CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

5-Chloro-2-methoxy-4-methyl-3-pyridyl)(4,5,6-trimethoxy-*o*-tolyl)methanone (Pyriofenone)

EC Number:

CAS Number: **688046-61-9**

Index Number:

Contact details for dossier submitter: **UK Competent Authority**
Chemicals Regulation Division
Health and Safety Executive
United Kingdom

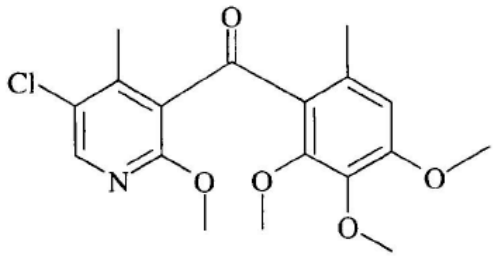
Version number: 2 **Date: February 2018**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*IUPAC:5-Chloro-2-methoxy-4-methyl-3-pyridyl)(4,5,6-trimethoxy-o-tolyl)methanone* *CA: Methanone, (5-chloro-2-methoxy-4-methyl-3-pyridinyl)(2,3,4-trimethoxy-6-methylphenyl)*
Other names (usual name, trade name, abbreviation)	Pyriofenone, IKF-309
ISO common name (if available and appropriate)	*Pyriofenone*
EC number (if available and appropriate)	Not assigned
EC name (if available and appropriate)	
CAS number (if available)	688046-61-9
Other identity code (if available)	
Molecular formula	$C_{18}H_{20}NO_5Cl$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	365.8
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	*Minimum purity: 96.5 %* *Maximum purity: 98.9 %*

1.2 Composition of the substance

Pyriofenone includes no isomers or additives. A number of confidential impurities are present; however none of these are relevant for the classification of the substance.

Table 2: Constituants (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Pyriofenone (CAS: 688046-61-9)	96.5 – 98.9 %	Not listed	Aquatic chronic 2 (H411 – toxic to aquatic life with long lasting effects)

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	-	*5-Chloro-2-methoxy-4-methyl-3-pyridyl)(4,5,6-trimethoxy-o-tolyl)methanone; pyriofenone*	-	688046-61-9	Carc. 2 Aquatic Chronic 1	H351 H410	GSH08 GSH09 Wng	H351 H410	-	M = 1	-
Resulting Annex VI entry if agreed by RAC and COM	-	*5-Chloro-2-methoxy-4-methyl-3-pyridyl)(4,5,6-trimethoxy-o-tolyl)methanone; pyriofenone*	-	688046-61-9	Carc. 2 Aquatic Chronic 1	H351 H410	GSH08 GSH09 Wng	H351 H410	-	M =1	-

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	Yes
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Data conclusive but not sufficient for classification	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	**Carc. 2; H351**	**Yes**
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic	**Aquatic chronic 1; H410**	**Yes**

Hazard class	Reason for no classification	Within the scope of public consultation
environment		
Hazardous to the ozone layer	Hazard class not assessed in this dossier. Not applicable as pyriofenone is not listed in Annex I to Regulation (EC) No. 1005/2009 (recognising the Montréal Protocol) and no Ozone Depleting Potential (ODP) is reported.	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Pyriofenone is a new pesticide active substance and has been reviewed in accordance with Directive 91/414/EEC with the UK as the Rapporteur Member State (RMS). There is no existing entry on Annex VI of CLP and there have been no previous classification and labelling discussions for this substance. In accordance with Article 36 (2) of the CLP Regulation, pyriofenone should now be considered for harmonised classification and labelling. Therefore, this CLH proposal considers all human health and environmental endpoints with the aim of achieving such.

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

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pyriofenone is a new active substance under Regulation 1107/2009.

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

5 IDENTIFIED USES

Pyriofenone is a fungicide, designed to treat mildew on crops such as wheat, rye, barley, spelt, oats, triticale and at larger concentrations, grapes.

6 DATA SOURCES

Pyriofenone is a new active substance under Regulation 1107/2009 and has not been placed on the market yet. The present evaluation exclusively relies on data submitted in the context of the application for approval as an active substance under Regulation 1107/2009 and any additional studies submitted directly by the applicant.

Draft assessment report (DAR): Volume 3 (B6) Toxicology (2012)

Draft assessment report (DAR): Volume 3 (B2) Physical Chemistry (2012)

Draft assessment report (DAR): Volume 3 (B8) Environmental fate (2012)

Draft assessment report (DAR): Volume 3 (B9) Ecoxtoxicology (2012)

Draft assessment report (DAR): Volume 4 Confidential information (2012)

7 PHYSICOCHEMICAL PROPERTIES

The physico-chemical properties of pyriofenone are summarised below. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.2; Physical and Chemical properties – November 2012.

All studies were conducted to appropriate quality standards and were considered adequate during the peer review.

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Crystalline powder	Turner, B., 2009a	Visual inspection Purity 99.19 % (w/w) DAR (B.2.1.7)
Melting/freezing point	Melting range: 93 – 95 °C	Turner, B., 2009a	EEC Method A1 (Metal block method) GLP Purity 99.19 % DAR (B.2.1.1)
Boiling point	Decomposes at temperatures > 100 °C	(Turner, 2009a)	EEC Method A2 (Siwoloboff method) GLP Purity 99.19 % DAR (B.2.1.2)
Relative density	1.33	(Turner, 2009a)	EEC Method A3 (Pycnometer) GLP Purity 99.19 % DAR (B.2.1.4)
Vapour pressure	1.9×10^{-6} Pa at 25 °C	(Turner, 2009b)	OECD 104/EEC Method A4 Vapour pressure balance GLP Purity 99.19 % DAR (B.2.1.5)
Surface tension	72.0 mN/m at 20 °C Not surface active	(Turner, 2009a)	EEC Method A5 90 % Saturated aqueous solution used with a surface tension torsion balance GLP Purity 99.19 % DAR (B.2.1.24)
Water solubility	1.56 mg/L at 20 °C and pH 6.6 Slightly soluble	(Turner, 2007)	OECD 105/EEC Method A6 (Column elution method) GLP Purity 99.19 % DAR (B.2.1.11) Not tested at pH range 4-10 as applicant asserts that pyriofenone is incapable of undergoing ionisation in this range, therefore water solubility would not be expected to be pH-dependent.
Partition coefficient n-octanol/water	$Log_{10}P_{ow}$ = 3.2 at 20 °C and pH 7.2-7.5	(Turner, 2009g)	OECD 107

Property	Value	Reference	Comment (e.g. measured or estimated)
	Potential for bioaccumulation		GLP Purity 99.19 % DAR (B.2.1.13) Not tested at pH range 4-10 as applicant asserts that pyriofenone is incapable of undergoing ionisation in this range, therefore LogP would not be expected to be pH-dependent.
Flash point	Not tested or required as the melting point is above 40 °C		DAR (B.2.1.21)
Flammability	Not highly flammable. In a preliminary test, pyriofenone burned locally with a yellow flame which extinguished 2 seconds after removal of the heat source.	(Turner, 2009c)	EEC Method A10 – flammability GLP Purity 97.88 % DAR (B.2.1.20)
Explosive properties	Not explosive	(Turner, 2009c)	EEC Method A14 (Koenen test apparatus used) GLP Purity 97.88 % DAR (B.2.1.22)
Self-ignition temperature	Autoflammability 378 °C	(Turner, 2009c)	EEC Method A15 GLP Purity 97.88 % DAR (B.2.1.20)
Oxidising properties	Not oxidising. Burn rates 2:1, 1:1, 1:2 mixtures of pyriofenone: cellulose were significantly less than for barium nitrate/cellulose reference mixtures.	(Turner, 2009c)	EEC Method A17 GLP Purity 97.88 % DAR (B.2.1.23)
Dissociation constant	The applicant asserts that pyriofenone does not possess a dissociation constant in the pH range 4-10. Spectrophotometric evidence supplies indicates little variation in the pH range 1-12.9.	(Turner, 2009h)	OECD 112 GLP Purity 99.19 % DAR (B.2.1.18)

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 6: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC Method A14 (explosive properties)	Not explosive	GLP	(Turner, 2009c)

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

Pyriofenone was tested for explosive properties using EEC Method A14 and was found not to be explosive. Further, experience in handling and use indicates that it is not a pyrophoric solid and does not emit flammable gas on contact with water.

8.1.2 Comparison with the CLP criteria

Pyriofenone did not meet the criteria for classification as an explosive substance.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – conclusive but not sufficient for classification

8.2 Self-heating substances

Table 7: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC Method A15 (autoflammability)	Autoflammability: 378 °C	GLP	(Turner, 2009c)

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

Pyriofenone was assessed for auto-flammability using EEC Method A15. The result showed no self-ignition up to the temperature of the melting point.

8.2.2 Comparison with the CLP criteria

Pyriofenone did not meet the criteria for classification as a self-heating substance.

8.2.3 Conclusion on classification and labelling for self-heating substances

Not classified – conclusive but not sufficient for classification

8.3 Oxidising solids

Table 8: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC Method A17 (oxidising properties)	Not oxidising	GLP	(Turner, 2009c)

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

Pyriofenone was tested for oxidising properties using a reference mixture of cellulose and barium nitrate. Burn rates for mixtures of pyriofenone: cellulose were found to be significantly less than the reference mixture. Therefore, pyriofenone is not oxidising.

8.3.2 Comparison with the CLP criteria

Pyriofenone does not meet the criteria for classification as an oxidising solid.

8.3.3 Conclusion on classification and labelling for oxidising solids

Not classified – conclusive but not sufficient for classification

8.4 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC Method A10 (flammability)	Not highly flammable	GLP	(Turner, 2009c)

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

In a standard study (EEC Method A10), a preliminary test showed that pyriofenone burned locally with a yellow flame which extinguished two seconds after removal of the heat source. Therefore, pyriofenone does not meet the classification criteria for classification as a flammable solid.

8.4.2 Comparison with the CLP criteria

Pyriofenone does not meet the criteria for classification as a flammable solid.

8.4.3 Conclusion on classification and labelling for flammable solids

Not classified – conclusive but not sufficient for classification

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 10: Summary table of toxicokinetic studies

Method	Results	Reference
Metabolism in rats EU88/302/EEC GLP	**Absorption:** 76 – 89 % following the low dose and 36-53 % following the high dose (saturation of absorption processes)	DAR: B.6.1.1 and B.6.1.2

Method	Results	Reference
Fischer 4-9/sex/dose (depending on the investigation) ^{14}C-(phenyl)-pyriofenone and ^{14}C-(pyridyl)-pyriofenone Single dose: 5 mg/kg bw or 200 mg/kg bw Repeat dose: 5 mg/kg bw/day, 14 days	**Distribution:** Widespread distribution in tissues after single and repeated dosing, declining with time. The concentration of pyriofenone in tissues was higher in males than females (generally 2-4 times higher). The pattern of distribution following repeated dosing was similar to that after a single dose but levels were 2-10 times higher suggestive of accumulation. The organs with the highest concentration of pyriofenone were the liver, kidney, whole blood and plasma. **Metabolism:** Metabolic pathway: After single and repeated dosing, demethylation of the methoxy groups at the 3- and 4-positions of the benzene ring to give two mono-hydroxy products and a di-hydroxy product. All three metabolites undergo glucuronide conjugation. In males – no individual metabolites accounted for > 3 % of the dose. In females – one urinary metabolite was present at 9.5 % of the dose; this was the glucuronide of the di-hydroxy metabolite. There were no unidentified metabolites at > 5 % of the dose. Evidence of enterohepatic recirculation **Excretion:** ***Following single dosing:*** 87-97 % eliminated in urine and faeces within 48 h (mainly via the faeces) ***Following repeated dosing:*** > 90 % of the dose was eliminated within 24 h after the final dose (mainly via the faeces) **Toxicokinetics:** ***Following single dosing:*** The AUC_{120} and $T_{1/2}$ were lower in females than males $T_{1/2}$ was shorter for plasma than red blood cells ***Following repeated dosing:*** The T_{max} and AUC_{120} were increased in males but largely unchanged in females. $T_{1/2}$ was shorter for plasma than red blood cells Whole blood: Plasma ratio was increased – increased distribution of radioactivity into red blood cells.	(Anon, 2009)

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

The toxicokinetics of pyriofenone has been investigated in male and female Fischer rats (4-9/sex/group) using ^{14}C-(phenyl)-pyriofenone and ^{14}C-(pyridyl)-pyriofenone using single oral doses of 5 mg/kg bw and 200 mg/kg bw and after repeated dosing for 14-days with 5 mg/kg bw.

Absorption

Pyriofenone was found to be rapidly absorbed following low dose administration (5 mg/kg bw) with approximately 76-89 % absorbed. Following administration with the higher dose of pyriofenone (200 mg/kg bw), the absorption processes appeared to become saturated with approximately 50 % less being absorbed (36-53 %).

Distribution

Distribution of pyriofenone in the tissues of rats was widespread in both sexes. In general, the concentrations of radioactivity in the tissues of males was higher than in females (2-4 times) and the majority of tissues concentrations declined with time. The highest concentrations of pyriofenone were found in the liver, kidneys, whole blood and abdominal fat.

Following repeated dosing, there was some evidence of accumulation with higher tissues concentrations of radioactivity than after a single dose. In all dose groups, tissue levels were low at 120 hours post dosing.

Metabolism

Pyriofenone was found to be extensively metabolised and the pathway involved de-methylation of the two methoxy groups in the 3- and 4-positions of the benzene ring. This gave rise to the 3-hydroxy, 4-hydroxy and 3,4-dihydroxy analogues. These three metabolites could then undergo conjugation to glucuronide conjugates. In females, the 3,4 dihydroxy glucuronide was the major metabolite found in urine (9.5 % of the dose) but in males, not one metabolite was present at levels > 3 % of the dose.

Excretion

Following a single dose of pyriofenone, 87-97 % of the dose was eliminated in rats. This occurred mainly by the faeces. Only minor differences in excretion patterns occurred after repeated dosing.

Administration of pyriofenone at the high dose led to increased rate and extent of exposure to radioactivity in rats when compared to the low dose, however they were not proportionate to size of the dose increase, indicating non-linear kinetics. These increases were greater in males than in females. After repeated dosing, the rate and extent of exposure was increased in the plasma and whole blood of males, but was essentially unchanged in the plasma of females (it was increased in the whole blood). The terminal half-life was longer after repeated dosing, indicating that changes in clearance and/or volume of distribution of radioactivity occurred during repeated dosing. Sequestation into red blood cells was evident after single and repeated dosing as the half-life was shorter for plasma than whole blood and also after a single dose, the whole blood: plasma ratio was higher in males than in females and increased with increasing dose. After repeated dosing, the whole blood: plasma ratio also increased, indicating an increased distribution of radioactivity into the red blood cells.

There were no findings from the toxicokinetic studies that might influence the proposed classification of pyriofenone. In general, pyriofenone was well-absorbed and fully eliminated. There was no evidence of tissue accumulation or the presence of any unidentified or toxic metabolites.

10 EVALUATION OF HEALTH HAZARDS

The human health hazards of pyriofenone are summarised below. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.6; Mammalian Toxicology – November 2012.

Acute toxicity

10.1 Acute toxicity - oral route

Table 11: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study OECD 423 GLP	Rat, strain not specified, 6 females	IKF-309 technical Purity 97.88 %	2000 mg/kg bw in 1 % aq. methyl cellulose (1 % w/v)	> 2000 mg/kg bw No mortalities. No gross internal abnormalities. Abnormal body position in 2/6 females.	DAR: B.6.2.1 (Anon., 2008a)

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

Pyriofenone was tested for acute oral toxicity in one guideline study using rats (strain not specified). Six females received a single limit dose of 2000 mg/kg bw in by oral gavage in aqueous methyl cellulose (1 % w/v) (10 ml/kg bw). There were no mortalities and no gross abnormalities found at necropsy. Clinical signs were limited to the finding of abnormal body position in two rats. The LD_{50} was > 2000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

In order to be classified with acute toxicity category 4 (oral), the lowest category for this endpoint, the LD_{50} must fall between the following range: $300 < LD_{50} \leq 2000$ mg/kg bw. The LD_{50} of pyriofenone for oral toxicity was found to be > 2000 mg/kg bw, therefore it does not require classification for acute toxicity by the oral route.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification – conclusive but not sufficient for classification

10.2 Acute toxicity - dermal route

Table 12: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity study	Rats, strain not specified,	IKF-309 technical (Purity	2000 mg/kg bw in 1 % aq. methyl cellulose (1 % w/v)	> 2000 mg/kg bw No mortalities.	DAR: B.6.2.2 (Anon., 2008b)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402 (1987) GLP	5males and 5 females	97.88 %)	Semi-occlusive 24 h topical application	No gross internal abnormalities. Slight erythema observed in all animals, resolved by day 7, scabbing in 1 female on Day 7 and 1 female from Day 4 – 14.	

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

One guideline study to assess acute toxicity via the dermal route is available. Male and female rats (strain not specified) received a single semi-occluded topical application of pyriofenone (2000 mg/kg bw) in aqueous methyl cellulose (1 % w/v) for 24 h. There were no mortalities in this study and no internal findings at necropsy. Clinical signs were limited to slight erythema in all animals and scabbing in one female on Day 7 and in another from Day 4 – 14. The LD_{50} was > 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

In order to be classified with acute toxicity category 4 (dermal), the LD_{50} should be between $1000 < LD_{50} \leq 2000$ mg/kg bw. The results from the guideline study in rats showed that the LD_{50} for acute dermal toxicity was > 2000 mg/kg bw, therefore, classification for this endpoint is not required.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification - conclusive but not sufficient for classification.

