test) 99.9% GLP
Relative density	1.256 at 20 °C	Badt-Tognucci A. 2007a	OECD 109/EEC A.3 (gas comparison pycnometer) 98.6% pure GLP
Vapour pressure	Extrapolated: 6.43×10^{-6} Pa at 25 °C Determination of the vapour pressure at 70 °C, 80 °C and at 90 °C and extrapolation to 25 °C by linear regression as the evaporation of the test substance could not be determined at lower temperatures 3.70×10^{-3} Pa at 70 °C 8.04×10^{-3} Pa at 80 °C 3.61×10^{-2} Pa at 90 °C	Tognucci A. 1999c	OECD 104/ EEC A.4 (gas saturation method) 99.9% pure GLP

Surface tension	56.7 mN/m at 20.6°C The a.s. is surface active according to the test method.	Walbert S. 2006b	O ECD 115/ EEC A.5 (Ring method) 98.6% pure, GLP
Water solubility	At 20 °C: 2.535 mg/L at pH 4 1.375 mg/L at pH 7 1.657 mg/L at pH 10 At 30 °C: 1.426 mg/L at pH 7 At 20°C: 7.53 mg/L at pH 5	Franke J. 2008a Tognucci A. 1999d	OECD 105/EEC A.6 (method) 98.6% pure GLP OECD 105/J MAFF 5092 99.8% pure GLP
Partition coefficient n-octanol/water	log P_{OW} = 3.9 at pH 5 and 20 °C: At 20 °C: log P_{OW} = 4.36 at pH 4: log P_{OW} = 4.62 at pH 7: log P_{OW} = 4.54 at pH 10: At 30 °C: log P_{OW} = 4.43 at pH 7:	Labano S. 2012 (Tognucci, A. 1999d) Franke J. 2008b (Franke J. 2008a)	Calculated from solubility in pure distilled water (Tognucci A. 1999d) and solubility in octanol – expert statement, 99.8% Calculated from solubility in water (buffered solutions Franke J. 2008a) and solubility in octanol. 98.6% pure GLP The difference in the Log Pow between the two references is attributed to the difference between the water solubility in distilled water (Tognucci 1999d) compared to that in buffered solutions (Franke 2008 a). The lower water solubility's in the Franke 2008a studies were considered to be due, in part, to a 'salt-out' effect in the buffer solutions and a lower purity of penthiopyrad. The Log Pow of 3.9 was considered to be valid in the peer review.
Flash point	Not applicable, substance is a solid at > 40 °C		
Flammability	Not highly flammable. In contact with the ignition source the test material melted and turned yellow, emitting some sparks and white fume. No ignition within 2 minutes. Experience in handling	Badt-Tognucci A. 2007c	EEC A.10 98.6% pure GLP

	and use indicates it is not pyrophoric and does not react with water to liberate flammable gases.		
Explosive properties	Not explosive. The molecule does not contain chemical groups associated with explosive properties. Decomposition energy (ΔH_{dec}) < 500 J/g	Walbert S. 2006a	
Self-ignition temperature	No auto-ignition up to 400 °C.	Badt-Tognucci A. 2007d	EEC A.16 98.6% pure GLP
Oxidising properties	Not oxidising. Consideration of the structure indicates that the molecule will not have oxidising properties.	Walbert S. 2006c	
Dissociation constant	$pK_a = 10.0 \pm 0.16$	Tognucci A. 1999f Schreitmüller, J. 2006b	OECD 112 (Titration method) 99.9% pure GLP

2 MANUFACTURE AND USES

2.1 Manufacture

The substance is manufactured outside of the EU for use as a pesticidal active substance.

2.2 Identified uses

The substance is used within the EU as a pesticidal active substance for use against pathogenic fungi on pome fruit, tomatoes, aubergines, cucurbits, cucumbers and courgettes. It is also used for foliar and ear disease control on cereals.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

3.1 *Physico-Chemical Properties*

3.1.1 Summary and discussion of physico-chemical properties

Refer to table 8.

3.1.2 Comparison with criteria

In a standard flammability study (EEC A10) penthiopyrad was found to be not flammable. In contact with the ignition source the test material melted and turned yellow, emitting some sparks and white fume. No ignition was observed within 2 minutes. Experience in handling and use indicates penthiopyrad is not pyrophoric and does not react with water to liberate flammable gases. Further, it was also tested in a standard self ignition temperature study (EEC A16) and no spontaneous ignition was observed below 400 ^{o}C.

Penthiopyrad does not contain chemical groups associated with explosive properties. Decomposition energy (ΔH_{dec}) < 500 J/g.

Consideration of the structure indicates that penthiopyrad will not have oxidising properties.

3.1.3 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4 HUMAN HEALTH HAZARD ASSESSMENT

A full reference list can be found in the publically available Report and Proposed Decision of the United Kingdom Made to the European Commission under Article 8 of Council Directive 91/414/EEC – January 2012 and subsequent addenda – September 2012

In particular, refer to Volume 3 Annex B Section B6: Toxicology and Metabolism Part A: Evaluation and Assessment of Data Submitted, January – 2012 and addenda September 2012 for the studies summarised below.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Single and multiple oral dose absorption, distribution, metabolism and excretion of penthiopyrad, and the single dose pharmacokinetic profile, have been evaluated in the Wistar rat (Mitsui Chemicals Agro, Inc., 2005a; 2009a, b, c). The studies were performed according to US-EPA Health Effects Test Guideline OCSPP 870.7485, OECD guideline no. 41, Japan MAFF guideline 12 NohSan no. 8147 and EU Directive 88/302/EEC, B.36.

Absorption

Single orally administered doses of 10 or 100 mg/kg penthiopyrad in aqueous dispersion were rapidly (T_{max} values: 0.4 – 1.3 hours) and extensively absorbed (>83% administered dose, AD) from the GI tract of the Wistar rat. The relative systemic exposure was dose-dependent in the range 10 – 100 mg/kg, as determined by the C_{max} and AUC ratios. There were no clear sex- or dose-related differences in the extent of absorption, although C_{max} and AUC tended to be slightly higher in females than in males. The extent of oral absorption was not affected by repeated dosing of up to 7 days. In the plasma, radioactivity plateaued at up to 3.3 times the single dose value but was subsequently cleared to levels below the limit of detection within 48 hours of the final dose.

Distribution

Following oral absorption of a single dose, penthiopyrad was rapidly and widely distributed to body tissues with maximum tissue concentrations achieved within 1 hour of dosing, at which time concentrations in the liver, fat, lymph nodes adrenals, pancreas, kidneys, urinary bladder and GI tract were higher than those in the plasma. There were no clear sex- or dose-related differences in tissue distribution or tissue residues. Repeated dosing lead to a small increase of residues in adrenals, blood, fat, kidney, liver, lung, lymph nodes, ovaries, pancreas and thyroid compared to a single dose. Concentrations of radioactivity decreased in all tissues following cessation of dosing. The overall tissue distribution in both sexes 24 hours after multiple doses was similar to that following a single dose.

Metabolism

Extensive *in vivo* metabolism occurred at numerous positions within the molecule by thienyl ring oxidation and conjugation with glutathione, N-demethylation, alkyl side-chain hydroxylation and oxidation to carboxylic acids, glucuronidation, thienyl ring opening and cleavage of the two ring structures. The most abundant metabolite in both urine and faeces was formed as the result of N-demethylation and oxidation of the terminal methyl moiety of the alkyl side chain to carboxylic acid. The intermediate demethylated and hydroxylated metabolites formed glucuronic acid conjugates that were mainly recovered in bile. The most abundant metabolites in bile were formed as a result of thienyl ring oxidation to 753-F-DO followed by conjugation with glutathione and catabolism of glutathione. N-demethylation and side chain hydroxylation probably occurred in parallel. Four metabolites containing the pyrazole moiety following cleavage from the thienyl moiety were excreted in both urine and faeces. The two acids (PCA and DM-PCA) are likely formed by amide hydrolysis from PAM and DM-PAM. The thienyl ring appears to be completely degraded and the radiocarbon incorporated into normal metabolic processes. There was no evidence of the induction of specific metabolic processes following repeat dosing. In common with the single dose metabolite profile, it is complex following multiple dosing. However, there was no significant impact on the levels of major or minor metabolites following multiple dosing.

Excretion

Excretion of penthiopyrad was dose-dependent and proceeded rapidly, predominantly in bile (62.8 – 81.1% AD) but with appreciable amounts excreted in the urine (5.0 – 18.3% AD). Excretion in faeces and urine was essentially complete within 48 hours after dosing. Elimination of penthiopyrad via respired volatiles and CO_2 was negligible. Residual tissue and carcass levels were very low at ≤ 0.10% and 0.41% AD, respectively. There were no clear sex- or dose-related differences in the extent, route or rate of elimination. Repeated dosing had no clear effect on elimination. Following repeated dosing of up to 7 days, faecal excretion predominated (71.8 and 65.0% AD, in males and females respectively), and elimination was rapid and essentially complete within 48 hours of the final dose.

4.1.2 Human information

No data are available

4.1.3 Summary and discussion on toxicokinetics

The toxicokinetics of penthiopyrad following oral administration have been investigated in single and repeat dose studies in rats. Following single and repeat administration, penthiopyrad was well absorbed and widely distributed. Penthiopyrad was extensively metabolised and excreted in the faeces and urine. Biliary excretion accounted for nearly all of the faecal excretion. There are no clear sex-related differences.

4.2 Acute toxicity

Information on the acute toxicity of penthiopyrad is available from one oral study in rats, one dermal study in rats and one inhalation study in rats.

Table 10: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and remarks**
Acute oral toxicity OECD 423, GLP compliant HanIbm: WIST (SPF) Wistar rats (3/sex/dose) Gavage Doses: 2000 mg/kg bw Penthiopyrad (purity 99.8%), aqueous suspension (vehicle: bi-distilled water)	Males > 2000 mg/kg bw Females > 2000 mg/kg bw	No deaths or clinical signs of an adverse reaction to treatment occurred, and there were no macroscopic findings at necropsy in any animal. One male exhibited a low overall weight gain during the 14 day observation period. Reference: Mitsui Chemicals Agro, Inc. (2000)
Acute Inhalation		
Method	**LC50**	**Observations and remarks**
Acute inhalation toxicity OECD 403, GLP compliant HanIbm: WIST (SPF) Wistar rats (5/sex/dose) Concentration: 5.59 mg/L air (mean), dust aerosol Exposure: nose-only, 4 hrs MMAD ± GSD = 2.71 ± 2.96 µm Penthiopyrad (purity 99.8%)	Males > 5.59 mg/L Females > 5.59 mg/L	No deaths occurred during either the exposure or the observation periods. Clinical signs of an adverse reaction to treatment did not occur during the exposure period, but were evident following exposure. All animals exhibited slightly hunched posture and a moderate decrease in spontaneous activity approximately one hour after exposure, and most animals (9/10) exhibited ruffled fur. Clinical signs persisted for up to 2 days. Transient marginal weight loss occurred in 5/10 animals during the 3 days following exposure. There were no macroscopic findings at necropsy in any animal. Reference: Mitsui Chemicals Agro, Inc. (2001a)
Acute Dermal		
Method	**LD50**	**Observations and remarks**
Acute dermal toxicity OECD 402, GLP compliant HanIbm: WIST (SPF) Wistar rats (5/sex/dose) Doses: 2000 mg/kg bw, semi-occlusive Penthiopyrad (purity 99.8%), vehicle: polyethylene glycol 300	Males > 2000 mg/kg bw Females > 2000 mg/kg bw	No deaths or systemic clinical signs of an adverse reaction to treatment occurred. There were no local signs of an effect of treatment at the application site, and there were no macroscopic findings at necropsy in any animal. Two females exhibited small weight losses (0.6 and 5.3%) from day 1 to day 8 of the 14 day observation period, with subsequent recovery. Reference: Mitsui Chemicals Agro, Inc. (2001b)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Via the oral route, data are available from one study in Wistar rats. The LD_{50} value (for males and females) was > 2000 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

Via the inhalation route, data are available from one study in Wistar rats. The LC_{50} value (for males and females) was > 5.9 mg/L for 4 hours

4.2.1.3 Acute toxicity: dermal

Via the dermal route, data are available from one study in Wistar rats. The LD_{50} value (for males and females) was > 2000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

No data are available.

4.2.3 Summary and discussion of acute toxicity

See Section 4.2.1.

4.2.4 Comparison with criteria

Via the oral route, the LD_{50} was > 2000 mg/kg bw. This is above the cut-off for classification under CLP (2000 mg/kg bw), therefore no classification is proposed.

Via the inhalation route, the LC_{50} was > 5.59 mg/L for a respirable dust. This is above the cut-off for classification under CLP (5 mg/L), therefore no classification is proposed.

Via the dermal route, the LD_{50} was > 2000 mg/kg bw. This is above the cut-off for classification under CLP, therefore no classification is proposed.

4.2.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

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

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

The relevant animal data are summarised in Table 10. No human data are available.

4.3.2 Comparison with criteria

STOT-SE is considered when there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality. Substances that produce 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 under the CLP Regulation.

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.

There was no clear evidence of any specific toxic effects on a target organ or tissue noted in the acute toxicity studies. Clinical signs of toxicity were observed after single, inhalation exposures to penthiopyrad, but these were transient in nature and are considered to be unspecific signs of general acute toxicity. No classification for STOT-SE 1 or 2 under CLP is proposed.

No definitive signs of respiratory tract irritation or narcotic effects were observed, therefore no classification for STOT-SE 3 is proposed.

4.3.3 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.4 Irritation

4.4.1 Skin irritation

Data are available from one guideline skin irritation study in the rabbit.

Table 11: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Acute dermal irritation study OECD 404, GLP compliant New Zealand White Rabbit (3f) Doses: 0.5g, occlusive Penthiopyrad (purity 99.8%), vehicle: de-ionised water (applied as a dry powder, but covered with a water-moistened gauze-patch)	No signs of erythema, eschar formation, edema or other evidence of skin irritation in any of the animals at 1, 24, 48 and 72 h after patch removal, at either the treated or the control site. All individual scores were 0.	No deaths or clinical signs reported	Mitsui Chemicals Agro , Inc. (2001c)

4.4.1.1 Non-human information

Data are available from one guideline skin irritation study in rabbits. No signs of irritation were observed in any of the animals during the study.

4.4.1.2 Human information

No data are available.

4.4.1.3 Summary and discussion of skin irritation

See Table 11 and Section 4.4.1.1.

4.4.1.4 Comparison with criteria

Under CLP, classification is required if mean scores of >2.3 are recorded for erythema/eschar or oedema in at least 2 out of 3 tested animals. As all individual scores were 0 for all effects at all time points, no classification for skin irritation is proposed.

4.4.1.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.4.2 Eye irritation

Data are available from one guideline eye irritation study in the rabbit.

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Acute eye irritation OECD 405, GLP compliant New Zealand White Rabbit (3f/group) Dose: 0.1 g Group 1: non-irrigated Group 2: irrigated: eyes irrigated with luke-warm water for 30-60 seconds 30 seconds after test substance instillation Penthiopyrad (purity 99.8%), no vehicle	Mean scores (24 – 48h) for each animal: <u>Non-irrigated animals:</u> Cornea: 0, 0, 0 Iris: 0, 0, 0 Conjunctivae (redness): 0.33, 0, 0.33 Conjunctivae (Chemosis): 0.33, 0, 0.33 <u>Irrigated animals:</u> Cornea: 0, 0, 0 Iris: 0, 0, 0 Conjunctivae (redness): 0, 0, 0.33 Conjunctivae (Chemosis): 0, 0, 0	No deaths or clinical signs were observed during the study in either group	Mitsui Chemicals Agro , Inc. (2001d)

4.4.2.1 Non-human information

Data are available from one guideline eye irritation study in the rabbit. The results of the study are presented in Table 12. In the non-irrigated group, there were no corneal or iridial lesions in any of the animals. At 1 hour, all three of the animals had conjunctival redness scores of 1 and chemosis scores of 2, together with discharge from the eye. At 24 h, one animal was completely free of all signs of irritation, whereas two of the animals had redness and chemosis scores of 1. All animals

were free of ocular discharge at 24 h after installation, and were free of all ocular reactions at 48 and 72 h after installation.

In the irrigated group, there were no corneal or iridial lesions in any of the animals. At 1 h, all three of the animals had conjunctival redness scores of 1; among these animals two had chemosis scores of 2 and the third had a chemosis score of 1. Apart from this reaction, there were no other ocular reactions at 24, 48 and 72 h after installation.

4.4.2.2 Human information

No data are available.

4.4.2.3 Summary and discussion of eye irritation

See Table 12 and Section 4.4.2.1.

4.4.2.4 Comparison with criteria

Penthiopyrad caused mild, transient eye irritation in rabbits, characterized by conjunctival redness and chemosis. None of the individual scores at any time point relevant for classification (24, 48 or 72 h) were greater than 1, and the observed irritation does not meet the appropriate criteria for classification under the CLP Regulation (in at least 2 out of 3 tested animals, a positive response of: corneal opacity ≥ 1, and/or iritis ≥ 1, and/or conjunctival redness ≥ 2, and/or chemosis ≥ 2, calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material and which fully reverses within 21 days). Therefore, no classification for eye irritation is proposed.

4.4.2.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

The respiratory tract irritation potential of penthiopyrad has not been directly investigated in animals. In an acute toxicity study via the inhalation route (Section 4.2), no effects consistent with respiratory tract irritation were observed. No repeated dose studies via the inhalation route are available.

4.4.3.2 Human information

No data are available.

4.4.3.3 Summary and discussion of respiratory tract irritation

See Section 4.4.3.1.

4.4.3.4 Comparison with criteria

Under the CLP Regulation, substances which cause transient respiratory tract irritation are classified as STOT SE Category 3 (H335 May cause respiratory irritation). No effects consistent with respiratory tract irritation were observed in the acute inhalation toxicity study, therefore no classification is proposed.

4.4.3.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.5 Corrosivity

Table 13: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
See Table 11			

4.5.1 Non-human information

Information about the corrosivity of penthiopyrad is provided by the skin irritation study (see Section 4.4.1).

4.5.2 Human information

No data are available.

4.5.3 Summary and discussion of corrosivity

See Section 4.5.1.

4.5.4 Comparison with criteria

Under the CLP Regulation, a substance is classified as corrosive if it produces destruction of skin tissue in at least one animal during a skin irritation study. Penthiopyrad did not lead to full thickness destruction or irreversible damage to the skin when tested for skin irritation, and therefore does not meet the criteria for classification as corrosive. No classification for corrosivity is proposed.

4.5.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.6 Sensitisation

4.6.1 Skin sensitisation

The skin sensitisation potential of penthiopyrad has been investigated in a guinea pig maximization test (Table 14).

Table 14: Summary table of relevant skin sensitisation studies

Species/Method	Doses	No. sensitised/total no.	Result	Reference
Skin sensitisation (GPMT) OECD 406, GLP compliant Hartley Guinea pig (20 f, plus 10f naïve control, 10f positive control and 5f naïve positive control) Penthiopyrad (purity 99.8%), vehicle: 1:1 emulsion of Freunds complete adjuvant and sterile physiological saline; paraffin oil (intradermal) and white petrolatum (topical)) Positive control: 2,4-dinitrochlorobenzene (DNCB)	Test Concentrations: *Induction:* Intradermal: 5% Topical: 50% *Challenge:* 50%	Test: 0/20 Naïve penthiopyrad Control: 0/10 Positive control DNCB: 10/10 Naïve DNCB Control: 0/5	Negative	Mitsui Chemicals Agro, Inc. (2001e)

4.6.1.1 Non-human information

In a standard guinea pig maximisation test, 20 test animals were treated with intradermal injections of 5% penthiopyrad (3 x 0.1 ml). 10 naïve control animals were injected with vehicle (paraffin oil) only. Another group of 10 animals was injected intradermally with 2,4-dinitrochlorobenzene (DNCB), the positive control, and a final group of 5 naïve control animals was injected with vehicle (white petrolatum) only. Topical induction applications were made 7 days after the intradermal induction. The topical induction sites received 0.4g of penthiopyrad (or DNCB), which was applied on filter paper and then secured with surgical tape to form a closed patch. Naïve control groups received white petrolatum only. The patches were removed after 48 hours. The challenge sites were scored for skin irritation reactions at 24 and 48 hours after patch removal.

The challenge skin reaction scores at 24 and 48 h for all 20 test (penthiopyrad) and naïve control animals were zero. None of the animals were considered to have been sensitised. The challenge skin reaction scores at 24 and 48 h for all 10 positive control (DNCB) animals was 3, whereas all reaction scores in the naïve control group were zero. Therefore, all of the DNCB-treated animals were considered to have been sensitised.

The concentrations of penthiopyrad applied during the main study were based on preliminary irritation studies. During the preliminary studies, no skin irritation reactions occurred at the highest concentration investigated (i.e., 50% w/w). Consequently, the highest induction dose that would result in mild to moderate skin irritation and the highest non-irritating challenge dose were not explicitly identified in the study. However, topical induction sites were pre-treated with 10% sodium lauryl sulphate (a primary irritant) in the main study, which is considered sufficient to produce a mild skin reaction.

4.6.1.2 Human information

No data are available.

4.6.1.3 Summary and discussion of skin sensitisation

See Section 4.6.1.1.

4.6.1.4 Comparison with criteria

Under the CLP Regulation, a substance is considered to be a skin sensitiser if it produces sensitization in ≥ 30% of the test animals in a guinea pig maximization test. In a standard guinea pig maximisation test, penthiopyrad led to skin sensitisation in 0/20 animals tested. Therefore, no classification is proposed based on the available data.

4.6.1.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.6.2 Respiratory sensitisation

Table 15: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No data are available			

4.6.2.1 Non-human information

No data are available.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available.

4.6.2.4 Comparison with criteria

No data are available.

4.6.2.5 Conclusions on classification and labelling

Not classified, data lacking.

4.7 Repeated dose toxicity

Information on repeated dose toxicity is available from nine studies; three 28 day oral studies (in the rat, mouse and dog), one 28 day dermal study (in the rat), three 90 day oral studies (in the rat, mouse and dog), and two chronic 1 year oral studies (one in the rat, and one in the dog).

Further to this, information on repeated dose toxicity is also provided by the available studies on carcinogenicity, reproductive toxicity, neurotoxicity and immunotoxicity. These studies are discussed in detail in Sections 4.10, 4.11 and 4.12 respectively

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat

Table 16: Summary table of relevant repeated dose oral toxicity studies in the rat.