10.3 Acute toxicity - inhalation route

Table 13: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity study OECD 403 (1981) GLP	Rat, Sprague Dawley, 5 males and 5 females	IKF-309 technical (purity 97.88 %) Aerosol MMAD 3.9 µm, GSD 2.25	5.18 mg/l for 4 h (nose only) No mortalities No gross internal abnormalities No significant clinical findings	> 5.18 mg/L	DAR: B.6.2.3 (Anon., 2008)

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

There is one guideline acute inhalation toxicity study available, carried out in Sprague Dawley rats. Rats of both sex were exposed to an aerosol of pyriofenone for four hours (nose only) to an analysed concentration of 5.18 mg/l. The MMAD was 3.9 µm and a GSD of 2.25 with at least 40 % of the particles in the respirable range. Clear nasal discharge was observed in 3 rats only immediately following exposure, however, all rats appeared active and healthy during the course of the study. No rats died and there were no macroscopic findings at necropsy. The LC_{50} was > 5.18 mg/l.

10.3.2 Comparison with the CLP criteria

In order to be classified with acute toxicity category 4 (inhalation), the LC_{50} should lie between $1.0 < LC_{50} \leq 5.0$ mg/l (dusts and mists). As the LC_{50} for pyriofenone was > 5.18 mg/l there is no requirement to classify for acute inhalation toxicity.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification - conclusive but not sufficient for classification.

10.4 Skin corrosion/irritation

Table 14: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Skin irritation study OECD 404 (2002) GLP	Rabbits, New Zealand White, 3 females	IKF-309 technical Purity 97.88 %	0.5 g, 4 h, semi-occlusive	No mortalities and no treatment-related signs. No dermal reactions in any animals	DAR: B.6.2.4 (Anon., 2008c)

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

In a single skin irritancy study, pyriofenone was applied to the clipped intact dorso-lumbar skin of three female New Zealand White rabbits for four hours, under a semi-occlusive dressing. There were no mortalities and no treatment-related clinical signs. No dermal reactions were observed in any rabbit, with a score of 0 across all timepoints.

10.4.2 Comparison with the CLP criteria

In a guideline skin irritation study with pyriofenone, there was no evidence of skin corrosion. In order to be classified with skin irritation, pyriofenone would be expected to cause erythema or oedema in at least two out of three rabbits with mean value of ≥ 2.3 - ≤ 4.0 from gradings at 24, 48 and 72 hours after patch removal. In all animals, at all timepoints, the gradings were 0 and therefore, pyriofenone does not meet the criteria for classification with skin irritation.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification - conclusive but not sufficient for classification.

10.5 Serious eye damage/eye irritation

Table 15: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Eye irritation study OECD 405 (2002) GLP	Rabbits, New Zealand White, 3 females	IKF-309 technical Purity 97.88 %	0.1 ml	No mortality or clinical signs Mean individual animal scores at 24,48 and 72 h: Corneal opacity: 0, 0, 0 Iritis; 0, 0, 0 Conjunctival redness: 0, 0.3, 0 Conjunctival erythema: 0, 0, 0 All effects were fully reversible by 48 h.	DAR: B.6.2.5 (Anon., 2008d)

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

There is one eye irritation study carried out with pyriofenone in rabbits. Three female New Zealand White rabbits each had pyriofenone (0.1 ml) instilled into the ocular sac of their right eye. The left eye remained untreated and acted as a control. Ocular reactions were assessed at 1, 24, 48 and 72 hours after treatment. There were no effects on the cornea or iris but slight irritation to the conjunctiva was observed. Redness of the conjunctiva was observed in 3/3 animals 1h post installation (grade 1) and in 1/3 animals at 24 h post-installation (grade 1). The redness disappeared in all animals by 48 h after application. Conjunctival chemosis was observed in 2/3 animals (grade 1) at 1 h after application but this was shown to be fully reversible within 24 h.

10.5.2 Comparison with the CLP criteria

In a guideline eye irritation study in rabbits there were no evidence of any serious eye damage. There was some mild irritation to the conjunctiva of all rabbits (maximum grading of 1), however the mean score of this over 24 – 72 hours was less than 1 and was reversible within 48 h. According to the classification criteria, for a substance that has the potential to cause irreversible eye irritation, classification in category 2 is required when the substance produces:

At least in 2/3 tested animals, a positive response of:

- Corneal opacity ≥ 1 and/or
- Iritis ≥ 1, and/or
- Conjunctival redness ≥ 2 and/or
- Conjunctival oedema ≥ 2

Therefore pyriofenone does not meet the criteria for classification with eye irritation and no classification is required.

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

No classification - conclusive but not sufficient for classification.

10.6 Respiratory sensitisation

No data are available on this endpoint.

10.7 Skin sensitisation

Table 16: Summary table of animal studies on skin sensitisation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Local Lymph Node Assay
OECD 429
GLP</td><td>Mice, CBA/JNCrlj, 5 females/group</td><td>IKF-309 technical
Purity 97.88 %
Positive control: α-hexylcinnamaldehyde</td><td>0, 5, 10 or 25 % (w/v)</td><td>No differences in stimulation index in any treatment groups:
<table>
<tr><th>Test material concentration (% w/v)</th><th>Lymph node weight (mg)</th><th>Test/control ratio (SI)</th></tr>
<tr><td>Vehicle control</td><td>4.6</td><td>1</td></tr>
<tr><td>Positive control</td><td>11.8</td><td>7.74</td></tr>
<tr><td>5</td><td>4.9</td><td>0.78</td></tr>
<tr><td>10</td><td>4.7</td><td>1.04</td></tr>
<tr><td>25</td><td>5</td><td>0.57</td></tr>
</table></td><td>DAR: B.6.2.6
(Anon., 2009a)</td></tr>
</table>

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

Pyriofenone was tested for skin sensitisation in a Local Lymph Node Assay (LLNA) using CBA mice. In this study, five females/group were given three consecutive daily topical applications of pyriofenone to the dorsal surface of both ears. The doses used were selected from a preliminary study and were 0, 5, 10 or 25 % w/v suspended in acetone/olive oil. The results showed there were no significant differences between the groups treated with pyriofenone and the vehicle control. The SI value was < 3 in all cases. Based on this study, pyriofenone is not a skin sensitiser.

10.7.2 Comparison with the CLP criteria

In a guideline LLNA, pyriofenone showed no evidence of skin sensisation in mice when tested up to a concentration of 25 % w/v. Therefore, it does not meet the criteria for classification for this endpoint.

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification - conclusive but not sufficient for classification.

10.8 Germ cell mutagenicity

Table 17: Summary table of mutagenicity/genotoxicity tests in vitro

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance,</th><th>Relevant information about the study including rationale for dose selection (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td>Bacterial reverse mutation test
OECD 471
GLP</td><td>IKF-309 technical
Purity 97.88 %</td><td>Test concentrations: 0, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate
± S9
Plate 1 – standard plate incorporation assay
Plate 2 – pre-incubation stage included
Strains tested:
S. typhimurium TA1535, TA1537, TA98 and TA100
E. coli WP2uvrA</td><td>Negative
No evidence of mutagenicity ±S9
No evidence of cytotoxicity
Precipitation was observed in both plates at 1500 µg and above</td><td>DAR: B.6.4.1a
(May, K. 2007</td></tr>
<tr><td>Mammalian chromosome aberration test in CHL cells
OECD 473
GLP
Study conducted in 2007</td><td>IKF-309 technical
Purity 97.88 %</td><td>Solubility in DMSO was assessed in a preliminary test (max solubility 2542 µg/ml). This was used as the maximum concentration in test 1 (initial test), however toxicity occurring ± S9 meant that the concentrations tested were reduced in test 1 (repeat test) and test 2.
Test concentrations:
Preliminary toxicity test: 18.86 – 2542</td><td>Positive
Preliminary toxicity test
Cytotoxicity at concentrations ≥ 79.44 µg/ml (-S9) and ≥ 158.88 µg/ml (+S9)
Test1 :
Metaphase analysis data (duplicate cultures)
No test-related effects on chromosome aberration frequency observed in the presence of S9. Results from the study without S9 are shown below:
<table>
<tr><td colspan="4">Without S9 (3 h exposure + 12 h recovery)</td></tr>
<tr><td></td><td colspan="2">Aberrant cells No. (% mean)</td><td>Relative mitotic index (%)</td></tr>
<tr><td>Concentration (µg/ml)</td><td>Excluding gaps</td><td>Including gaps</td><td></td></tr>
<tr><td>0</td><td>1,1 (1)</td><td>3,3 (3)</td><td>100</td></tr>
<tr><td>60</td><td>2,3 (2.5)</td><td>3, 5 (4)</td><td>65</td></tr>
<tr><td>65</td><td>4, 5 (4.5)</td><td>6,5 (5.5)</td><td>68</td></tr>
</table></td><td>DAR: B.6.4.1b
(Pritchard, L. 2008)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance,</th><th>Relevant information about the study including rationale for dose selection (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td>µg/ml
Test 1: 0, 60, 65 and 70 µg/ml (-S9)
0, 90, 110 and 120 µg/ml (+S9)
Test 2: 0, 20, 30 and 40 µg/ml (-S9)
0, 100, 110 and 130 µg/ml (+S9)
Test 1 – treatment for 3 h followed by 12 h recovery period (± S9)
Test 2 – treatment for 15 h (no recovery) (-S9), treatment for 3 h followed by a 12 h recovery period (+S9)
Positive controls: Mitomycin C or cyclophosphamaide</td><td>
<table>
<tr><td>70</td><td>6,5 (5.5)**</td><td>6, 8 (7)</td><td>63</td></tr>
<tr><td>MTC (0.1 µg/ml)</td><td>24, 21 (22.5)***</td><td>30, 27 (28.5)***</td><td>47</td></tr>
</table>
HCD (excluding gaps): range 1.0 – 2.75, mean 1.9 (0.6 %) [Oct 2005-2007 studies]
Test 2:
Metaphase analysis data (duplicate cultures)
No test-related effects on chromosome aberration frequency observed in the presence of S9. Results from the study without S9 are shown below:
<table>
<tr><td colspan="4">Without S9 (15 h continuous exposure)</td></tr>
<tr><td></td><td colspan="2">Aberrant cells No. (% mean)</td><td>Relative mitotic index (%)</td></tr>
<tr><td>Concentration (µg/ml)</td><td>Excluding gaps</td><td>Including gaps</td><td></td></tr>
<tr><td>0</td><td>2, 2 (2.0)</td><td>4, 5 (4.5)</td><td>100</td></tr>
<tr><td>20</td><td>3, 3 (3.0)</td><td>3, 4 (3.5)</td><td>87</td></tr>
<tr><td>30</td><td>3, 3 (3.0)</td><td>4, 6 (5.0)</td><td>49</td></tr>
<tr><td>40</td><td>1, 3 (2.0)</td><td>10, 8 (9.0)</td><td>54</td></tr>
<tr><td>MTC (0.1 µg/ml)</td><td>32, 37 (34.5)***</td><td>37, 39 (38.0)***</td><td>96</td></tr>
</table>
HCD (including gaps): range 2.5 – 4.5, mean 3.8 (0.6 %) [Oct 2005-2007 studies]</td><td></td></tr>
<tr><td>In vitro mutagen test using mouse lymphoma L5178Y cells
OECD 476
GLP</td><td>IKF-309 technical
Purity 97.88 %</td><td>Solubility in DMSO was assessed in a preliminary test (max solubility 1271 µg/ml). This was used as the maximum concentration in a preliminary test to determine toxicity. The concentrations used in the main tests (test 1 and 2) were based on</td><td>Negative
No evidence of mutagenicity ±S9 with test substance but appropriate results with the positive controls were observed.</td><td>DAR: B.6.4.1c
(Hynes, L. 2008)</td></tr>
</table>

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		the results of the preliminary study. Test 1 – cell exposure for 3 h at concentrations of: 0, 19.86, 39.72, 79.44, 58.88, 317.75, 635.5 and 1271 µg/ml (± S9) Test 2 – cell exposure for 24 h at concentrations of: 0, 20, 40, 50, 60, 70 and 80 µg/ml (± S9) Positive controls: Methanesulphonate (MMS) (-S9) Benzo(a)pyrene (BaP) (+S9)		

Table 18: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mouse micronucleus test OECD 474 GLP	IKF-309 technical Purity 97.88 %	Mice, CD-1, (5/sex/dose) Oral gavage 0, 500, 1000 or 2000 mg/kg bw in aq. Methyl cellulose (1 % w/v) Positive control: Mitomycin C (12 mg/kg bw)	**Negative:** - No increase in the number of micronucleated polychromatic erythrocytes - No increase in the incidence of normochromatic erythrocytes - No significant decreases in the proportion of polychromatic erythrocytes - No bone marrow cell toxicity	DAR: B.6.4.2a (Anon., 2008e)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			No mortalities or clinical signs.	
Unscheduled DNA synthesis (UDS) test OECD 486 GLP	IKF-309 technical Purity 97.88 %	Rats, Sprague Dawley, (3 males/dose) Oral gavage 0, 500, 1000 or 2000 mg/kg bw in aq. methyl cellulose (1 % w/v) Positive controls: Dimethylnitroamine (DMN) (10 mg/kg bw) or 2-acetylaminofluorene (50 mg/kg bw) Hepatocytes were isolated from livers at 2 and 16 h	**Negative:** - No induction of UDS in rat hepatocytes No mortalities or clinical signs.	DAR: B.6.4.2b (Anon., 2010)
Comet assay in rats OECD 489 GLP	IKF-309 technical Purity 97.88 %	Rats, Fischer (5 males/dose for pyriofenone and negative controls, 3 males/positive control) Oral gavage 0, 500, 1000 or 2000 mg/kg bw in aq. methyl cellulose (0.5 % w/v) Positive control: ethyl methanesulphonate (EMS) (200 mg/kg bw) The liver only was investigated.	**Negative:** - There was no induction of DNA damage in liver cells of rats.	Study report submitted to CA 2017 (Anon., 2017)
Comet assay in mice Non-guideline Non-GLP	IKF-309 technical Purity 97.88 %	Mice, CD-1, (5/sex/dose) Oral gavage 0, 500, 1000 or 2000 mg/kg bw/day suspended in corn oil Positive control: ethyl methanesulphonate (EMS) (200 mg/kg bw) The liver only was investigated.	**Negative:** - No induction of single strand DNA damage in mouse liver	DAR: B.6.4.2c (Anon., S., 2011)

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

In vitro studies

The potential of pyriofenone to induce gene mutations in bacterial cells and gene mutation or chromosome damage in mammalian cells has been investigated in three well-conducted *in vitro* studies.

In bacteria, pyriofenone gave a clear negative result of mutagenicity in both the presence or absence of metabolic activation. Equally, when pyriofenone was assessed for its mutagenic potential in mouse lymphoma L5178Y cells, there were no increases in mean mutant frequency at the tk locuss in treated cells.

Reproducible, negative results were found when pyriofenone was tested for the potential to induce chromosome aberrations in Chinese Hamster Lung (CHL) cells in the presence of S9. In the absence of S9, when CHL cells were treated for 3 hours up to a concentration of 70 µg/ml, followed by a 12 hour recovery period, the number of aberrant cells both excluding gaps and including gaps increased in a concentration-dependent manner, reaching statistical significance at 70 µg/ml (excluding gaps). Given that mitotic index was reduced by only approximately 35%, this was not considered to have been influenced by the toxicity of the test substance to the cells. . In contrast, in a second test without S9, involving 15 h continuous exposure up to a concentration of 40 µg/ml, there was no increase in the number of aberrant cells (excluding gaps). The top dose produced a 50% redulction in mitotic index and is therefore considered to have been sufficiently high. An increase in aberration frequency including gaps was seen at the top dose, but this finding was not statitstically significant and, given the absence of an effect minus gaps, is considered of uncertain biological relevance. There is no explanation for the different results seen without S9 for the 3 h and 15 treatment periods, respectively; the positive result in the first test cannot be dismissed. Consequently, pyriofenone appears to have potential to induce chromosome damage in cultured mammalian cells, specifically in the absence of exogenous metabolic activation.

In vivo studies

Three guideline studies and one non-guideline study have been conducted to assess the potential of pyriofenone to induce chromosomal or DNA damage *in vivo*, in rodents.

In a micronucleus test in male and female CD-1 mice (5/dose), pyriofenone was administered in a single oral dose of 500, 1000 or 2000 mg/kg bw in aqueous methyl cellulose (1 % w/v) by oral gavage. The vehicle served as the negative control and mitomycin C (12 mg/kg bw) as the positive control. The animals were sacrificed 24 or 48 hours after administration, with the bone marrow of the two femora being prepared from each animal of each group at 24 hours and from animals of the vehicle and top dose group at 48 hours. For each animal, 2000 polychromatic erythrocytes were evaluated for the presence of micronuclei.

Administration of pyriofenone did not lead to any biologically relevant increase in the number of polychromatic erythrocytes that contained micronuclei and the rate of micronuclei was close to the concurrent negative control data. The positive-control, mitomycin C, led to the expected increase in the rate of polychromatic erythrocytes that contained micronuclei.

In an *in vivo-in vitro* unscheduled DNA synthesis test in male Sprague Dawley rats (3/dose), pyriofenone was administered at 0 (vehicle control), 500, 1000 or 2000 mg/kg bw in aqueous methyl cellulose (1 % w/v). Positive control groups were administered dimethylnitroamine (DMN) or 2-acetylaminofluorene (2-AAF) at 10 or 50 mg/kg bw, respectively. Hepatocytes were isolated from the livers at 2 and 16 hours post-administration of pyriofenone and were incubated in the presence of ^{3}H-thymidine before evaluation of UDS using autoradioagraphy.

In the pyriofenone-treated animals, there were no increases in net nuclear grains or percentage of cells in repair. Results were all comparable to the negative control data. The positive controls behaved

appropriately. Therefore, under the conditions of this study, pyriofenone did not induce UDS in rat hepatocytes.