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes. Where two percentages or values are indicated in parentheses, the first value relates to the effects seen in males, and the second value to effects seen in females. If only one value is indicated in parentheses, then this value applies to both sexes, unless otherwise stated.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Observations and Remarks
28 day repeated dose oral study OECD 407 GLP Compliant Wistar rat (5/sex/dose) Penthiopyrad (>97%) in diet Reference: Mitsui Chemicals Agro, Inc. (2001f,g,h)	0, 25, 65, 160, 400 and 1000 mg/kg bw/day	**In a 28 day study in the rat, the guidance value for classification is ≤ 300 mg/kg bw/d. No adverse effects were noted at 25, 65 or 160 mg/kg bw/d. The adverse effects seen at higher doses, although not considered relevant for classification, are included below for information.** **400 mg/kg bw/d** *Clinical Chemistry:* ↑ cholesterol (47%, 107%), phospholipid levels (30%, 69%) and Gamma-Glutamyl Transferase (G-GT) activity (139%, 682%) *Liver:* ↑ liver weight (13%, 19%, relative to bw) **1000 mg/kg bw/d** *Body weight/body weight gain:* ↓ mean body weight (22%, 13%) ↓ mean body weight gain (47%, 30%) *Haematology:* Prolongation of activate partial thromboplastin time (APTT) (males only, ↑25.3%) *Clinical Chemistry:* ↑ cholesterol (52%, 153%), ↑phospholipid levels (74%, 113%), ↑ G-GT activity (2100%, 12500%) , ↑ triglycerides (104%, females only) *Liver:* ↑ liver weight (45%, 73%, relative to bw)

Method	Dose Levels	Observations and Remarks
		Slight to moderate hepatic micro/macrovesicular fatty change in all animals Kupffer cell proliferation (5/5, 3/5) and hepatic hypertrophy (2/5, 1/5) *NOAEL = 160 mg/kg bw/d, LOAEL = 400 mg/kg bw/d
90 day repeated dose oral study OECD 407 GLP compliant Wistar rat (10/sex/dose) Penthiopyrad (>99.8%) in diet Reference: Mitsui Chemicals Agro, Inc. (2001i, 2005, 2007)	0, 40, 100, 250 and 625 mg/kg bw/d Additional groups dosed with 0 and 625 mg/kg bw/d were dosed for 13 weeks and then left untreated for 4 weeks to assess reversibility of effects	**In a 90 day study in the rat, the guidance value for classification is ≤ 100 mg/kg bw/d. No adverse effects were observed at 40 mg/kg bw/d. The adverse effects at 100 mg/kg bw/d (after 13 weeks of treatment) were as follows:** *Haematology:* ↑ APTT (11%, males only), ↓ mean corpuscular haemoglobin concentration (MCHC) (2%, males only) *Clinical Chemistry:* ↑ phospholipid (17%, males only) *Organ weights:* ↑ liver weight, relative to bw (11%, males and females) *Histopathological findings:* Peripherolobular, macrovesicular fatty changes in the liver (1/10 females, mean severity grade (MSG) 1.0) Hepatocellular hypertrophy (6/10 males, MSG 1.3, 5/10 females, MSG 1.0) Hepatocellular degeneration (1/10 males, MSG 1.0) Kupffer cell proliferation (3/10 males, MSG 1.0) **The following effects were observed at doses > 100 mg/kg bw/d, and are therefore not relevant for classification. They are included for information.** **250 mg/kg bw/d** *Haematology:* ↓ MCHC (6%, males only), ↑ APTT (19%, 6%), ↓ Hb (4%, females only), PT (6%, females only) *Clinical Chemistry:* ↓ total bilirubin (31%, 44%), ↑phospholipid (18%, 30%), ↑ total cholesterol (40%, females only) *Organ weights:* ↑ liver weight, relative to bw (18%, 26%) *Macroscopic findings:* Accentuated lobular pattern of the liver (3/10 males) *Histopathological findings:* Peripherolobular, macrovesicular fatty changes in the liver (4/10 females, MSG 1.0) Hepatocellular hypertrophy (10/10 males, MSG 1.7, 8/10 females, MSG 1.9) Hepatocellular degeneration (2/10 males, MSG 1.5) Kupffer cell proliferation (2/10 males, MSG 1.5)

Method	Dose Levels	Observations and Remarks
		625 mg/kg bw/d *Clinical signs/survival:* One treatment-related death (male) *Body weight gain:* ↓ body weight gain (males only, 13.2%) *Functional Observational Battery:* ↓ locomotor activity (week 13, 21%, 27%) *Haematology:* ↑Red blood cell (RBC) count (5%, females only), ↓ haemoglobin concentration (Hb) (5%, 3%), ↓ mean corpuscular haemoglobin (MCH) (7%, 8%), ↓MCHC (6%), ↑ prothrombin time (PT) (16%, males), ↓ PT (8%, females), ↑ APTT (35%, 16%), ↓ Low Fluorescence reticulocytes (12% females only) *Clinical Chemistry:* ↓ total bilirubin (20%, 48%), ↑total cholesterol (62%, 105%), ↑ triglycerides (25%, 59%), ↑ phospholipids (57%, 66%), ↑ alkaline phosphatase (41%, 21%), ↑ G-GT (1900, 1700%) *Organ weights:* ↑ liver weight, relative to bw (44%, 46%) ↓ spleen weight, relative to bw (20%, females only) ↑ ovaries weight, relative to bw (26%) *Macroscopic findings:* Accentuated lobular pattern of the liver (5/10 males), thickened/enlarged liver (2/10 males) *Histopathological findings:* Peripherolobular, macrovesicular fatty changes in the liver (9/9 males, MSG 2.8, 3/10 females, MSG 1.8) Hepatocellular hypertrophy (9/9 males, MSG 2.9, 10/10 females, MSG 1.9) Hepatocellular degeneration (9/9 males, MSG 1.8, 7/10 females MSG 1.0) Kupffer cell proliferation (9/9 males, MSG 1.8, 6/10 females, MSG 1.0) *NOAEL = 40 mg/kg bw/d, LOAEL = 100 mg/kg bw/d
Chronic (52 week) oral toxicity OECD 452, GLP compliant Wistar rat, 20/sex/dose, plus 10/sex/dose for interim sacrifice at 26 weeks Penthiopyrad (98.8% purity), in the diet Reference: (2005), Mitsui Chemicals Agro	0, 6.25, 25, 100 and 400 mg/kg bw/d	**In a 52 week study in the rat, the guidance value for classification is calculated as ≤ 25 mg/kg bw/d. No adverse effects were observed at 6.25 or 25 mg/kg bw/d. The adverse effects seen at doses > 25 mg/kg bw/d after 52 weeks of treatment, although not considered relevant for classification, are included below for information:** **100mg/kg bw/d** *Clinical chemistry:* ↓ bilirubin (20%, 30%), ↑ cholesterol (females only, 35%), ↑ phospholipids (females only, 28%) *Organ weights (relative to body weight):* ↑ liver weight (9%, 15%) *Histopathological changes:*

Method	Dose Levels	Observations and Remarks
, Inc. (2006a)		Adrenal gland - diffuse hypertrophy of zona glomerulosa (minimal to slight, 5/20 females, 12/20 males), cortical lipid vacuolation (minimal to marked, 12/20 females, 20/20 males). **400 mg/kg bw/d** *Body weight:* ↓ body weight gain in males (↓10%) *Haematological parameters:* ↑ Partial thromboplastin time (PTT) (males only, 45%), ↓ relative prothrombin time (PT) (males only, 9%) *Clinical chemistry:* ↑ Potassium (9%, 12%), ↓ bilirubin (25%, 36%), ↑ cholesterol (21%, 81%), ↑ phospholipids (21%, 55%), ↓ GLDH (males only, 64%), ↑ AP (males only, 19%), ↑ GGT (4.20-10.56 U/L, cf. 0.0 U/L in controls), ↓ A/G ratio (females only, 10%) *Organ weights (relative to body weight):* ↑ liver weight (28%, 49%), ↑ adrenal weight (females only, 14%), ↑ kidney weight (males only, 11%) *Histopathological changes:* Adrenal gland – cortical lipid vacuolation (minimal to marked, 20/20 males, 20/20 females), diffuse hypertrophy of zona glomerulosa (minimal to slight, 17/20 males, 16/20 females) Thyroid – diffuse follicular hypertrophy (minimal to moderate, 20/20 males, 20/20 females) Liver – periportal fat vacuolation (minimal to marked, 20/20 males), periportal cell swelling (minimal to moderate, 16/20 males), periportal single cell necrosis (minimal to slight, 4/20 males), centrilobular hypertrophy (8/20 females) Ovaries – interstitial cell hypertrophy (minimal to moderate, 17/20 females) *NOAEL = 25 mg/kg bw/d, LOAEL = 100 mg/kg bw/d

In the available studies in the rat, adverse effects were noted in the liver of both sexes at doses relevant for classification i.e., 100 mg/kg bw/d in the 90 day study. Effects were also noted on some clinical chemistry and haematological parameters at this dose level. The effects seen in the liver included fatty changes, hepatocellular hypertrophy, hepatocellular degeneration and Kupffer cell proliferation. However, due to the low severity of these effects at this dose level, they do not warrant classification for STOT-RE under the CLP Regulation.

At higher doses (i.e., not relevant for classification), adverse effects were also observed in the thyroid, adrenal gland and ovaries.

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the studies is available in the DAR. The following summaries are based on the information in the DAR.

28 day repeated dose (oral) study in Wistar rats

The following doses were investigated: 0, 25, 65, 160, 400 and 1000 mg/kg bw/d. There were no deaths and no adverse clinical signs reported at any dose level, apart from soft faeces at 1000 mg/kg bw/d. Mean body weights and body weight gains were significantly reduced in both sexes at 1000 mg/kg bw/d. A decrease in mean body weight was also noted in males at 400 mg/kg bw/d, although to a lesser extent. Food consumption was not affected at any dose level. No effects on the Functional Observational Battery (FOB), locomotor activity or grip strength (all performed during week 4) were noted.

Haematological parameters were unaffected by treatment, other than a slight prolongation of APTT in males at 400 and 1000 mg/kg bw/d. Dose-related increases in plasma total cholesterol and phospholipid levels and G-GT activity occurred in both sexes at 400 and 1000 mg/kg bw/d, and increased triglycerides in females at 1000 mg/kg bw/d. Total bilirubin concentrations were reduced at ≥ 65 mg/kg bw/d (both sexes, 27-48%), but this finding, in isolation, is not considered to be an adverse effect. There were no treatment related effects on macroscopic pathology at necropsy, but relative and/or absolute liver weights showed a dose-related increase in both sexes at 400 and 1000 mg/kg bw/d. Treatment-related histopathological alterations were slight to moderate hepatic micro/macrovesicular fatty change mainly in the peripherolobular region in all animals at 1000 mg/kg bw. The fatty change was accompanied in most animals by slight Kupffer cell proliferation, and hepatic hypertrophy in a few animals.

90 day repeated dose (oral) study in Wistar rats

The following doses were investigated: 0, 40, 100, 250 and 625 mg/kg bw/d. No adverse clinical signs were noted at any dose level, apart from soft faeces at 625 mg/kg bw/d. One treatment-related death (male) occurred at 625 mg/kg bw/d. Prior to death, the animal exhibited uncoordinated movements, laboured respiration, ruffled fur and general poor condition. The animal had an enlarged and dark red hepatic papillary process, moderate hepatocellular hypertrophy and a congested thymus.

Body weight gain was significantly reduced in males at 625 mg/kg bw/d (↓13.2% after 13 weeks, ↓7.7% after the 4 week recovery period), but food consumption was unaffected by treatment at all dose levels. In week 13, no treatment-related effects were noted on the ocular architecture, FOB or grip strength assessments; however locomotor activity was reduced at 625 mg/kg bw/d.

Haematological parameters were affected in males at doses ≥ 100 mg/kg bw/d and in females at doses ≥ 250 mg/kg bw/d (see Table 16). All parameters affected at 625 mg/kg bw/d had recovered by the end of the 4 week recovery period, with the exception of increased RBC count in females.

Treatment-related effects on clinical chemistry parameters occurred in males at dose levels ≥ 100 mg/kg bw/d, and in females at dose levels ≥250 mg/kg bw/d (see Table 16). All effects at 625 mg/kg bw/d were reversible. There were no treatment-related effects at any dose level on the chemical or cellular constituents of urine.

Relative liver weights were increased in both sexes from 100 mg/kg bw/d, whereas absolute liver weights were increased in females at 250 and 625 mg/kg bw/d, and in males at the top dose only. Relative liver weights showed some recovery, but were still slightly elevated at the end of the 4 week recovery period (↑4-7%). Absolute spleen weights were reduced in both sexes at the top dose, however relative spleen weight was only reduced in females. Absolute and relative ovary weights were increased at the top dose.

Treatment-related macroscopic findings at necropsy were noted in the liver of males at 250 and 625 mg/kg bw/d, consisting of accentuated lobular pattern, thickened or enlarged liver. Treatment-related histopathological alterations occurred in the liver in both sexes at all dose levels; hepatocellular degeneration (minimal to slight) and/or Kupffer cell proliferation (minimal to slight) and/or macrovesicular fatty change (minimal to marked). Hepatocellular hypertrophy (graded as minimal to moderate) was evident in some or all animals at all dose levels of penthiopyrad, however at 40 mg/kg bw/d, in the absence of other hepatic alterations, the hypertrophic response alone was considered not to be an adverse reaction to treatment. At the end of the recovery period, hepatocellular hypertrophy remained but at reduced severity, and the other histopathological alterations had largely regressed.

52 week repeated dose (oral) study in Wistar rats

The following doses were investigated: 0, 6.25, 25, 100 and 400 mg/kg bw/d. No treatment-related deaths or clinical signs were noted at any dose level. There were no treatment-related effects on food consumption, and reduced body weight gain occurred in males only (↓10%) at 400 mg/kg/day. Minimal body weight changes (↓ < 6%) were seen in both sexes after 52 weeks.

Treatment-related effects on the haematological parameters at 52 weeks were confined to increased PTT, slightly reduced relative PT and slightly reduced relative reticulocyte count in males only at 400 mg/kg/day.

Treatment-related effects on the clinical chemistry parameters after 26 or 50 weeks occurred in one or both sexes at 100 and/or 400 mg/kg/day. Increased levels of cholesterol, phospholipids, potassium ion, globulin, GGT and alkaline phosphatase, reduced levels of bilirubin, GLDH and glucose, and reduced A/G ratio occurred at 400 mg/kg/day. Effects at 100 mg/kg/day were increased plasma globulin, potassium ion, and decreased A/G ratio. There were no treatment-related alterations in the urinalysis profile at any dose level.

Treatment-related changes in organ weights (relative to body weight) were noted at 100 and 400 mg/kg bw/d (see Table 16). Liver weights were increased in both sexes treated with 100 and 400 mg/kg bw/d at both the interim and terminal sacrifices. Males at 100 and 400 mg/kg bw/d showed higher adrenal weights than controls after 26 weeks of treatment, but not after 52 weeks. Conversely, females at 400 mg/kg bw/d showed slightly higher adrenal weights after 52 weeks of treatment, but not after 26 weeks. The kidney weights of males at 100 and 400 mg/kg bw/d were increased after 26 weeks of treatment. After 52 weeks of treatment, increased kidney weights were seen in males treated with 400 mg/kg bw/d only.

There were no treatment-related macroscopic findings at necropsy at any dose level (at either the interim or terminal sacrifice point). After 26 weeks, treatment-related histopathological changes were noted in the adrenal gland of females at 100 mg/kg/day, and in the liver (males only), thyroid (females only) and adrenal glands (both sexes) at 400 mg/kg/day. In the adrenal gland, there was an increased incidence and severity of adrenal diffuse hypertrophy in the zona glomerulosa, and an increased severity of cortical lipid vacuolation. In the liver of males, there was an increased severity of periportal fat vacuolation, and periportal cell swelling (hydropic degeneration) occurred in some animals. In the thyroid of females, there was an increased incidence and severity of diffuse follicular hypertrophy.

After 52 weeks of treatment, histopathological changes were noted in the thyroid (males only) and adrenal gland at 100 mg/kg bw/d, and in the adrenal gland, liver and thyroid of both sexes, as well as in the ovaries, at 400 mg/kg bw/d. The effects on the adrenals and liver were similar to those at 26 weeks. In the ovaries, there was an increased incidence and severity of ovarian interstitial cell hypertrophy. An increased incidence of thyroid follicular cell hypertrophy was seen in both sexes at

400 mg/kg/day and in males at 100 mg/kg/day. However, the differences noted were only statistically significant in females at 400 mg/kg/day and not supported by corroborating toxicity.

Mice

Table 17: Summary table of relevant repeated dose oral toxicity studies in mice

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes. Where two percentages or values are indicated in parentheses, the first value relates to the effects seen in males, and the second value to effects seen in females. If only one value is indicated in parentheses, then this value applies to both sexes, unless otherwise stated.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Observations and Remarks
28 day oral study OECD 407 GLP compliant CD-1 mice, 6/sex/group Penthiopyrad (>99.8%) in diet Reference: Mitsui Chemicals Agro , Inc. (2001j)	0, 30, 100, 300 and 1000 mg/kg bw/d	**In a 28 day study in the mouse, the guidance value for classification is s ≤ 300 mg/kg bw/d. No adverse effects were noted at 30 or 100 mg/kg bw/d.** **300 mg/kg bw/d** *Clinical chemistry:* Males only: ↑ triglycerides (78%) *Organ weights:* ↑ liver weight (16%, 17%) **1000 mg/kg bw/day** *Haematology:* Females only: ↓ haemoglobin concentrations (9%) and RBC counts (8%) *Clinical chemistry:* ↓ plasma albumin (11%, males only) and ↑ globulin concentrations (9%), ↑ triglycerides (79%) (males only) *Organ weights:* ↑ liver weight (22%, 17%) *Histopathology:* Hepatocellular hypertrophy (slight, diffuse) *NOAEL = 100 mg/kg bw/d, LOAEL = 300 mg/kg bw/d
90 day oral study OECD 408 GLP compliant CD-1 mice, 10/sex/group Penthiopyrad (>99.8%) in diet Reference: Mitsui Chemicals Agro , Inc. (2002a)	0, 30, 100, 300 and 1000 mg/kg bw/d	**In a 90 day study in the mouse, the guidance value for classification is ≤ 100 mg/kg bw/d. No adverse effects were observed at 30 or 100 mg/kg bw/d. The adverse effects at higher doses, although not considered relevant for classification, are included below for information:** **300 mg/kg bw/day** *Clinical chemistry:* Males only: ↑ BUN (36%) *Organ weights:* ↑ relative liver weight (13%, 11%) **1000 mg/kg bw/day** *Body weight gain/body weight:*

		Males only: ↓ bw gain (26%), ↓ bw (10%) *Haematology:* ↓ RBC (7%, 9%), ↓ Hb (7%, females only) *Clinical chemistry:* Males only: ↓ A/G ratio (14%), ↑ BUN (28%) *Organ weights:* ↑ relative liver weight (24%, 16%) Males only: ↑ relative thyroid weight (50%) *Histopathology:* Diffuse hepatocellular hypertrophy (slight-moderate), 5 m and 5 f Thyroid follicular cell hypertrophy (slight), 8 m and 6 f *NOAEL = 100 mg/kg bw/d, LOAEL = 300 mg/kg bw/d

In the available studies in the mouse, an increase in relative liver weight (16-17%) was observed at a dose relevant for classification, i.e., 300 mg/kg bw/d in a 28 day repeated dose study. This effect on its own, in the absence of any associated histopathology findings, does not warrant classification for STOT-RE under the CLP Regulation.

At higher doses (i.e., not relevant for classification), adverse effects were also noted in the thyroid, and on some haematological and clinical chemistry parameters.

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the studies is available in the DAR. The following summaries are based on the information in the DAR.

28 day repeated dose (oral) study in CD-1 mice

The following doses were investigated: 0, 30, 100, 300 and 1000 mg/kg bw/d. No deaths or clinical signs were reported, and there were no treatment-related effects on food consumption, body weights or body weight gains.

After 4 weeks of treatment, haemoglobin concentration (Hb) and red blood cell count (RBC) were significantly reduced in females at 1000 mg/kg bw/d. Clinical chemistry parameters were affected in males at 300 mg/kg bw/d and above, and in females at the top dose only.

No treatment-related gross pathological findings were noted at any dose level. At 300 and 1000 mg/kg bw/d, liver weights (relative to body weight) were significantly increased in both sexes. The only histopathological finding was diffuse hepatocellular hypertrophy (graded as 'slight') which was observed in all animals at 1000 mg/kg bw/d.

90 day repeated dose (oral) study in CD-1 mice

The following doses were investigated: 0, 30, 100, 300 and 1000 mg/kg bw/d. No deaths or clinical signs were reported. In males, lower mean body weights were noted at the top dose (↓10%). Body weights were unaffected by treatment in females.

Reduced RBC counts were noted in both sexes at 1000 mg/kg bw/d, and a decrease in Hb was also noted in females at this dose level. Elevated urea-nitrogen levels were observed in males at 300 and 1000 mg/kg bw/d, however there were no histopathological findings in the kidneys of these animals. A reduced A/G ratio was also noted in males at the top dose.

Relative liver weights were increased in both sexes at 300 and 1000 mg/kg bw/d. Relative thyroid weights were increased in both sexes at the top dose, however the increase was statistically significant in males only.

There were no treatment-related macroscopic findings at necropsy at any dose level. Histopathological findings were noted in the liver and thyroid gland. In the liver, diffuse hepatocellular hypertrophy (graded as slight to moderate) was noted in 5 males and 5 females at 1000 mg/kg bw/day, but not at lower dose levels or in the controls of either sex. Thyroid follicular cell hypertrophy (slight) was noted in 8 males and 6 females at 1000 mg/kg bw/d, but not at lower dose levels or in the controls of either sex.

Dog

Table 18: Summary table of relevant repeated dose oral toxicity studies in the dog

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes. Where two percentages or values are indicated in parentheses, the first value relates to the effects seen in males, and the second value to effects seen in females. If only one value is indicated in parentheses, then this value applies to both sexes, unless otherwise stated.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Observations and Remarks
28 day oral study Non-guideline GLP compliant Beagle dogs, 1/sex/group Penthiopyrad (>99%) in diet Reference: Mitsui Chemicals Agro , Inc. (2001k)	0, 1000, 3000, 10000 or 32000 ppm	**1000 ppm (27.1 mg/kg bw/d in males; 29.1 mg/kg bw/d in females)** Male: ↑ relative thyroid weight (219%) Female: ↓ relative thyroid weight (12%) **3000 ppm (79.6 mg/kg bw/d in males; 94.1 mg/kg bw/d in females)** *Organ weights:* Male: ↑ relative thyroid weight (47%) Female: ↓ relative thyroid weight (31%) *Histopathology:* Male only: Diffuse hepatocellular hypertrophy (grade: slight) Both sexes: Slight mucosal edema of the lamina propria of the gallbladder **10000 ppm (269 mg/kg bw/d in males; 316 mg/kg bw/d in females)** *Clinical chemistry:* Female only: ↑ total cholesterol (31%) *Organ weights:* Female only: ↑ relative liver weight (20%) Both sexes: ↑ relative thyroid weight (67%, 8%) *Histopathology:* Female only: Diffuse hepatocellular hypertrophy (grade: slight) Both sexes: Slight mucosal edema of the lamina propria of the gallbladder **32000 ppm (920 mg/kg bw/d in males; 982 mg/kg bw/d in females)** *Body weight gain/body weights:* ↓ bw gain (50%, 43%) *Haematology:* Male only, after 2 weeks: ↓ haematocrit (Hct) (10%), ↓ Hb (9%), ↓RBC (11%)

		Clinical chemistry: ↑plasma alkaline phosphatase (142%, 180%), total cholesterol (47%, 54%) *Organ weights:* ↑ relative liver weight (23%, 15%), ↑ relative thyroid weight (64%, 32%) *Histopathology:* Diffuse hepatocellular hypertrophy (grade: slight) *NOAEL = 27.1(m), 29.1(f) mg/kg bw/d, LOAEL = 79.6(m), 94.1(f) mg/kg bw/d
90 day oral study OECD 409 GLP compliant Beagle dogs, 4/sex/group Penthiopyrad (99.1%) in diet Reference: Mitsui Chemicals Agro , Inc. (2001L)	0, 300, 3000 or 30000 ppm (equivalent to 0, 8.0, 76.7 or 811 mg/kg bw/d in males, and 0, 8.2, 80.9 or 864 mg/kg bw/d in females)	**No adverse effects were observed at 300 or 3000 ppm. Adverse effects noted at higher doses were as follows:** **30000 ppm (811 mg/kg bw/d in males, 864 mg/kg bw/d in females)** *Body weight gain/body weights:* ↓ body weight gain (44%), ↓ mean body weight (7-9%) *Haematology:* ↓ APTT at week 7 (~13%) *Clinical chemistry:* ↑ alkaline phosphatase (AP) (327%, 263%), ↑ total bilirubin (50%, 35%), ↓albumin (19%, 23%), ↓ albumin/globulin ratio (35%, 29%), ↑ triglycerides (62%, 37%) Females only: ↑ G-GT (67%) *Organ weights:* ↑ relative liver weight (50%, 34%) Females only: ↑ relative thyroid weight (42%) *Histopathology:* Diffuse hepatocellular hypertrophy (slight-moderate) in all animals Cholecystitis (slight or moderate, 3/4 m, 4/4 f) *NOAEL = 76.7(m), 80.9 (f) mg/kg bw/d, LOAEL = 811 (m), 864 (f) mg/kg bw/d
52 week oral study OECD 452 GLP compliant Beagle dogs, 4/sex/group Penthiopyrad (99.8%) in diet Reference: Mitsui Chemicals Agro , Inc. (2006b)	0, 310, 2150 or 15000 ppm (equivalent to 0, 7.9, 54.4 and 461 mg/kg bw/d in males and 0, 8.1, 56.6 and 445 mg/kg bw/day in females)	**No adverse effects were noted at 310 or 2150 ppm. Adverse effects observed at the top dose were as follows:** **15000 ppm (461 mg/kg bw/d in males, 445 mg/kg bw/d in females)** *Food consumption, body weight gain and body weight:* ↓ food consumption (14%, 15%), ↓ body weight gain (88%, 62%) and ↓ body weight (22%, 17%) *Haematological parameters:* ↓ APTT (~20%) Males only: ↓ RBC (~12%), ↓ haemoglobin concentration (12%), ↑ platelets (~65%) *Clinical chemistry:* ↑ AP (991%, 962%), ↑ G-GT (250%, 460%) and ↓Alb (16%, 4%)

		Males only: ↓A/G ratio (45%), ↓ triglycerides (24%) and ↑total cholesterol (54%). *Organ weights:* ↑ relative liver weight (94%, 64%), ↑relative adrenal weight (63%, 64%) *Histopathology:* Diffuse hepatocellular hypertrophy (slight) Mucosal epithelial hyperplasia of the gallbladder (slight to moderate, 3/4 males, all females) Cholecystitis (1/4 males, 1/4 females) Adrenal cortical cell hypertrophy (slight, all animals) *NOAEL = 54.4(m), 56.6(f) mg/kg bw/d, LOAEL = 461(m), 445(f)

In dogs, the toxicological profile was similar to that seen in rats and mice, and the target organs were the liver and thyroid. Adverse effects were also noted in the gallbladder. In the non-guideline 28 day study which used 1 dog/sex/dose, effects were noted in the thyroid from ~27 mg/kg bw/d, and in the liver from 80 mg/kg bw/d. However, in the guideline 90 day and 52 week studies, effects in the liver were only noted at very high doses (from ~811 and ~445 mg/kg bw/d respectively). Effects in the thyroid were only noted at high doses in females in the guideline 90 day study (~811 mg/kg bw/d), and were not observed at all in the guideline 52 week study. On this basis, the effects seen in the liver and thyroid do not warrant classification for STOT-RE under the CLP Regulation.