Two Comet assays are available. A recent, well-performed guideline test in rats and a non-guideline assay in mice. In both studies, only the liver was investigated.

Fischer rats (5 males/dose) received two doses of pyriofenone (21 h interval) by gavage (0, 500, 1000 and 2000 mg/kg bw). Three hours after the final dose, the rats were sacrificed and the liver was removed. A portion of the left lateral lobe was excised and evaluated for a change in mean % tail DNA. The results of this study were negative with no change in the range of mean % tail DNA any of the treatment groups. The positive control behaved accordingly. Therefore it was concluded that pyriofenone does not induce DNA damage in the liver cells of rats.

A similar study was carried out in mice. Male CD-1 mice (5/dose) were treated with pyriofenone (oral gavage) for 48 h ((0, 500, 1000 or 2000 mg/kg bw/day). Three hours after the final dose, the mice were sacrificed and the liver was removed. The results of the study showed no evidence of single-strand DNA damage in mouse liver following administration of pyriofenone. All % Tail DNA values were comparable to the vehicle control and within the historical control data provided by the laboratory. Pyriofenone does not induce DNA damage in the liver cells of mice under the conditions of this study.

10.8.2 Comparison with the CLP criteria

Pyriofenone has been tested for its potential genotoxic properties in a battery of *in vitro* assays and *in vivo* tests.

The exposure of *S. typhimurium* and *E. coli* tester strains to pyriofenone up to and including the limit concentration of 5000 µg/plate did not produce an increased number of reversions, either with or without metabolic activation. In an *in vitro* assay for chromosomal aberrations in Chinese Hamster Lung cells there was evidence of an increase in cells containing chromosomal aberrations when incubated in the absence of metabolic activation. There was no evidence of mutagenic potential with pyriofenone in a mouse lymphoma cell mutation assay.

From the available studies it appears that pyriofenone has the ability to damage chromosomes *in vitro*. In accordance with the CLP regulation, positive results from *in vitro* studies alone are not sufficient to classify for germ cell mutagenicity. As there was no evidence of mutagenicity in a micronucleus test in mice, an unscheduled DNA synthesis test in rats or in Comet assays in rats and mice, pyriofenone does not meet the requirements for classification for germ cell mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification - conclusive but not sufficient for classification.

10.9 Carcinogenicity

Table 19: Summary table of animal studies on carcinogenicity

↑↓ denote an increase or decrease in a parameter with respect to the control value
Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$
abs. = absolute
rel. = relative

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
OECD 451 GLP Rat, Fischer F344, 50/sex/dose Purity 97.88 % (w/w) Year of study: 2007 DAR: B.6.5.1b (Anon., 2010d)	0, 200, 1000 or 5000 ppm for 104 weeks Equivalent to: ♂ 7.25, 36.4 and 197 mg/kg bw/day ♀ 9.13, 46.5 and 254 mg/kg bw/day	Data includes all animals from the terminal kill and those found dead or sacrificed in extremis. **Non-neoplastic findings** **<u>5000 ppm (197/254 mg/kg bw/day):</u>** ***Observations:*** ↑ Cumulative mortality in males in weeks 101 (14 %)* and 104 (17 %)* ↓ Body weight in females week 104 (13 %)** ***Organ weights:*** ↑ Liver, males 30 %** (rel.) and females 13 %* (abs.) and 32 %** (rel.) ↑Kidneys, males 18 %** (rel.) and 39 %** (abs.) and females 36 %** (rel.) ↑Adrenals, males 46 %* (rel.) ↑Caecum, males 1.5 fold** (abs.) and 1.8 fold** (rel.) and females 2.1 fold** (abs.) and 2.5 fold** (rel.) ***Histopathology:*** **Liver** Necrosis: 8/50** males versus 0 in controls Fatty change: 23/50** males and 33/50** females versus 2/50 and 7/50 in controls (males and females respectively) Hypertrophy: 34/50** males and 37/50** females versus 0 in controls Focal congestion: 13/50** females versus 1/50 in controls **Kidneys** Coarse surface: 12/50* males versus 4/50 in controls Chronic nephropathy: 45/50** females versus 17/50 in controls **Large intestines** Caecum, distension: 15/50** males, 14/50** females versus 0/50 in controls Black contents: 5/50 males* versus 0/50 in controls **Testis** Atrophy: 15/50 males versus 9/50 in controls **Skin** Loss of fur, 8/50* males and 35/50** females versus 2/50 and 12/50 in controls (males

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th></tr>
<tr><td></td><td></td><td>
and females respectively)
Atrophy of hair follicles, 6/50* males versus 0 in controls
Perifolliculitis, 8/50* females versus 2/50 in controls

Lymph node (mesenteric)
Sinus dilation: 17/49** males versus 0 in controls

<u>1000 ppm (36.4/46.5 mg/kg bw/day):</u>
Histopathology:
Kidneys
Chronic nephropathy: 35/50** females versus 17/50 in controls

<u>200 ppm (7.25/9.13 mg/kg bw/day):</u>
No treatment-related findings

Neoplastic findings

Males:
<table>
<tr><td></td><td></td><td colspan="4">Dose (ppm)</td><td>HCD (2007 +/- 5 years)</td></tr>
<tr><td colspan="2">Number of animals affected (percentage)</td><td>0</td><td>200</td><td>1000</td><td>5000</td><td>Untreated F344 male rats</td></tr>
<tr><td>Liver:</td><td>Hepatocellular adenoma</td><td>4 (8 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>6 (12 %)</td><td>0 – 4 %</td></tr>
<tr><td></td><td>Hepatocellular carcinoma</td><td>0</td><td>1 (2 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>0 %</td></tr>
<tr><td></td><td>Combined total</td><td>4 (8 %)</td><td>2 (4 %)</td><td>3 (6 %)</td><td>8 (16 %)</td><td>-</td></tr>
</table>
HCD: Laboratory historical control data on hepatocellular tumour incidence in control male F344 rats 1978 – 2011 was provided by the Applicant. Hepatocellular adenoma incidence ranged from 0 – 12 % and hepatocellular carcinoma ranged from 0 – 4% over the 33 year span. In the 5 years before or after the study year of 2007, the incidence of adenoma was 0 –4 % and there were no incidence of carcinoma in control animals.

No statistical significance was observed.

There were no neoplastic findings in females.
</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
OECD 451 GLP Mouse, CD-1, 52/sex/dose Purity 97.88 % (w/w) Year of study: 2010 DAR: B6.5.2 (Anon., 2010e)	0, 600, 1800 or 5400 ppm (males) 0, 300, 1000 or 3000 ppm (females) for 78 weeks Equivalent to: ♂ 77.6, 237 and 716 mg/kg bw/day ♀ 49.4, 167 and 486 mg/kg bw/day	Data include all animals from the terminal kill and those found dead or sacrificed in extremis. **Non-neoplastic findings** **<u>5400 ppm (716 mg/kg bw/day) (males only):</u>** ***Observations:*** ↑ Incidence of perigenital staining 24/52 versus 7/52 in controls ***Histopathology:*** **Kidneys** Granular: 10/52** versus 1/52 in controls Cortical scarring: 28/52* versus 16/52 in controls Cortical tubular basophilia: 47/52* versus 37/52 in controls **Liver** Masses: 12/52* versus 4/52 in controls Hypertrophy: 12/52** versus 0 in controls Necrosis (individual hepatocytes): 7/52* versus 1/52 in controls **Prostate** Hyperplasia (acinar cells): 5/52* versus 0/51 in controls **<u>3000 ppm (486 mg/kg bw/day) (females only):</u>** ***Observations:*** ↓ Body weight gain week 76 (17 %) ***Histopathology:*** **Liver** Pigment in macrophages: 18/52* versus 8/52 in controls **<u>1800 ppm (237 mg/kg bw/day) (males only):</u>** ***Observations:*** ↑ Incidence of perigenital staining 13/52 versus 7/52 in controls ***Histopathology:*** **Kidneys** Cortical tubular basophilia: 47/52* versus 37/52 in controls

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results

Liver

Masses: 11/52* versus 4/52 in controls

Hypertrophy: 16/52** versus 0 in controls

Necrosis (individual hepatocytes): 8/52* versus 1/52 in controls

1000 ppm (167 mg/kg bw/day) (females only):

No treatment-related findings at this dose and below.

600 ppm (77.6 mg/kg bw/day) (males only):

Histopathology:

Liver

Masses: 10/52 versus 4/52 in controls

Hypertrophy: 13/52** versus 0 in controls

Neoplastic findings

Males:

	Dose ppm (mg/kg bw/day)	0	600 (77.6)	1800 (237)	5400 (716)	HCD range (Sept 1997 - Jun 2007)
Liver	Hepatocellular adenoma	3 (5.8 %)	7 (13.5 %)	6 (11.5 %)	9 (17.3 %)	7.8 - 26 %
	Hepatocellular carcinoma	1 (1.9 %)	2 (3.8 %)	3 (5.8 %)	3 (5.8 %)	0 - 8.0 %
	Combined total	4 [7.7 %]	9 [17.3 %]	9 [17.3 %]	12* [23.1 %]	9.8 - 36 %

The historical control data provided was in the date range September 1997 to June 2007, whilst the study itself was performed from October 2007 – January 2010. A detailed assessment of the data provided by the applicant indicates the percentages of adenoma and carcinoma showed no particular trends over the years. Many of the studies which resulted in percentages towards the higher end of the range fell in the years closest to the current study year.

There were no neoplastic findings in females.

10.9.1 Carcinogenicity study in the rat

In a well-conducted and reliable study (Table 19), Fischer rats (50/sex/dose) were administered pyriofenone in the diet for 104 weeks at doses of 0, 200, 1000 or 5000 ppm [equivalent to 0, 7.25/9.13, 36.4/46.5, 197/254 mg/kg bw/day (males/females)].

The main target organs for non-neoplastic effects were the liver, kidneys and large intestines, with the majority of effects occurring in animals of the top dose group [5000 ppm (197/254 mg/kg bw/day). There were no treatment-related findings at the low dose of 200 ppm (7.25/9.13 mg/kg bw/day).

In males and females, liver weights were increased [males 30 % (relative) and females 32 % (relative) and 13 % (absolute) when compared to controls]. Associated histopathology included an increased incidence in liver necrosis (8/50 males versus 0 in controls), fatty changes (23/50 males versus 2/50 in controls and 33/50 females versus 7/50 in controls), hypertrophy (24/50 males and 37/50 females versus 0 in controls) and focal congestion (13/50 females versus 1/50 in controls).

Kidney weights were also increased in animals of the top dose group [males 18 % (relative) and 39 % (absolute) and females 36 % (relative)]. The surface of the kidneys were found to be coarse in a number of males and chronic nephropathy was more prevalent in females of the top and mid dose groups than in controls.

Caecum weight was increased in males and females (approximately 2-fold when compared to control animals) and distension was observed as well as black contents in some males.

Other findings included a slight increase in the number of males of the top dose group with testicular atrophy, loss of fur in both males and females and sinus dilation of the mesenteric lymph node in males.

The only significant neoplastic findings were observed in the liver of male rats. There was a dose-related increase in the incidence of hepatocellular adenoma (2 %, 4 % and 12 % in the low, mid and high dose groups respectively). However, the biological significance of these findings is uncertain given that hepatocellular adenoma was also seen in 8 % of control males. The incidence of hepatocellular carcinoma was increased marginally at all doses (0, 2%, 2% and 4 % in the control, low, mid and high dose groups respectively). These small increases in the frequencies of adenoma and carcinoma were not found to be statistically significant.

Increased mortality was seen among top dose male rats during the last 3 weeks of this study when compared to all the other dose groups. In week 101, cumulative mortality was 14 %; in the final week 104, cumulative mortality was 17 %. Strictly, this top dose was therefore above the MTD recommended for a carcinogenicity study. However, on the basis of the individual animal data, there was no link seen between increased mortality and the incidence of liver tumours. In the animals found dead before the end of the study, 3/17 (18 %) were found to have liver adenoma and 1/17 (6 %) had carcinoma of the liver. In those surviving to the end of the study, 3/33 (9 %) had adenoma of the liver and 1/33 (3 %) had liver carcinoma.

Historical control data

Historical control data (HCD) were provided from the laboratory where the carcinogenicity study in rats was carried out. This included the incidences of hepatocellular adenoma and carcinoma in control male F344 rats in studies carried out from 1978 – 2011. The incidence ranges of adenoma and carcinoma during this period were 0 – 12 % and 0 – 4 %, respectively. The findings in the concurrent study are within these ranges, however according to CLP, HCD should be contemporary to the study being evaluated (e.g. within a period of up to 5 years of the study) and data older than this should be used with caution and acknowledgement of its lower relevance and reliability. Further, closer analysis of the HCD showed that the majority of the higher incidences of adenoma and carcinoma occurred between the years 1980 and 1986, which indicates that tumour incidences in control animals may have changed with time. Taking this into account, and utilising only the studies within a 5 year time period of the concurrent study, the incidence of adenoma ranged from 0 – 4 % and carcinoma incidence was 0. Thus the finding of adenoma (12 %) at the top dose of

5000 ppm was above the HCD data range. It is noted that the control incidence of 8 % in this study was also above the HCD. The findings of carcinoma in the low, mid and top dose group (2, 2 and 4 %) were also all above the HCD range.

The Applicant provided further examples of HCD for spontaneous hepatocellular adenoma and carcinoma in male F344 rats taken from national databases. These included a paper by the US National Toxicology Program that indicated maximum incidences of adenoma and carcinoma in this strain of male rats of 10% and 6%, respectively (Haseman, et al, 1998) and a report by Charles River showing incidences of hepatocellular adenoma and carcinoma of 4.3% and 3.3%, respectively (Lang, ., 1990). These HCD indicate that spontaneous incidences of adenoma and carcinoma have been shown to occur in males of this strain of rat, however, given the relatively low incidences in this study, the Dossier Submitter believes that more weight should be given to the laboratory control data.

Overall, pyriofenone appears to have produced a weak carcinogenic response in the liver of male rats. No response was seen in female rats. Among males, there were dose-related increases in adenoma, carcinoma and adenoma/carcinoma combined, but the response rates were small and, in the case of the adenomas, the incidence in the control group was greater than the HCD provided, giving less weight to the increase at the top dose. The incidence of carcinoma in the low and mid dose groups was limited to only one animal per dose group, but the incidence of 4 % in the top dose group was above the contemporary laboratory HCD data provided. Overall, the slight dose-response seen in males cannot be dismissed entirely as a chance finding.

10.9.2 Carcinogenicity study in the mouse

In a well-conducted and reliable study, CD-1 mice (52/sex/group) were administered pyriofenone in the diet for 78 weeks (Table 19) at doses of 0, 600, 1800 or 5400 ppm in males and 0, 300, 1000 or 3000 ppm in females [equivalent to 0, 77.6/49.4, 237/167 or 716/486 mg/kg bw/day (males/females)].

Non-neoplastic findings in this study were generally limited to the liver and the kidneys. There were no treatment-related findings at doses of 1000 ppm and below in females.

In males livers were found to have an increase in masses (10/52, 11/52 and 12/52 in the low, mid and high groups respectively versus 4/52 in controls). Hypertrophy was also observed (13/52, 16/52 and 12/52 in the low, mid and high groups respectively versus 0 in controls) and necrosis of individual hepatocytes was seen in an increased number of males (5400 ppm: 7/52 and at 1800 ppm: 8/52 versus 1/52 in controls). The only finding related to the liver in females was an increase in animals with macrophage pigmentation (18/52 versus 8/52 in controls) following a dose of 3000 ppm.

In top dosed males, kidneys were observed to be granular in an increased number of animals and corticol scarring was also seen. There was an increase in cortical tubular basophilia in males of the mid and top dose groups.

There was no increased mortality among mice at the highest dose level. No significant tumour findings were evident in females. In males there was an increase in hepatocellular adenoma incidence at all doses when compared to controls (6%, 13 %, 11 and 17 % in the control, low, mid and high dose groups respectively). However, the relationship to dose was weak: 13 % adenoma incidence at 600 ppm, yet only 17 % at 5400 ppm, an almost 10-fold increase in dose. There was also a small increased incidence of liver cell carcinoma at all doses when compared to controls (2 %, 4 %, 6 %, 6 %) but statistical significance was only reached when combined incidences of adenoma and carcinoma were compared. Furthermore, the incidence rates of these tumours were well within the historical control data (HCD) provided for this laboratory (combined rate: 9.8 – 36 %). The HCD appear to be relevant for this study; they derived from comparable studies carried out between 1997 and 2007. Analysis of the historical data showed no concern about the range of values or any particular trends in percentages of tumours found over the years.

To conclude, small increases in liver adenoma, carcinoma and adenoma/carcinoma combined were observed in male mice only. However, the relationship to dose was also weak and the findings in all treatment groups were within the HCD provided. Therefore, pyriofenone does not appear to be carcinogenic in the mouse.

10.9.3 Mechanistic studies relevant to findings in the liver

A consideration of potential modes of action that could attribute for the weak carcinogenic effect seen in the rat study is provided in Section 10.9.4. In wanting to understand the biological events occurring in the rat liver, the Applicant undertook a series of studies that focused on the possibility of pyriofenone being an activator of the constitutive androstane receptor (CAR). Several non-guideline, non-GLP mechanistic studies were conducted in order to investigate this mode of action. These included an assessment of cytochrome P450 (CYP P450) gene expression and replicative DNA synthesis in isolated rat and human hepatocytes and enzyme induction and cell proliferation in rats and mice. Also available is a mechanistic study comparing CAR-knock-out rats to wild-type rats. The main findings are summarised in Tables 20-23.