The effects in the gallbladder consisted of slight mucosal oedema of the lamina (at doses ≥ 79.6 mg/kg bw/d in a 28 day study), cholecystitis (at doses ≥ 811 mg/kg bw/d in the 90 day study, and at doses ≥445 mg/kg bw/d in the 52 week study) and mucosal epithelial hyperplasia (at doses ≥445 mg/kg bw/d in the 52 week study). The severity of these effects does not warrant classification.

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the studies is available in the DAR. The following summaries are based on the information in the DAR.

28 day repeated dose (oral) study in beagle dogs

The following doses were investigated: 0, 1000, 3000, 10000 and 32000 ppm. No deaths or treatment-related clinical signs were noted at any dose level. Body weight gains were reduced in both sexes at the top dose, however actual body weights were unaffected by treatment. This was due to slightly higher initial weights of the high dose animals compared to controls. No treatment-related effects were noted on food consumption.

Small, transient decreases in haematocrit (Hct), haemoglobin (Hb) and red blood cell count (RBC) were noted in the male after 2 weeks at 32000 ppm, with subsequent partial recovery at 4 weeks. Plasma alkaline phosphatase activity and total cholesterol were increased in both sexes at 32000 ppm. Total cholesterol was also increased in the female at 10000 ppm. At 32000 ppm, the male showed slightly elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities at 4 weeks, but an effect of treatment was considered equivocal.

Increased liver weights (relative to body weight) were noted in the female at 10000 ppm and both sexes at 32000 ppm. Relative thyroid weights were increased in males at all dose levels, and females at the top two doses. The very large increase (219%) in relative thyroid weight in the male treated with 1000 ppm appears to be an anomaly. Relative thyroid weights were decreased in

females treated with 1000 and 3000 ppm. No trends towards dose-related changes were observed in any of the other organs investigated.

There were no treatment-related gross pathological lesions at necropsy at any dose level. Treatment-related histopathological findings were noted in the liver and the gallbladder – no findings were noted in the thyroid. In the liver, diffuse hepatocellular hypertrophy, graded as slight, occurred in both sexes in 32000 ppm and in the female at 10000 ppm. It was also noted in the male at 3000 ppm, but not at 10000 ppm. Slight mucosal oedema of the lamina propria of the gallbladder occurred in both sexes treated at doses of 3000 ppm and above, but in the male at 32000 ppm severe hypoplastic change in the gall-bladder precluded normal histological evaluation. Slight vacuolation in the pars recta of the proximal tubules of the kidney occurred in both sexes at 32000 ppm, but a relationship to treatment was considered equivocal.

90 day repeated dose (oral) study in beagle dogs

The doses investigated were: 0, 300, 3000 and 30000 ppm. No deaths or treatment-related clinical signs were noted at any dose level. Body weight gain was reduced in both sexes at 30000 ppm (~44%), resulting in lower mean body weights at the end of the treatment period (7-9%). Marginally lower group mean food consumption was also noted at this dose.

After 7 weeks of treatment, a small but statistically significant decrease was noted in activated partial thromboplastin time (APTT) in both sexes at 30000 ppm and in females at 3000 ppm. The effect was transient and was not present at the end of 13 weeks of treatment. Given that the differences were small and the toxicological significance of reduced APTT is not clear, the effect was not considered to be an adverse effect of treatment. Females at 30000 ppm had significantly lower haemoglobin concentrations than females, but in the absence of effects on other parameters (e.g., red blood cell count, Hct) the effect was not considered to be toxicologically relevant.

Plasma alkaline phosphatase (AP) activity was increased in both sexes at 30000 ppm, after 7 and 13 weeks of treatment. In females, gamma glutamyl transpeptidase (GGT) was significantly increased at 30000 ppm at weeks 7 and 13. GGT levels were also slightly raised in males at this dose level; however the effect was not statistically significant. Plasma albumin concentrations and the albumin/globulin ratio were also reduced in both sexes at the top dose, whereas total cholesterol and triglyceride concentrations were raised. The changes in the clinical chemistry parameters were considered to reflect altered hepatic function associated with increased liver weight and moderate hepatocellular hypertrophy.

There were no treatment-related gross pathological lesions at necropsy at any dose level. Relative liver weights were increased in both sexes at 30000 ppm (34-50%), and relative thyroid weights were increased in females only (42%) at this dose level. Treatment-related histopathological findings were noted in the liver and gallbladder of both sexes at 30000 ppm, and in the adrenal gland of males only. In the liver, slight or moderate diffuse hepatocellular hypertrophy was observed in all animals. Together with the changes in the clinical chemistry parameters, this could be indicative of hepatic functional change. In the gallbladder, slight or moderate cholecystitis was noted in all females and 3/4 males, and consisted of infiltration of the lamina propria mucosae with foamy macrophages and loss of long mucosal folds. Moderate adrenal cortical cell hypertrophy was observed in all males. No other treatment-related histopathological alterations were observed in other tissues, including the thyroid.

52 week repeated dose (oral) toxicity study in beagle dogs

The doses investigated were: 0, 310, 2150 or 15000 ppm. There were no deaths and no treatment-related clinical signs recorded at any dose level. At 15000 ppm, food consumption and body weight

gain were reduced in both sexes, which resulted in lower mean body weights in these animals at the end of 52 weeks (↓ 17-22%).

Treatment-related effects on some haematological parameters and clinical chemistry parameters were noted at 15000 ppm (see Table 18). No treatment-related gross pathological lesions were identified at necropsy at any dose level, although 2/4 males at 15000 ppm showed ascites. The significance of this finding isn't clear, although it may be secondary to chronic liver damage. Absolute and relative liver weights were increased in both sexes at 15000 ppm (in males, abs/rel weights +51%/94% and in females abs/rel weights +38%/64%), although the effect in females was not statistically significant. Adrenal weights were also increased in both sexes (abs/rel 44/63% in males and 30/64% in females). The male group mean absolute weights of the heart and epididymides were significantly lower than control values, but relative weights were only slightly (about 9%) lower than control values, suggesting an indirect effect of reduced body weight gain. Furthermore, there were no associated microscopic lesions.

Treatment-related histopathological alterations occurred in the liver, adrenal glands and gall bladder of both sexes at 15000 ppm. In the liver, slight diffuse hepatocellular hypertrophy was noted in all males and females. Slight or moderate mucosal epithelial hyperplasia of the gall bladder occurred in 3 males and all females, which was accompanied by cholecystitis in one male and one female. These changes were not evident at lower dose levels or in control animals. Slight adrenal cortical cell hypertrophy occurred in all males and females at 15000 ppm, but not at lower dose levels or in the controls.

4.7.1.2 Repeated dose toxicity: inhalation

No data are available.

4.7.1.3 Repeated dose toxicity: dermal

Table 19: Summary table of relevant repeated dose dermal toxicity studies in the rat

*The values for the NOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Observations and Remarks
28 repeated dose dermal study OECD 410 GLP compliant Crl:CD(SD) rat, 10/sex/group Penthiopyrad (98.6%) Reference: Mitsui Chemicals Agro , Inc. (2008a)	0, 40, 200 and 1000 mg/kg bw/d	**In a 28 day dermal study in the rat, the guidance value for classification is ≤ 600 mg/kg bw/d. No adverse effects were noted at any of the doses investigated in this study.** *NOAEL (local effects) = 1000 mg/kg bw/d NOAEL (systemic effects) = 1000 mg/kg bw/d

No adverse effects were noted up to doses of 1000 mg/kg bw/d in the available repeated dose dermal toxicity study, therefore no classification is proposed.

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the study is available in the DAR. The following summary is based on the information in the DAR.

28 day repeated dose (dermal) study in Crl:CD(SD) rats

The following doses were investigated: 0, 40, 200 and 1000 mg/kg bw/d. No deaths or treatment-related clinical signs were reported, and there were no reactions at the dermal application sights at any dose level. There were no treatment related effects on food consumption, body weight, body weight gain, urinalysis or haematology parameters. During the clinical chemistry investigations, a slightly higher total protein concentration occurred in males in the 1000 mg/kg bw/d group. No effects were observed on albumin concentration. The A/G ratio was marginally, but not significantly, lower than controls. Since the difference from controls was only 6.2% and occurred in isolation, without histopathological changes in the liver, the higher total protein concentration in males was not considered to be an adverse finding. A number of other minor changes in clinical chemistry parameters were noted, but these were considered to be due to unusually low values in the control animals, rather than an effect of treatment.

There were no treatment-related gross lesions at necropsy at any dose level, and there were no effects on organ weights. No histopathological findings were noted in any of the organs investigated.

4.7.1.4 Repeated dose toxicity: other routes

No data are available.

4.7.1.5 Human information

No data are available.

4.7.1.6 Other relevant information

Information on repeated dose toxicity is also provided by the available studies on carcinogenicity, reproductive toxicity, neurotoxicity and immunotoxicity. These studies are discussed in detail in Sections 4.10, 4.11 and 4.12 respectively. In these studies, no adverse effects were noted at or below doses relevant for classification for repeated dose toxicity.

4.7.1.7 Summary and discussion of repeated dose toxicity

See Section 4.8.1.

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

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE

Under the CLP Regulation, STOT RE may be assigned on the basis of a substance demonstrating evidence of significant or severe specific organ toxicity, at or below a guidance value of 100 mg/kg bw/d (for classification in Category 2) obtained in a 90 day oral study (in the rat). For 90 day dermal exposures, the equivalent guidance value is ≤ 200 mg/kg bw/d. These guidance values are adjusted to take into account studies of different durations. ‘Significant’ toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are

toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

Repeated dose toxicity has been evaluated in 28-day and 13-week oral studies in the rat, mouse and dog, 52 week oral studies in the rat and dog, and a 28 dermal study in the rat.

Oral exposure

Following oral administration, the target organs were as follows: liver, thyroid (in rats, mice & dogs), adrenal gland (rats & dogs), ovaries (rats) and gallbladder (dog). Haematological perturbations also occurred in all 3 species after 28 and/or 90 days of treatment, and in the rat and dog after 52 weeks. However, the changes occurred at high dose levels, were mild and frequently did not present a consistent picture of anaemia.

Liver

Following repeated oral exposure, the liver was a common target organ in all 3 species. However, at doses relevant for classification, effects were only seen in the rat and dog. In the rat, these effects were seen at 100 mg/kg bw/d in the 90 day study, and consisted of increased relative liver weight (↑11%), fatty changes (1/10 females), hepatocellular hypertrophy (6/10 males, 5/10 females), hepatocellular degeneration (1/10 males) and Kupffer cell proliferation (3/10 males). All of these histopathological alterations were graded by the study authors as minimal to slight. In the dog, increased liver weight and diffuse hepatocellular hypertrophy (slight) was noted in the female at 316 mg/kg bw/d in the 28 day study. Given the low severity and incidence of the effects observed, and the fact that they were seen only at the cut-off value for classification, they do not provide a strong basis for classification for STOT-RE under the CLP Regulation.

Thyroid

The thyroid was also a common target organ in all 3 species. However, in the rat and mouse, no adverse effects were seen in this organ at doses relevant for classification. In the non-guideline 28 day study in the dog, in which 1 dog/sex/dose was used, increased thyroid weight was noted in the male from 27.1 mg/kg bw/d, whereas decreased thyroid weight was noted in the female from 29.1 mg/kg bw/d. Increased thyroid weight was noted in the females at doses ≥316 mg/kg bw/d. No histopathological changes were noted in this organ at any dose level. Increased thyroid weight was also noted in the guideline 90 day study, but only in females at very high doses (864 mg/kg bw/d). No effects in the thyroid were reported in either sex in the guideline 52 week study at doses up to 461 mg/kg bw/d. Given the absence of any associated histopathological changes, and the absence of effects in the 52 week study, the organ weight changes seen in the 28 day and 90 day study do not warrant classification for STOT-RE under the CLP Regulation.

Adrenal Gland

The adrenal gland was a target organ in rats and dogs after 52 weeks of treatment at high doses. In the rat, cortical lipid vacuolation (minimal to marked) and diffuse hypertrophy of the zona glomerulosa (minimal to slight) was observed at 400 mg/kg bw/d. In the dog, adrenal cortical cell hypertrophy (slight) was observed at 445 mg/kg bw/d. At doses relevant for classification, no effects were seen in this organ in either species.

Ovaries

The ovaries were a target organ in rats after 52 weeks of treatment at high doses (minimal to moderate interstitial cell hypertrophy at 400 mg/kg bw/d). At doses relevant for classification, no effects were observed in this organ.

Gallbladder

The gallbladder was a target organ in dogs after 28 days (slight mucosal edema of the lamina of the gallbladder at doses ≥ 79.6 mg/kg bw/d), 90 days (cholecystitis at 811/864 mg/kg bw/d in males and females respectively) and 52 weeks (cholecystitis and mucosal epithelial hyperplasia at 461/445 mg/kg bw/d in males and females respectively). As the effects seen in the 28 day study were of low severity, and effects seen in the longer term studies were only observed at very high doses (i.e., much higher than the guidance values set for rodents), they do not warrant classification for STOT-RE under the CLP Regulation.

Dermal exposure

No adverse effects/target organs were identified in the 28 day dermal study in rats.

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

The findings relevant for classification as STOT RE were the effects seen in the liver of rats at 100 mg/kg bw/d during the 90 day study. These effects consisted of increased relative liver weight (↑11%), fatty changes (1/10 females), hepatocellular hypertrophy (6/10 males, 5/10 females), hepatocellular degeneration (1/10 males) and Kupffer cell proliferation (3/10 males). All of these histopathological alterations were graded by the study authors as minimal to slight. Given the low severity and incidence of the effects observed, and the fact that they were seen only at the cut-off value for classification, they do not provide a strong basis for classification for STOT-RE under the CLP Regulation.

Furthermore, the following criteria are given as a guide in Annex I (part 3.9.2.7.3) of CLP to assist in making a decision on classification.

A classification for STOT-RE is required when toxic effects that may include the following descriptions occur at or below the guidance values (100 mg/kg bw/d for oral exposures, 200 mg/kg bw/d for dermal exposures, based on 90 day studies in the rat; the guidance values are adjusted to take into account studies of different durations).

a) Morbidity or death resulting from repeated or long-term exposure

There were no treatment-related deaths or cases of moribund animals at doses equal to or below the guidance values.

b) Significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell).

There were no treatment-related functional changes in the central or peripheral nervous systems or other organ systems at doses equal to or below the guidance values.

c) Any consistent and significant adverse changes in clinical biochemistry, haematology or urinalysis parameters

There were no consistent and significant adverse changes in clinical biochemistry, haematology or urinalysis parameters at doses equal to or below the guidance values.

d) Significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination

There was no significant organ damage noted at necropsy or subsequently seen or confirmed at microscopic examination at doses equal to or below the guidance values.

e) Multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity

No such effects were noted at doses equal to or below the guidance values.

f) Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver) *and* g) Evidence of appreciable cell death (including cell degeneration and reduced cell numbers) in vital organs incapable of regeneration

In the rat, effects were seen in the liver at 100 mg/kg bw/d in the 90 day study, and included fatty changes (1/10 females), hepatocellular hypertrophy (6/10 males, 5/10 females), hepatocellular degeneration (1/10 males) and Kupffer cell proliferation (3/10 males). All of these histopathological alterations were graded by the study authors as minimal to slight. Given the low severity and incidence of the effects observed, they are not considered to provide clear evidence of marked organ dysfunction or appreciable cell death.

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

Not classified – conclusive but not sufficient for classification

4.9 Germ cell mutagenicity (Mutagenicity)

Table 20: Summary table of relevant in vitro and in vivo mutagenicity studies

In Vitro Data				
Method	**Organism/strain**	**Concentrations tested**	**Result**	**Reference**
Bacterial gene mutation assay OECD 471, GLP compliant Penthiopyrad (purity 99.8%) in DMSO	*S. typhimurium:* TA100, TA98, TA1535 and TA1537 *E. coli:* WP2*uvr*A	8.19 - 5000µg/plate (preliminary cytotoxicity assay), 2.43 – 2400 µg/plate	Negative (± S9)	August 2000a
Bacterial DNA damage or repair test GLP compliant Penthiopyrad (purity 99.8%) in DMSO	*B. subtilis:* M45 Rec-, H17 Rec+	177 – 22,650 µg/disc without S9, 88.5 – 11,325 µg with S9	Negative (± S9)	August 2000b
Mammalian chromosome aberration test OECD 473, GLP compliant Penthiopyrad (purity 99.8%) in DMSO	Chinese hamster lung fibroblasts (CHL/IU cells)	6 hours -S9: 52.4*, 65.5*, 81.9*, 102, 128, 160, 200 6 hours +S9: 65.5, 81.9,*, 102*, 128*, 160*, 200, 250 24 hours –S9: 41.9, 52.4*, 65.5*, 81.9*, 102, 128, 160 *dose levels examined for chromosomal aberrations	Positive (+S9) Negative (-S9)	August 2000c
Gene mutation assay OECD 476 (short, 3hr exposure protocol), GLP compliant Penthiopyrad (purity 99.8%) in DMSO	Mouse lymphoma cells (L5178Y, TK locus)	3 hours –S9: 0, 6.18, 8.82, 12.6, 18.0, 25.7, 36.8, 52.5, 75.0 µg/mL 3 hours + S9: 0, 4.32, 6.18, 8.82, 12.6, 18.0, 25.7, 36.8, 52.5 µg/mL	Negative (± S9)	August 2000

In vivo Data				
Method	**Organism/strain**	**Concentrations tested**	**Result**	**Reference**
Micronucleus assay OECD 474, GLP compliant Penthiopyrad (purity 99.8%)	BDF_1 mice (6m/dose)	0, 250, 500 or 1000 mg/kg bw/d, 2 doses, 24 hours apart (total doses = 0, 500, 1000 or 2000 mg/kg bw/d)	Negative Significant decrease in the proportion of PCE relative to total erythrocytes, indicating adequate exposure of target cell population.	Mitsui Chemicals Agro, Inc. (2000b)
Unscheduled DNA synthesis assay Non-guideline GLP compliant Penthiopyrad (purity 99.8%) in 0.5% aqueous carboxymethyl cellulose Sampling times: 2hrs, 4 hrs	Crj:CD(SD)IGS rats (8m/dose)	0, 1000 and 2000 mg/kg bw	Negative	Mitsui Chemicals Agro, Inc. (2000c)

4.9.1 Non-human information

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the available studies is available in the DAR. The following summaries are based on the information in the DAR.

4.9.1.1 In vitro data

A guideline bacterial gene mutation assay is available (August 2000a). In the main assay, concentrations of penthiopyrad up to and including those producing precipitation and/or cytotoxicity were assayed with and without metabolic activation. There was no increase in the incidence of revertant colonies at any non-cytotoxic level of penthiopyrad, in any strains, both with and without S9. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased.

A bacterial DNA damage or repair test is available (August 2000b). In the main assay, penthiopyrad did not induce DNA damage in *B. subtilis*, either with or without metabolic activation, at concentrations near the solubility limit in the nonactivated phase of testing or up to and including the limit of solubility in the S9-activated phase of testing. The positive control substances, Mitomycin C (MitC) - S9, and Trp-P-1 +S9, showed marked differences in growth inhibition of the repair-competent vs. the repair-deficient strain (11.2 and 13.6 mm, respectively). The negative control substance, Kanamycin (KM), produced no relevant difference in growth inhibition between the strains.

A guideline mammalian chromosome aberration test is available (August 2000c). There were no biologically relevant increases in the incidences of aberrant cells for any treatment condition at

levels producing >50% cell survival. In the 6-hour exposure (-S9) study, the incidence of structurally aberrant cells less gaps at 81.9 µg/ml penthiopyrad (4.5%) slightly exceeded the solvent control incidence of 1%, but remained within the range of a negative response. In the 6-hour exposure (+S9), the incidence of structurally aberrant cells less gaps at 160 µg/mL penthiopyrad (17.5%) exceeded the solvent control incidence of 0.5% (cell survival at this concentration was 40.1%). The increase in aberrant cells was due entirely to higher incidences of chromatid breaks and exchanges. In the 24 hour continuous exposure study, the following frequencies of aberrations (less gaps) were seen: 4.0% (52.4µl/mL), 4.0% (65.5 µl/mL) and 5.0% (81.9 µl/mL), compared to the solvent control incidence 0%. The differences were due largely to a higher incidence of chromatid breaks. Cell survival at 81.9 µl/mL was 21.5%.

Based on the results, penthiopyrad is considered to induce chromosomal aberrations in mammalian cultured cells in the presence of S9.

A guideline gene mutation assay in mouse lymphoma cells is available (August 2000). On the basis of a dose-finding study, the gene mutation assay was performed with penthiopyrad concentrations ranging from 4.32 - 52.5 µg/mL and 6.18 to 75 µg/mL, with and without S9 respectively. At the highest dose tested in the main assay, no cells survived treatment with 75 µg/mL –S9. Relative survival (RS) was 17.8 and 18.8% at 52.5 µg/mL –S9 or +S9 activation, respectively. No significant differences relative to the solvent control groups in the mutation frequencies (MFs) were noted at any concentration of penthiopyrad, either with or without metabolic activation. The proportions of small colonies formed at all levels of penthiopyrad both with and without S9 were comparable to the solvent control values. The positive controls induced gene mutations at high frequencies, the fold induction ratios being 4.64 and 5.26, respectively. The MFs of both solvent and positive controls were within the historical control ranges of the performing laboratory.

4.9.1.2 In vivo data

A guideline micronucleus assay is available in mice. There were no deaths and no clinical signs of toxicity in any of the animals treated with penthiopyrad, although most treated animals lost a small amount of weight during the experimental period. The group mean incidences of micronucleated polychromatic erythrocytes (MNPCE) in the treated groups were similar to the vehicle control incidence, and none were statistically significantly different from the control incidence. The individual incidences of MNPCE in all treated animals were within the range of concurrent control incidences. In contrast, the mean incidence of MNPCE in the positive control group (MitC) was significantly ($p<0.01$) higher than the control group by a factor > 50-fold.

The proportion of PCE relative to total erythrocytes was significantly lower than the control in the group treated with 2000 mg/kg bw (42.1% ± 6.3, cf. 52.9% ± 0.9), indicating that the target cell population had been adequately exposed. The proportion of PCE relative to total erythrocytes was also lower than the control at 1000 mg/kg bw (48.3 ± 3.4), but the difference was not statistically significant.

A non-guideline *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay is available in male CD (SD) IGS rats. Three groups of 8 male CD (SD) IGS rats were treated once by gavage with penthiopyrad at 0, 1000 or 2000 mg/kg bw. Animals were sacrificed 2 and 16 hours post treatment; hepatocytes were isolated, allowed to attach to coverslips, placed in tritiated thymidine, and prepared for autoradiographs. UDS was determined by comparing radioactive labelling in hepatocyte nuclei from treated animals to cells recovered from the vehicle control group. Two additional groups (4 rats/group) received the positive control for the 2-hour harvest (5 mg/kg DMN)

or the 16-hour harvest (100 mg/kg 2-AAF). Body weights and the general condition of the animals were recorded.

Grain counts were performed on coded slides with 50 hepatocytes/chamber (150/animal) being counted. Net nuclear grains (NNG) were determined by subtracting grains from one nuclear-sized area of cytoplasm from the nuclear grains. The percentage of heavily radiolabeled cells was recorded as an indicator of cells undergoing replicative repair.

No adverse clinical signs or effects on body weight were noted at either dose (1000 or 2000 mg/kg bw/d) of penthiopyrad. There was no evidence of an increase in the net nuclear grain (NNG) counts or cells in repair at either dose or at either sampling interval. By contrast, the positive control substances (DMN at 2 hours and 2-AAF at 16 hours), produced NNG counts of 32.2 and 30.1, respectively, and both induced repair in 100% of cells. The mean number of NNG and the incidence of cells in repair in both the vehicle and positive control groups were within the laboratory historical data ranges.

It was concluded that penthiopyrad did not induce DNA repair, as assessed by NNG counts and proportion of cells in repair, at doses up to and including the limit dose of 2000 mg/kg, under the conditions employed in this study.

4.9.2 Human information

No data are available.

4.9.3 Other relevant information

No data are available.

4.9.4 Summary and discussion of mutagenicity

Penthiopyrad has been evaluated in a comprehensive battery of six genotoxicity assays, comprising *in vitro* (bacterial and mammalian gene mutation, mammalian cytogenetics, DNA repair) assays, a UDS consisting of *in vivo* dosing followed by *in vitro* culture and assay, and *in vivo* clastogenicity. The *in vitro* assays were performed with and without an exogenous metabolic activation system.

In an *in vitro* mammalian chromosome aberration test, penthiopyrad caused an increase in the incidence of structurally aberrant cells in the presence of S9. However, a well conducted, guideline *in vivo* micronucleus test was negative. Furthermore, penthiopyrad did not demonstrate any genotoxic potential in any of the other available studies. Based on the weight of evidence, penthiopyrad is not considered to have any genotoxic potential.

4.9.5 Comparison with criteria

Based on the weight of evidence, penthiopyrad is not considered to have any genotoxic potential, therefore classification for mutagenicity is not warranted.