Table 20: *In vitro* studies in rat hepatocytes

<table>
<tr><th>Type of study/data</th><th>Relevant information about the study (as applicable)</th><th>Observations</th></tr>
<tr><td>Expression of CYP genes in rat hepatocytes (PCR measurement of mRNA)

Non-guideline
Non-GLP

Taken from a study report submitted directly to the CA:

(Shikama, H., 2013a)</td><td>F344 rat hepatocytes (freshly isolated and pooled from male rats)

Pyriofenone: 1.25, 2.5 and 5 ppm

Positive control: PB 3, 30 and 300 ppm</td><td>Altered gene expression of CYP2B is a marker for activation of CAR whereas altered expression of CYP1A is a marker for activation of the aryl hydrocarbon receptor (AhR)
<table>
<tr><td>Concentration (ppm)</td><td colspan="2">Rat</td></tr>
<tr><td></td><td>CYP2B1</td><td>CYP1A2</td></tr>
<tr><td></td><td colspan="2">Relative quantity of CYP mRNA</td></tr>
<tr><td>Pyriofenone</td><td colspan="2"></td></tr>
<tr><td>Vehicle control (DMSO 0.005 %)</td><td>1</td><td>1</td></tr>
<tr><td>1.25</td><td>1</td><td>2</td></tr>
<tr><td>2.5</td><td>3</td><td>3.5</td></tr>
<tr><td>5</td><td>11</td><td>4.5</td></tr>
<tr><td>PB</td><td colspan="2"></td></tr>
<tr><td>Vehicle control (dH_20)</td><td>1</td><td>1</td></tr>
<tr><td>3</td><td>8</td><td>0.5</td></tr>
<tr><td>30</td><td>9</td><td>0.5</td></tr>
<tr><td>300</td><td>14</td><td>1</td></tr>
</table>
Little study information was given, it was unclear as to how many replicates were performed. No statistical analysis was performed.

There was a concentration-dependent increase of both CYP2B1 and CYP1A2 following exposure to pyriofenone indicating activation of both CAR and AhR.</td></tr>
<tr><td>Effect of pyriofenone on DNA replication in rat hepatocytes (measured by BrdU incorporation)

Non-guideline
Non-GLP</td><td>F344 rat hepatocytes (freshly isolated from male rats, number and age unknown)

Pyriofenone: 5 and 10 ppm

PB 30 and 300 ppm

Positive control: epidermal growth factor (EGF) 25 ng/ml</td><td>Table showing BrdU incorporation into rat hepatocytes (average of three replicates from a single study):
<table>
<tr><td></td><td></td><td>EGF (ng/mL)</td><td colspan="2">PB (ppm)</td><td></td><td colspan="2">Pyriofenone (ppm)</td></tr>
<tr><td></td><td>control</td><td>25</td><td>300</td><td>30</td><td>control</td><td>5</td><td>10</td></tr>
<tr><td>% of Control</td><td>100</td><td>132.4</td><td>125.8</td><td>112.9</td><td>100</td><td>99</td><td>107.2</td></tr>
</table>
No statistics were performed on the results of this study.

Increased DNA replication occurred with EGF and PB (30 and</td></tr>
</table>

Type of study/data	Relevant information about the study (as applicable)	Observations
Taken from a study report submitted directly to the CA: (Shikama, H., 2013b)		300 ppm) A "slight" increase in DNA replication occurred with pyriofenone 10 ppm – however no clear conclusion can be reached.

In vitro studies in rat hepatocytes

A study to investigate the effects of pyriofenone on the expression of the CYP genes CYP2B6 and CYP1A2 in rat hepatocytes was conducted in isolated male F344 rat hepatocytes (Shikama, H., 2013a). A preliminary cytotoxicity test was first carried out to determine appropriate test concentrations. PB caused no cytotoxicity at the highest dose tested (300 ppm); whereas pyriofenone caused no cytotoxicity at concentrations of 12.5 ppm and below. The test concentrations used in the main study were PB: 3, 30 and 300 ppm and pyriofenone: 1.25, 2.5 and 5 ppm. Cells were pooled from an unspecified number of rats and exposed to pyriofenone or PB for 24 h. RNA was extracted and gene expression was measured using PCR.

The results of the study showed that pyriofenone increased levels of CYP2B1 expression genes concentration-dependently from 2.5 ppm and also increased CYP1A2 concentration-dependently from 1.25 ppm. Treatment with the known CAR-activator PB resulted in a concentration-dependent increase of CYP2B1 levels only from 3 ppm. However, the study consisted of only one experiment with only one replicate, and so the results can offer only a preliminary view of the effects of pyriofenone on the expression of CYP2B1 and 1A2 and it was not possible to perform any statistical analysis.

A second study assessed the effect of pyriofenone on DNA replication (a measurement of cell proliferation) in isolated male F344 rat hepatocytes, by measurement of incorporation of the DNA precursor 5-bromo-2'-deoxyuridine (BrdU) (Shikama, H., 2013b). Test concentrations used were PB: 30 and 300 ppm, pyriofenone: 5 and 10 ppm and epidermal growth factor (EGF): 25 ng/mL. Both PB and the positive control, EGF were clearly shown to cause DNA replication. At 10 ppm, pyriofenone showed, what was described by the study authors as, a "slight" increase in DNA replication. This increase of 7 % above the control value compared to the increases of about 25 – 30 % seen with the positive control substances appears to be very small. The result of this study is inconclusive with respect to the potential of pyriofenone to induce increased DNA replication in rat liver. In the absence of any statistical analysis the Dossier Submitter believes the results can only show that pyriofenone *may* induce increased DNA replication in rat liver.

Table 21: *In vivo* studies in rats

↑↓ denote an increase or decrease in a parameter with respect to the control value
Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$
abs. = absolute
rel. = relative

Type of study/data	Relevant information about the study (as applicable)	Observations
Hepatocyte proliferation in rats (Cell proliferation measurement using immunohistochemical staining of histopathology slides for BrdU of duodenum and liver sections. The	Fischer Rats (males/5/dose) Pyriofenone: 0, 200 and 20000 ppm (dietary administration) Equivalent to 0, 15.7/14.4 and 849/1109 mg/kg bw/day (3 day group/7 day group)	**After 3 days:** **<u>Pyriofenone (20000 ppm/849 mg/kg bw/day):</u>** ↑Mean RDS (replicative DNA Synthesis) index 2.98 versus 1.23 in control (no statistical significance) **<u>Pyriofenone (200 ppm/15.7 mg/kg bw/day):</u>** No increase in liver weight and no increase in mean RDS index

Type of study/data	Relevant information about the study (as applicable)	Observations
duodenum sections were used to validate the immunostaining method. Nuclei were stained using haematoxylin and eosin). Non-guideline Non-GLP DAR: B.6.8.3b (Anon., 2009b)	The difference in average intake in the top dose, 7-day treated animals is thought to be due to palatability of the test diet. Positive control: Chloroform 1000 mg/kg bw/day Treatment time: 3 or 7 days (2 days for positive control)	compared to controls. **After 7 days:** **<u>Pyriofenone (20000 ppm/1109 mg/kg bw/day):</u>** ↑Mean RDS index 3.91** versus 1.42 in controls ↑ Liver weight 16 % (abs.) and 27 % (rel.)** No adverse liver pathology **<u>Pyriofenone (200 ppm/14.4 mg/kg bw/day):</u>** No increase in liver weight and no increase in mean RDS index compared to controls. **Chloroform (1000 mg/kg bw/day):** ↑Mean RDS index 14.3** versus 1.42 in controls
Hepatic enzyme induction in rats Non-guideline Non-GLP DAR: B.6.8.3a (Anon., 2011a)	Fischer, F344 rats (males/5/dose) Pyriofenone: 0, 200 and 20000 ppm Equivalent to 0, 14.3 and 1300/1289 mg/kg bw/day (Group 1/2) Positive control: Phenobarbital sodium (PB) 500 ppm Group 1: 14 days treatment Group 2: 14 days treatment + 14 days recovery Livers were excised and homogenised. The microsomal suspension was then used to determine microsomal protein content, total CYP 450 content and ECOD and PROD activities Ethoxycoumarin O-dealkylase (ECOD) is a marker for CYP1A2 activity and penoxyresorufin O-dealylase (PROD) is a marker for CYP2B1 activity	**Pyriofenone** **<u>20000 ppm (1300/1289 mg/kg bw/day):</u>** ↑Liver weight (rel.) 43 % ↑PROD 20 fold**, ↑ CYP2B1 content, 8 fold** ↑ECOD 1.5 fold**, ↑ CYP1A2 content, 1.6 fold Effects were all reversible within 14 days. **<u>200 ppm (14.3 mg/kg bw/day):</u>** No effects observed. **PB** **<u>500 ppm:</u>** ↑Liver weight (rel.) 35 % ↑ Total cytochrome P450 2.6 fold ↑ PROD 71 fold**, ↑ CYP2B1 content, 40 fold** ↑ ECOD 4.7 fold**, no effect on CYP1A2 levels
In vivo mechanism analysis study in CAR-knock-out (KO)	CAR-KO rats (5 males/dose) Wild type (WT) rats (Sprague-Dawley) (5	**<u>5000 ppm (357.2/363.6 mg/kg bw/day):</u>** <u>Food consumption:</u>

Type of study/data	Relevant information about the study (as applicable)	Observations
rats Non-guideline Non-GLP Taken from a study report submitted directly to the CA: (Anon.., 2017a)	males/dose) Pyriofenone (purity 97.88 %) Dietary administration: 0 or 5000 ppm for 7 days (Equivalent to 357.2 mg/kg bw/day in CAR-KO rats and 363.6 mg/kg bw/day in WT rats) Livers were removed, weighed and then the left lateral lobe was used for measurement of CYP2B1 gene expression, measurement of CYP2B1 enzyme activity and hepatocyte proliferation. Ki-67 is a protein marker of cellular proliferation, present during all active phases of the cell cycle	↓ Food consumption: WT 16 % (days 1-3 only) KO 12 % (days 1-3 only) <u>Clinical chemistry:</u> ↑ Total cholesterol 20 % (WT only) ↓ Triglyceride 50 % (WT only) <u>Findings in the liver:</u> ↑ Weight WT: 16 % (abs. and rel.) KO: 20 % and 19 % (abs. and rel. respectively) Hypertrophy (minimal): WT: 1/5 (versus 0 in controls) KO: 1/5 (versus 0 in controls) <u>CYP2B1 gene expression:</u> WT: ↑ 130-fold (Normalised mRNA amount was 129.0 versus 0.99 in controls)* KO: ↑ 120-fold (Normalised mRNA amount was 14.4 versus 0.12 in controls)** <u>CYP2B1 activity:</u> WT: ↑ 8-fold** KO: No increase. <u>Hepatocyte proliferation:</u> Ki-67 positive ratio: there was no evidence of hepatocyte proliferation in WT or KO rats.

In vivo studies in rats

An *in vivo* study, carried out in male F344 rats, investigated the effect of pyriofenone on hepatic cell proliferation. Rats (5/group) received either pyriofenone (0, 200 or 20000 ppm) for 3 or 7 days in their diet or the positive control, chloroform (1000 mg/kg bw/day) for 2 days. Two hours before necropsy, each animal received a dose of the DNA precursor 5-bromo-2'-deoxyuridine (BrdU) (100 mg/kg bw) *(i.p.*). At the higher concentration of pyriofenone, after three days, there was no increase in liver weight but there was a very slight increase in hepatocellular proliferative activity. However, this was not statistically significant (mean RDS was increased to 2.98 versus 1.23 in controls). After seven days of treatment, liver weight was statistically significantly increased (absolute liver weight 16 % and relative liver weight 27 % of controls) and there was a statistically significant increase in replicating DNA following treatment with 20000 ppm (1109 mg/kg bw/day) pyriofenone (mean RDS was increased to 3.91 versus 1.42 in controls). Chloroform also increased cell proliferation in this study.

The results of this study showed that pyriofenone can cause an increase in replicative proliferation in Fischer rats at high doses.

Pyriofenone was also tested for its effects on hepatic enzyme induction in male F344 rats.

In this study, the rats were divided into two groups; group one received pyriofenone in the diet at either 0, 200 or 20000 ppm for 14 days and group two received pyriofenone in the diet at either 0 or 20000 for 14 days with a 14 day recovery period to assess the reversibility of any effects observed. Liver microsomes taken from treated rats were then used to determine the microsomal protein content, the total P450 content and ethoxycoumarin O-dealkylase (ECOD), a marker for CYP1A2 and penoxyresorufin O-dealylase (PROD), a marker for CYP2B1 activities.

The results showed that following 14 days of treatment with pyriofenone (20000 ppm) or PB (500 ppm), liver weight was increased (pyriofenone 43 % and PB 35 % with respect to controls). Phase I liver enzymes were induced following treatment with PB, with total CYP450 content, increasing 2.6-fold that of controls and PROD and ECOD activities increasing 71 and 4.7-fold of controls (respectively). Pyriofenone also increased PROD and ECOD activities, somewhat less markedly than PB at 20 and 1.5 fold of controls (respectively). The results from group two indicated that this effect was reversible. Treatment with pyriofenone caused an increase in CYP2B1 content (8-fold) and a small increase in CYP1A2 (1.6-fold). PB induced CYP2B1 (40-fold) but CYP1A content was not affected. Induction of other P450 isoforms were not assessed in this study.

Therefore, pyriofenone has been shown to cause enzyme induction *in vivo* in Fischer rats.

An *in vivo* mechanistic study was carried out comparing the effects of pyriofenone in CAR-knock out (KO) and wild-type (WT) Sprague Dawley rats (5/males/dose). Animals received pyriofenone in the diet at a concentration of either 0 or 5000 ppm (357.2/363.6 mg/kg bw/day KO/WT) for 7 days. At the end of the study period body weight, food consumption, clinical chemistry, liver weight, CYP2B1 gene expression, CYP2B1 activity and hepatocyte proliferation were all measured and statistically analysed.

No deaths occurred during the dosing period and there were no abnormal clinical signs. Body weight in the 5000 ppm groups remained comparable to the 0 ppm groups. There was a reduction in food consumption in both WT and KO animals treated with 5000 ppm on days 1-3 (12/16 % KO/WT compared to untreated groups) which was resolved during days 3-8. Some changes to blood chemistry were noted in WT animals. These were an increase in total cholesterol (20 % compared to controls) and a decrease in triglycerides (50 % compared to controls). Minimal hypertrophy of the liver was noted in 1/5 WT and 1/5 KO animals only.

Measurement of the Ki-67 positive ratio in hepatocytes, a cellular marker for proliferation (Scholzen, T. and Gerdes, J., 2000), did not show any increase in either WT or KO rats. In fact, the ratio actually decreased in both treated groups compared to controls. However, the observation associated with increased cell proliferation of increased liver weight was noted in both WT and KO treated animals. In WT rats the increase was 16 % greater than controls (absolute and relative) and in KO rats the increase was 20 % and 19 % greater than controls (absolute and relative respectively).

CYP2B1 gene expression was increased in both WT and KO groups. In WT rats the increase was 130-fold of the controls and in the KO rats expression increased 120-fold of the controls. Pyriofenone did not increase in CYP2B1 activity in the CAR-KO rats, whilst in WT rats CYP 2B1 activity was found to increase by 8-fold of the controls.

Overall, the observation of increased CYP2B1 activity in WT but not KO rats is consistent with CAR activation. However, the observation of increased CYP2B1 gene expression in both WT and KO animals suggests that there may be other mechanisms of action by which pyriofenone effects the liver.

Table 22: *In vivo* studies in mice

↑↓ denote an increase or decrease in a parameter with respect to the control value
Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$

abs. = absolute
rel. = relative

Type of study/data	Relevant information about the study (as applicable)	Observations
Hepatic enzyme induction in mice and assessment of hepatocyte proliferation Non-guideline Non-GLP DAR: B.6.8.4 (Anon., 2010f)	CD-1 mice (males, 12/dose) Dietary administration for 4 weeks Pyriofenone: 0, 5000 or 10000 ppm Equivalent to 0, 854 or 1714 mg/kg bw/day Liver sections from all animals were stained for proliferating cell nuclear antigen (PCNA) to demonstrate presence of proliferating cells. Enzyme assays were performed on pooled livers of the 3 dose groups to measure microsomal protein concentration, EROD activity, testosterone hydroxylase and dehydrogenase activities and lauric acid hydroxylase activities.	No clinical signs of toxicity, no reductions in body weight and no macroscopic findings at necropsy. **10000 ppm/1714 mg/kg bw/day** ↑ Liver weight 14 % (rel.)** ↑ Cytochrome P450 1.5 fold ↑EROD activity 1.4 fold **5000 ppm/854 mg/kg bw/day** ↑ Liver weight 12 % (rel.)** ↑ Cytochrome P450 1.3 fold ↑EROD activity 1.37 fold

In vivo studies in mice

Hepatic enzyme induction and cell proliferation were studied *in vivo* in male CD-1 mice. Groups of 5 mice received pyriofenone in the diet at doses of 0, 5000 or 10000 ppm for 4 weeks. Liver sections were taken from all animals and stained for proliferating cell nuclear antigen (PCNA) to demonstrate presence of proliferating cells. Enzyme assays were performed on pooled livers of each dose group and the following parameters measured: i) microsomal protein content, ii) cytochrome P450 concentration, iii) 7-ethoxyresorufin O-deethylase activity (EROD) (a marker for CYP1A1 and 1A2), iv) testosterone hydroxylase and dehydrogenase activities (a monitor for CYP2A, 2B, 2C and 3A) and v) lauric acid hydroxylase activities (a monitor for CYP2E and 4A). The results showed an increase in relative liver weight following both doses of pyriofenone (12 % and 14 % at 5000 and 10000 ppm respectively) and a small increase in cytochrome P450 in both dose groups, indicating Phase I enzyme induction. There was no evidence of induction of CYP2A, CYP 2B, CYP2C, CYP 2E, CYP 3A or CYP4A. A small increase in EROD activity was observed (approximately 1.4 fold) following treatment with 10000 and 5000 ppm pyriofenone. There was no evidence of any increase in the rate of cell proliferation in the liver. The results of this study indicate that pyriofenone has little effect of on hepatic phase I enzyme induction or cell proliferation in mice.

Table 23: *In vitro* studies in human hepatocytes

<table>
<tr><th>Type of study/data</th><th>Relevant information about the study (as applicable)</th><th>Observations</th></tr>
<tr><td>Expression of CYP genes in human hepatocytes (PCR measurement of mRNA)

Taken from a study report submitted directly to the CA:
(Shikama, H., 2013a)</td><td>Human hepatocytes (Cryopreserved cells from males, number and health status unknown)
Pyriofenone: 1.25, 2.5 and 5 ppm

Positive control: PB 3, 30 and 300 ppm</td><td>Altered gene expression of CYP2B is a marker for activation of CAR whereas altered expression of CYP1A is a marker for activation of the aryl hydrocarbon receptor (AhR)

<table>
<tr><td>Concentration (ppm)</td><td colspan="2">Human</td></tr>
<tr><td></td><td>CYP2B6</td><td>CYP1A2</td></tr>
<tr><td></td><td colspan="2">Relative quantity of CYP mRNA</td></tr>
<tr><td>Pyriofenone</td><td colspan="2"></td></tr>
<tr><td>Vehicle control (DMSO 0.005 %)</td><td>1</td><td>1</td></tr>
<tr><td>1.25</td><td>Not tested</td><td>Not tested</td></tr>
<tr><td>2.5</td><td>1</td><td>0.5</td></tr>
<tr><td>5</td><td>7</td><td>1</td></tr>
<tr><td>PB</td><td colspan="2"></td></tr>
<tr><td>Vehicle control (dH_20)</td><td>1</td><td>1</td></tr>
<tr><td>3</td><td>Not tested</td><td>Not tested</td></tr>
<tr><td>30</td><td>5</td><td>0.5</td></tr>
<tr><td>300</td><td>14</td><td>0.5</td></tr>
</table>

Little study information was given, it was unclear as to how many replicates were performed. No statistical analysis was performed.

An increase in CYP2B6 was seen with 5 ppm pyriofenone and with increasing concentrations of phenobarbital indicative of CAR activation.

There was no increase in CYP1A2 activity in the presence of pyriofenone or phenobarbital, suggesting no activation of AhR.</td></tr>
<tr><td>Effect of pyriofenone on DNA replication in human hepatocytes (measured by BrdU incorportation)

Taken from a study report submitted directly to the CA:
(Shikama, H., 2013b)</td><td>Human hepatocytes (sex, number and health status unknown)
Pyriofenone: 0.6, 2.5 and 10 ppm

PB 30 and 300 ppm

Positive control: epidermal growth factor (EGF) 25 ng/ml</td><td>Table showing BrdU incorporation into human hepatocytes(average of four replicates from a single study):
<table>
<tr><td></td><td></td><td>EGF (ng/mL)</td><td colspan="2">PB (ppm)</td><td></td><td colspan="3">Pyriofenone (ppm)</td></tr>
<tr><td></td><td>control</td><td>25</td><td>300</td><td>30</td><td>control</td><td>10</td><td>2.5</td><td>0.6</td></tr>
<tr><td>% of Control</td><td>100</td><td>148.1</td><td>95.1</td><td>92.2</td><td>100</td><td>99.9</td><td>96.6</td><td>97</td></tr>
</table>

No statistical analysis was performed.

DNA replication was evident following exposure to the positive control EGF

No DNA replication was observed with PB or pyriofenone.</td></tr>
</table>

In vitro studies in human hepatocytes

Pyriofenone was tested in human hepatocytes for its ability to cause changes in expression of CYP genes and to assess cell proliferation.

In the first study, pyriofenone was tested for its ability to cause changes in expression of the genes CYP2B6 and CYP1A2 using cryopreserved human hepatocytes (males, number of donors, age and health status unknown) (Shikama, H., 2013a). The test concentrations used in the main study were PB: 30 and 300 ppm and pyriofenone: 2.5 and 5 ppm and cells were exposed for a period of 24 h. RNA was extracted and gene expression was measured using PCR.

The results showed that pyriofenone, at the higher concentration, increased CYP2B6 gene expression. PB also caused increased expression of CYP2B6. There was no effect on CYP1A2 levels following exposure to either substance. There was no statistical analysis of the results. Consistent with a CAR mode of action, pyriofenone caused an increase in CYP2B6 expression.

A second study assessed the effect of pyriofenone on DNA replication in human hepatocytes by measurement of incorporation of BrdU (Shikama, H., 2013b). Test concentrations used were PB; 30 and 300 ppm, pyriofenone: 0.6, 2.5 and 10 ppm and epidermal growth factor (EGF): 25 ng/mL. There was no evidence of DNA replication following treatment with pyriofenone or PB. However, an increase of 48 % was seen in cultures treated with EGF, confirming the potential of the cells to under S-phase DNA synthesis. This finding was consistent with the hypothesis that a CAR mode of action is not relevant to humans.

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

The carcinogenic potential of pyriofenone has been investigated in rats and mice. In a carcinogenicity study in rats, a marginal increase in the number of hepatic adenoma and carcinoma was observed in males treated with pyriofenone (5000 ppm). The increases observed were above that of the concurrent control and above the contemporary historical control data provided. No such increase was observed in females or in mice.

The increase in liver tumours in rats were associated with an increase in mortality towards the end of the study. Non-neoplastic findings in the liver included an increase in relative organ weight, fatty changes and hypertrophy in both males and females and an increase in the number of males with necrosis of the liver and increase in the number of females with focal congestion. The increase in mortality and hepatic cell death at the highest dose might be indicative of excessive toxicity, although body weight was largely unaffected in males. If this were the case, it could be argued that the tumours observed were a non-specific consequence of the hepatoxicity of pyriofenone under extreme dosing conditions, making them of less relevance to humans. However, no excess toxicity was observed at 200 or 1000 ppm where tumours were also seen. There are other potential modes of action that should be considered, as discussed below.

10.9.4.1 Potential Modes Of Action (MOA)

Alongside the possibility of the tumour findings in male rats being related to the toxicity of pyriofenone, there are various possible mechanistic explanations that can be considered for this weak carcinogenic response in rats (summarised in the Table 24).

Table 24: Potential modes of action (MOA) relating to tumour formation in the liver of rats

Mode of Action	Data relating to pyriofenone	Conclusion
Genotoxicity	Assessed in a battery of standard *in vitro* and *in vivo* mutagenicity studies (Section 10.8). Although induction of chromosomal damage was observed in an *in vitro* test, the weight of evidence from a micronucleus test in mice, an unscheduled DNA	Unlikely

	synthesis test in rats (targeting the liver) and two Comet assays investigating the liver of rats and mice, suggests that this is not the mode of action of pyriofenone.	
Cytotoxicity	There was an increase in mortality in male rats of the top dose group in the rat carcinogenicity study and also an increase in single cell necrosis. Therefore, there is a possibility that cytotoxicity could be a potential mode of action of pyriofenone. However, no significant liver toxicity was observed in the low or mid dose groups of the rat carcinogenicity study at which there were single incidences of liver adenoma and carcinoma. Other findings in the liver included increases in relative liver weight, fatty changes and hypertrophy.	Plausible, but not definitive
Peroxisome proliferator-activated receptor alpha (PPARα) activation	Investigated by substance-mediated induction of CYP4A mRNA levels or enzymatic activity. There were no studies conducted to investigate the induction of CYP 4A1 gene transcription in rodent hepatocytes. In mice, there was no change in expression of genes coding for CYP4A indicating activation of the PPAR α receptor does not occur. There were no equivalent studies in rats. From the repeated dose studies in rats and mice, there was no evidence of peroxisome proliferation (a key marker of PPARα receptor activators) following histopathological examinations.	Unlikely
Constitutive androgen receptor (CAR)/Pregnane X receptor (PXR) activation	*In vitro* and *in vivo* mechanistic studies indicate that pyriofenone induces changes in rats and humans consistent with this mode of action (see details in Table 27). A recent study designed to definitively address the CAR-dependence of pyriofenone showed that whilst CYP2B1 enzyme activity was increased in WT rats, it was not increased in CAR-KO rats following administration of pyriofenone in the diet. However, CYP2B1 gene expression was increased in KO rats as was liver weight. However, the study did not demonstrate the ability of pyriofenone to cause proliferation in either WT or KO rats. Therefore, the results of this study leaves uncertainties as to whether the effects of pyriofenone are entirely due to CAR activation. See Section 10.9.4.2 for further details. There were no studies carried out in rats to assess CYP3A transcription (associated with CAR/PXR activation), although in mice, there was no evidence of an increase in CYP3A activity (there was no substance-related increases in liver tumours in mice).	Plausible, but not definitive

Aryl hydrocarbon receptor (AhR) activation	In rat hepatocytes, pyriofenone was shown to induce expression of CYP1A genes in a concentration-dependent manner. *In vivo* studies in rats with pyriofenone showed induction of ECOD activity (a marker of CYP1A activity) and an increase in CYP1A2 content. Sodium phenobarbital also increased ECOD activity, however there were no associated changes to CYP1A content. These results indicate that pyriofenone has potential to activate the AhR, however the magnitude of the increase in ECOD was much lower than the increase in PROD activity (associated with the CAR MOA). There was a small increase in EROD activity in mice (a marker of CYP1A activity). No such increase in expression was observed for CYP1A2. A change in expression of CYP1A2 might be under some influence of CAR, however, it is widely recognised as a marker of the aryl hydrocarbon receptor (AhR). This receptor is involved in various signalling pathways and dysregulation of these cellular processes may also provoke a carcinogenic response. It can occur in rats and humans. The absence of an induction of this enzyme therefore suggests this MoA is not of relevance to pyriofenone.	Plausible, but unlikely
Porphyria	There were some modest changes to red blood cell parameters in many of the repeated dose studies, although in most cases the changes were less than 5 % when compared to control values. Wainstok de Calmanovici et al (1984) indicated that the precursors of haem synthesis, aminolevulinic acid (ALA) and porphobilinogen (PBG) would be elevated in the development of porphyria. This was not the case in any of the studies available. In the 2-generation study in rats, brown deposition was described in the Glisson's capsule of the liver and necrosis of the liver was observed in mice in the carcinogenicity study.	Unlikely
Endocrine Activity	There were no adverse effects observed in the ovaries or testes of rats, mice or dogs in any of the repeated dose or reproduction studies. In one 2-generation reproduction study in rats, there was an increase in the incidence of follicular cell hypertrophy of the thyroid of parental female rats dosed with 5000 ppm (307 - 677 mg/kg bw/day) pyriofenone. This finding alone is not considered to warrant an endocrine effect following treatment with pyriofenone. There were no hormonal disturbances observed.	Unlikely
Immunosuppression	In the short term and chronic studies provided, there was no evidence of changes to the immune system or immune cells.	Unlikely

The potency of pyriofenone is low, borderline responses are seen: in male rats it appears to be a weak carcinogen; in female rats there was no clear carcinogenic response. From the data available, summarised in the table above, the most plausible modes of action that could account for the weak carcinogenic response to pyriofenone in male rats would appear to be non-genotoxic, involving either cytotoxicity or activation of the constitutive androstane receptor (CAR). Evidence to show that this carcinogenic response was driven by the hepatotoxicity of pyriofenone is limited. A clear link between exposure to pyriofenone, toxicity and the formation of pre-neoplastic lesions in the liver is lacking. The Applicant has therefore considered CAR activation to be the most likely explanation for the tumour response seen.

10.9.4.2 Activation of the Constitutive Androstane Receptor (CAR)

A pathway of changes in the liver stemming from activation of the constitutive androstane receptor (CAR) has been well characterised in recent years as a potential mechanism of action for some rodent liver carcinogens. Whilst there is strong evidence for this pathway being relevant to rats and mice, data relating to the rodent liver carcinogen phenobarbital (PB) suggest it to be qualitatively not plausible for humans (Elcombe et al 2014).

The MOA involves activation of CAR which results in changes in expression of a wide range of genes, including genes involved in phase I and phase II xenobiotic metabolism; induction of phase III transporters and regulation of genes associated with various physiological processes such as cell proliferation, apoptosis and metabolism, eventually leading to liver tumour formation. Many of the substances that activate the CAR receptor also activate the pregnane X receptor (PXR), producing a combined response pattern of gene expression and functional changes. The key and associative events involved in this process are illustrated below (Figure 1):

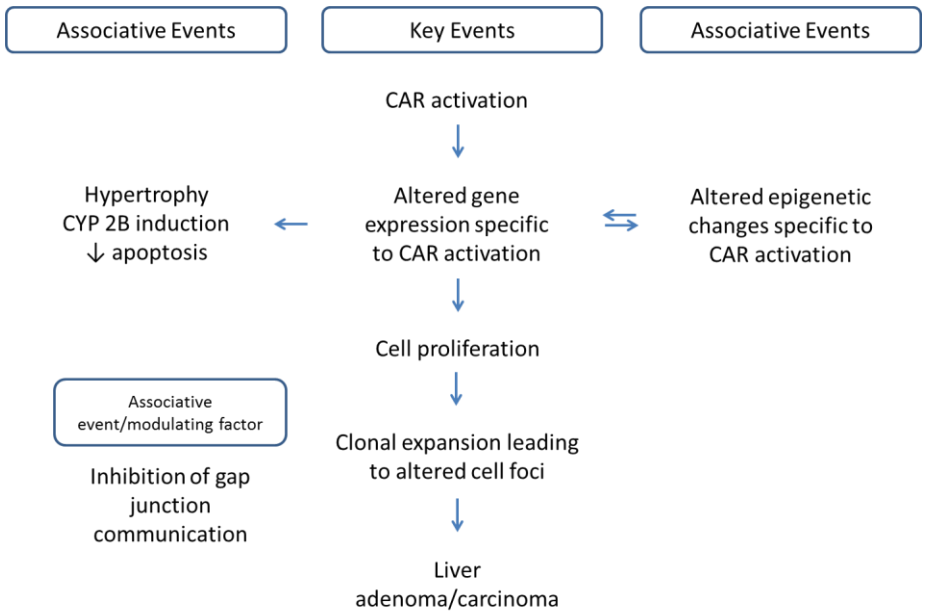


Figure 1. Key and associative events involved in CAR-mediated liver toxicity and tumour formation

A number of the effects produced in the rodent liver by these substances have also been seen in humans or model systems that are representative of the human liver. These include activation of CAR and induction of the appropriate forms of cytochrome P450 (e.g. CYP2B) and liver hypertrophy. However, evidence from humans who have been exposed to phenobarbital for long periods do not seem to be at increased risk of developing liver cancer. Similarly, in experimental systems, the substances concerned have been shown to induce cell proliferation in rodent liver cells, but not in human cells. Therefore, if the CAR activation can be proven to be key to the slightly increased liver tumour incidence in male rats following treatment with

pyriofenone, then this would show that no classification for carcinogenicity would be justified in the absence of relevance to humans.

10.9.4.3 Mechanistic study findings related to key and associative events of CAR-activation

The results obtained from the mechanistic studies carried out *in vitro* in rats and humans and *in vivo* in rats and mice have been critically assessed in relation to the key and associative events involved in the CAR-mediated formation of tumours in rodents. Reference is made to the model CAR-activating substance PB. The evidence is summarised in Table 25.

10.9.4.3.1 Key event 1 – CAR activation

Induction of the key enzymes CYP2B and CYP3A occur largely via activation of CAR and the pregnane X receptor (PXR) (respectively). It is known that many of the molecules that activate CAR, also activate PXR, producing a combined response pattern of gene expression and functional changes (Moore JT *et al*. 2000). Therefore, induction of CYP2B (and CYP3A) enzymes are indicative of CAR activation.

The activation of CAR is the molecular initiating event in this pathway. It is possible, using genetically engineered CAR "knock-out" rats (or mice) to show the CAR-dependency of the increase in liver weight, induction of CYP2B enzymes and cellular proliferation seen in wild type animals.

In a recently performed mechanistic study comparing the effects of pyriofenone in CAR knock-out (KO) rats and wild-type (WT) rats CYP2B enzyme activity was increased only in the group of WT rats (by 8-fold of controls). However, liver weight was found to increase in both groups and an increase in CYP2B gene expression occurred to a similar extent in both WT and KO rats. There was no evidence of hepatocyte proliferation in either WT or KO rats at the dose and timeframe used.

Therefore, there is some evidence to indicate that CAR activation can occur in male rats following exposure to pyriofenone but it is not definitive.

10.9.4.3.2 Key event 2 – Altered gene expression specific to CAR activation

Altered gene expression specific to CAR activation is considered to be the second Key event in the mechanism of action for phenobarbital-induced rodent liver formation.

In rat hepatocytes, a concentration-dependent increase of CYP2B1 and CYP1A2 gene expression was found following exposure of cells to pyriofenone (concentrations of 2.5 ppm and above). In a similar experiment using human hepatocytes, there was a similar increase in expression of CYP2B6 (at 5 ppm). However, in an *in vivo* study comparing CAR-KO and WT rats, expression of CYP 2B1 genes was observed in both treated groups, indicating strictly the lack of CAR-dependency for this finding.

Associated events

Induction of CYB 2B isoforms is considered an associative event to key event 2. In a study using CAR-null rats, it was found that the CAR receptor was essential for induction of CYP 2B1 activity following treatment with pyriofenone (5000 ppm/~360 mg/kg bw/day). The results of this study showed no increase in CYP2B1 activity in KO rats, whilst a small (8-fold) increase in activity was observed in WT rats.