4.9.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Carcinogenicity has been investigated in two guideline carcinogenicity studies by the oral route; one in rats, and one in mice.

Table 21: Summary table of relevant carcinogenicity studies

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes. Where two percentages or values are indicated in parentheses, the first value relates to the effects seen in males, and the second value to effects seen in females. If only one value is indicated in parentheses, then this value applies to both sexes, unless otherwise stated.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Carcinogenicity study (104 weeks) OECD 451, GLP compliant Wistar rat, 50/sex/dose Penthiopyrad (purity 98.8%), in the diet Reference: Mitsui Chemicals Agro, Inc. (2006c)	0, 9, 27, 83 and 250 mg/kg bw/d	**Non-neoplastic effects:** **There were no non-neoplastic effects observed in the thyroid at any dose-level.** **No adverse effects were observed at 9 or 27 mg/kg bw/d. Adverse effects noted at higher dose levels were as follows:** **83 mg/kg bw/d** *Macroscopic lesions:* Lungs (females only) - focus/foci (13/50) *Histopathological effects:* Liver (males only) – periportal fatty degeneration (7/50) **250 mg/kg bw/d** *Organ weights:* ↑ liver weight, relative to body weight (18%, 25%) *Macroscopic lesions:* Liver (males only) - accentuated lobular pattern (8/50) Adrenal gland (both sexes) – discolouration Lungs (females only) - focus/foci (13/50) nb. An increased incidence of nodules was noted in the thyroid of males (5/50 cf. 2/50 in controls), however this was not statistically significant. *Histopathological effects:* Liver – hepatocellular hypertrophy (7/47 females, 11/50 males), fatty change (26/47 females), periportal fatty degeneration (33/50 males) Kidneys (males only) – tubular basophilia (45/50), interstitial fibrosis (17/50), pyelitis (9/50), glomerulosclerosis (23/50), chronic nephropathy (41/50) Lungs (females only) – interstitial inflammation (6/48)

<table>
<tr><td></td><td></td><td><u>Neoplastic effects:</u>
<table>
<tr><td rowspan="2">Sex</td><td rowspan="2">Thyroid</td><td colspan="5">Incidence at (mg/kg bw/d):</td></tr>
<tr><td>0</td><td>9</td><td>27</td><td>83</td><td>250</td></tr>
<tr><td rowspan="2">Male</td><td>Follicular adenoma</td><td>3/50</td><td>1/50</td><td>6/48</td><td>2/49</td><td>#9/49</td></tr>
<tr><td>Follicular carcinoma</td><td>2/50</td><td>1/50</td><td>0/48</td><td>0/49</td><td>3/49</td></tr>
<tr><td rowspan="2">Female</td><td>Follicular adenoma</td><td>3/50</td><td>1/50</td><td>2/49</td><td>0/50</td><td>0/48</td></tr>
<tr><td>Follicular carcinoma</td><td>0/50</td><td>0/50</td><td>1/49</td><td>0/50</td><td>1/48</td></tr>
</table>
#At the top dose, 18% of males had follicular adenoma.
Laboratory historical controls (males): thyroid follicular adenoma: 3.92 % (range: 0.00-14.29%), thyroid follicular carcinoma: 0.80% (range: 0.00-6.00%) from 40 studies on 2741 animals from 1982-2004 conducted under same conditions (in-life phase of current study 2003-2005).
NOAEL = 27 mg/kg bw/d, LOAEL = 83 mg/kg bw/d</td></tr>
<tr><td colspan="3">Mice</td></tr>
<tr><td>Carcinogenicity study (78 weeks)
OECD 451, GLP compliant
CD1 mice, 52/sex/dose
Penthiopyrad (purity 98.8%), in the diet
Reference: Mitsui Chemicals Agro, Inc. (2006d)</td><td>0, 20, 60, 200 and 600 mg/kg bw/d</td><td><u>Non-neoplastic effects:</u>
200 mg/kg bw/d:
Gross lesions:
↑ incidence of liver masses (males only, 17/31)
Organ weights:
↑ liver weight (78%, relative to body weight, males only)
Histopathological findings:
Thyroid: ↑ incidence of thyroid follicular cell hypertrophy (both sexes, 14/52 males, 12/52 females), altered colloid (males only, 11/52)

600 mg/kg bw/d:
Clinical signs
Pale coloured skin, pale coloured eyelids (females only)
Gross lesions
Males only: dark coloured liver (5/34) and spots on liver (6/34)
Organ weights
↑ thyroid weight (88%, 85%), relative to body weight
↑ liver weight (91%, relative to body weight, males only)
Histopathological findings:
Thyroid: ↑ incidence of thyroid follicular cell hypertrophy (28/52 males, 34/52 females), altered colloid (41/52 males, 44/52 females), brown pigment (lipofuscin) deposition (19/52 males, 28/52 females)
Lungs (females only): ↑ incidence of alveolar foamy cell accumulation (11/52)</td></tr>
</table>

Neoplastic effects

Sex	Lesion in liver	Incidence at (mg/kg bw/d):				
		0	20	60	200	600
		All animals				
Male	hepatocellular adenoma	7/52	13/52	10/52	13/52	15*/52 (29%)
	hepatocellular carcinoma	2/52	1/52	1/52	5/52	6/52
	Total (adenoma + carcinoma)[#]	9/52	14/52	11/52	15/52	19*/52 (37%)
Female	- hepatocellular adenoma	4/52	2/52	2/52	4/52	2/52
	- hepatocellular carcinoma	0/52	0/52	0/52	0/52	0/52
	Total (adenoma + carcinoma)[#]	4/52	2/52	2/52	4/52	2/52
		Animals sacrificed at 78 weeks				
Male	- hepatocellular adenoma	5/36	8/32	7/34	11*/31 (35%)	12*/34 (35%)
	- hepatocellular carcinoma	1/36	1/32	1/34	4/31	2/34
	Total (adenoma + carcinoma)[#]	6/36	9/32	8/34	13*/31 (42%)	13*/34 (38%)
Female	- hepatocellular adenoma	4/42	2/42	2/41	4/31	2/42
	- hepatocellular carcinoma	0/42	0/42	0/41	0/31	0/42
	Total (adenoma + carcinoma)[#]	4/42	2/42	2/41	4/31	2/42

*$p < 0.05$

[#] Number of animals with adenoma or carcinoma or both adenoma and carcinoma

Historical control data::

	Organ - lesion	Laboratory historical control incidence, from 20 studies conducted between 1999 and 2006:	Laboratory historical control incidence, from 20 studies conducted between 1991 and 2009
Male	- hepatocellular adenoma	17.31 – 34.62 %	17.31 – 38.46 %
	- hepatocellular carcinoma	0.00 – 9.62 %	0.00 – 21.15 %
	- hepatocellular adenoma and/or carcinoma	23.08 – 36.54 %	23.08 – 46.15 %

See Table 22 for further detail on the historical control data.

** NOAEL = 59.8 and 60.3 mg/kg bw/d in males and females respectively, LOAEL = 200 mg/kg bw/d

The following summaries are based on information provided in the DAR.

A guideline carcinogenicity study is available in Wistar rats. The following doses were investigated: 0, 9, 27, 83 and 250 mg/kg bw/d. There were no treatment-related clinical signs and no effect of treatment on survival.

Statistically significant increases in liver weight (relative to body weight) were noted in both sexes at 250 mg/kg bw/d. Treatment-related macroscopic lesions occurred in the liver (accentuated lobular pattern), lungs (foci) and adrenal gland (discoloration) in one or both sexes at 250 mg/kg/day, and in the lungs of females at 83 mg/kg/day. Nodules were observed in the thyroid of males at 250 mg/kg bw/d, however the difference compared to the controls was not statistically significant.

Treatment-related, non-neoplastic histopathological alterations occurred in the liver, kidneys, lungs, ovaries, and adrenal glands. Hepatocellular hypertrophy was increased in females at 27 and 83 mg/kg/day and in both sexes at 250 mg/kg/day. Periportal fatty degeneration was noted in some males treated at 83 and 250 mg/kg/day, and fatty change was evident in some females at 250 mg/kg/day. The incidence of chronic progressive nephropathy (CPN) was increased in males at 250 mg/kg/day. Single elements of CPN (interstitial fibrosis, pyelitis and glomerulosclerosis), showed higher incidences than the controls at lower dose levels, but since the group mean severity of these findings was generally less than the severity in the controls, and the incidence of CPN was not affected at dose levels up to 83 mg/kg/day, the higher incidences were not considered to be adverse effects of treatment. The incidences of alveolar macrophages, graded minimal or mild, were increased in all treated female groups but this was not considered to be an effect of treatment. The study author attributed the effect to dust inhalation from feed or bedding, possibly with test material. However, there was a treatment-related increase in the incidence of interstitial inflammation in females at 250 mg/kg/day. There was also a shift towards increased severity of ovarian senile atrophy in this group. The incidence of focal fatty change in the adrenals of females at 250 mg/kg/day was significantly increased and diffuse hypertrophy of the zona glomerulosa occurred in 4/49 females at 250 mg/kg/day, but incidence of the latter was not statistically significant. Increases in glomerulosa and fatty change were also seen at 83 mg/kg/day. There were no other non-neoplastic findings considered to be treatment-related.

The incidence of thyroid follicular adenoma in males at 250 mg/kg bw/d (18.4%) exceeded the concurrent control incidence of 6.0%, and slightly exceeded the historical control range high value of 14.2% (range 0.00 - 14.29% in males, derived from 40 studies on 2741 animals during the period February 1982 - June 2004). All diagnosed follicular adenomas and carcinomas occurred in survivors sacrificed at the end of the treatment period, with the exception of a single case of adenoma in a male treated at 27 mg/kg/day. The incidence of follicular adenomas in all males at 250 mg/kg/day was not statistically significant (Fishers exact test), but the incidence in survivors sacrificed at the end of the treatment period (9/34) was significantly higher than the control incidence of 6/37 ($p = 0.0395$). The incidences of adenoma in male groups treated at lower dose levels and in all female groups were comparable to control. There was no effect on the incidences of follicular carcinoma in either sex at any dose level.

There were no dose-related, statistically significant excess incidences of tumors, relative to the controls, in any other tissues.

Mice

A guideline carcinogenicity study is available in mice, in which animals were treated for 78 weeks. The following doses were investigated: 0, 20, 60, 200 and 600 mg/kg bw/d.

There were no treatment-related effects on mortality at any dose level. Treatment-related clinical signs occurred in females at the top dose only, and consisted of pale coloured skin (8/52) and eyelids (7/52) compared to the controls (2/52 and 1/52, respectively). There was no effect on food consumption at any dose level, however reduced body weight gain was noted in both sexes at 600 mg/kg bw/d.

Treatment-related gross lesions at necropsy were noted in males treated with 200 and 600 mg/kg bw/d. At 200 mg/kg bw/d, males showed an increased incidence of liver masses (17/31) compared to the control incidence (10/36). Eleven of the seventeen cases at 200 mg/kg bw/d were confirmed histologically as hepatocellular adenoma, the overall incidence of which was statistically significant in this group (see below). At 600 mg/kg bw/d, males showed dark coloured liver (5/34) and spots on the liver (6/34). Three of the six cases of liver spots were confirmed histologically as foci of cellular alteration (eosinophilic cell type), the incidence of which was not significantly increased.

Treatment-related effects on organ weights occurred at 200 and 600 mg/kg/day. Males and females at 600 mg/kg/day showed markedly increased absolute and relative thyroid weights (up to 88%), and males at 200 and 600 mg/kg/day showed increased liver weights (up to 91%).

Treatment-related non-neoplastic histopathological alterations occurred in the thyroid of both sexes at 200 and 600 mg/kg/day, and in the lungs of females at 600 mg/kg/day. There was a dose-related increase in the incidence of thyroid follicular cell hypertrophy in both sexes at 200 and 600 mg/kg/day, accompanied in males at 200 mg/kg/day and in both sexes at 600 mg/kg/day by altered colloid, and in both sexes at 600 mg/kg/day by lipofucsin deposition. Females at 600 mg/kg/day also showed an increased incidence of alveolar foamy cell accumulation, often associated with lymphocyte infiltration.

There was no effect of treatment on the numbers of benign and malignant neoplasms and the number of animals with neoplasm(s), with the exception of the overall incidence of neoplasms in male mice (all animals) treated at 600 mg/kg/day, which was higher than the male control incidence. The difference in this group was attributable, in part, to a significantly increased incidence of hepatocellular neoplasms, but there was no evidence of an earlier occurrence (unscheduled deaths) of any specific type of neoplasm including hepatic adenoma.

Males at 200 and 600 mg/kg/day sacrificed after 78 weeks of treatment had significantly higher incidences of hepatocellular adenomas, and adenoma + carcinoma, than the concurrent control group, although the incidences of carcinoma alone were not significantly increased in either group. The incidences of hepatocellular adenomas and adenoma + carcinoma, were also significantly higher in all males (incidental deaths + terminal sacrifice) at 600 mg/kg/day. Early development of these tumours did not occur since the first occurrence of hepatocellular adenoma or carcinoma was in weeks 52, 74 and 59 (adenoma), or weeks 71, 74 and 65 (carcinoma), at 0, 200 and 600 mg/kg/day, respectively.

Although the total incidences (all animals) of hepatocellular adenoma in males at 200 and 600 mg/kg/day (25 and 29%, respectively) were higher than the concurrent control incidence (13%), they were within the laboratory historical control incidence range of 17.31 – 34.62% from studies conducted between 1999 and 2006 (see Table 22). The concurrent control incidence was lower than the lowest historical control incidence. Furthermore, there was no clear dose-response relationship, no increase in the incidence of pre-neoplastic alterations, no similar effect in females and no effect on tumour latency.

There were no other statistically significant differences between treated and control male groups in the incidences of individual tumour types. Statistically significant differences between treated and control female groups in the incidences of individual tumour types were confined to lower incidences in the treated groups.

Table 22. Historical control data: tumour incidences in control ICR (CRj:CD-1) mice (males) in studies conducted under the same study conditions as the penthiopyrad study

Male mice	Study ID		3	4	7	9	12	14	15	16	17	18	19*	20	21	22	23	24	25	26	27	28	29
	Year study started		1991	1991	1993	1995	1996	1999	2001	2002	2003	2003	2003	2004	2004	2005	2005	2006	2006	2008	2009	2009	2009
	Total	%																					
No. examined	1096		51	52	52	50	52	52	52	52	52	52	52	51	56	52	52	56	52	52	52	52	52
Hepatocellular adenoma	307	28.01	16	18	20	14	12	17	9	14	16	14	7	14	16	18	15	18	14	16	10	12	17
%			31.37	34.62	38.46	28.00	23.08	32.69	17.31	26.92	30.77	26.92	13.46	27.45	28.57	34.62	28.85	32.14	26.92	30.77	19.23	23.08	32.69
Hepatocellular carcinoma	64	5.84	1	4	11	0	2	3	3	4	1	0	2	3	1	2	5	3	1	3	9	6	0
%			1.96	7.69	21.15	0.00	3.85	5.77	5.77	7.69	1.92	0.00	3.85	5.88	1.79	3.85	9.62	5.36	1.92	5.77	17.31	11.54	0.00
Hepatocellular adenoma and/or carcinoma	344	31.39	16	21	24	14	13	19	12	15	16	14	9	16	16	19	19	19	15	18	16	16	17
(%)			31.37	40.38	46.15	28.00	25.00	36.54	23.08	28.85	30.77	26.92	17.31	31.37	28.57	36.54	36.54	33.93	28.85	34.62	30.77	30.77	32.69

*Penthiopyrad study

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4.10.1.2 Carcinogenicity: inhalation

No data are available.

4.10.1.3 Carcinogenicity: dermal

No data are available.

4.10.2 Human information

No data are available.

4.10.3 Other relevant information

In the carcinogenicity study in rats, a small increase in the incidence of thyroid tumours compared to the historical control was observed in males. These thyroid tumours require further consideration for classification purposes. The applicant has made available several mechanistic studies intended to enable a thorough evaluation of the cancer findings in rodents. The relevant study with regards the thyroid tumours is summarised in Table 23.

Table 23: Summary table of study on mechanism of action and supporting data

Method	Dose levels	Observations and remarks
Two week investigation of thyroid function Non-guideline, GLP compliant Wistar rats, 6m/dose/group Penthiopyrad (98.6% purity), in the diet Ref: Mitsui Chemicals Agro, Inc. (2008b)	0, 400, 4000 and 16000 ppm Group 1: treated for 7 days then sacrificed Group 2: treated for 14 days then sacrificed Group 3: treated for 14 days, sacrificed after a 28 day recovery period Mean achieved dose levels (based on an average of all animals including the recovery group): 37.8, 371 and 1453 mg/kg/day	Numbers in brackets indicate values after 7 and 14 days of treatment, unless otherwise stated. All effects were reversible during the 28 day recovery period, unless otherwise stated. **4000 ppm (371 mg/kg bw/d)** ↑ TSH (29%, 13%) ↑ relative liver weight (16%, 13%) ↑ cytochrome P450 content (44%, 23%) ↑ UDPGT activity towards 4-hydroxybiphenyl (153%, 140%) ↑ microsomal protein content (after 7 days only, 26%) ↑ PCNA LI (after 7 days only, 2.06-fold) Mild thyroid follicular cell hypertrophy (2/6 animals) **16000 ppm (1453 mg/kg bw/d)** ↓ food consumption during 1st week of study ↓ body weights (12-15%) ↓ T4 (51%, 42%) ↑ TSH (38%, 13%) ↑ relative liver weight (37-40%) ↑ cytochrome P450 content (56%, 35%) ↑ UDPGT activity towards 4-hydroxybiphenyl (310%, 310%) and 4-

		nitrophenol (222%, 318%) ↑ microsomal protein content (after 7 days only, 40%) (reversible) ↑ PCNA LI (after 7 days only, 2-fold) ↑ expression of Prop-1 (after 7 days only, 49%) Diffuse hepatocellular hypertrophy (all animals), mild thyroid follicular cell hypertrophy (3/6) No NOAEL/LOAELs identified

Thyroid

A non-guideline study is available which investigates the effects of penthiopyrad on hepatic drug-metabolising enzyme induction and cell proliferation in rats following dietary administration for 2 weeks, and supports the development of a proposed mechanism of action for possible effects on the thyroid gland.

Two groups of 6 young male Wistar rats/dose were treated for 7 or 14 days with 0, 37.8, 371 or 1453 mg/kg bw/d penthiopyrad in the diet and then sacrificed and subjected to necropsy. A further group of 6 animals/dose were treated for 14 days and then sacrificed after a 28-day recovery period.

Circulating serum levels of T4, T3 and TSH were measured at the end of the treatment or recovery periods. Hepatic microsomal UDP-glucuronosyl transferase (UDPGT) activities, protein content and total cytochrome P_{450} activity were also measured. Quantitative analysis of gene expression related to TSH in the pituitary was performed and thyroid follicular cell proliferation activity was measured. Pituitary, thyroid and liver tissues were stained with H&E and examined microscopically. In addition, further paraffin sections of the pituitary from each animal were stained immunohistochemically for TSH-secreting cells.

There were no deaths or adverse clinical signs in any of the treatment groups. Reduced body weights were noted in the animals treated with 1453 mg/kg bw/d (12-15%) at the end of the treatment period, but not at the end of the recovery period. Food consumption at 1453 mg/kg bw/d ppm was markedly reduced during the first week of treatment.

At dietary concentrations of 371 and/or 1453 mg/kg bw/d ppm penthiopyrad elicited a pattern of effects comprising hepatocellular hypertrophy, increased liver weight, increased hepatic cytochrome P_{450} and UDPGT activities, reduced circulating T4 levels, up-regulation of the pituitary Prop-1 gene, increased TSH levels, increased thyroid follicular cell proliferation, and thyroid follicular cell hypertrophy. The effects of treatment were fully reversible on withdrawal of treatment for 28 days. Based on these results it is postulated that penthiopyrad is a phenobarbital-type hepatic UDPGT inducer with a potential to enhance biliary excretion of T4, thereby lowering circulating T4 levels, which results in an increase in circulating TSH through negative feedback leading to thyroid follicular cell hypertrophy and subsequently thyroid adenomas.

A non-guideline study (not summarized in Table 23) is also available which investigates the effects of penthiopyrad on hepatic drug-metabolising enzyme induction and cell proliferation in male Wistar rats following dietary administration (Mitsui Chemicals Agro, Inc., 2002b, 2006e). Groups of 18 rats were treated orally for up to 14 days with 6.47, 66.7 or 632 mg/kg bw/d penthiopyrad, or 62.9 mg/kg bw/d phenobarbitone (PB) or 177 mg/kg bw/d clofibrate (CF) as reference materials. Six animals/group were sacrificed after 3, 7 and 14 days of treatment and subjected to necropsy, liver weight recording, measurement of hepatic cell proliferation (by PCNA immunohistochemical staining), analysis of gap junction protein (Cx32), as an indicator of cell-to-cell communication and

routine H&E liver histopathology. In addition, drug metabolising enzyme activities in both the microsomal fraction and the 700xg supernatant fraction containing peroxisomes were measured in animals sacrificed after 14 days:

Hepatic 700xg supernatant fraction containing peroxisomes: protein content, palmitoyl-CoA oxidase and carnitine acyltransferase activities.

Microsomal fraction: protein content, cytochrome P450 content, pentoxyresorufin O-dealkylase, UDP-glucuronyl transferase activity and CYP1A1, CYP2B1, CYP3A2 or CYP4A1 contents.

Electron microscopy was performed on liver sections from two control and two high dose penthiopyrad animals.

The results of this study support the findings in Mitsui Chemicals Agro, Inc.(2008b). Penthiopyrad produced enhanced hepatic cell proliferation during the early stages of treatment. Hepatic POD, UDPGT and cytochrome p450 isozymes activities were also increased (6, 1.5 and 6.45-fold respectively), although the effect of penthiopyrad was milder than that of phenobarbitone. Centrilobular hepatocellular hypertrophy and proliferation of smooth endoplasmic reticulum also occurred in response to penthiopyrad, but it had no effect on hepatic cell-to-cell communication. Enlarged liver in some animals and increased microsomal CYP4A1 activity occurred in response to penthiopyrad at 66.7 mg/kg bw/d, but there were no effects of treatment on any parameters at 6.47 mg/kg bw/d.

4.10.4 Summary and discussion of carcinogenicity

Carcinogenicity has been investigated in two guideline carcinogenicity studies by the oral route; one in rats, and one in mice. Mechanistic studies are also available.

Thyroid Tumours (male rats)

In the carcinogenicity study in rats, the incidence of thyroid follicular adenoma in males at the top dose (18.4% at 250 mg/kg bw/d) exceeded the concurrent control incidence of 6.0%, and slightly exceeded the historical control range high value of 14.29% (range 0.00 – 14.29%). The total incidence of follicular adenoma in males at this dose level was not statistically significant (Fishers exact test), but the incidence in survivors sacrificed at the end of the treatment period (9/34) was statistically significantly higher than the control incidence of 6/37 ($p = 0.0395$). No excessive toxicity was noted at this dose level.

There was no increase in the incidence of follicular adenoma in males at lower dose levels, or in females at any dose level, and there was no increase in the incidence of follicular carcinoma in either sex at any dose level. There was no increase in the incidence of follicular adenoma or carcinoma in mice.

The available genotoxicity studies (Section 4.9) show that penthiopyrad is non-genotoxic. A mechanistic study is available in rats which provides a plausible explanation for the slight increase in follicular adenomas observed in males at the top dose. It is postulated that penthiopyrad is a hepatic UDPGT inducer which enhances biliary excretion of T4, thereby lowering circulating T4 levels. This results in a compensatory increase in circulating TSH from the pituitary through negative feedback, resulting in sustained stimulation of the thyroid to produce thyroid hormone. This leads to proliferation of thyroid follicular cells (hyperplasia), which can eventually lead to neoplasia.

A number of policies have been developed by regulatory agencies and other authoritative bodies on the relevance of thyroid tumours in rodents produced by perturbations of thyroid hormone homeostasis to hazard and risk assessment in humans. For example, the US EPA noted that although the rodent model provides a qualitative indicator of a potential human thyroid cancer hazard, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid-pituitary status (EPA, 1998). IARC stated that agents which induce thyroid follicular-cell tumours in rodents by interfering with thyroid hormone homeostasis can be assumed not to be carcinogenic in humans at concentrations that do not lead to alterations in thyroid hormone homeostasis (IARC, 2001). In the EU, significantly, the Committee of Specialised Experts agreed that there is convincing scientific evidence that humans are considerably less sensitive than rodents (especially rats) to perturbation of thyroid hormone homeostasis and the subsequent development of thyroid follicular tumours induced by non genotoxic compounds (ECBI, 1999).

Liver tumours (male mice)

In the 78-week mouse carcinogenicity study, there was an increased incidence of hepatocellular adenoma in males at the top dose compared to the concurrent control. However, the incidence at the top dose (29%) fell well within the historical control range for this tumour type (17.31 – 34.62 %). Furthermore, the historical control data indicate that the concurrent control value was unusually low (in fact, the lowest value recorded during 21 studies conducted between 1991 and 2009 – refer to table 22). The apparent increase in hepatocellular adenoma in males is therefore considered to be an artefact of the low concurrent control value, and not an effect of treatment with penthiopyrad.