CYP450 enzyme induction was assessed in a second *in vivo* study in rats and also in mice. Following treatment with pyriofenone (20000 ppm/~1300 mg/kg bw/day) in rats for 14 days, there was an increase in PROD activity (a marker of CYP 2B activity) and also an increase in transcription of CYP 2B1 (8-fold when compared to controls). The magnitude of this effect was much lower than that seen with PB. BROD activity (a marker of both CYP2B and CYP3A) was not measured nor was transcription of CYP3A enzymes. In a similar study in mice, there was no such increase in CYP2B1 content. However, this could be considered consistent with the findings in the main carcinogenicity study in mice, where there were no treatment-related increases in liver tumours.

Liver enlargement, due to both hypertrophy and hyperplasia is also considered an associative event in the MOA for CAR activation leading to tumour formation. Centrilobular hypertrophy and liver weight increases were observed in all of the standard repeated dose rat studies (and also the mouse and dog studies) from doses of 226 mg/kg bw/day and above. Liver weight was also increased in the CAR-KO mechanistic study, occurring in CAR-KO rats as well as the WT rats.

Therefore, whilst pyriofenone can causes changes to gene expression and liver weight associated with CAR activation, it has also been shown to cause these changes in the absence of the CAR receptor. Enzyme induction of CYP 2B1 caused by pyriofenone appeared to be dependent on CAR.

10.9.4.3.3 Key event 3 – Cell proliferation

Increased cell proliferation is considered the third Key event in the CAR mode of action leading to the formation of tumours in rodents. It is known that in humans, following CAR activation by PB, altered gene expression and enzyme induction of CYP2B6 occurs, however there is no increase in hepatic cell proliferation. In contrast to rats and mice, there is no decrease in apoptosis or increase in the incidence of liver tumours.

As an indicator of cell proliferation, increased DNA replication was measured in an *in vitro* mechanistic study using rat hepatocytes. The results of the study showed a clear, dose-dependent increase in uptake of BrdU by the cells after exposure to PB. Following treatment with pyriofenone (10 ppm) there was a small increase in BrdU uptake. However, the magnitude of this response was considerably less than seen with PB. In a similar experiment using human hepatocytes, a clear negative result was obtained indicating no DNA replication following exposure of the cells to a maximum concentration of 10 ppm pyriofenone.

In *in vivo* mechanistic studies in rats, a statistically significant increase in liver cell proliferation was observed following treatment with 20000 ppm (1109 mg/kg bw/day) pyriofenone administered *via* the diet for 7 days [mean replicative DNA synthesis (RDS) index 3.91 versus 1.42 in controls] but at a dose of 5000 ppm (~360 mg/kg bw/day) for 7 days no cell proliferation was noted in either WT or CAR-KO rats. There was no evidence of cell proliferation in the livers of mice.

Therefore, at high doses only, pyriofenone does have potential to cause hepatocellular proliferation in rats but in a study to determine a definitive CAR mode of action, no proliferation was demonstrated in either WT or CAR-KO rats. Consequently, it is uncertain whether pyriofenone causes cell proliferation by activation of CAR.

10.9.4.3.4 Key event 4 – Clonal expansion leading to altered cell foci

Altered liver foci are precursor lesions for subsequent tumour formation. In the standard and mechanistic studies provided by the applicant, the only evidence of focal congestion was in the livers of top-dosed female rats of the 2-year carcinogenicity study. It is possible that pyriofenone can induce these changes in male rats, but a study optimised to show this was not conducted.

10.9.4.3.5 Key event 5 – Liver adenoma/carcinoma

In the 2-year carcinogenicity study in rats, a small, dose-related increase in the incidence of liver adenoma and carcinoma was observed in males, but not females. The number of rats with tumours was above the concurrent control and were above the contemporary HCD, therefore the possibility of a weak carcinogenic response cannot be excluded.

Table 25: Summary of evidence in rodents and humans associated with the key and associative events involved in the CAR-mediated formation of tumours in rats

Key and Associative events	Evidence in rats and mice	Evidence in humans
Activation of CAR	Some evidence In an *in vivo* mechanistic study using CAR-KO rats, CYP2B1 enzyme activity was increased in WT but not KO out rats. However, changes in gene expression and liver weight were noted in both KO and WT rats and there was no evidence of hepatocellular proliferation at the doses used.	No data
Altered gene expression	Strong positive In an *in vitro* study using male Fischer rat hepatocytes, there was a concentration-dependent increase in gene expression of CYP2B1 after exposure to pyriofenone. An increase in CYP2B1 gene expression was observed in both WT and KO rats indicating that the effect is not CAR-dependent.	Positive evidence In an *in vitro* study using human male hepatocytes there was an increase in gene expression of CYP2B6 only.
Hypertrophy	Strong positive Observed in male and female rats and in male mice *in vivo*.	No data
Increased hepatocellular proliferation	Some evidence *In vitro*, slight increases in DNA replication in rat hepatocytes were indicative of increased proliferation. In rats, there was a small increase in hepatocyte proliferation after 7 days of exposure to pyriofenone (1109 mg/kg bw/day). The magnitude of this was lower than with the positive control, chloroform and was not seen at all at a dose of ~15 mg/kg bw/day). Hepatocellular proliferation was not observed at a cellular level in either WT or KO rats (administered with the lower dose of ~ 360 mg/kg bw/day), however liver weight was increased in both groups of animals.	Negative In an *in vitro* study using human male hepatocytes there was no evidence of DNA replication.
Altered hepatic foci	Some evidence Only observed in female rats in the *in vivo* carcinogenicity study. However, absence of an effect in rats has not been established.	No data
Liver tumours	Positive evidence A slight increase in the number of adenoma and carcinoma in male rats	No data

	only, observed following two years of treatment with pyriofenone. No such increase was observed in females or in mice.	

Following a critical assessment of all mechanistic data provided, it appears that the CAR mode of action is a plausible explanation for the increase in liver tumours observed in male rats treated with pyriofenone. However, some uncertainties remain:

- In a study intended to provide definitive evidence of the CAR-dependence in the mechanism of action of pyriofenone, the key event of altered gene expression occurred in both wild-type and knock-out rats, indicating a lack of CAR-dependence.
- In the same study, liver weight was found to be increased in both CAR-KO and WT rats – this indicates pyriofenone might cause induction of P450 enzymes and cell proliferation independently of CAR activation.
- *In vitro* studies in rat hepatocytes, investigating the effect of pyriofenone on DNA replication - the magnitude of the BrdU incorporation into the cells following exposure to pyriofenone (10 ppm) was very small. The experiment was performed in isolation and there was no statistical analysis of the data performed.
- *In vivo* studies in rats to investigate enzyme induction and hepatocyte proliferation - the top concentration tested was four times higher than that causing liver tumours *in vivo*. Whilst use of a more representative dose did lead to enzyme induction in WT rats, it did not lead to increased cell proliferation in either WT or CAR-KO rats.
- In the *in vitro* studies in human hepatocytes investigating expression of CYP genes and the effect of pyriofenone on DNA replication cells used were pooled cryopreserved cells from males. The number of donors, age and health status remain unknown. Therefore, the results should be used with caution before reaching conclusions about the relevance to the human population as a whole.
- Inhibition of apoptosis and other associative events in the CAR-mediated tumour model have not been investigated, however there is some debate as to the importance of inhibition of apoptosis as a determinant of tumour promotion in rodents, therefore, the lack of studies on this do not represent a major knowledge gap.
- No data was provided on the ability of pyriofenone to cause an increase in gene transcription and enzyme activity consistent with activity of the PXR - many of the substances that activate the CAR receptor also activate the PXR receptor, producing a combined response pattern of gene expression and functional changes. Whilst this doesn't form a data gap in itself, it would have been more informative and added more weight to the evidence.

Given these study limitations and data gaps, the weight of evidence supporting the view that pyriofenone induced liver cancer in male rats via a non-genotoxic pathway solely involving CAR-activation is not entirely persuasive.

10.9.4.4 Conclusion

In a carcinogenicity study in rats, a small increase in the incidence of liver tumours was observed in males treated with pyriofenone (5000 ppm in the diet). The increases observed were above the concurrent control values; and above the contemporary laboratory control incidence of 0. There was no carcinogenic effect seen in female rats or in male or female mice.

The most likely mechanism of action underpinning this activity is considered to involve CAR-activation. However, based on the evidence currently available there remains some key areas of uncertainty. As such, in the absence of a definitive conclusion about the MoA, the relevance of the rat liver tumour findings for humans cannot be discounted completely.

10.9.5 Comparison with the CLP criteria

An increase in tumours has been observed in the livers of male rats following treatment with pyriofenone.

As there is no evidence to suggest that pyriofenone causes carcinogenicity in humans, classification with Category 1A is not considered appropriate.

In order to be classified in Category 1B, the evidence provided must be considered sufficient to presume the substance has carcinogenic potential in humans. As the liver tumours observed occurred in just one species (rats), in one sex (males), in one tissue (the liver) and the increased incidences were slight, only just over the concurrent control level yet within the literature historical control levels for this species (laboratory control data was not provided) the data is not considered sufficiently convincing for this category.

The strength of evidence relating to a carcinogenic effect following exposure to pyriofenone is considered limited and not sufficiently convincing to place the substance in categories 1A or 1B.

Consequently, pyriofenone could either be classified in category 2 for carcinogenicity, based on limited evidence of carcinogenicity in rats, or be not classified for this endpoint, based on the tumour findings being explained as no relevance to humans.

Pyriofenone is non-genotoxic. A clear non-genotoxic mechanistic basis to account for the increased tumour incidence in male rats is lacking. The possibility is that this could have occurred via a CAR-mediated mode of action, which is generally agreed to be of limited relevance to humans. However, for pyriofenone this mode of action has not been definitively established. A cytotoxic mode of action is also plausible but, again, definitive evidence is lacking. There appears to be no other plausible mechanisms of action.

In accordance with the criteria provided in Annex I of the CLP Regulation, "limited evidence" of carcinogenicity in animals is provided for pyriofenone:

(a) The evidence is limited to a single experiment

(b) There are unresolved questions about the interpretation of the study results

It is possible, on the basis of the total weight of evidence, that the overall likelihood that pyriofenone poses a carcinogenic hazard to humans is low. However, as discussed above, there is currently insufficient evidence to underpin the proposed species-specific mechanism of action. This provides sufficient uncertainty to support a category 2 classification for this endpoint.

10.9.6 Conclusion on classification and labelling for carcinogenicity

Category 2 carcinogen; H351 – Suspected of causing cancer

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 26: Summary table of animal studies on adverse effects on sexual function and fertility

↑↓ denote an increase or decrease in a parameter with respect to the control value
Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$
abs. = absolute
rel. = relative

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
Multi-generation toxicity study in rats (Dose-range finding study) OECD 416 GLP Rats, Wistar 8/sex/group DAR: B.6.6.1a (Anon., 2009d)	IKF-309 technical Purity 97.88 % 0, 300, 3000, 10000 or 20000 ppm Equivalent to: ♂ 17.9, 185, 591 and 1159 mg/kg bw/day ♀ 31.9, 328, 1004 and 1828 mg/kg bw/day	***General toxicity:*** ***Parents*** **20000 ppm (1159/1828 mg/kg bw/day):** ↓Bodyweight gain: 21 %* in males at week 10, 10 % in females during weeks 0-3 (pre-mating) and 24 % during weeks 14-20 (gestation) ↓ Food consumption: 13 %** during weeks 0-3, 17 % during weeks 14-20 and 20 %* during weeks 14-21 (lactation) in females ***Organ Weights:*** ↑ Liver weight: 36 %** (abs.) and 50 %** (rel.) in males and 80 %** (abs.) and 88 %** (rel.) in females ↑ Kidneys weight: 27 %** (abs.) and 39 %** (rel.) in males and 11 %** (rel.) in females ↑ Caecum weight: 2.4 fold** (abs.) and 1.6 fold** (rel.) in males and 1.9 fold* (abs. and rel.) in females ↓ Ovaries weights: 38 %** (abs.) and 33 %** (rel.) ***Histopathology:*** **Liver** Diffuse hepatocyte hypertrophy:8/8*** males and 8/8*** females Enlargement: 8/8*** males and 8/8*** females (versus 0 in controls) Dark colour: 8/8*** males and 5/8 females (versus 0 in controls) **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 5/8* males ***Clinical Chemistry:*** ↑ γ-Glutamyl transpeptidase 4-fold** in males, 14-fold** in females ↓ Creatinine 18 %** in males, 24 %** in females ↓ Glucose 16 %** in males, 17 %** in females ↑ Total Cholesterol 77 %** in females ↓ Total bilirubin 63 %** in females Haematology: ↓ Haemoglobin 12 %** in females, (5 %** in males) ↓ Haematocrit 11 %** in females, (4 %* in males) ↑ Platelet count 40 %** in males, 55 %** in females

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		<u>10000 ppm (591/1004 mg/kg bw/day):</u> ***Organ Weights:*** ↑ Liver weight: 27 %** (abs.) and 30 %** (rel.) in males and 62 %** (abs. and rel.) in females ↑ Kidneys weight: 26 %** (abs.) and 29 %** (rel.) in males ↑ Caecum weight: 2.2 fold** (abs. and rel.) in males and females ↓ Ovaries weights: 24 %** (abs.) and 23 %** (rel.) ***Histopathology:*** **Liver** Diffuse hepatocyte hypertrophy:8/8*** males and 8/8*** females (versus 0 in controls) Enlargement: 6/8** males and 8/8*** females (versus 0 in controls) Dark colour: 8/8*** males and 4/8* females (versus 0 in controls) **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 5/8* males (versus 0 in controls) ***Clinical Chemistry:*** ↑ γ-Glutamyl transpeptidase 2-fold** in males, 5-fold** in females ↓ Creatinine 15 %** in males, 18 %** in females ↓ Glucose 15 %** in females ↑ Total Cholesterol 48 %** in females ↓ Total bilirubin 43 %** in females ***Haematology:*** ↓ Haemoglobin 10 %** in females ↓ Haematocrit 10 %** in females ↑ Platelet count 28 %** in males, 25 %* in females **<u>3000 ppm (185/328 mg/kg bw/day);</u>** ***Organ Weights:*** ↑ Liver weight: 13 %* (abs.) and 12 %** (rel.) in males and 24 %* (abs.) and 22 % (rel.) in females ↑ Kidneys weight: 17 %** (abs.) and 15 %** (rel.) in males ↑ Caecum weight: 1.7-fold (abs. and rel.) in males and 1.6-fold* (abs. and rel.) in

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		females ***Clinical Chemistry:*** ↑ Total Cholesterol 35 %* in females ↓ Total bilirubin 43 %** in females **300 ppm (17.9/31.9 mg/kg bw/day):** No treatment-related findings ***F_1 generation:*** **20000 ppm (1159/1828 mg/kg bw/day):** ↓Bodyweight [25-26 days of age]: 36 %** in males and 35 %** in females ***Organ Weights:*** ↓ Brain weight: 10 %** (abs.) and 41 %** (rel.) in males and 10 %** (abs.) and 38** % (rel.) in females ↓ Spleen weight: 54 %** (abs.) and 30 %** (rel.) in males and 60 %** (abs.) and 37 %** (rel.) in females ↓ Thymus weight: 44 %** (abs.) and 14 % (rel.) in males and 47 %** (abs.) and 19 % (rel.) in females ↓ Uterus weight: 16 % (abs.) and 27 %* (rel.) **10000 ppm (591/1004 mg/kg bw/day):** ↓Bodyweight [25-26 days of age]: 21 %** in males and 12 %** in females ***Organ Weights:*** ↓ Brain weight: 22 %** (rel.) in males ↓ Spleen weight: 35 %** (abs.) and 18 %* (rel.) in males and 23 %** (abs.) (rel.) in females ↓ Thymus weight: 25 %** (abs.) in males and 424 %** (abs.) and 13 % (rel.) in females **≤ 3000 ppm (185/328 mg/kg bw/day):** No treatment-related findings ***Reproductive effects:*** There were no treatment-related effects on the oestrous cycle, mating index, fertility index, gestation index, duration of gestation or number of implantations in the parent generation or the F_1 generation.

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th></tr>
<tr><td></td><td></td><td><u>Parents</u>
<u>20000 ppm (1159/1828 mg/kg bw/day):</u>
1/8 females was non-pregnant
1/8 females lost the entire litter by lactation Day 4

<u>≤ 10000 ppm (591/1004 mg/kg bw/day:</u>
No treatment-related findings

<u>F_1 generation:</u>
No effects observed at any doses

NOAELs were not set for this study.</td></tr>
<tr><td>Two-generation reproduction toxicity study in rats
OECD 416
GLP
Rats, Wistar
(24/sex/dose)

DAR: B.6.6.1b

(Anon., 2009c)</td><td>IKF-309 technical
Purity 97.88 %
0, 150, 1000 or 5000 ppm</td><td>There were no effects on reproductive indices or on the development of offspring in this study.

Mean intake of test material:

<table>
<tr><td></td><td>Parental Males</td><td colspan="3">Parental Females</td></tr>
<tr><td>Concentration (ppm)</td><td>Pre-mating (weeks 0-10)</td><td>Pre-mating (weeks 0-10)</td><td>Gestation (Days 0-20)</td><td>Lactation (Days 1-14)</td></tr>
<tr><td>150</td><td>9.61</td><td>11.9</td><td>9.3</td><td>21.2</td></tr>
<tr><td>1000</td><td>64.1</td><td>79.2</td><td>62</td><td>138.1</td></tr>
<tr><td>5000</td><td>334</td><td>395</td><td>307</td><td>677</td></tr>
<tr><td></td><td>F1 males</td><td colspan="3">F1 Females</td></tr>
<tr><td>150</td><td>11.4</td><td>13</td><td>9.6</td><td>20.6</td></tr>
<tr><td>1000</td><td>76.8</td><td>84.4</td><td>61.6</td><td>130</td></tr>
<tr><td>5000</td><td>393</td><td>434</td><td>321</td><td>709</td></tr>
</table>

<u>General toxicity:</u>
<u>Parents:</u>
<u>5000 ppm (334/307-677 mg/kg bw/day):</u>
Organ Weights:
↑ Liver weight: 27 %** (abs.) and 29 %** (rel.) in males and 42 %** (abs.) and 38 %** (rel.) in females</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		↑ Kidneys weight: 22 %** (abs.) and 24 %** (rel.) in males and 13 %** (abs.) and 10 %** (rel.) in females ↑ Thyroids weight: 21 %** (abs.) and 23 %** (rel.) in males ↑ Caecum weight: 1.8 fold** (abs. and rel.) in males and 1.9 fold** (abs. and rel.) in females ***Histopathology:*** **Liver** Diffuse hepatocyte hypertrophy:13/23** males and 20/23** females (versus 0 in controls) Brown deposition in Glisson's capsule: 13/23** males (versus 0 in controls) Dark colour: 14/23** males and 20/23** females (versus 0 in controls) **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 11/23** males (versus 0 in controls) **Thyroid** Follicular cell hypertrophy: 14/23** females (versus 3/21 in controls) ***Haematology:*** ↓ Haemoglobin 10 %** in females ↓ Haematocrit 9 %* in females ↑ Platelet count 11 % in females ↓ Lymphocyte count 20 %* in females ↓ Basophil count 67 %** in females **1000 ppm (64.1/62-138.1 mg/kg bw/day):** ***Organ Weights:*** ↑ Liver weight: 11 %** (abs.) and 9 %** (rel.) in females **150 ppm (9.6/9.3-21.2 mg/kg bw/day):** No treatment-related findings. ***F_1 generation:*** **5000 ppm (393/321-709 mg/kg bw/day):** ***Organ Weights:*** ↑ Liver weight: 31 %** (abs.) and 27 %** (rel.) in males and 56 %** (abs.) and 48 %** (rel.) in females ↑ Kidneys weight: 24 %** (abs.) and 21 %** (rel.) in males and 18 %** (abs.) and 10

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		%** (rel.) in females ↑ Caecum weight: 1.9 fold** (abs. and rel.) in males and 1.5 fold** (abs. and rel.) in females ***Histopathology:*** **Liver** Diffuse hepatocyte hypertrophy:22/24** males and 22/24** females (versus 0 in controls) Brown deposition in Glisson's capsule: 8/24** males (versus 0 in controls) **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 11/24** males (versus 0 in controls) **Thyroid** Follicular cell hypertrophy: 19/24** males (versus 5/22 in controls) and 16/23** females (versus 5/22 in controls) **Caecum** Distension: 18/24** males and 6/23* females (versus 0 in controls) ***Haematology:*** ↑ Platelet count 18 %** in females NOAEL general toxicity (parental):150 ppm (9.6/9.3-21.2 mg/kg bw/day) NOAEL reproductive toxicity: > 5000 ppm (> 334/307-677 mg/kg bw/day) NOAEL general toxicity (F_1 generation): 1000 ppm (76.8/61.6-130 mg/kg bw/day)

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

Pyriofenone was tested in a preliminary dose range-finding study and a definitive two-generation study in rats in order to assess its effects on sexual function and fertility.

Preliminary Study

In the preliminary study, male and female Wistar rats (8/sex/group) were administered pyriofenone in their diet at concentrations of 0, 300, 10000 or 20000 ppm for three weeks prior to mating, throughout gestation and lactation until weaning of the F_1 offspring (doses were equivalent to 17.9/31.9, 185/328, 591/1004 and 1159/1828 mg/kg bw/day males/females).

No mortalities occurred during the study and there were no clinical signs. Body weight gain was reduced in males of the top dose group throughout the ten week study (21 % lower than controls) and in females of the

top dose group during the premating period (weeks 0-3, 17 % lower than controls) and throughout the gestation period (weeks 14-20, 24 % lower than controls). The reduction in weight gain was accompanied with reduced food consumption during weeks 0-3, 14-20 and during the lactation period, days 14-21.

In the parental generation, the main organs affected in this study were the liver, kidneys and caecum. From a dose of 3000 ppm (185/328 mg/kg bw/day) the weights of these organs were increased, dose-dependently. In males and females of the top dose group, absolute and relative liver weights were increased by 36 and 50 % in males and 80 and 88 % in females compared to controls (absolute and relative weights respectively). Histopathology revealed diffuse hepatocyte hypertrophy and enlargement in all males and females of the 10000 ppm and 20000 ppm dose groups.

All males of the top two dose groups had darkened livers as did 5/8 females of the 20000 ppm group and 4/8 females of the 10000 ppm dose group. Kidney weight was increased in males of the top dose group by 27 and 36 %, compared to controls, (absolute and relative respectively) with increased hyaline droplet deposition in proximal tubule cells (5/8 males) whilst females were shown to be slightly less sensitive with a 11 % relative increase in weight when treated with 20000 ppm pyriofenone only. The large intestine was affected, with the weight of the caecum increasing approximately 2-fold in both males and females of the top two dose groups.

Females treated with 20000 ppm and 10000 ppm were also found to have a decrease in ovaries weight (20000 ppm: 38 % absolute and 33 % relative; 10000 ppm: 24 % absolute and 23 % relative). In the absence of any associated histopathology or effects to sexual function, this decrease in weight was likely to be associated with the decrease in body weight gain.

Changes in clinical chemistry were consistent with the toxicity occurring in the liver and kidney. In females of the top dose and those dosed with 10000 ppm, there were decreases to haemoglobin and haematocrit levels. Both males and females dosed with 20000 and 10000 ppm had elevated platelet counts.

In the F_1 generation, body weight was reduced in males and females treated with doses ≥ 10000 ppm (20000 ppm: 36 % in males and 35 % in females, 10000 ppm: 21 % in males and 12 % in females). Organs affected following treatment with pyriofenone were the brain, spleen, thymus and uterus with dose-dependent reductions in weight observed from a dose of 10000 ppm. There was no associated histopathology with the organ weight changes and in the absence of such, they are considered to be a consequence of generalised toxicity and the reduction in body weight.

There were no treatment-related effects to oestrous cycle, mating index, fertility index, gestation index, the duration of gestation or the number of implantations. There was also no effect on the development of the offspring. At the top dose of 20000 ppm, one female was non-pregnant and one female lost her entire litter by lactation Day 4. The cause was likely due to ill-health; however no firm conclusions can be drawn from this study due to the low numbers of animals used and the decreased pre-mating period.

Two-generation study

In the main study, male and female Wistar rats (24/sex/dose) received pyriofenone in their diet at concentrations of 0, 150, 1000 or 5000 ppm [approximately 10-20, 70-130, 320 – 700 mg/kg bw/day (exact doses in mg/kg bw/day can be found in Table 26, depending on sex and study period)]. There were no treatment-related mortalities, clinical findings or effects on body weight or body weight gain in P or F_1 parental animals.

In P and F_1 parental animals the main organs affected following treatment with pyriofenone were the liver, kidneys, thyroids and caecum.

In the P generation, liver weight was increased from a dose of 1000 ppm (64.1/62-138.1 mg/kg bw/day) in males and females. At the top dose of 5000 ppm (334/307-677 mg/kg bw/day) the increased liver weight [27 % and 29 % in males and 42 % and 38 % in females (absolute and relative respectively) compared to controls] was accompanied by diffuse hepatocyte hypertrophy (13/23 males and 20/23 females versus 0 in controls), brown deposition in the Glisson's capsule in males (13/23 males versus 0 in controls) and dark colouring (14/23 males and 20/23 females).

Kidney weight (absolute and relative) was increased in males and females of the top dose by up to 24 % and in males, this was accompanied by increased hyaline droplet deposition in proximal tubule cells (11/23 males versus 0 in controls).

The thyroid weight was increased in males of the top dose [21 % (abs.) and 23 % (rel.) compared to controls] but an increase in the incidence of follicular cell hypertrophy was observed in females only (14/23 versus 3/21 in controls), indicating that whilst the increase in thyroid weight might be due to stress and general toxicity, the hypertrophy was more likely to be due to the effects of the ingested substance.

Caecum weight was increased in both males and females of the top dose by just under 2-fold (absolute and relative). Similarly to the preliminary study, haemoglobin and haematocrit levels were decreased in females of the top dose and the female platelet count was increased (11 % compared to controls). Changes to white blood cell parameters were noted, with a decrease in lymphocyte count and a decrease in the basophil count in females of the top dose.

Males and females of the top dose of the F_1 parental generation also had increased liver weights [31 % and 27 % in males and 56 % and 48 % in females (absolute and relative respectively)] with accompanying diffuse hepatocyte hypertrophy (22/24 males and females, versus 0 in controls) and brown deposition in the Glisson's capsule in males only (8/24 males versus 0 in controls).

Kidney weight was also increased in both sexes [24 % and 21 % in males and 18 % and 10 % in females (absolute and relative)]. In males, increased hyaline droplet deposition in proximal tubule cells was also noted (11/24 males versus 0 in controls).

The caecum weight was increased in both males and females (1.5 – 1.9 fold) and distension was noted (18/24 males and 6/23 females, versus 0 in controls).

Follicular cell hypertrophy of the thyroid was also noted in both males and females (19/24 males and 16/23 females versus 5/22 in controls) and haematology revealed an 18 % increase in platelet count in females compared to controls.

There were no treatment-related changes to sexual function or fertility in males or females in both the P and F_1 generations. There were no developmental adverse effects to F_1 or F_2 offspring.

The results indicate that pyriofenone does not cause adverse effects to sexual function or fertility in rats.

10.10.3 Comparison with the CLP criteria

Pyriofenone has been well-tested in a guideline-compliant two-generation study in Wistar rats. Further information has also been provided by a preliminary dose range-finding study, also in Wistar rats. A specific effect on fertility, reproduction and pregnancy outcome was not demonstrated by these studies. At the top dose of 20000 ppm in the preliminary study, one female failed to become pregnant and another lost all of her litter by lactation Day 4, however these effects occurred only at a dose that also resulted in marked parental toxicity (decreased food consumption and body weight gain and significant changes to liver, kidney and caecum weight with associated histopathogical adverse effects).

Pyriofenone is not known as a human reproductive toxicant; therefore classification in Category 1A is not necessary.

From the animal data available, there was no clear evidence to suggest that pyriofenone should be presumed to be a human reproductive toxicant; therefore classification in Category 1B is not appropriate.

Classification in Category 2 is reserved for substances where there is some evidence from human or experimental animals of an adverse effect on sexual function and fertility. Such effects should be observed in the absence of other toxic effects. On the basis that there is no evidence that pyriofenone causes any adverse effects to sexual function or fertility, it should not be classified in this category. Therefore no classification for this endpoint is required.

10.10.4 Adverse effects on development

Table 27: Summary table of animal studies on adverse effects on development

↑↓ denote an increase or decrease in a parameter with respect to the control value
Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$
abs. = absolute
rel. = relative

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th></tr>
<tr><td>Teratogenicity study in rats
(Dose-range finding study)
OECD 414
GLP
Rats, Wistar
(8 females/ group)

DAR: B.6.6.2a

(Anon., 2009f)</td><td>IKF-309 technical
Purity 97.88 %
0, 30, 100, 300 or 1000 mg/kg bw/day
In aq. Carboxymethyl cellulose (1 %)</td><td><u>Maternal effects</u>
<u>1000 mg/kg bw/day):</u>
↑ Caecum weight: 23%* (rel.)

There were no other treatment-related findings or effects on pregnancy or fetal abnormalities.

NOAELs were not set for this study.</td></tr>
<tr><td>Teratogenicity study in rats
OECD 414
GLP
Rats, Wistar
(24 females/ group)

DAR: B.6.6.2b

(Anon., 2009e)</td><td>IKF-309 technical
Purity 97.88 %
0, 30, 300 or 1000 mg/kg bw/day
In aq. Carboxymethyl cellulose (1 %)</td><td><u>Maternal effects</u>
<u>1000 mg/kg bw/day):</u>
↑ Liver weight: 16%** (abs.) and 14 %** (rel.)
↑ Caecum weight: 1.3 fold** (abs. and rel.)

There were no treatment-related effects on pregnancy.
<u>Foetal effects</u>
There were no treatment-related increases in malformations observed in this study. However, a number of variations were observed as shown in the table below:
<table>
<tr><td>Dose mg/kg bw/day</td><td></td><td>0</td><td>30</td><td>300</td><td>1000</td><td>HCD range</td></tr>
<tr><td></td><td></td><td colspan="5"></td></tr>
<tr><td>No. foetuses [litters]</td><td></td><td>284 [24]</td><td>290 [24]</td><td>284 [24]</td><td>301 [24]</td><td></td></tr>
<tr><td>No. of litters with variations</td><td></td><td>24</td><td>23</td><td>23</td><td>23</td><td></td></tr>
<tr><td>No. of foetuses with variations:</td><td>Visceral</td><td>30</td><td>29</td><td>35</td><td>18*</td><td></td></tr>
</table>
</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		<table><tr><td></td><td>Skeletal</td><td>84</td><td>83</td><td>105*</td><td>121**</td><td></td></tr><tr><td colspan="7"></td></tr><tr><td rowspan="2">Discontinuous rib cartilage</td><td>Foetuses</td><td>38 (25.5 %)</td><td>45 (54.2 %)</td><td>47 (31.8 %)</td><td>62 (39.5 %)*</td><td>29.7 - 44.8 %</td></tr><tr><td>Litters</td><td>15 (62.5 %)</td><td>17 (70.8 %)</td><td>20 (83.3 %)</td><td>23 (95.8 %)*</td><td></td></tr><tr><td rowspan="2">Supernumeracy ribs:</td><td>Foetuses</td><td>64 (43.0 %)</td><td>51 (34.0 %)</td><td>85 (57.4 %)*</td><td>98 (62.4 **)</td><td>35.7 - 61.0 %</td></tr><tr><td>Litters</td><td>21 (87.5 %)</td><td>18 (75.0 %)</td><td>23 (95.8 %)</td><td>22 (91.7 %)</td><td></td></tr><tr><td rowspan="2">Full supernumerary ribs:</td><td>Foetuses</td><td>5 (3.4 %)</td><td>3 (2.0 %)</td><td>10 (6.8 %)</td><td>14 (8.9 %)#</td><td>5.6 - 6.8 %</td></tr><tr><td>Litters</td><td>3 (12.5 %)</td><td>3 (12.5 %)</td><td>7 (29.2 %)</td><td>7 (29.2 %)</td><td></td></tr></table># statistically significant (Fischers test p = 0.03) NOAEL general toxicity: 300 mg/kg bw/day NOAEL developmental toxicity: 30 mg/kg bw/day NOAEL teratogenicity: 1000 mg/kg bw/day
Teratogenicity study in rabbits (Dose-range finding study) OECD 414 GLP Rabbits, Japanese White (8 females/ group) DAR: B.6.6.3a (Anon., 2009g)	IKF-309 technical Purity 97.88 % 0, 30, 100, 300 or 1000 mg/kg bw/day In aq. Carboxymethyl cellulose (1 %)	**<u>Maternal effects:</u>** **<u>1000 mg/kg bw/day):</u>** ***General toxicity*** ↓ Body weight gain: 10 % ↑ Liver weight: 27% (abs.) and 35 % (rel.) ***Pregnancy*** No. of abortions/premature deliveries: 4/8 (versus 0 in controls) Resorptions and foetal deaths: 20.1 % (versus 7.4 % in controls) **<u>300 mg/kg bw/day):</u>** ***General toxicity*** Body weight loss: -130 g (versus gain of 280 g in control group) ***Pregnancy*** Resorptions and foetal deaths: 24.1 % (versus 7.4 % in controls) **<u>100 mg/kg bw/day):</u>** ***Pregnancy*** Resorptions and foetal deaths: 21.4 % (versus 7.4 % in controls)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		Foetal effects: There were no toxicologically relevant findings at any doses. NOAELs were not set for this study.
Teratogenicity study in rabbits OECD 414 GLP Rabbits, Japanese White (25 females/ group) DAR: B.6.6.3b (Anon., 2009h)	IKF-309 technical Purity 97.88 % 0, 30, 100 or 300 mg/kg bw/day In aq. Carboxymethyl cellulose (1 %)	There were no effects on body weight or body weight gain in any treated groups. **Maternal effects:** **300 mg/kg bw/day:** ***Pregnancy*** No. of abortions/premature deliveries: 2/25 (versus 0 in controls) **≤100 mg/kg bw/day:** No treatment-related findings **Foetal effects:** There were no toxicologically relevant findings at any doses (including the frequency of resorptions and foetal deaths). NOAEL general toxicity: 100 mg/kg bw/day NOAEL developmental toxicity:300 mg/kg bw/day NOAEL teratogenicity: 300 mg/kg bw/day

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

The effects of pyriofenone on development following exposure during pregnancy have been well tested in a preliminary (dose range-finding) teratogenicity study, in rats and rabbits and a definitive teratogenicity study, also in rats and rabbits.

Studies in rats

In a preliminary teratogenicity study, female Wistar rats received pyriofenone in aqueous sodium carboxymethylcellulose (1 %) by oral gavage (8/group). Doses used were 0, 30, 100, 300 or 1000 mg/kg bw/day. There were no treatment-related deaths, nor any effects on body weight or clinical signs. At the top dose of 1000 mg/kg bw/day there was an increase in relative caecum weight in dams (23 % greater than controls). There were no other treatment-related findings, effects on pregnancy or foetal abnormalities in any dose group.