4.10.5 Comparison with criteria

The apparent increase in the incidence of liver tumours in male mice is considered to be an artefact of the very low concurrent control value, and not an effect of treatment. Therefore, only the follicular cell adenomas seen in male rats will be considered further to decide whether penthiopyrad should be classified for carcinogenicity.

Classification of a substance as a carcinogen is based on a weight of evidence approach and expert judgment.

The implications of the increased incidence of thyroid follicular tumours in male rats for hazard classification is evaluated using the ECHA Guidance on the Application of the CLP Criteria in Regulation (EC) No. 1272/2008 (ECHA, 2009), and Specialised Experts guidance on non-genotoxic thyroid carcinogens (ECBI, 1999).

Specialised Experts (ECBI, 1999)

The main conclusion from the Specialised Experts' report was:

Essentially, it was agreed that non-genotoxic carcinogenic substances producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the thyroid hormone axis, in general, do not need to be classified.

Inhibition of T4 release was listed as one of the clearly established mechanisms for perturbation of the pituitary-thyroid axis and the proposed MOA for penthiopyrad is consistent with this mechanism.

The T_{25} approach can be used as a simple and convenient index of carcinogenic potency, and is the daily dose inducing a tumour incidence of 25% upon lifetime exposure assuming a linear dose response between and above the experimental doses. The T_{25} of penthiopyrad can be calculated as follows:

$Dose_{adjusted}$ = $Dose_{experimental}$ x $(D/7)(W_1/S)(W_2/S)(E/S)^2$

= 250 mg/kg bw/d x (7/7)(1)(1)(1)

= <u>250</u> mg/kg bw/d

D = Number of days animals dosed per week

W_1 = Dosing duration (weeks)

W_2 = Study duration (weeks)

S = standard lifespan of the animals under test

E = duration of experiment (weeks)

$$\text{Net tumour incidence} = 100 \times \frac{\%\text{ tumours in test group} - \%\text{ tumours in the control group}}{100 - \%\text{ tumours in the control}}$$

= 100 x (18% - 6%) / (100% - 6%)

= <u>12.8%</u>

T_{25} = $Dose_{adjusted}$ x (25/% net tumour incidence)

= 250 mg/kg bw/d x (25/12.8)

<u>= 488.3</u>

The potency classifications are:

Carcinogens of high potency: T_{25} value < 1 mg/kg bw/day

Carcinogens of medium potency: 1 mg/kg bw/day < T25 value < 100 mg/kg bw/day

Carcinogens of low potency: T_{25} value > 100 mg/kg bw/day.

On this basis, penthiopyrad can be considered to be a carcinogen of <u>low</u> potency.

<u>CLP Regulation (EC) No 1272/2008 and guidance (ECHA, 2009)</u>

Consideration of the animal carcinogenicity data on penthiopyrad indicates that there is “limited evidence of carcinogenicity” based on:

- "The agent increases the incidence only of benign neoplasms" (penthiopyrad induced a marginal, statistically significant increase in follicular cell adenomas in male rats at the top dose tested).
- "The evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs" (tumours were only observed in the thyroid gland).

Additional considerations for classification in the case of penthiopyrad include:

- "Tumour type and background incidence". Rodents, particularly the rat, are known to be susceptible to the induction of thyroid tumours associated with perturbations of thyroid hormone homeostasis. This tumour type is less relevant for humans.
- "Mode of action and its relevance for humans, such as mutagenicity, cytoxicity with growth stimulation, mitogenesis, immunosuppression". There is convincing evidence for a non-genotoxic MOA for thyroid follicular tumours in male rats. Humans are shown to be far less sensitive to this MOA.
- The ECHA CLP guidance specifically lists some mechanisms of tumour formation considered not relevant for humans, one of which is:

"Certain thyroid tumours in rodents mediated by UDPGT induction (IARC, 1999; EU Specialised Experts, 1999)".

- Mutagenicity: "Evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects". Based on the available genotoxicity studies, penthiopyrad is not considered to be genotoxic.

Considering the weight of evidence, it is concluded that penthiopyrad does not meet the criteria for carcinogenicity classification according to Regulation (EC) No. 1272/2008.

4.10.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

Data are available from a 2 generation reproductive toxicity study in the rat.

4.11.1.1 Non-human information

Table 24: Summary table of relevant reproductive toxicity studies – Fertility

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Two generation reproductive toxicity study, oral route OECD 416 Wistar rat, 24/sex/dose Penthiopyrad (98.8%), in diet Ref: Mitsui Chemicals Agro, Inc. 2004, 2005c)	0, 200, 1000 or 5000 ppm (equivalent to overall mean dose values in the P males, F1 males, P females and F1 females of: 200ppm: 11.0, 12.8, 18.1 and 19.0 mg/kg bw/d; 1000 ppm: 54.0, 64.2, 90.5 and 95.6 mg/kg bw/d; 5000 ppm; 278, 340, 439 and 480 mg/kg bw/d)	**P/F1 Parental animals** **No adverse effects were observed at 200 ppm.** **1000 ppm** *Organ weights:* P/F1 females: ↑ relative liver weight (6.3/9.7%) F1 females: ↑ relative adrenal weight (10.7%) *Histopathology:* F1 females: adrenal cortical hypertrophy (10/24) **5000 ppm** *Gross pathology:* P/F1 females: dark liver colour P/F1 males & females: ↑ incidence thyroid enlargement *Organ weights:* P/F1 males & females: ↑ relative liver weight (15.1-21.9%) P/F1 males & females: ↑ relative adrenal weight (16-27%) P males & females, F1 females: ↑ relative thyroid weight (44-46%) *Histopathology:* P/F1 males & females: follicular hypertrophy in thyroid (8-11/24), hepatocyte hypertrophy (7-14/24) **F1/F2 Offspring** **No adverse effects were noted at 200 or 1000 ppm.** **5000ppm**

		Body weight gain/body weights: F1 males: ↓ body weight gain from day 4 F2 males & females: ↓ body weight gain from day 14 ↓ mean body weights at weaning, day 21 (13.6%, 11.8% in F1 males & females, 9.4% and 9% in F2 males and females) *Organ weights (at 25-27 days old):* F1 males, F2 males & females: ↑ relative brain weight (5.3-9%) F2 males & females: ↓ relative thymus weights (8.9-11%) F1 males & females, F2 females: ↓relative spleen weight (9.8-13%) *Parental NOAEL = 200 ppm (11.0 mg/kg bw/d), parental LOAEL = 1000 ppm (54.0 mg/kg bw/d). Reproduction NOAEL = 5000 ppm (278 mg/kg bw/d) Offspring NOAEL = 1000 ppm (54.0 mg/kg bw/d), offspring LOAEL = 5000 ppm (278 mg/kg bw/d)

No adverse effects on fertility were noted at doses of up to 5000 ppm (278-480 mg/kg bw/d) in a guideline two generation repeated dose toxicity study, therefore no classification is proposed based on the results of this study.

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the study is available in the DAR. The following summary is based on the information in the DAR.

A guideline two generation reproductive toxicity study by the oral route is available in Wistar rats. The following doses were investigated: 0, 200, 1000 and 5000 ppm. No treatment-related clinical signs were noted in either generation at either dose level.

P/F1 parental animals:

Two P generation females died prematurely, but neither death was considered to be treatment-related. The mean age at completion of preputial separation or vaginal patency in F1 parental animals was slightly prolonged at 5000 ppm, however this was attributed to low body weight at 5000 ppm. The estrous cyclicity in both generations was unaffected by treatment. All treated males and females of both generations successfully mated, and the mean time to mating was unaffected by treatment. There was no effect of treatment on female fertility and gestation indices, duration of gestation or the mean number of implantation sites. Male fertility was also unaffected by treatment at all dose levels, and there was no effect of treatment in either generation on testicular sperm head count, epididymal sperm count, sperm motility or sperm morphology.

Treatment-related gross pathology alterations comprised dark liver colour in P and F1 females at 5000 ppm (incidence: 15/24 and 16/24, respectively, compared to 0/24 in controls and all other dose levels), and increased incidences of thyroid enlargement in both sexes of both generations at 5000 ppm (P males: 8/24, F1 males: 7/24, P females: 6/24, F1 females: 5/24, cf. 1-3/24 in controls). The latter change was largely due to hydropic (vacuolar) follicular degeneration, a change that occurs spontaneously in this strain of rat. Treatment-related effects on organ weights occurred in the liver, adrenals and thyroids. Absolute and/or relative liver weights were increased in both sexes of both generations at 5000 ppm and in females of both generations at 1000 ppm. Absolute and relative adrenal weights were increased in both sexes of both generations at 5000 ppm. Absolute and

relative thyroid weights were increased in both sexes of both generations, although in F1 males the differences were not statistically significant.

There were no treatment-related histopathological alterations in the reproductive organs and pituitary gland at 5000 ppm. Treatment-related hypertrophic alterations were noted in the liver, adrenals and thyroids, which could be correlated with the increases in organ weights. Female F1 animals at 1000 ppm also showed an increased incidence of adrenal cortical hypertrophy. The increases in organ weight and hypertrophy were considered to be treatment-related but not adverse. There was no effect of treatment at 5000 ppm on the F1 female group mean ovarian follicle count.

F1/F2 offspring:

There were no treatment-related effects on the number of pups delivered or the sex ratio in either generation. The viability indices for the treated groups were comparable to controls, and there were no treatment-related effects on pup body weights measured the day after birth. However, at 5000 ppm, F1 males showed reduced body weight gain from day 4, and F1 females, F2 males and F2 females showed reduced weight gain from day 14 of lactation. At weaning on day 21, the group mean body weights of pups at this dose were up to 13.6% lower than control values. The effect in the F1 generation was slightly greater than in the F2 generation pups.

No treatment-related gross pathological findings were noted in F1 and F2 weanlings at any dose level. Reduced absolute and/or relative thymus weight occurred in F1 and F2 males and F2 females at 5000 ppm, and reduced absolute and relative spleen weights occurred in F1 and F2 males and F1 females. Relative brain weights were significantly increased in F1 and F2 males at 5000 ppm and in F2 females at 1000 and 5000 ppm. These differences at 5000 ppm were considered to reflect reduced body weights at the two highest dose levels. No treatment-related histopathological alterations were evident in the spleen and thymus of F1 and F2 weanlings at 5000 ppm.

4.11.1.2 Human information

No data are available.

4.11.2 Developmental toxicity

Data are available from two guideline developmental studies, one in the rat and one in the rabbit.

Table 25: Summary table of relevant reproductive toxicity studies – Development

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Developmental toxicity study, oral OECD 414 Wistar rat, 22f/dose Penthiopyrad (98.6%), gavage, at 10 mL/kg in aqueous carboxymethylcellulose,	0, 62.5, 250 or 1000 mg/kg bw/d	**No adverse effects were noted at 62.5 or 250 mg/kg bw/d.** **1000 mg/kg bw/d:** **Maternal effects** ↓ body weight gain (37.5%) and ↓ food consumption (9.5%) during days 6-9 after mating ↓ gravid uterine weight

<table>
<tr>
<td>0.5% w/v/Tween 80, 0.1% w/v</td>
<td></td>
<td>
<u>Offspring effects</u>
↑ early resorptions (180%) and post-implantation loss (13%, cf. 4% in controls)
<table>
<tr><th rowspan="2">Dose</th><th rowspan="2">Corpora Lutea</th><th rowspan="2">Implantations</th><th colspan="3">Resorptions</th><th colspan="3">Live Young</th><th rowspan="2">Sex ratio (% M)</th><th colspan="2">Implant. Loss (%)</th></tr>
<tr><th>Early</th><th>Late</th><th>Total</th><th>Male</th><th>Female</th><th>Total</th><th>Pre-</th><th>Post-</th></tr>
<tr><td>0</td><td>13.3</td><td>12.1</td><td>0.5</td><td>0.0</td><td>0.5</td><td>5.4</td><td>6.2</td><td>11.6</td><td>46.3</td><td>10.5</td><td>4.0</td></tr>
<tr><td>62.5</td><td>13.8</td><td>12.4</td><td>0.8</td><td>0.0</td><td>0.8</td><td>5.7</td><td>5.8</td><td>11.5</td><td>49.3</td><td>11.1</td><td>7.0</td></tr>
<tr><td>250</td><td>12.5</td><td>11.3</td><td>0.5</td><td>0.0</td><td>0.5</td><td>4.9</td><td>5.9</td><td>10.8</td><td>45.1</td><td>9.2</td><td>5.3</td></tr>
<tr><td>1000</td><td>13.1</td><td>11.7</td><td>1.4*</td><td>0.0</td><td>1.5*</td><td>5.3</td><td>4.9*</td><td>10.2</td><td>50.7</td><td>10.8</td><td>13.0**</td></tr>
</table>
* p < 0.05; ** p <0.01

*Maternal NOAEL = 250 mg/kg bw/d, maternal LOAEL = 1000 mg/kg bw/d
Foetal NOAEL = 250 mg/kg bw/d, foetal LOAEL = 1000 mg/kg bw/d
</td>
</tr>
<tr>
<td>Developmental toxicity study, oral
OECD 414
NZW rabbits, 24f/dose
Penthiopyrad (98.6%), gavage, at 5 mL/kg in aqueous carboxymethylcellulose, 0.5% w/v/Tween 80, 0.1% w/v

Ref: Mitsui Chemicals Agro , Inc. (2006f,g)</td>
<td>0, 25, 75 or 225 mg/kg bw/d</td>
<td>
No adverse effects were noted at 25 or 75 mg/kg bw/d.

<u>Maternal effects</u>
250 mg/kg bw/d
One female killed prematurely – see text
↓ gravid uterine weight

<u>Offspring effects</u>
250 mg/kg bw/d
↓ mean foetal weight (7.8%)
↓ mean litter weight (13.5%)

*Maternal NOAEL = 75 mg/kg bw/d, maternal LOAEL = 225 mg/kg bw/d
Foetal NOAEL = 75 mg/kg bw/d, foetal LOAEL = 225 mg/kg bw/d
</td>
</tr>
</table>

4.11.2.1 Non-human information

No toxicologically significant effects on development were noted at doses of up to 1000 mg/kg bw/d in the rat, or up to 225 mg/kg bw/d in the rabbit, therefore no classification is required.

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the study is available in the DAR. The following summaries are based on the information in the DAR.

<u>Rats</u>

A guideline developmental toxicity study is available in Wistar rats. The following doses were investigated: 0, 62.5, 250 and 1000 mg/kg bw/d. There were no treatment-related deaths or adverse clinical signs at any dose level. At 1000 mg/kg bw/day, body weight gain and food consumption were marginally reduced (↓37.5% and ↓9.5%, respectively) following the onset of treatment

(Days 6-9 after mating), but subsequently weight gain and food consumption were similar to the controls. By Day 20, the group mean bodyweight was still lower than controls, but the difference was not statistically significant.

At 1000 mg/kg/day, the gravid uterine weight was significantly lower than the controls, but the overall maternal weight gain after adjustment for the gravid uterine weight was unaffected by treatment. On Day 20 of gestation, macroscopic examination of females at necropsy revealed no findings that could be associated with treatment at any dose level.

All treated and control females had a live litter at necropsy on Day 20 of gestation, with the exception of one non-pregnant female at 1000 mg/kg/day. At 1000 mg/kg/day, there was a statistically significant increase in the number of early resorptions and post-implantation losses. Consequently, the live litter size was marginally less than the controls in this dose group (12%), although the difference was not statistically significant. The statistical significance of the increase in post implantation loss is considered to be an artifact of the way in which implantation loss is calculated (100 x (Number of implants – Number of live fetuses)/number of implants). In the study, a slight (not statistically significant) decrease was seen in the number of implants and the number of viable foetuses at the top dose. When these values are combined and manipulated in the calculation used for implantation loss, the result is statistically significant. For the purposes of classification, this effect is not considered to be toxicologically significant.

Placental and foetal weights were unaffected by treatment at all dose levels, but litter weight at 1000 mg/kg/day was lower than the controls by (↓12.0%) as a consequence of the smaller litter size. The nature and incidence of major abnormalities, minor visceral and skeletal abnormalities and skeletal variants among the treated and control groups did not indicate an effect of treatment on foetal development at any dose level.

Rabbits

A guideline developmental study is also available in New Zealand White rabbits. The following dose levels were investigated: 0, 25, 75 and 225 mg/kg bw/d. A decrease in group mean gravid uterine weights was noted at the top dose (225 mg/kg bw/d) only. One female at the top dose was killed prematurely because of evidence of abortion on day 26 of gestation, which followed a period of markedly reduced food consumption and weight loss (↓0.69kg between days 18 and 26). Due to a number of factors (occurrence at the top dose, the closeness of this dose to the maximum tolerated dose, and a low incidence of spontaneous abortion in this strain of rabbit), the effect was considered to be treatment-related. Macroscopic examination of the dam did not reveal any abnormalities, and five empty implantation sites were noted during uterine examination. Three dead foetuses were found in the cage, which were grossly normal at macroscopic examination. There were no other maternal clinical signs that could be related to treatment with penthiopyrad.

There was no effect of treatment on body weight gain or food consumption during gestation at any dose level. There were no treatment-related gross lesions at necropsy at any dose level and the majority of females were pregnant and had a live litter on Day 29 of gestation. Litter data (number of corpora lutea, implantations, early and late resorptions and live young), sex ratio and extent of pre- and post-implantation loss showed no adverse effect of treatment at any dose level.

At 225 mg/kg/day, the overall mean foetal weight was lower (↓7.8%) than the control value. This was mainly attributable to a statistically significant reduction (↓12.1%) in female mean foetal weight, although male foetal weight was also marginally lower than the controls. There was no associated evidence of retarded foetal development identified during detailed visceral and skeletal

examinations. Mean litter weight at 225 mg/kg/day was also reduced (↓13.5%) relative to the controls.

4.11.2.2 Human information

No data are available.

4.11.3 Other relevant information

A post natal developmental neurotoxicity study is available (see Section 4.12). No effects relevant for classification for developmental toxicity were observed during this study.

4.11.4 Summary and discussion of reproductive toxicity

Fertility

In a guideline 2-generation reproductive toxicity study, no treatment-related effects on fertility were observed.

Development

In a pre-natal developmental toxicity study in the rat, resorptions and slight decreases in post-implantation survival, litter size and gravid uterine weight were observed at 1000 mg/kg bw/d. However, these effects were only observed in the presence of maternal toxicity (indicated by reduced body weight gain and food consumption), and so do not provide evidence of a specific effect on development.

In a pre-natal developmental toxicity study in the rabbit, slightly reduced foetal and litter weights were noted at the top dose of 225 mg/kg bw/d at which maternal toxicity was observed (one abortion accompanied by weight loss and reduced mean gravid uterus weight). Overall, mild developmental toxicity was seen in the rabbit only in the presence of maternal toxicity.

4.11.5 Comparison with criteria

Fertility

Classification of a substance in Category 1A is largely based on evidence from humans. As there is no human data available, classification in category 1A is not appropriate.

The potential for penthiopyrad to adversely affect fertility has been well investigated in a standard 2-generation reproductive toxicity study, with supporting information available from a number of repeated dose studies. No evidence of any adverse effects on reproductive performance or reproductive organs and tissues was observed. Therefore, no classification for fertility is proposed.

Development

Classification of a substance in Category 1A is largely based on evidence from humans. As there is no human data available, classification in category 1A is not appropriate. Classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an effect on development in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on development is considered not to be a secondary non-

specific consequence of other toxic effects. No evidence of developmental toxicity was observed in rabbits or rats in the absence of maternal toxicity. Therefore, classification in Category 1B is not appropriate,

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. Mild developmental toxicity was observed in the rabbit, but this was regarded as the unspecific, secondary consequence of maternal toxicity. Relatively minor changes were observed in the rat, which were considered to be secondary to maternal toxicity, reducing concern for human health. Therefore, classification in Category 2 is not considered appropriate.

4.11.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Table 26: Summary table of relevant neurotoxicity studies

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes. Where two percentages or values are indicated in parentheses, the first value relates to the effects seen in males, and the second value to effects seen in females. If only one value is indicated in parentheses, then this value applies to both sexes, unless otherwise stated.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Acute neurotoxicity OECD 424 SD rats, 10/sex/dose Penthiopyrad (98.6%), orally by gavage 14 day observation period Ref: Mitsui Chemicals Agro, Inc. (2008c,d)	0, 125, 500 or 2000 mg/kg bw	**No adverse effects were observed at 125 mg/kg bw** **500 mg/kg bw** *FOB (effects seen on Day 1 only)* ↓ reactivity to handling scores (females only), ↑ incidence of slight to moderate abnormal gait (females only), hunched posture (2m, 1f), ↓ body temperature (0.4-1.5°C), ↑ landing footsplay values, increased incidence of 'no response' in the approach and touch tests (males only), ↓ high beam scores (rearing activity), ↓ low beam scores (cage-floor activity) **2000 mg/kg bw** *FOB (effects seen on Day 1 only)* Piloerection, ↓ reactivity to handling scores, ↓ body tone, ↑

		incidence of slight to moderate abnormal gait, hunched posture (5m, 5f), slight, whole body tremor (7f), chewing mouth movements (5f), slow breathing (3m), ↓ body temperature (0.7-1.8°C), ↑ landing footsplay values, increased incidence of 'no response' in the approach and touch tests, increased incidence of a weak response in the tail pinch test (males only), ↓ high beam scores (rearing activity), ↓ low beam scores (cage-floor activity) *NOAEL = 125 mg/kg bw, LOAEL = 500 mg/kg bw
Subchronic neurotoxicity OECD 424 SD rats, 10/sex/dose Penthiopyrad (98.6%), orally in the diet Ref: Mitsui Chemicals Agro, Inc. (2008e)	0, 10, 40, 160 or 640 mg/kg bw/d	**160 mg/kg bw/d** *Body weight gain:* ↓ bw gain, females only (13%) **640 mg/kg bw/d** ↓ bw gain (17%, 11%) *NOAEL = 160 mg/kg bw/d, LOAEL = 640 mg/kg bw/d
Postnatal developmental neurotoxicity 22f/dose (parental animals), 12f/dose for FOB assessment, up to 22/sex/dose offspring for FOB assessment, 10/sex/dose offspring for detailed neuropathlogical evaluation OECD 426 SD rats Penthiopyrad (99.2%), gavage Ref: Mitsui Chemicals Agro, Inc.(2009d)	0, 100, 250 or 500 mg/kg bw/d	**Effects in the Offspring:** **250 mg/kg bw/d** *Body weight gain:* ↓ mean body weight gain, lactation day 1-4, males only (17%) ↓ mean body weight gain, day 7-13 (14%, 9%) *Observations on physical examination::* Perianal staining **500 mg/kg bw/d** *Body = weight gain:* ↓ mean body weight gain, lactation day 1-4 (17%) ↓ mean body weight gain, day 7-13 (32%, 33%) *Observations on physical examination::* Perianal staining *FOB observations:* ↑ incidence of occasional slight whole body tremors on day 21 ↑ motor activity (rearing and cage floor activity) ↓ peak startle amplitude values in females on Day 61/62 of age *NOAEL for maternal effects = 500 mg/kg bw/d, no LOAEL identified NOAEL for offspring effects = 100 mg/kg bw/d, LOAEL = 250 mg/kg bw/d

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As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the studies is available in the DAR. The following summaries are based on the information in the DAR.

Acute neurotoxicity study in rats

The following doses were investigated: 0, 125, 500 or 2000 mg/kg bw. No deaths or adverse clinical signs were observed. Body weight gain was reduced during the first week of the observation period in both sexes at 2000 mg/kg bw.

In the FOB, home cage observations were unaffected by treatment at all dose levels on all testing occasions. During the in-the-hand, arena, manipulation and motor activity assessments on the day of dosing, a number of treatment-related effects occurred in one or both sexes treated at 500 or 2000 mg/kg (see Table 26). None of these effects were apparent at the subsequent evaluations on days 8 and 15.

Macroscopic examination at necropsy revealed no treatment-related lesions at any dose level. Brain weights and dimensions were unaffected by treatment at all dose levels. There were no treatment-related histopathological findings in any of the tissues of the central and peripheral nervous system and skeletal muscle examined at 2000 mg/kg penthiopyrad.

Subchronic neurotoxicity study in rats

The following doses were investigated: 0, 10, 40, 160 or 640 mg/kg bw/d. There were no treatment-related deaths or clinical signs. A treatment-related decrease in bodyweight gain occurred in both sexes at 640 mg/kg bw/d (11-17%) and in females only at 160 mg/kg bw/d (13%). There were no treatment-related effects on food consumption in either sex.

Home cage, in-the-hand, in the arena and manipulation observations did not reveal any treatment-related effects at any of the observation intervals during the treatment period. Quantitative assessment of motor activity revealed no effects of treatment at any dose level at any observation interval on rearing activity or cage floor activity.

The macroscopic examination performed at termination revealed no treatment-related lesions at any dose level; brain weight, length and width were also unaffected by treatment at all dose levels. There were no treatment-related histopathological findings in any of the central and peripheral nerve tissues or in skeletal muscle, eyes and optic nerve in animals treated at 640 mg/kg/day.

Post natal developmental neurotoxicity study in rats

The following doses were investigated: 0, 100, 250, 500 mg/kg bw/d. There were no treatment-related reproductive effects, and no treatment-related deaths or clinical signs were observed in the pregnant or lactating females. There were no treatment-related effects on body weight in the maternal animals during gestation or lactation, however food consumption was reduced during lactation at 250 and 500 mg/kg bw/d. The effect on food consumption was not considered to be adverse, as mean bodyweights for all treatment groups were not significantly different to controls.

On Day 1 of age, mean offspring body weights were marginally lower than controls at 500 or 250 mg/kg/day, and subsequent bodyweight gain during days 1-4 was reduced in both sexes at 500 mg/kg/day and in males at 250 mg/kg/day. Between Day 7 (the start of offspring treatment) and Day 13, mean bodyweight gain of both sexes was lower than the controls at 500 or 250 mg/kg/day, and signs of perianal staining were observed for many offspring in these dose groups.

Effects on the FOB were confined to offspring receiving 500 mg/kg/day, in which an increased incidence of occasional slight whole body tremors was noted on Day 21 of age, but not

subsequently after discontinuation of treatment. Motor activity (rearing and cage floor activity) for males and females at 500 mg/kg/day was consistently high relative to the controls on all test days. There was no effect of treatment on auditory startle response pre-pulse inhibition, but peak startle amplitude values with and without a pre-pulse in female offspring at 500 mg/kg/day group were significantly lower than the controls at day 61/62 of age. There were no effects at any dose level on the learning and memory capacity of the offspring as assessed by a swimming maze. Sexual maturation, assessed by the time of vaginal opening or balano-preputial separation was unaffected by treatment.

There were no treatment-related macroscopic findings in the offspring at scheduled termination, no effects on brain weights, no histopathological changes in the tissues of the central and peripheral nervous systems presented for neuropathological examination on day 21 or day 66 of age and no changes in brain morphometry on day 21 or day 66 of age.

4.12.1.2 Immunotoxicity

Table 27: Summary table of relevant immunotoxicity studies

↑/↓ = increased/decreased compared to control.

*The values for the NOAELs and LOAELs are provided for information only. All NOAEL and LOAEL values provided in this CLH Report have been taken from the Draft Assessment Report (DAR), produced in accordance with Directive 91/414/EEC.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Immunotoxicity study Modified OECD 407 SD rats, 10m/dose Penthiopyrad (98.6%), orally in the diet Ref: Mitsui Chemicals Agro, Inc. (2009e)	0, 45, 170 and 700 mg/kg bw/d for 4 weeks Positive control group: intraperitoneal injection of cyclophosphamide (CP) at 50 mg/kg, 2 days before termination	**700 mg/kg bw/d** *Body weight, food consumption and body weight gain* ↓ food consumption (1st week of treatment, 11.9%) ↓ body weight (11%) ↓ body weight gain (34.6%) *Macroscopic findings* Enlarged liver *Organ weights* ↑ relative liver weight (24%) ↓ relative spleen weight (17%) *NOAEL for immunotoxic effects = 700 mg/kg bw/d (no LOAEL identified) NOAEL for systemic effects = 175 mg/kg bw/d, LOAEL = 700 mg/kg bw/d
Immunotoxicity study Modified OECD 407 SD CD-1 mice, 10m/dose Penthiopyrad (98.6%), orally in the diet	0, 62.5, 250 and 1000 mg/kg bw/d Positive control group: daily oral (gavage) doses of 20 mg/kg bw/d cyclophosphamide for 5 days (7 to 3	**250 mg/kg bw/d** *Organ weights* ↑ absolute/relative liver weight (15%/12%) **1000 mg/kg bw/d**

Ref: Mitsui Chemicals Agro, Inc. (2009f)	days before termination)	*Organ weights* ↑ absolute/relative liver weight (20%/21%) ↓ numbers of plaque-forming cells *NOAEL for immunotoxic effects = 250 mg/kg bw/d, LOAEL = 1000 mg/kg bw/d NOAEL for systemic effects = 250 mg/kg bw/d, LOAEL = 1000 mg/kg bw/d

As penthiopyrad has been reviewed under the pesticides legislation, a detailed evaluation of the studies is available in the DAR. The following summaries are based on the information in the DAR.

Rats

An immunotoxicity study (modified OECD 407) is available in Sprague-Dawley rats. The following doses were investigated: 0, 45, 170 and 700 mg/kg bw/d. A group of rats, given a single intraperitoneal injection of 50 mg/kg cyclophosphamide (CP) 2 days before termination, acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells (SRBCs) in 0.9% saline 4-days prior to termination. In addition to the standard test protocol, the following investigations were carried out. Bone marrow smears were prepared and examined from all control and high dose animals. A semi-quantitative histopathological assessment of lymphoid tissue compartments, with respect to both the lymphocyte and non-lymphocyte components was performed on thymus, lymph nodes, spleen, mucosa-associated lymphoid tissue (MALT) and bone marrow from the control and all penthiopyrad dose group animals. All splenic tissue not required for histology from all test, control and positive control animals, was used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T-cell-dependent immunogen, SRBCs, using a modification of the Jerne Plaque Forming Cell (PFC) assay. The number of lytic plaques for each animal was determined and group mean responses were calculated and expressed as group mean number of PFC/spleen and per 10^6 splenocytes.

There were no treatment-related deaths or clinical signs. Bodyweight loss and reduced food consumption, followed by reduced weight gain, were noted at the top dose only. Water intake was also increased at the top dose, possibly related to diet palatability.

There were no treatment-related changes in total and differential white blood cell counts at any dose level or in the positive control group. Treatment-related gross lesions identified at necropsy were confined to liver enlargement, correlated with increased weight, at 700 mg/kg/day. Absolute and relative spleen weights were significantly lower than control values at 700 mg/kg/day, however there was no effect of treatment at any dose level on the weights of the lymph nodes and thymus. The absolute and relative weights of the spleen, thymus and all weighed lymph nodes were lower than control values in the cyclophosphamide positive control group.

There were no treatment-related changes in the cellularity, distribution and morphology of bone marrow cells at 700 mg/kg/day, and treatment-related histopathological changes were confined to the thymus. A dose-related increase in the incidence of minimal thymic cortical tingible body macrophages occurred at 175 or 700 mg/kg/day. This change was attributed to engulfed nuclear material from B-lymphocytes, and was not considered to be an adverse effect of treatment or evidence of immunotoxicity. There were no treatment-related changes identified during the semi-quantitative assessment of lymphoid tissue components, with respect to both lymphocyte and non-lymphocyte components.

A significant decrease was observed in the number of cells recovered from the spleen of animals at 700 mg/kg/day, and there was a collateral, non-significant decrease in the total number of PFC/spleen at 700 mg/kg/day. However, there was no effect of treatment at any dose level on the number of PFC/10^6 spleen cells. Since there was no effect of treatment on the functional assessment of the humoral immune response to a T-lymphocyte-dependent antigen, and in the absence of any other treatment-related changes in hematology or histopathology of the lymphatic tissues, the decrease in total spleen cell numbers at 700 mg/kg/day was considered not to be of toxicological significance. The mean numbers of cells/spleen, PFC/10^6 cells and PFC/spleen at 45 or 175 mg/kg/day were comparable to, or greater than, the control values. The PFC response for the animals treated with cyclophosphamide was significantly ablated. The cyclophosphamide treated group also showed a marked reduction in the total number of spleen cells.

Mice

An immunotoxicity study (modified OECD 407) is also available in mice. The following doses were investigated: 0, 62.5, 250 and 1000 mg/kg bw/d. A similar group of mice, given daily oral (gavage) doses of 20 mg/kg/day cyclophosphamide (CP) for 5 days (7 to 3 days before termination), acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells (SRBCs) in 0.9% saline 4-days prior to termination. In addition to the standard OECD 407 protocol, bone marrow smears were prepared and examined from all control and high dose animals. A semi-quantitative histopathological assessment of lymphoid tissue compartments, with respect to both the lymphocyte and non-lymphocyte components was performed on the thymus, lymph nodes, spleen, mucosa-associated lymphoid tissue (MALT) and bone marrow from the control and all penthiopyrad-treated animals. All splenic tissue not required for histology from all test, control and positive control animals, was used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T-cell-dependent immunogen, SRBCs, using a modification of the Jerne Plaque Forming Cell (PFC) assay. The number of lytic plaques for each animal was determined and group mean responses were calculated.

There were no treatment-related deaths or clinical signs, and no effects on bodyweight gain or food consumption at any dose level. Overall water intake was increased by 20 or 26% at 250 or 1000 mg/kg/day, but this was considered not to be an adverse effect.

There were no treatment-related changes in total and differential white blood cell counts at any dose level or in the positive control group. No treatment-related gross lesions were identified at necropsy. Absolute and relative liver weights were slightly higher than control values at 250 or 1000 mg/kg/day. There were no effects on the weights of the spleen or thymus.

There were no treatment-related changes in the cellularity, distribution and morphology of bone marrow cells at 1000 mg/kg/day, and treatment-related histopathological changes were confined to the spleen of animals treated at 1000 mg/kg/day. In the spleen, there was an increased incidence of minimal or slight extramedullary haemopoiesis which was considered not to be an adverse effect of treatment, and a higher incidence of increased cellularity/size of the periarteriolar lymphocyte sheath (PALS) at 1000 mg/kg/day. The latter finding was considered to be of uncertain relationship to treatment, and is also considered not to be an adverse response to treatment, but rather a functional change suggesting an expansion of T-lymphocyte numbers. There were no other treatment-related changes identified during the semi-quantitative assessment of lymphoid tissues, with respect to both lymphocyte and non-lymphocyte components.

There was a statistically significant decrease in the numbers of plaque-forming cells, expressed both as PFC/spleen and PFC/10^6 splenocytes, at 1000 mg/kg/day, but not at lower dose levels. However, there was no histopathological evidence of a decrease in splenic B-cell numbers. Treatment with

cyclophosphamide resulted in statistically significant decreases in the numbers of plaques per spleen and per 10^6 cells, and the number of cells/spleen, demonstrating the sensitivity of the test system.

A NOAEL of 250 mg/kg bw/d has been identified for immunotoxicity and systemic effects, based on a decrease in plaque-forming ability observed at the LOAEL of 1000 mg/kg/day.

4.12.1.3 Specific investigations: other studies

No data are available.

4.12.1.4 Human information

No data are available

4.12.2 Summary and discussion

For neurotoxicity, the following guideline studies are available in the rat by the oral route: an acute neurotoxicity study, a sub chronic neurotoxicity study and a post-natal developmental neurotoxicity study. In the acute study, a number of transient functional alterations (e.g., hunched posture, unsteady gait, reduced body temperature, and increased landing footsplay) and decreased motor activity at the estimated time-to-peak-effect (4 hours) were noted on the day of administration at 500 mg/kg bw/d. These effects were not seen at any other time during the observation period. In the sub chronic study, penthiopyrad did not elicit functional or morphological evidence of neurotoxicity at target dose levels of up to the maximum tolerated dose of 640 mg/kg/day. In the post-natal developmental neurotoxicity study, increased motor activity and whole body tremors (on Day 21) were observed at 500 mg/kg bw/d.

For immunotoxicity, the following guideline studies are available: an immuotoxicity study in rats by the oral route, and an immunotoxicity study in mice by the oral route. There were no indications of an immunotoxic effect of penthiopyrad in rats. In mice, a decrease in plaque-forming ability was noted, but only at the top dose tested (1000 mg/kg bw/d).

4.12.3 Comparison with criteria

In the available studies on rats, transient functional alterations and decreased motor activity were noted on the day of dosing during an acute study at 500 mg/kg bw/d. Such effects do not warrant classification under CLP. In the sub chronic study, penthiopyrad did not elicit functional or morphological evidence of neurotoxicity at target dose levels of up to the maximum tolerated dose of 640 mg/kg/day. In the post-natal developmental neurotoxicity study, increased motor activity and whole body tremors (on Day 21) were observed at 500 mg/kg bw/d. Such effects do not warrant classification under CLP.

According to the available studies, there was no indication of an immunotoxic effect of penthiopyrad in rats. In mice, a decrease in plaque-forming ability was noted, but only at the top dose tested (1000 mg/kg bw/d). This effect on its own does not warrant classification for immunotoxicity.

4.12.4 Conclusions on classification and labelling

No classification and labelling is proposed for penthiopyrad regarding neurotoxicity or immunotoxicity.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Penthiopyrad is a pesticide for use as a foliar fungicide on pome fruit, tomato and cereals. Available environmental fate and hazard studies have been considered under EU Directive 91/414/EEC and summarised in the publically available Report and Proposed Decision of the United Kingdom Made to the European Commission under Article 8 of Council Directive 91/414/EEC – January 2012 and subsequent addenda – September 2012

In particular refer to Volume 3 Annex B Section B8: Environmental Fate and Behaviour Part A: Evaluation and Assessment of Data Submitted Data – 2012 and
Volume 3 Annex B Section B9: Ecotoxicology Part A: Evaluation and Assessment of Data Submitted Data -2012. The key information pertinent to determining a classification is presented below.

The outcome of the pesticide peer review and agreed endpoints from this process were summarised in the EFSA Conclusion, 2013.

Where available information on degradation products is included – full details of degradant names and structures are presented in Annex 1.

5.1 Degradation

Table 28: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis OECD Guideline 111, GLP	Stable at pH 4, 7 and 9 at 50 ^{o}C	Valid	Tognucci, 1999a
Aquatic photolysis OECD Guideline 21, GLP	No significant photolysis	Valid	Burgener, 1999
Ready biodegradation OECD Guideline 301F, GLP	Not rapidly biodegradable -1% degradation after 28 days	Valid	Seyfried, 2007
Water/sediment simulation OECD Guideline 308, GLP	DT_{50} 242 to 296 days based on whole system Mineralisation: minimal with <5% Applied Radioactivity	Valid Aerobic system	Adam, 2008
Water/sediment simulation OECD Guideline 308, GLP	DT_{50} 34.8 days based on water phase only Mineralisation: minimal with <1% Applied Radioactivity	Valid Anaerobic system Whole system DT_{50} unavailable due to limited sediment degradation.	Adam, 2007

5.1.1 Stability

Aqueous hydrolysis

An aqueous hydrolysis study (Tognucci, 1999a) is available following GLP and OECD Guideline 111. The study used penthiopyrad (99.9% purity) at initial concentrations of less than half the water solubility. Test solutions were incubated at 50 ^{o}C ± 0.5 ^{o}C. No significant degradation was observed

and analysis showed less than 10% degradation after 5 days. On this basis, the DT_{50} at 25 ^{o}C is considered greater than one year and penthiopyrad is considered hydrolytically stable.

Aqueous photolysis

An aqueous photolysis study (Burgener, 1999) is available following GLP and OECD Guideline 21. The study used penthiopyrad (99% purity) at nominally 2.0 mg/l. Test solutions were incubated at pH 7 for 15 days at 25 ^{o}C under constant irradiation ('Suntest' apparatus with a xenon burner and filter system to absorb UV radiation below 290 nm). No significant degradation was observed and penthiopyrad is considered photolytically stable.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available.

5.1.2.2 Screening tests

A ready biodegradation study (Seyfried, 2007) is available following OECD Guideline 301F (Manometric Respirometry Test) and GLP using penthiopyrad (purity 98.6%). Test solutions were prepared with 100 mg test item meaning the substance was tested above the water solubility of 1.375 mg/l at pH 7 and 20 ^{o}C (Franke, 2008a). Validation criteria for the Reference and Toxicity Controls were met. Ultimate biodegradation reached a maximum of -1% over 28 days. Overall, the substance is considered not readily biodegradable.

5.1.2.3 Simulation tests

Aerobic System

A degradation in aerobic water-sediment system study (Adam, 2008) is available following OECD Guideline 308 and GLP. The study used ^{14}C-penthiopyrad (100% purity) with two labels: pyrazole [P-^{14}C]-penthiopyrad and thienyl [T-^{14}C]-penthiopyrad. Two aerobic systems were used: 'River' and 'Pond'. The water and sediment test conditions are included in Table 29 below. The system was treated with 0.1 mg penthiopyrad per litre of water via the water surface.

Table 29: Water-sediment system test conditions

Criteria	River	Pond
Water properties	pH: 7.05 at start to 8.31 at end Total organic carbon: 0.98% at start Oxygen: 8.1 mg/l at start to 8.6 mg/l at end Redox potential: 139 mV at start to 214 mV at end	pH: 7.65 at start to 8.13 at end Total organic carbon: 5.49% at start Oxygen: 8.7 mg/l at start to 8.4 mg/l at end Redox potential: 130 mV at start to 232 mV at end
Sediment properties	75.2% sand; 18.5% silt; 6.3% clay Organic carbon 0.87% at start pH: 7.39 at start to 7.14 at end Redox potential: -116 mV at start to -45 mV at end	14.9% sand; 57.7% silt; 27.4% clay Organic carbon 3.4% at start pH: 6.96 at start to 7.16 at end Redox potential: -142 mV at start to -63 mV at end

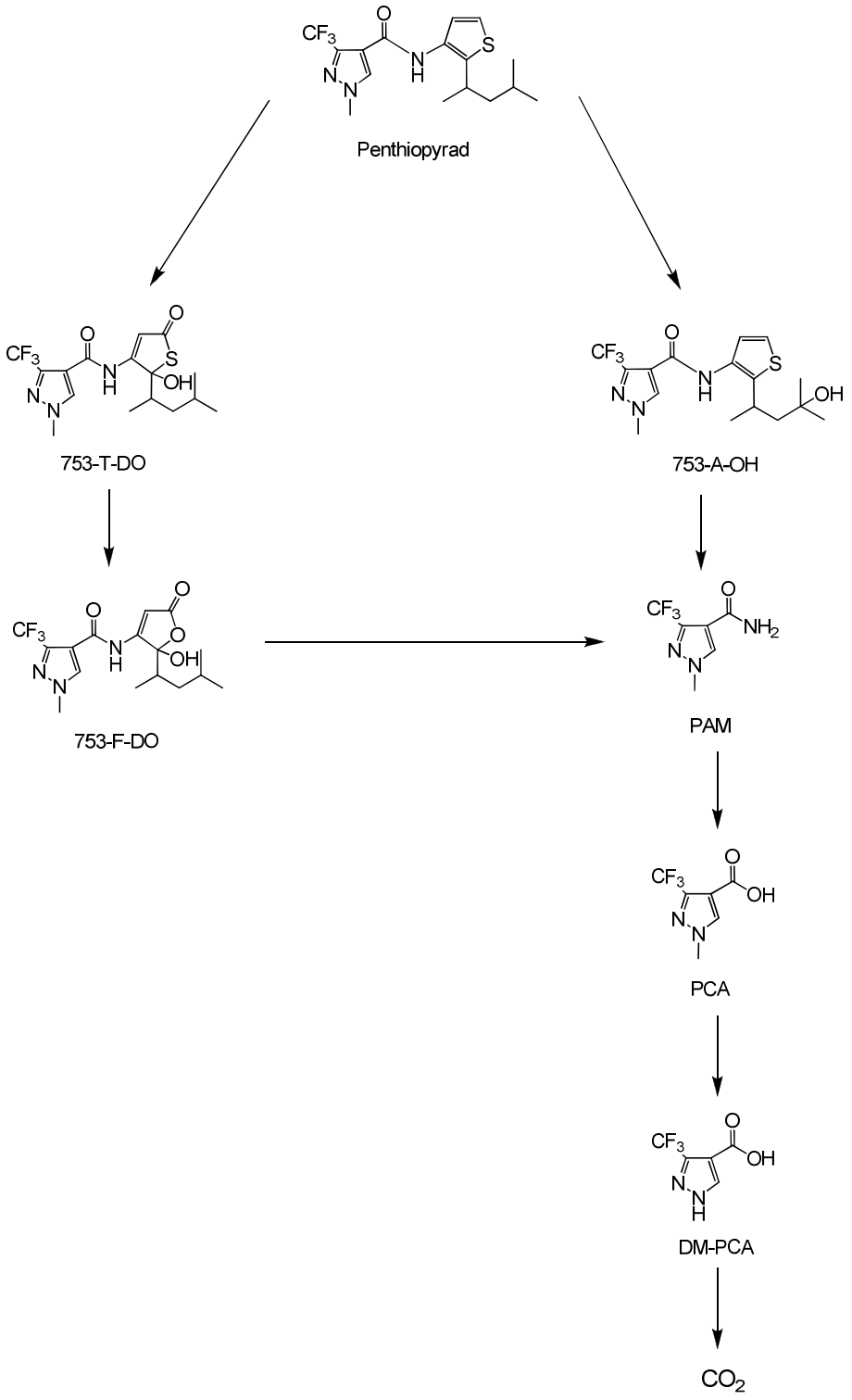
The study was conducted at 20°C, in the dark under aerobic conditions. The guideline recommendation of study duration 100 days was extended to 185 days as sediment microbial activity was still viable. Radioactivity was determined by Liquid Scintillation Counting (LSC) and subsequent analysis by High Performance Liquid Chromatography (HPLC) was undertaken. Total mean recoveries for both systems were >99% Applied Radioactivity (AR) for both labels.

Penthiopyrad dissipated from the water phase to the sediment phase in both systems. In water penthiopyrad decreased from initial 91-94% AR to 8-9% AR on day 185. In sediment penthiopyrad increased from initial 5-6% AR to a maximum of 63.3% AR and 68.1% AR ('Pond' system) on day 56. From this point, levels declined with 46.8% AR ('River' system) and 58% AR ('Pond' system) by day 185.

Three principle degradants were observed at >3% AR: PCA, DM-PCA and 753-A-OH[1]. PCA was the major degradant reaching 9.3% AR ('River' system) and 10.5% AR ('Pond' system) by day 185. DM-PCA reached 5.5% AR in the 'River' system but was <5% AR in the 'Pond' system. 753-A-OH reached 7.5% AR in the 'River' system but was <5% AR in the 'Pond' system. Figure 1 shows the proposed aerobic degradation pathway.

[1] For full details refer to Annex 1

Figure 1: Proposed degradation pathway of penthiopyrad in water-sediment systems under aerobic conditions



Degradation rates (DT_{50}) for penthiopyrad were calculated using Single First Order (SFO) for sediment and whole system, and Double First Order Parallel (DFOP) for water using MicroCal Origin (v. 6.1). Due to insufficient data, reliable sediment DT_{50} values could not be determined and whole system values were based on extrapolation. The values are presented in Table 30.

Table 30: DT_{50} half-lives for penthiopyrad in aerobic water-sediment systems

System	Phase	DT_{50} (days)
River	Water	10.9
	Whole system	242*
Pond	Water	8.9
	Whole system	296*

*Value extrapolated beyond length of study and should be treated with caution

Overall, penthiopyrad was observed to dissipate from the water column in both systems to sediment where subsequent decline was also noted. However, mineralisation was minimal with <5% AR observed for both systems.

Anaerobic System

A degradation in anaerobic water-sediment system study (Adam, 2007) is available following OECD Guideline 308 and GLP. The study used ^{14}C-penthiopyrad (100% purity) with two labels: pyrazole [P-^{14}C]-penthiopyrad and thienyl [T-^{14}C]-penthiopyrad. In deviation from the guideline only one anaerobic system was used: 'Pond'.

The water and sediment test conditions are included in table 31 below.

Table 31: Water-sediment system test conditions

Criteria	Pond
Water properties	pH: 8.01 at start to 7.19 at end Total organic carbon: 11.19 mg C/l at end Oxygen: 0.03 mg/l at start to 0.1 mg/l at end Redox potential: -250 mV at start to -189 mV at end
Sediment properties	14.9% sand; 57.7% silt; 27.4% clay Organic carbon 3.4% at start pH: 7.95 at start to 7.19 at end

The study was conducted at 20°C, in the dark under anaerobic conditions for 100 days. Radioactivity was determined by LSC and subsequent analysis by HPLC was undertaken. Total mean recoveries for both systems were >100% Applied Radioactivity (AR) for both labels.

Penthiopyrad dissipated from the water phase to the sediment phase. In water, penthiopyrad decreased from initial 100% AR to 19.8% AR on day 100. In sediment penthiopyrad increased from initial 4.2% AR to a maximum of 84.3% AR day 100.

Minimal formation of degradants were observed with none >3% AR. The major degradant, DM-PCA reached 2.5% AR in water and 0.7% AR in sediment.

A DT_{50} half life of 34.8 days was calculated using single first-order kinetics for the water phase. Given the limited degradation in sediment, it was not possible to calculated sediment or whole system DT_{50} values.

Overall, penthiopyrad was observed to dissipate from the water column to sediment under anaerobic conditions at a slower rate than under aerobic conditions. Minimal mineralisation was observed with <1% AR.

5.1.3 Summary and discussion of degradation

Penthiopyrad is considered hydrolytically and photolytically stable.

In a ready biodegradation study no degradation (-1%) was observed over 28 days.

In an aerobic water-sediment study penthiopyrad was observed to dissipate from the water column to sediment in both systems where subsequent decline was also noted. Estimated whole system DT_{50} values were between 242 and 296 days. Minimal mineralisation was observed.

In an anaerobic water-sediment study penthiopyrad was observed to dissipate from the water column to sediment. The water phase DT_{50} value was 34.8 days. Sediment and whole systems DT_{50}s were not calculated due to limited degradation in sediment. Minimal mineralisation was observed.

Overall, the degradation information does not provide sufficient data to show penthiopyrad is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non classifiable products. Consequently, penthiopyrad is considered not rapidly degradable for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

One GLP study (Völkel, 2007) is available investigating the adsorption of penthiopyrad (purity 100%). The study followed OECD Guideline 106 and used 5 soils. The K_{oc} values ranged between 610 and 1050 ml/g. This equates to $\log K_{oc}$ values between 2.79 and 3.02. The average Koc value was 800 ml/g equating to $\log K_{oc}$ 2.9. As also indicated by the water-sediment studies at 5.1.2.3, these K_{oc} data indicate a propensity for penthiopyrad to dissipate from the water column into sediment. In soil, these results would indicate that penthiopyrad has only low or slight mobility and that the mobility would be influenced by soil organic carbon and clay content.

5.2.2 Volatilisation

Experimental data (Tognucci, 1999c) indicate the vapour pressure for penthiopyrad is 6.43×10^{-6} Pa at 25 °C based on extrapolation following OECD Test Guideline 104. The Henry's Law Constant (Labano, 2009a) was calculated at pH 7 to be 7.66×10^{-3} Pa m^3 mol^{-1} at 20 °C indicating penthiopyrid is unlikely to partition from the water phase to air.

5.2.3 Distribution modelling

Not relevant for classification and labeling.

5.3 Aquatic Bioaccumulation

Table 32: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water Calculation based on solubility in water and *n*-octanol	Log K_{ow} 4.36 at pH 4, 20°C Log K_{ow} 4.62 at pH 7, 20°C Log K_{ow} 4.54 at pH 10, 20°C	Valid Log K_{ow} is not pH dependant Water solubility in buffered solutions	Franke, (2008a and 2008b)
Partition coefficient *n*-octanol/water: calculated based on solubility in *n*-octanol and pure water	Log K_{ow}: 3.9 at pH 20 °C	'Expert statement/case' considered valid in peer review. Water solubility in distilled water	Labano, 2012
Experimental aquatic BCF OECD Guideline 305, GLP	Steady state whole fish BCF: 155-186 l/kg Depuration half-life DT_{50} whole fish: 0.65 days	Flow through, 14 days exposure, 14 days depuration	Mitsui Chemicals Agro, Inc., 2008

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

As relevant experimental data are available, estimations are not included.

5.3.1.2 Measured bioaccumulation data

An experimental aquatic BCF for penthiopyrad (purity 97%) is available following GLP and OECD Guideline 305 (Mitsui Chemicals Agro , Inc.,2008). The study used ^{14}C-penthiopyrad, a flow-through system with Rainbow Trout (*Oncorhynchus mykiss*) and two exposure concentrations; 0.1 and 10 µg/l. The exposure period ran for 14 days followed by a 14-day depuration period.

Review under Directive 91/414/EEC highlighted some study limitations as listed below. These resulted in recalculated BCFs presented as 1-1.18x the values quoted in the study (see below). While there is some uncertainty due to the study limitations (see below) key validity criteria were met and the study was considered reliable for use.

Summary of study limitations[2]:

- Total organic carbon concentrations were 2.68-6.08 mg/l exceeding the guideline 2 mg/l. Such excess carbon was considered to have the potential to sorb up to 15% of the test compound. To account for this BCFs are quoted as ranges representing 1-1.18x quoted study values.
- On day 9, the test item concentration in the high dose exposure medium was over 20% less than the mean value.

[2] For full details refer to Penthiopyrad DAR, Volume 3, B.9 Hazard Assessment under Directive 91/414/EEC

- Sampling intervals were insufficiently timed to allow an uptake rate to be calculated. While this may lead to uncertainty regarding steady state, given the low BCFs it is considered unlikely to result in BCFs greater than 500 l/kg.
- Fish were acclimatized for 11 days where the guideline recommends a minimum of 2 weeks.
- The study temperature range was 12-13.8 ^{o}C, which is below the test guideline range of 13-17 ^{o}C.
- The flow-through volume was approximately 3.3 tank volume replacement/day where the guideline recommends 5.

Insufficient information was available to calculate BCFs based on parent residues. Steady state whole fish mean BCF values based on Total Radioactive Residues (TRR) of 158-186 l/kg for the 0.1 µg/l nominal exposure concentration and 155-182 l/kg for the 10 µg/l nominal exposure concentration were calculated. Kinetic BCFs were not calculated as an uptake rate could not be determined due to sampling intervals.

The study measured fish body weight and lipid content on day 4 and 14 of the uptake phase. It is recommended (Environment Agency, unpublished) that the mean lipid content at the end of the depuration phase or the time-weighted average lipid content over the depuration phase is used to calculate a lipid normalised BCF. As these values are not available and the uncertainty associated with using uptake measurements is unknown for penthiopyrad, a lipid normalised BCF is not included. However, given the low study BCFs (below 200 l/kg), it is unlikely lipid normalisation would result in BCF values greater than 500 l/kg.

The study report and DAR include BCF values based on the lipid content in control fish. These values are essentially lipid weight BCFs as a ratio of test item in lipid and in water – they are not lipid normalised BCFs.

Extensive metabolism was observed with penthiopyrad accounting for 35% Total Radioactive Residues (TRR) in fish at day 10 and 42% TRR in fish at day 14. The depuration half-lives (DT_{50}) for whole fish were 0.65 days for the high dose and 0.86 days for the low dose.

5.3.2 Summary and discussion of aquatic bioaccumulation

The substance is surface active (56.7 mN/m). Given this, log K_{ow} values for penthiopyrad have been calculated based on solubility in *n*-octanol and water (Franke, 2008a and Franke 2008b): 4.36 at pH 4, 4.62 at pH 7 and 4.54 at pH 10 (all at 20 ^{o}C). These are based on water solubility in buffered solutions which could have induced a 'salt-out' effect and in combination with impurities may have reduced the water solubility leading to slightly higher log K_{ow} values (Labano, 2012).

Review under Directive 91/414/EEC recommended a log K_{ow} value based on penthiopyrad solubility in distilled water (Tognucci, 1999d) and *n*-octanol. This calculated value (Labano, 2012) is 3.9 at pH 5 and 20 ^{o}C. The review also noted that the partition coefficient is not significantly affected by pH.

The 3.9 log K_{ow} value is just below the CLP log K_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate under CLP. Other values in buffered solutions were just greater than 4. Nevertheless, an experimental bioconcentration study in fish is available to consider bioaccumulation further.

In the experimental study, whole fish BCF values for penthiopyrad were less than 500 indicating a low potential for bioaccumulation. The parent substance was also observed to be extensively

metabolised and rapid depuration was observed with depuration DT_{50}s of 0.65-0.86 days (based on total radioactivity). On this basis, the substance does not meet CLP criteria as a bioaccumulative substance.

5.4 Aquatic toxicity

A summary of available valid information on the aquatic toxicity of penthiopyrad is presented in Table 33. Where available, a summary of valid information for degradants is also included in Annex 2, Table 1.

Studies were reviewed under Directive 91/414/EEC and considered valid. Further details are presented for studies conducted on the active substance penthiopyrad but not for its degradants as these are less toxic and not considered further for classification of penthiopyrad.

Table 33: Summary of relevant information on aquatic toxicity

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	0.386* (mm)	Mitsui Chemicals Agro , Inc., 2007a
Acute toxicity to fish OECD Guideline 203, GLP	Common Carp (*Cyprinus carpio*)	Mortality	Flow-through	96 hours	LC_{50}	0.572* (mm)	Mitsui Chemicals Agro , Inc.,, 2005
Acute toxicity to fish OECD Guideline 203, GLP	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Static	96 hours	LC_{50}	>0.604* (mm)	Mitsui Chemicals Agro , Inc.,, 2007b
Acute toxicity to fish OECD Guideline 203, GLP	Fathead Minnow (*Pimephales promelas*)	Mortality	Semi-static	96 hours	LC_{50}	**0.290* (mm)**	Mitsui Chemicals Agro , Inc.,, 2009a
Acute toxicity to fish OECD Guideline 203, GLP	Sheepshead Minnow (*Cyprinodon variegates*)	Mortality	Semi-static	96 hours	LC_{50}	1.38* (mm)	Mitsui Chemicals Agro , Inc., 2007e
Fish Early Life-Stage (FELS) toxicity OECD Guideline 210, GLP	Fathead Minnow (*Pimephales promelas*)	Time to hatch, hatching success, survival and growth (length, wet weight and dry weight)	Flow-through	33 days	NOEC	**0.051 (mm)** Based on length and wet weight	Mitsui Chemicals Agro , Inc.,, 2008
Daphnia sp Acute Immobilisation OECD Guideline, 202 GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>1.375 (experimental water solubility limit	Maeda, 2005

Daphnia magna Reproduction OECD Guideline 211, GLP	*Daphnia magna*	Survival; reproduction; growth	Flow-through	21 days	NOEC	0.471 (mm)	Palmer *et al*, 2007c
Mysid Acute Toxicity Test OPPTS 850.1035 GLP	Mysid Shrimp *Americamysis bahia*	Acute immobilisation	Semi-static	96 hours	LC_{50}	>1.7 (mm)	Palmer *et al*, 2007d
Oyster Acute Toxicity Test (Shell Deposition) OPPTS 850.1025 GLP	Eastern Oyster (*Crassostrea virginica*)	Shell deposition	Flow-through	96 hours	EC_{50}	1.2 (mm)	Palmer, 2008
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudo-kirchneriella subcapitata*	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>4.0 (twa) 0.45 (twa)	Sueta, 2005
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudo-kirchneriella subcapitata*	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>1.5 (gmm) 0.788 (gmm)	Palmer *et al*, 2009a
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Skeletonema costatum*	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>1.576 (gmm) 0.373 (gmm)	Palmer *et al*, 2009b
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Anabaena flos-aquae*	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>1.240 (gmm) 1.240 (gmm)	Palmer *et al*, 2009c
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Navicula pelliculosa*	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>1.429 (gmm) 1.429 (gmm)	Palmer *et al*, 2009d
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP	*Lemna gibba*	Growth	Static	7 days	ErC_{50} NOErC	>1.205 (mm) 1.205	Sindermann *et al*, 2008

Notes:
mm refers to mean measured concentrations
twa refers to time-weighted mean measured concentrations
gmm refers to geometric mean measured concentrations
*Based on binomial method due to lack of two or more concentrations where mortality (%) was between 0 and100
Key endpoints used in acute and chronic classification are highlighted in **bold**.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Five acute toxicity to fish studies using penthiopyrad (purity >98%) are available following GLP and OECD Guideline 203.

Study 1 (Mitsui Chemicals Agro , Inc., 2007)

Using Rainbow Trout (*Oncorhynchus mykiss*), the nominal exposure range was 38, 75, 150, 300 and 600 µg/l. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) at 0.1 ml/l and a solvent control was included. Analytical measurement used High Performance Liquid Chromatography (HPLC) with UV detection. Results were based on mean

measured values: 34, 71, 146, 276 and 540 µg/l. A static system was used and some exposure solutions were not within 20% of nominal and results are based on mean measured concentrations. Validity criteria were met and the test is considered reliable. The 96-hour LC_{50} was calculated to be 386 µg/l (equating to 0.386 mg/l) with 95% confidence intervals of 276 to 540 µg/l.

Study 2 (Mitsui Chemicals Agro , Inc., 2005)

Using Common Carp (*Cyprinus carpio*), the nominal exposure range was 98.8, 296, 444, 667 and 1,000 µg/l in a flow-through system. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) at 0.1 ml/l and a solvent control was included. Analytical measurement at study start and end used High Performance Liquid Chromatography (HPLC) with a UV detection. It is noted that analytical measurements of renewal stock solutions were not undertaken. However, penthiopyrad is anticipated to be stable over the study duration and results were based on mean measured values: 96.9, 274, 434, 685 and 1,010 µg/l. Validity criteria were met although it is not clear if reported organic carbon measurements were based on total or dissolved carbon. The laboratory provided sampling information demonstrating it was unlikely the Total Organic Carbon (TOC) content would have exceeded the test guideline. The 96-hour LC_{50} was calculated to be 572 µg/l (equating to 0.572 mg/l) with 95% confidence intervals of 434 to 685 µg/l. Overall, the test is considered reliable supplementary information.

Study 3 (Mitsui Chemicals Agro , Inc, 2007b)

Using a static system and Bluegill Sunfish (*Lepomis macrochirus*), the nominal exposure range was 260, 430, 720, 1,200 and 2,000 µg/l. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) at 0.1 ml/l and a solvent control was included. Analytical measurement at study start and end used High Performance Liquid Chromatography (HPLC) with a UV detection. Results were based on mean measured values: 237, 369, 604, 938 and 1,540 µg/l. A white precipitate was observed at the two highest exposure concentrations at study initiation indicating the test item was present in excess of solubility in test media. It is noted that test solutions were not centrifuged to remove the precipitate. The study 96-hour LC_{50} was calculated to be 1,224 µg/l (equating to 1.224 mg/l) with 95% confidence intervals of 938 to 1,540 µg/l. To account for the uncertainty that the two higher concentrations do not represent soluble concentrations, the Directive 91/414/EEC review presented the LC_{50} as >0.604 µg/l. Overall, the test is considered reliable supplementary information.

Study 4 (Mitsui Chemicals Agro , Inc, 2009)

Using a semi-static system and Fathead Minnow (*Pimephales promelas*), the nominal exposure range was 50, 100, 200, 400 and 800 µg/l. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) at 0.1 ml/l and a solvent control was included. Analytical measurement at study start and end used High Performance Liquid Chromatography (HPLC) with a UV detection. Results were based on mean measured values: 54.2, 136, 203, 414 and 753 µg/l. Validity criteria were met although it is not clear if reported organic carbon measurements were based on total or dissolved carbon. The laboratory provided sampling information demonstrating it was unlikely the Total Organic Carbon (TOC) content would have exceeded the test guideline. The Directive 91/414/EEC review noted variability in the 100 µg/l exposure solutions based on analytical measurements. This is not anticipated to impact the study outcome as mortality was not observed at this treatment level. The 96-hour LC_{50} was calculated to be 290 µg/l (equating to 0.290 mg/l) with 95% confidence intervals of 203 to 414 µg/l.

Overall, the test is considered reliable and given it presents the lowest LC_{50} value from the dataset, it is considered the key study for acute toxicity.

Study 5 (Mitsui Chemicals Agro , Inc, 2007e)

Using a semi-static system and Sheepshead Minnow (*Cyprinodon variegatus*), the nominal exposure range was 260, 430, 720, 1,200 and 2,000 µg/l. Exposure solutions were prepared with saltwater (salinity 20%) and the aid of the solvent dimethylformamide (DMF) at 0.1 ml/l , a solvent control was included. Analytical measurement at study start and end used High Performance Liquid Chromatography (HPLC) with UV detection. It is noted that analytical measurements of renewal stock solutions were not undertaken. However, penthiopyrad is anticipated to be stable over the study duration and results were based on mean measured values: 238, 370, 630, 996 and 1,447 µg/l. Validity criteria were met although it is not clear if reported organic carbon measurements were based on total or dissolved carbon. The laboratory provided sampling information demonstrating it was unlikely the Total Organic Carbon (TOC) content would have exceeded the test guideline. The 96-hour LC_{50} was calculated to be 1,381 µg/l (equating to 1.38 mg/l) with 95% confidence intervals of 996 µg/l to no upper limit. While test solutions are close to or exceed the experimental water solubility limit, test solutions were observed to be clear and colourless throughout the study. Overall, the test is considered reliable supplementary information.

5.4.1.2 Long-term toxicity to fish

A 33-day flow-through chronic toxicity to fish study (Mitsui Chemicals Agro , Inc., 2008) using penthiopyrad (purity 98.6%) following GLP and OECD Guideline 210 is available. The study used Fathead Minnow (*Pimephales promelas*) and the following endpoints: time to hatch, hatching success, survival and growth. The nominal exposure range was 13, 25, 50, 100 and 200 µg/l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Analytical measurement used HPLC with a UV detector. Results were based on mean measured values: 13, 26, 51, 100 and 188 µg/l. Validity criteria were met and the test is considered reliable. No significant effect on time to hatch, hatching success or toxicity following hatching were observed. The study report identified a statistically significant difference in growth endpoints compared to controls. It also considered the most sensitive endpoints were length and dry weight and a NOEC of 100 µg/l was determined.

Review under Directive 91/414/EEC resulted in a revised NOEC of 51 µg/l (see DAR Addendum 5). This resulted from statistical analysis showing that the 100 µg/l exposure concentration induced significant reductions in fish total length and wet weight compared to control fish. Therefore the 51 µg/l value was agreed as the revised study NOEC and is considered valid for the purpose of classification. Overall, it is the lowest chronic endpoint.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1 (Maeda, 2005)

A static acute toxicity to *Daphnia magna* study using penthiopyrad (purity 98.8%) is available following GLP and OECD Guideline 202. Exposure solutions were prepared by direct addition without the aid of a solvent resulting in higher treatments above the experimental water solubility limit of 1.375 mg/l at 20 ^{o}C and pH 7. The nominal exposure range was 0.525, 0.839, 1.34, 2.15, 3.44 and 4.89 mg/l. Analytical measurement used HPLC with a UV detector. Results were based on mean measured values: 0.53, 0.835, 1.32, 2.16, 3.41 and 4.89 µg/l. Validity criteria were met and the test is considered reliable. The study 48-hour EC_{50} was calculated to be 2.531mg/l with 95% confidence intervals of 2.251 to 2.829 mg/l. While no precipitate was observed during the study,

review under Directive 91/414/EEC considered it was not appropriate to quote an EC_{50} significantly above the experimental water solubility. No effects were observed at the 1.32 mg/l treatment (below the water solubility) and only 25% immobility was observed at the 2.16 mg/l treatment (above the water solubility). On this basis, the EC_{50} is quoted as >1.375 mg/l reflecting the experimental water solubility.

Study 2 (Palmer, 2008)

An acute toxicity (shell deposition) to the Eastern Oyster (*Crassostrea virginica*) study using penthiopyrad (purity 98.6%) is available following GLP and US EPA OPPTS 850.1025 guideline. The test used flow-through conditions using natural seawater (salinity 20 ‰). Test solutions were prepared with the aid of a solvent (DMF at 0.1 ml/l) and a solvent control was included. The nominal exposure range was 130, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement was determined using HPLC with UV detector. Results were based on soluble mean measured values: 83, 150, 310, 610 and 1,200 µg/l. Validity criteria were met and test is considered reliable. Based on visual assessment, the study 96-hour EC_{50} was 1,200 µg/l due to 50.63% shell growth inhibition at the highest test concentration equating to 1.2 mg/l based on measured concentrations.

Study 3 (Palmer, 2007d)

A semi-static acute toxicity to the saltwater mysid (*Americamysis bahia*) study using penthiopyrad (purity 98.6%) is available following GLP and US EPA OPPTS 850.1035 guideline. The test used flow-through conditions using natural seawater (salinity 20 ‰). Test solutions were prepared with the aid of a solvent (DMF at 0.1 ml/l) and a solvent control was included. The nominal exposure range was 130, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement used HPLC with a UV detector. Results were based on mean measured values: 150, 270, 490, 850 and 1,700 µg/l. Validity criteria were met and the test is considered reliable. No mortality was observed and sub-lethal effects were only observed at the highest treatment. The study 96-hour EC_{50} was >1,700 µg/l (>1.7 mg/l) based on measured concentrations.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A flow-through chronic toxicity to *Daphnia magna* study (Palmer *et al*, 2007c) using penthiopyrad (purity 98.6%) is available following GLP and OECD Guideline 211. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. The nominal exposure range was 30, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement used HPLC with a UV detector. Results were based on mean measured values: 130, 236, 471, 818 and 1,515 µg/l. Validity criteria were met and test is considered reliable. The most sensitive endpoint was reproduction with a NOEC of 471 µg/l (equivalent to 0.471 mg/l) determined.

5.4.3 Algae and aquatic plants

Algae:

Five algal growth inhibition studies using penthiopyrad (purity >95%) are available.

Where effects values are quoted as 96-hour results, 72-hour effects values are not cited in the study reports. While 72-hour values are preferred, in this instance algae are significantly less sensitive than fish (algal EC_{50} values are greater than the experimental water solubility limit; lowest algal NOECs are a factor of 10 lower than the fish chronic NOEC) and it is not considered to impact the proposal.

Study 1 (Sueta, 2005)

A static algal growth inhibition test using penthiopyrad (purity 98.8%) and *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is available following GLP and OECD Guideline 201. Exposure solutions were prepared by direct addition without the use of a solvent although no precipitate was observed. The nominal exposure range was based on 4.27, 9.39, 20.7 45.5 and 100% of a saturated solution. Analytical measurement used HPLC with a UV detector. Results were based on time-weighted mean measured values: 199, 449, 960, 2,090 and 4,020 µg/l. Validity criteria were met and test is considered reliable. As 28% inhibition of growth was observed at the highest treatment, the 72-h E_rC_{50} is >4,020 µg/l (4.02 mg/l) which is above the experimental water solubility limit. The 72-hour NOE_rC was determined to be 449 µg/l (0.449 mg/l).

Study 2 (Palmer *et al*, 2009a)

A static algal growth inhibition test using penthiopyrad (purity 98.6%) and *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is available following GLP and OECD Guideline 201 under static conditions. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. The nominal exposure range was 130, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement was determined using HPLC with a UV detector. Results were based on geometric mean measured values: 104, 192, 387, 788 and 1,533 µg/l. Validity criteria were met and test is considered reliable. As 10% inhibition of growth was observed at the highest treatment, the 96-h E_rC_{50} is >1,533 µg/l (equating to 1.533 mg/l). The 96-hour NOE_rC was determined to be 788 µg/l (equating to 0.788 mg/l).

Study 3 (Palmer *et al*, 2009b)

A static algal growth inhibition test using penthiopyrad (purity 98.6%) and the diatom *Skeletonema costatum* is available following GLP and OECD Guideline 201 under static conditions. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. The nominal exposure range was 63, 130, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement used HPLC with a UV detector. Results were based on geometric mean measured values: 59, 110, 213, 373, 710 and 1,576 µg/l. Validity criteria were met and the test is considered reliable. As 22% inhibition of growth was observed at the highest treatment, the 96-h E_rC_{50} is >1,576 µg/l (1.576 mg/l). The 96-hour NOE_rC was determined to be 373 µg/l (0.373 mg/l).

Study 4 (Palmer *et al*, 2009c)

A static algal growth inhibition test using penthiopyrad (purity 98.6%) and the cyanobacteria *Anabaena flos-aquae* is available following GLP and OECD Guideline 201 under static conditions. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. The nominal exposure range was 130, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement used HPLC with a UV detector. Results were based on geometric mean measured values: 91, 165, 334, 621, and 1,240 µg/l. Validity criteria were met and test is considered reliable. As less than 50% inhibition of growth was observed at the highest treatment, the 96-h E_rC_{50} is >1,240 µg/l (1.24 mg/l). The 96-hour NOE_rC was determined to be 1,240 µg/l (1.24 mg/l).

Study 5 (Palmer *et al*, 2009d)

A static algal growth inhibition test using penthiopyrad (purity 98.6%) and *Navicula pelliculosa* is available following GLP and OECD Guideline 201 under static conditions. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. The nominal exposure range was 130, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement used HPLC with a UV detector. Results were based on geometric mean measured values: 105, 188, 368, 695 and 1,429 µg/l. Validity criteria were met and the test is considered reliable. As less than 50%

inhibition of growth was observed at the highest treatment, the 96-h E_rC_{50} is >1,429 µg/l (1.429 mg/l). The 96-hour NOE_rC was determined to be 1,429 µg/l (1.429 mg/l).

Aquatic plants:

A static 7-day toxicity to *Lemna gibba* study (Sindermann *et al*, 2008) using penthiopyrad (purity 98.6%) is available following GLP and OECD Guideline 221. Exposure solutions were prepared with the aid of the solvent DMF (0.02ml/l) and a solvent control was included. The nominal exposure range was 130, 250, 500, 1,000 and 2,000 µg/l. Analytical measurement used HPLC with a UV detector. Results were based on mean measured values: 126, 267, 502, 686 and 1,205 µg/l. Validity criteria were met and the test is considered reliable. The study endpoints were percentage reduction in frond number, frond yield, biomass, growth rate based on frond number and growth rate based on biomass.

At 7 days, less than 50% inhibition was observed for all endpoints so all 7-day EC_{50} values were > 1,205 µg/l (equating to 1.205 mg/l). As no significant growth reduction compared to controls was observed for all endpoints, the 7-day growth NOE_rC was 1,205 µg/l (1.205 mg/l).

5.4.4 Other aquatic organisms (including sediment)

No further relevant aquatic effects data are available.

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

For the purpose of classification, penthiopyrad is considered not rapidly degradable.

Penthiopyrad has a calculated log K_{ow} value of 3.9. In an experimental BCF study, penthiopyrad (as total radioactive residue) had whole fish BCFs less than 500 and was observed to be extensively metabolised and rapidly depurated. It is therefore not considered bioaccumulative.

Identified degradants are considered less toxic and not considered further for classification of penthiopyrad.

Aquatic acute toxicity data on penthiopyrad are available for fish, invertebrates, algae and aquatic plants. Fish are the most acutely sensitive trophic group with $L(E)C_{50}$ values in the range 0.1 to 1.0 mg/l. The lowest value is 0.290 mg/l for Fathead Minnow (*Pimephales promelas*). On this basis penthiopyrad should be classified as Aquatic Acute 1 with an M factor of 1.

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

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M factor = 1

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M factor = 1

6 OTHER INFORMATION

Abbreviations

Alk P	Alkaline phosphatase
ALT	alanine aminotransferase
AP	alkaline phosphatase
APTT	activate partial thromboplastin time
AST	aspartate aminotransferase
bw	bodyweight
CPN	chronic progressive nephropathy
DAR	Draft Assessment Report
G-GT	Gamma-Glutamyl Transferase
Hb	haemoglobin concentration
Hct	haematocrit
LOAEL	lowest observed adverse effect level
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MF	mutation frequencies
MSG	Mean severity grade
NOAEL	no observed adverse effect level
PT	relative prothrombin time
PTT	Partial thromboplastin time
RBC	red blood cell
LF Retic	low fluorescence reticulocytes

7 REFERENCES

A full reference list can be found in the publically available Report and Proposed Decision of the United Kingdom Made to the European Commission under Article 8 of Council Directive 91/414/EEC – January 2012 and subsequent addenda – September 2012

Specifically:
Volume 3 Annex B Section B1: Identity - 2012
Volume 3 Annex B Section B2 (B.2.1): Physical and Chemical Properties - 2012
Volume 3 Annex B Section B6: Toxicology and Metabolism Part A: Evaluation and Assessment of Data Submitted, January - 2012
Volume 3 Annex B Section B8: Environmental Fate and Behaviour Part A: Evaluation and Assessment of Data Submitted Data - 2012
Volume 3 Annex B Section B9: Ecotoxicology Part A: Evaluation and Assessment of Data Submitted Data -2012

Conclusion on the peer review of the pesticide risk assessment of the active substance penthiopyrad, EFSA Journal 2013;11(2):3111

Physical and Chemical Properties

Tognucci, A. 1999a, Determination of the melting point/melting range of MTF-753, RCC Ltd, Report No. 728346, GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.1.1/01)

Tognucci, A., 1999b, Determination of the boiling point/boiling range of MTF-753, RCC Ltd., Report No. 728357, GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.1.2/01)

Badt-Tognucci, A., 2007a, Determination of the relative density and the bulk density of MTF-753, RCC Ltd., Report No. A59398, GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.2/01)

Tognucci, A., 1999c, Determination of the vapor pressure of MTF-753, RCC Ltd., Report No. 728368, GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.3.1/01)

Labano, S., 2009a, Penthiopyrad: Calculation of Henry's Law Constant, LKC Switzerland Ltd, Report No. 08-LKC-03, Not GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.3.2/01)

Tomiya, K. , 2008a, Physical state of purified active substance, penthiopyrad (MTF-753)., Mitsui Chemicals, Inc., Agrochemicals Division, Report No. not specified, Not GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.4.1/01)

Tomiya, K., 2008b, Physical state of penthiopyrad technical (MTF-753)., Mitsui Chemicals, Inc., Agrochemicals Division, Report No. not specified, Not GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.4.1/02)

Tomiya, K., 2008c, Color of purified active substance, penthiopyrad (MTF-753)., Mitsui Chemicals, Inc., Agrochemicals Division, Report No. not specified, Not GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.4.1/03)

Tomiya, K., 2008d, Color of penthiopyrad technical (MTF-753). Mitsui Chemicals, Inc., Agrochemicals Division, Report No. not specified Not GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.4.1/04)

Tomiya, K., 2008e, Odour of purified active substance, penthiopyrad (MTF-753). Mitsui Chemicals, Inc., Agrochemicals Division, Report No. not specified, Not GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.4.2/01)

Tomiya, K., 2008f, Odour of penthiopyrad technical (MTF-753)., Mitsui Chemicals, Inc., Agrochemicals Division, Report No. not specified, Not GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.4.2/02)

Franke, J., 2008a, Determination of the water solubility of MTF-753 including effect of pH and temperature, RCC Ltd., Report No. A79435, GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.6/01)

Tognucci, A., 1999d, Determination of the solubility of MTF-753 in water and in organic solvents

RCC Ltd., Report No. 728370, GLP, unpublished, Mitsui Chemicals Agro, Inc. (DAR IIA 2.6/02)

Human Health Hazard Assessment

ECBI (1999). EU Specialised Experts. Commission group of specialised experts in the fields of carcinogenicity, mutagenicity and reprotoxicity. 3.1 Non-genotoxic thyroid carcinogens in the rodent bioassay. ECBI/49/99 – Add.1 Rev 2.

ECHA (2009). ECHA Guidance on the Application of the CLP Criteria in Regulation (EC) No. 1272/2008. ECHA-09-G-02-EN.

EPA (1998). Assessment of thyroid follicular cell tumour. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. Report No. EPA/630/R-97/002, March 1998.

EU (1999). Guidelines for setting specific concentration limits for carcinogens in Annex I of directive 67/548/EEC Inclusion of potency considerations. Commission Working Group on the Classification and Labelling of Dangerous Substances.

(2005) Validation of an analytical method for the determination of MTF-753 in diet, RCC Ltd., 4452 Itingen, Switzerland, unpublished report no. 851875

(2000) Gene mutation assay with MTF-753 in mouse lymphoma cells (MLA); Biosafety Research Center, Shizuoka, 437-1213, Japan; report no 4849 (077-016); August 1, 2000; unpublished. MRID 47614895

Mitsui Chemicals Agro,, Inc. (2000a) Acute oral toxicity in rats. Report No. 760061. Unpublished report.

Mitsui Chemicals Agro,, Inc. (2000b) Micronucleus study with MTF-753 in mice. Report No. 4850(077-017)

Mitsui Chemicals Agro,, Inc. (2000c) *In vivo/in vitro* unscheduled DNA synthesis (UDS) assay with MTF-753 in rat hepatocytes. Report No. 4851(077-018)

Mitsui Chemicals Agro,, Inc. (2001a) MTF-753: 4-hour acute inhalation toxicity study in rats. Report No. 783292. Unpublished.

Mitsui Chemicals Agro,, Inc. (2001b) MTF-753: Acute dermal toxicity in rats. Report No. 783900. Unpublished.

Mitsui Chemicals Agro,, Inc. (2001c) MTF-753: dermal irritation study in rabbits. Report No. IET 00-0115

Mitsui Chemicals Agro,, Inc. (2001d) MTF-753: eye irritation study in rabbits. Report No. 00-0114. Unpublished.

Mitsui Chemicals Agro, Inc. (2001e) MTF-753: Skin sensitisation study in guinea pigs – maximisation test. Report No. 00-0116. Unpublished.

Mitsui Chemicals Agro, Inc. (2001f) MTF-753: subacute 28-day dose-range finding (feeding) study in the rat. Report No. 762658. Unpublished.

Mitsui Chemicals Agro, Inc. (2001g) First amendment to report – MTF-753: subacute 28-day dose-range finding (feeding) study in the rat. Report No. 762658. Unpublished.

Mitsui Chemicals Agro, Inc. (2001h) Statistical analyses of data from project 762658. Unpublished.

Mitsui Chemicals Agro, Inc. (2001i) Statistical analysis from study number 781503, MTF-753: subchronic 90-day toxicity study (feeding) in the rat. Report No. 781503. Unpublished.

Mitsui Chemicals Agro, Inc. (2001j) MTF-753: 28-day dose-range finding study in mice. Report No. 00-0020

Mitsui Chemicals Agro, Inc. (2001k) MTF-753: 28-day dose-range finding study in dogs. Report No. 00-0050

Mitsui Chemicals Agro, Inc. (2001L) MTF-753: 90-day oral (dietary) toxicity study in the dog. Report No. 00-0119

Mitsui Chemicals Agro, Inc. (2002a) MTF-753: 90-day oral (dietary) toxicity study in mice for dose range finding. Report No. 00-0101

Mitsui Chemicals Agro, Inc. (2002b) MTF-753: 2-week hepatic drug metabolising enzyme induction and cell proliferation study in rats. Report No. 00-051

Mitsui Chemicals Agro, Inc. (2004) A reproduction toxicity study in rats with MTF-753 – preliminary study. Report No. 02-01004

Mitsui Chemicals Agro, Inc. (2005a) Pilot study of the routes of elimination and pharmacokinetics of [^{14}C]MTF-753 in rats. Report No. 015187-1. Unpublished.

Mitsui Chemicals Agro, Inc. (2005b) MTF-753: subchronic 90-day toxicity study in the rat. Report No. 781503. Unpublished.

Mitsui Chemicals Agro, Inc. (2005c) A reproduction toxicity study in rats with MTF-753. Report No. 03-0085

Mitsui Chemicals Agro, Inc. (2006a) MTF-753: 52-week oral toxicity (feeding) study in the rat and first amendment dated January 17, 2007. Report No. 851875. Unpublished.

Mitsui Chemicals Agro, Inc. (2006b) MTF-753: Chronic oral (dietary) toxicity study in dogs. Report No. 02-0105

Mitsui Chemicals Agro, Inc. (2006c) MTF-753: 104-week oncogenicity (feeding) study in the rat and first amendment dated January 16, 2007. Report No. 847154

Mitsui Chemicals Agro, Inc. (2006d) MTF-753: carcinogenicity study in mice. Report No. 02-0007

Mitsui Chemicals Agro, Inc. (2006e) MTF-753: 2 week hepatic drug metabolising enzyme induction and cell proliferation study in the rat. Revision of final report. Report No. 00-0051

Mitsui Chemicals Agro, Inc. (2006f) MTF-753: preliminary embryo-foetal toxicity study in the rabbit by gavage administration. Report No. MTU352/050139

Mitsui Chemicals Agro, Inc. (2006g) MTF-753: embryo-foetal toxicity study in the rabbit by gavage administration. Report No. MTU351/053490

Mitsui Chemicals Agro, Inc. (2007) First amendment to report – MTF-753: subchronic 90-day toxicity study (feeding) in the rat. Report No. 781503. Unpublished.

Mitsui Chemicals Agro, Inc. (2008a) MTF-753: 4-week dermal toxicity study in the rat. Report No. LDA0079/073706\

Mitsui Chemicals Agro, Inc.(2008b) MTF-753: effect on thyroid function and its reversibility in rats. Report No. 0010

Mitsui Chemicals Agro, Inc.(2008c) MTF-753: dose range and time to peak effect in rats by acute oral administration. Report No. LDA0076/070069

Mitsui Chemicals Agro, Inc.(2008d) MTF-753: Acute neurotoxicity study by a single oral administration to the rat. Report No. LDA0077/072863

Mitsui Chemicals Agro, Inc.(2008e) MTF-753: 13-week dietary neurotoxicity study in rats. Report No. 0078/073100

Mitsui Chemicals Agro, Inc. (2009a) Amended report – Metabolism of [^{14}C]MTF-753 in rats. Report No. 015188-1-2. Unpublished.

Mitsui Chemicals Agro, Inc. (2009b) Identification of metabolites of [^{14}C-Thienyl] penthiopyrad (MTF-753) in the male rat. Report No. 1900W. Unpublished.

Mitsui Chemicals Agro, Inc. (2009c) Multiple dose excretion and tissue distribution study of [^{14}C]MTF-753 in rats. Report No. 020780-1. Unpublished.

Mitsui Chemicals Agro, Inc. (2009d) MTF-753: preliminary developmental neurotoxicity study by oral gavage administration to CD rats. Report No. MTU0417

Mitsui Chemicals Agro, Inc. (2009e) MTF-753: 4-week dietary immunotoxicity study in the rat. Report No. LDA/0080/073719.

Mitsui Chemicals Agro, Inc. (2009f) MTF-753: 4-week dietary immunotoxicity study in the mouse. Report No. MTU0418

(August 2000a) Bacterial reversion assay with MTF-753; Biosafety Research Center, Foods, Drugs and Pesticides, 437-1213 Shizuoka, Japan, report no. 4847 (077-014); August 1, 2000; unpublished. MRID 47614892

(August 2000b) DNA repair assay (rec-assay) with MTF-753 in *Bacillus subtilis* spores; Biosafety Research Center, Shizuoka, 437-1213, Japan; report no 4846 (077-013); August 1, 2000; unpublished. MRID 47614894

(August 2000c) Cytogenetic assay with MTF-753 in Chinese hamster lung (CHL/IU) cells; Biosafety Research Center, Foods, Drugs and Pesticides, 582-2, Arahama Shioshinden Fukude-Cho Iwata-Gun, Shizuoka 437-1213, Japan; report no. 4848 (077-015); August 1, 2000, unpublished, MRID 47614893

Environmental Hazard Assessment

Tognucci, A. (1999a) Hydrolysis determination of MTF-753 at different pH values. RCC Ltd., CH-4452 Itingen, Switzerland; unpublished. MRID 47614837.

Burgener, A. (1999) Aqueous photolysis of MTF-753 under laboratory conditions. RCC Ltd., CH-4452 Itingen, Switzerland; unpublished. MRID 47614839.

Seyfried B. (2007) MTF-753: Ready biodegradability in a manometric respirometry test. RCC Ltd, unpublished. MRID 47614974.

Adam D. (2008) ^{14}C-MTF-753: Route and Rate of Degradation in Aerobic Aquatic Sediment Systems. RCC Ltd, Itingen, Switzerland; unpublished. MRID 47614975.

Adam D. (2007) ^{14}C-MTF-753: Route and Rate of Degradation in Anaerobic Aquatic Sediment System. RCC Ltd, Itingen, Switzerland; unpublished. MRID 47614976.

Mitsui Chemicals Agro, Inc. (2008) Bioconcentration: Flow-through Fish Test with [^{14}C]MTF-753 in Rainbow Trout (*Oncorhynchus mykiss*; unpublished, Report No A59545

Mitsui Chemicals Agro, Inc. (2007a) MTF-753: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)., unpublished report no. 236A-112A.

Mitsui Chemicals Agro, Inc. (2005) A 96-hour Acute Toxicity Test of MTF-753 with Common Carp. unpublished report no. 93440.

Mitsui Chemicals Agro, Inc. (2007b) MTF-753: A 96-Hour Static Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*), unpublished report No. 236A-111.

Mitsui Chemicals Agro, Inc. (2009a) Penthiopyrad: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*).unpublished report No. 236A-120.

Mitsui Chemicals Agro, Inc. (2007e) MTF-753: A 96-Hour Static-Renewal Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). unpublished report no. 236A-116.

Mitsui Chemicals Agro, Inc. (2008) MTF-753: An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*), Wildlife International, Ltd., Easton, Maryland, USA, unpublished report No. 236A-119.

Mitsui Chemicals Agro, Inc. (2008a) Assessment of Toxic Effects of DM-PCA in Rainbow Trout (Oncorhynchus mykiss) using the 96 Hour Static, Acute Limit Test; unpublished report no. 20071562/01-AAOm.

Mitsui Chemicals Agro, Inc. (2008a) Report Amendment No.1 to Study 20071562/01-AAOm - Assessment of Toxic Effects of DM-PCA in Rainbow Trout (Oncorhynchus mykiss) using the 96 Hour Static, Acute Limit Test; unpublished report no. 20071562/01-AAOm.

Mitsui Chemicals Agro, Inc. (2008a) PCA: Acute Toxicity to Zebra Fish (*Brachydanio rerio*) in a 96 Hour static test; unpublished report no. B57374.

Mitsui Chemicals Agro, Inc. (2008a) Acute Toxicity Study of PAM in Rainbow Trout; unpublished report no. B130 (077-075).

Mitsui Chemicals Agro, Inc. (2009b) 753-A-OH: A 96 Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*), unpublished report No. 236A-129.

Mitsui Chemicals Agro, Inc. (2009c) 753-T-DO: A 96 Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). unpublished report No. 236A-126.

Maeda M. (2005) A 48-hour Acute Immobilization Test of MTF-753 with *Daphnia magna*. Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, unpublished report no. 93439.

Borrmann K. (2008b) Assessment of Toxic Effects of DM-PCA on Daphnia magna using the 48 Hour Static, Acute Limit Test. eurofins-GAB GmbH, Niefern-Öschelbronn, Germany; unpublished report no. 20071562/01-AADm.

Borrmann K. (2008b) First amendment to report - Assessment of Toxic Effects of DM-PCA on Daphnia magna using the 48 Hour Static, Acute Limit Test. eurofins-GAB GmbH, Niefern-Öschelbronn, Germany; unpublished report no. 20071562/01-AADm.

Schmidt T. (2008) PCA Acute toxicity to *Daphnia magna* in a 48 Hour Immobilization Test. RCC Ltd., Itingen, CH 4452, Switzerland; unpublished report no. B57396.

Oishi N. (2008b) Acute Immobilization Study of PAM in *Daphnia magna.* Biosafety Research Center, Foods, Drugs and Pesticides 582-2, Shioshinden, Iwata, Shizuoka 437-1213, Japan, unpublished report no. B131 (077-076).

Minderhout T., Kendall T.Z. and Krueger H.O. (2009d) 753-A-OH: A 48 Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*), Wildlife International Ltd.; Easton, Maryland, USA, unpublished report no. 236A-128.

Minderhout T., Kendall T.Z. and Krueger H.O. (2009e) 753-T-DO A 48 Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*), Wildlife International Ltd.; Easton, Maryland, USA, unpublished report no. 236A-125.

Sueta S. (2005) Algal Growth Inhibition Test of MTF-753 with *Pseudokirchneriella subcapitata*. Kurume Laboratory Chemicals Evaluation and Research Institute, Japan, unpublished report no. 93438.

Palmer S.J., Cartee T., Kendall T.Z. and Krueger H.O (2009a) Penthiopyrad: A 96-Hour Toxicity Test with the Freshwater Alga *Pseudokirchneriella subcapitata*. Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 236A-121.

Palmer S.J., Cartee T., Kendall T.Z. and Krueger H.O, (2009b) Penthiopyrad: A 96-Hour Toxicity Test with the marine diatom *Skeletonema costatum*. Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 236A-122.).

Palmer S.J., Cartee T., Kendall T.Z. and Krueger H.O (2009c) Penthiopyrad: A 96-Hour Toxicity Test with the Freshwater Alga *Anabaena flos-aquae*. Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 236A-123.

Palmer S.J., Cartee T.L., Kendall T.Z. and Krueger H.O. (2009d) Penthiopyrad: A 96-Hour Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 236A-124.

Sindermann A.B.; Kendall T.Z. and Krueger H.O. (2008) MTF-753: A 7-Day Toxicity Test with Duckweed (Lemna gibba G3). Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 263A-118.

Dengler D. (2008a) Assessment of Effects of DM-PCA on the growth of the Single Cell Green Alga Desmodesmus subspicatus, using the 96 Hour Static, Limit Test. eurofins-GAB GmbH, Niefern-Öschelbronn, Germany; unpublished report no. 20071562/01-AADs.

Dengler D. (2008a) First amendment to report - Assessment of Effects of DM-PCA on the growth of the Single Cell Green Alga Desmodesmus subspicatus, using the 96 Hour Static, Limit Test. eurofins-GAB GmbH, Niefern-Öschelbronn, Germany; unpublished report no. 20071562/01-AADs.

Peither A. (2008b) PCA: Toxicity of *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 96-Hour Algal Growth Inhibition Test. RCC Ltd., Itingen, CH 4452, Switzerland; unpublished report no. B57418.

Oishi N. (2008c) Growth Inhibition Study of PAM in a Green Alga. Biosafety Research Center, Foods, Drugs and Pesticides 582-2, Shioshinden, Iwata, Shizuoka 437-1213, Japan, unpublished report no. B132 (077-077).

Palmer S.J., Cartee T., Kendall T.Z. and Krueger H.O. (2009e) 753-A-OH: A 96-Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*). Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 236A-130.

Palmer S.J., Cartee T., Kendall T.Z. and Krueger H.O (2009f) 753-T-DO: A 96-Hour Toxicity Test with the Freshwater alga *Pseudokirchneriella subcapitata,* Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 236A-127.

Palmer S.J, Kendall T.Z and Krueger H.O (2007d) MTF-753: A 96-Hour Static-Renewal Acute Toxicity Test with the Saltwater Mysid (*Americamysis bahia*). Wildlife International, Ltd. Maryland, USA, unpublished report no. 236A-115.

Palmer S.J., Kendall T.Z. and Krueger H.O. (2008) MTF-753: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*). Wildlife International, Ltd., Easton, Maryland, USA, unpublished report no. 236A-117.

Völkel, W. (2007): ^{14}C-MTF-753: Adsorption/desorption on Soils. RCC Ltd., Itingen, Switzerland; unpublished report no. A59556

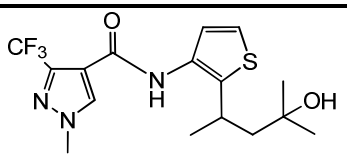
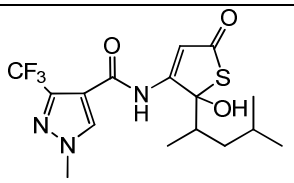
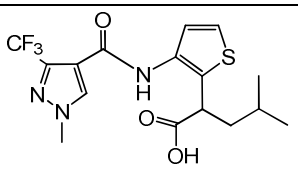
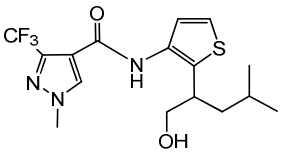
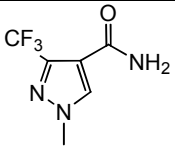
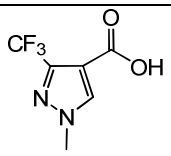
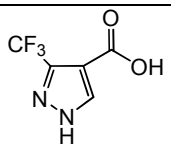
Environment Agency (unpublished) Bioaccumulation of chemicals in fish: Investigation of the dependence of depuration rate constant on lipid content of fish.

ANNEXES

ANNEX 1 - Degradant code, chemical name and structure.

ANNEX 2 – Aquatic toxicity data for penthiopyrad degradants.

ANNEX 1 – Degradant code, chemical name and structure.

Code name	Chemical name	Structural formula
753-A-OH	*N*-[2-(3-hydroxy-1,3-dimethyl-butyl) thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide	
753-T-DO	*N*-[5-hydroxy-5-(1,3-dimethylbutyl)-2-oxo-2,5-dihydrothiophen-4-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide	
M9	Not characterized	Not characterized
M11	3-methyl-1-{3-[(1-methyl-3-trifluoromethyl-1H-pyrazole-4-carbonyl)amino]thiophen-2-yl}pentanoic acid	
M12 (753-A-OH isomer)	N-[2-(1-hydroxymethyl-1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide	
PAM	1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide	
PCA	1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid	
DM-PCA	3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid	

ANNEX 2 – Aquatic toxicity data for penthiopyrad degradants.

Table 1: Summary of relevant information on aquatic toxicity for penthiopyrad degradants

Degradant / Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
DM-PCA (99.87%) Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>99.2 (mm)	Mitsui Chemicals Agro, Inc, 2008a
PCA (99.1%) Acute toxicity to fish OECD Guideline 203, GLP	Zebra fish (*Brachydanio* rerio)	Mortality	Static	96 hours	LC_{50}	>96.7 (mm)	Mitsui Chemicals Agro, Inc, 2008a
PAM (100%) Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Semi-tatic	96 hours	LC_{50}	>100.3 (mm)	Mitsui Chemicals Agro, Inc, 2008a
753-A-OH (98.91%) Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>9.3 (mm)	Mitsui Chemicals Agro, Inc, 2009b
753-T-DO (99.84%) Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>4.11 (mm)	Mitsui Chemicals Agro, Inc, 2009c
DM-PCA (99.87%) *Daphnia* sp Acute Immobilisation OECD Guideline, 202 GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>99.4 (mm)	Borrmann, 2008b
PCA (99.1%) *Daphnia* sp Acute Immobilisation OECD Guideline, 202 GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>91.8 (mm)	Schmidt, 2008
PAM (100%) *Daphnia* sp Acute Immobilisation OECD Guideline, 202 GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>100.5 (mm)	Oishi, 2008b
753-A-OH (98.91%) *Daphnia* sp Acute Immobilisation OECD Guideline, 202 GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>8.7 (mm)	Minderhout *et al*, 2009d
753-T-DO (99.84%) *Daphnia* sp Acute Immobilisation OECD Guideline, 202 GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>4.48 (mm)	Minderhout *et al*, 2009e
DM-PCA (99.87%) Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Desmodesmus subspicatus*	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>85.5 (mm) 85.5 (mm)	Dengler, 2008a

PCA (99.1%) Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>94.1 (mm) 48.9 (mm)	Peither, 2008b
PAM (100%) Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>100.4 (mm) 100.4 (mm)	Oishi, 2008c
753-A-OH (98.91%) Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>8.43 (mm) 8.43 (mm)	Palmer *et al*, 2009e
753-T-DO (99.84%) Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>0.871 (mm) 0.627 (mm)	Palmer *et al*, 2009f

Notes:
mm refers to mean measured
*formerly *Selenastrum capricornutum*