In the main, guideline-compliant, teratogenicity study, pyriofenone was administered to time-mated female Wistar rats, (24/group), by oral gavage at 0, 30, 300 or 1000 mg/kg bw/day. Dams dosed with 1000 mg/kg bw/day were found to have increased liver weight [16 % and 14 % greater than controls (absolute and

relative respectively)] and also increased caecum weight [1.3 fold greater than controls (absolute and relative)]. There were no treatment-related findings affecting pregnancy. From a dose of 300 mg/kg bw/day there was a statistically significant and dose-dependent increase in the number of foetuses with skeletal variations. These were an increase in incidence of supernumaracy of ribs and discontinuous rib cartilage. At 300 mg/kg bw/day the number of foetuses affected was 105/284 and at 1000 mg/kg bw/day the number was 121/301 (versus 84/284 in controls). At 1000 mg/kg bw/day the percentage of foetuses with discontinuous rib cartilage was statistically significantly increased to 39.5 % (versus 25.5 % in controls). Whilst this value is above the concurrent control value for this variation, it was within the laboratory historical control data (HCD) provided: range 29.7 – 44.8 %. At the same dose there was a statistically significant increase in the foetuses with supernumaracy ribs (62.4 % versus 43 % in controls). At 300 mg/kg bw/day the percentage of foetuses affected was also increased (57.4 %), however, the value was within the range of the given HCD (35.7 – 61.0 %). At 1000 mg/kg bw/day the percentage of foetuses affected with supernumaracy ribs was above the concurrent control and also above the HCD. Given that the increase occurred in a dose-dependent manner and is above the HCD, it could be considered treatment-related.

Following administration of pyriofenone to rats, an increase in skeletal variations, including an increased incidence of supernumaracy ribs noted in top-dose animals. This increase was above the concurrent control and above the laboratory historical control data.

Studies in rabbits

A preliminary teratogenicity study was carried out in Japanese White rabbits in order to determine dose levels for the main experiment. In this study, artificially inseminated females (8/group) were given an oral dose of pyriofenone (by gavage) at 0, 30, 100, 300 or 1000 mg/kg bw/day suspended in aqueous sodium carboxymethyl cellulose (1 %). There were no maternal deaths during this study. At doses of 300 mg/kg bw/day and above, body weight gain was affected with reduced body weight gain of 10 % in dams treated with 1000 mg/kg bw/day and body weight loss occurring in dams treated with 300mg/kg bw/day. It was noted that six animals in the top dose group had ceased eating during the study and three animals dosed with 300 mg/kg bw/day. Of the six animals in the top dose group that had ceased eating, 4 aborted or delivered prematurely. At the top dose, liver weight was also increased, 27 % (absolute) and 35 % (relative) in comparison to controls. This was accompanied by a marginal increase in the incidence of fatty change (8/8 animals versus 6/8 in controls), however the extent of the fatty change was found to be more severe in the livers of animals dosed with 1000 mg/kg bw/day. From a dose of 100 mg/kg bw/day and above, the percentage of resorptions and foetal deaths appeared to be higher than controls, however, with such small animal numbers no statistics were performed and no definitive conclusions can be drawn as to whether this was treatment-related. There were no treatment-related increases in the number of variations or malformations in foetuses of this study.

In the main teratogenicity study, artificially inseminated female Japanese White rabbits (25/group) were orally gavaged with pyriofenone suspended in aqueous sodium carboxymethylcellulose (1 %) at concentrations of 0, 30, 100 or 300 mg/kg bw/day (as determined from the preliminary study) on days 6 - 27 of gestation. Two females from the top dose group were sacrificed on gestation day 18 as they showed signs of abortion. Gross pathology of these females revealed that one had white spots on the liver and the other had a coarse-surfaced and enlarged spleen. These incidences of abortion are considered to have occurred by chance and not related to treatment with the test substance. There were no treatment-related gross abnormalities in the remaining animals. There were no treatment-related increases in variations or malformations in foetuses of this study.

Pyriofenone did not appear to be a developmental toxicant in rabbits.

10.10.6 Comparison with the CLP criteria

Developmental toxicity has been assessed in both rats and in rabbits.

Classification in Category 1A for effects on development is required when a substance is a known human reproductive toxicant. Pyriofenone is not a known human reproductive toxicant, therefore, classification in this category is not required.

In order to be classified in Category 1B, there must be clear evidence from animal studies of an adverse effect on development occurring in the absence of other toxic effects. As there was no clear evidence to suggest pyriofenone should be a presumed human reproductive toxicant, classification in this category is not appropriate.

Classification in Category 2 is required if a substance is considered a suspected human reproductive toxicant. In a guideline-compliant study in rats, there was an increased incidence in the number of skeletal variations (supernumeracy ribs) in animals of the top dose group that was marginally above the laboratory control data. This finding is considered a variation and not a malformation as the growth of supernumeracy ribs is similar to the other thoracic ribs but unlike extra cervical ribs, extra complete lumbar ribs in humans are not associated with congenital abnormalities. Therefore, they cannot represent a permanent structural change. This finding was not observed in the dose-range finding study carried out in advance of the main study. There were no adverse findings on foetal development in rabbits. Overall, it can be concluded that pyriofenone is not a developmental toxicant. Classification for this endpoint is not required.

10.10.7 Adverse effects on or via lactation

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

There were no adverse effects on or *via* lactation observed in the two-generation studies in rats or the teratogenicity studies in rats and rabbits.

10.10.9 Comparison with the CLP criteria

There was no evidence to suggest that pyriofenone had an adverse effect on lactation or via lactation, therefore classification with this endpoint is not appropriate.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Not classified. Data conclusive but not sufficient for classification.

10.11 Specific target organ toxicity-single exposure

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

Pyriofenone has been well investigated in a number of acute studies by the oral, dermal and inhalation routes using the limit dose of 2000 mg/kg. In these studies there were few clinical signs and no macroscopic abnormalities. Therefore, there is no evidence of any effects that might be concluded to be due to specific target organ toxicity.

10.11.2 Comparison with the CLP criteria

Pyriofenone did not cause specific organ toxicity following a single exposure in rats by the oral, inhalation or dermal routes. There are no reports of specific target organ toxicity following a single exposure in humans either. Therefore, pyriofenone should not be classified for this endpoint.

10.11.3 Conclusion on classification and labelling for STOT SE

Not classified. Data conclusive but not sufficient for classification.

10.12 Specific target organ toxicity-repeated exposure

The specific target organ toxicity of pyriofenone upon repeated exposure has been investigated in 28-day and 90-day studies in rats, mice and dogs, in one-year studies in rats and dogs. Additional information is provided by chronic/carcinogenicity studies in rats and mice, which are reported in section 10.9 (Table 19) and from a two-generation reproduction study, reported in section 10.10 (Table 26).

Table 28: Summary table of animal studies on STOT RE

↑↓ denote an increase or decrease in a parameter with respect to the control value
Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$
abs. = absolute
rel. = relative

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels and guidance values for classification**	**Results**
Oral studies		
28-Day oral dietary study OECD 407 GLP Rats, Fischer 6/sex/dose DAR: B.6.3.1a (Anon., 2010g)	IKF-309 technical Purity 98.04 % 0, 300, 3000, 10000 or 20000 ppm Equivalent to: ♂ 24.2, 251, 823 and 1657 mg/kg bw/day ♀ 26.1, 261, 841 and 1660 mg/kg bw/day STOT-RE 1: ≤ 30 mg/kg bw/day STOT-RE 2: > 30, ≤ 300 mg/kg bw/day	**<u>20000 ppm (1657/1660 mg/kg bw/day):</u>** ***Organ weights:*** ↑ Liver 38 %** (abs), 45 %** (rel.) in males and 59 %** (abs.and rel.) in females ↑Kidneys 22 %** (abs.), 28 %** (rel.) in males and 22 %** (abs.), 21 %** (rel.) in females ↑Thyroids 38 %** (rel.) in males ***Histopathology:*** **Liver** Diffuse hepatocyte hypertrophy: 6/6 males** and 6/6 females** (versus 0 in controls) Dark in colour: 6/6 males** and 6/6 females** (versus 0 in controls) **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 6/6 males** (versus 0 in controls) Increased calcification, corticomedullary junction: 4/6 females * (versus 0 in controls) **Caecum** Distended with contents: 6/6 males** and 6/6 females** (versus 0 in controls)

		Clinical chemistry: ↓ Alkaline phosphatase 29 %** in males and 31 %** in females ↑ γ-Glutamyl transpeptidase 20 %** in males, 40 %** in females ↓ Creatinine 19 %** in males, 17 %* in females ↑ Total Cholesterol 27 %** in males, 29%** in females ↓ Total bilirubin 33 %** in males and 75 %** in females ***Haematology:*** ↑ Platelet count 16.7 %** in males, 15 %** in females ↑ Lymphocyte count 17 %* in males **<u>10000 ppm (823/841 mg/kg bw/day):</u>** ***Organ weights:*** ↑ Liver 22 %** (abs), 28 %** (rel.) in males and 36 %** (abs.and rel.) in females ↑ Kidneys 16 %** (abs.), 18 %** (rel.) in males and 15 %** (abs.and rel.) in females ***Histopathology:*** **Liver** Diffuse hepatocyte hypertrophy: 6/6 males** and 6/6 females** (versus 0 in controls) Dark in colour: 6/6 males** (versus 0 in controls) **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 6/6 males** (versus 0 in controls) **Caecum** Distended with contents: 6/6 males** and 6/6 females** (versus 0 in controls) ***Clinical chemistry:*** ↓ Alkaline phosphatase 19 %** in males and 13 % in females ↑ γ-Glutamyl transpeptidase 10 %* in males, 20 %* in females ↓ Creatinine 13 %* in males, 11 % in females ↑ Total Cholesterol 25 %** in males, 11%** in females ↓ Total bilirubin 33 %** in males and 75 %** in females **<u>3000 ppm (251/261 mg/kg bw/day):</u>** ***Organ weights:*** ↑ Liver 10 %** (rel.) in males ***Histopathology:*** **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 6/6

		males** (versus 0 in controls) **Caecum** Distended with contents: 6/6 males** and 6/6 females** (versus 0 in controls) ***Clinical chemistry:*** ↓ Alkaline phosphatase 10 %** in males ↓ Total bilirubin 50 %** in females **<u>300 ppm (24.2/26.1 mg/kg bw/day):</u>** No treatment-related findings NOAEL: 300 ppm (24.2/26.1 mg/kg bw/day)
90-Day oral dietary study OECD 408 GLP Rats, Fischer 10/sex/dose DAR: B.6.3.1b (Anon., 2010h)	IKF-309 technical Purity 98.04 % 0, 300, 1000, 2500 or 5000 ppm Equivalent to: ♂ 17.9, 60.5, 150 and 305 mg/kg bw/day ♀ 20.6, 69.0, 171 and 350 mg/kg bw/day STOT-RE 1: ≤ 10 mg/kg bw/day STOT-RE 2: >10, ≤ 100 mg/kg bw/day	**<u>5000 ppm (305/350 mg/kg bw/day):</u>** ***Organ weights:*** ↑ Liver 21 %** (abs), 20 %** (rel.) in males and 13 %** (abs), 16 %** (rel.) in females ↑ Kidneys 15 %** (abs.), 16 %** (rel.) in males and 10 %** (rel.) in females ↑ Caecum 2.6 fold** (abs. and rel.) in males and 2 fold** (abs. and rel.) in females ***Histopathology:*** **Liver** Diffuse hepatocyte hypertrophy: 9/10** males and 6/10** females (versus 0 in controls) **Kidney** Increased hyaline droplet deposition in proximal tubule cells: 9/10**males (versus 0 in controls) Tubular basophilic change: 7/10** males **Caecum** Distended with contents: 10/10** males and 5/10* females (versus 0 in controls) ***Clinical chemistry:*** ↓ Aspartate aminotransferase 20 %** in males ↓ Alanine aminotransferase 19 %** in males ↑ Blood urea nitrogen 20 %** in males ↓ Creatinine 11 % in males, 26 %** in females ↑ Total cholesterol 24 %** in males ↓ Total bilirubin 50 %** in males and none detected** in females ***Haematology:*** ↑ Lymphocyte count 24 %** in males

		2500 ppm (150/171 mg/kg bw/day): ***Organ weights:*** ↑ Liver 14 %** (abs), 10 %** (rel.) in males ↑ Caecum 1.3fold (abs. and rel.) in males and 1.4 fold** (abs. and rel.) in females ***Clinical chemistry:*** ↓ Aspartate aminotransferase 14 %** in males ↓ Alanine aminotransferase 20 %** in males ↓ Creatinine 11 % in males ↓ Total bilirubin 25 %** in males and 50 %** in females **1000 ppm (60.5/69 mg/kg bw/day):** ***Clinical chemistry:*** ↓ Alanine aminotransferase 10 %** in males ↓ Total bilirubin 25 % in males and 25 %** in females **300 ppm (17.9/20.6 mg/kg bw/day):** No treatment-related findings NOAEL: 300 ppm (17.9/20.6 mg/kg bw/day)
13-Weeks oral dietary study OECD 408 GLP Mice, CD-1 10/sex/dose DAR: B.6.3.2a (Anon., 2009h)	IKF-309 technical Purity 97.88 % 0, 300, 1000, 3000 or 7000 ppm Equivalent to: ♂ 53, 176, 515 and 1318 mg/kg bw/day ♀ 61, 214, 695 and 1504 mg/kg bw/day STOT-RE 1: ≤ 10 mg/kg bw/day STOT-RE 2: >10, ≤ 100 mg/kg bw/day	**7000 ppm (1318/1504 mg/kg bw/day):** ***Organ weights:*** ↑ Liver 12 %** (rel.) in males and 14 %** (abs.), 18 %** (rel.) in females ***Histopathology:*** **Liver** Periportal hepatocytes hypertrophy: 3/10 males and 7/10 females (versus 0 in controls) ***Clinical chemistry:*** ↓ Aspartate aminotransferase 38 %* in males **3000 ppm (515/695 mg/kg bw/day):** ***Organ weights:*** ↑ Liver 9.5 %* (rel.) in males and 8.5 %* in females **1000 ppm (176/214 mg/kg bw/day):** ***Organ weights:*** ↑ Liver 11.5 %* (rel.) in females **300 ppm (53/61 mg/kg bw/day):** No treatment-related findings

		NOAEL: 300 ppm (53/61 mg/kg bw/day)
90-Day oral dietary study OECD 409 GLP Dogs, Beagle 4/sex/dose DAR: B.6.3.3b (Anon., 2010k)	IKF-309 technical Purity 97.88 % ♂ 0, 500, 3000 or 25000 ppm ♀ 0, 500, 3000 or 15000 ppm Equivalent to: ♂ 15.0, 90.3 and 776 mg/kg bw/day ♀ 15.3, 89.9 and 475 mg/kg bw/day Based on a 90 day study in rats: STOT-RE 1: ≤ 10 mg/kg bw/day STOT-RE 2: > 10, ≤ 100 mg/kg bw/day	**<u>25000 ppm (776 mg/kg bw/day) (males only):</u>** ***Observations:*** ↓ Body weight gain 35 % ***Organ weights:*** ↑ Liver 32 %** (abs), 40 %** (rel.) ↓ Testis 19 % (abs), 15 % (rel.) ↓ Prostate 27 % (abs) 24 % (rel.) ***Histopathology:*** **Liver** Centrilobular hypertrophy: 3/4 males (versus 0 in controls) ***Clinical chemistry:*** ↑Alkaline phosphatase 5.8 fold ↑ Triglyceride 84 %** **<u>15000 ppm (475 mg/kg bw/day) (females only):</u>** ***Organ weights:*** ↑ Liver 23 % (abs), 26 % (rel.) ↓ Spleen 28 % (rel.) ↓ Ovaries 53 % (abs), 52 % (rel.) ***Histopathology:*** **Liver** Centrilobular hypertrophy: 3/4 females (versus 0 in controls) ***Clinical chemistry:*** ↑Alkaline phosphatase 4.9 fold ***Haematology:*** ↑ Platelet count 42 %* in females **<u>3000 ppm (90.3/89.9 mg/kg bw/day):</u>** ***Clinical chemistry:*** ↑Alkaline phosphatase 2.2 fold in females **<u>500 ppm (15.0/15.3 mg/kg bw/day):</u>** No treatment-related findings NOAEL: 500 ppm (15.0/15.3 mg/kg bw/day)
1-Year oral dietary study	IKF-309 technical Purity 97.88 %	**<u>5000 ppm (226/275 mg/kg bw/day):</u>** ***Observations:***

OECD 452 GLP Rats, Fischer 20/sex/dose DAR: B.6.5.1a (Anon., 2010i)	0, 200, 1000 or 5000 ppm Equivalent to: ♂ 8.5, 42.9 and 226 mg/kg bw/day ♀ 10.6, 53.5 and 275 mg/kg bw/day STOT-RE 1: ≤ 2.5 mg/kg bw/day STOT-RE 2: > 2.5, ≤ 25 mg/kg bw/day	↑ Soiled fur 12/20** females (versus 0 in controls) ***Organ weights:*** ↑ Liver 16 %** (abs), 20 %** (rel.) in males and 29 %** (abs), 27 %** (rel.) in females ↑ Kidneys 15 %** (abs.), 20 %** (rel.) in males and 12 %** (abs) and 21 %** (rel.) in females ↑ Caecum 2.1 fold** (abs. and rel.) in males and 1.9 fold** (abs. and rel.) in females ↑ Adrenals 11 %** (abs.), 20 %** (rel.) in males and 13 %** (rel.) in females ***Histopathology:*** **Liver** Centrilobular hypertrophy: 18/20** males (versus 0 in controls) **Kidney** Tubular basophilic change: 10/20** males (versus 1/20 in controls) Increased deposition of brown pigment in tubular cells: 20/20** females (versus 0/20 in controls) **Bone marrow (sternum)** Increased haematopoiesis: 9/20** males (versus 0 in controls) **Bone marrow (femur)** Increased haematopoiesis: 9/20** males (versus 0 in controls) **Caecum** Distended large intestine: 5/20* males and 10/20** females ***Clinical chemistry:*** ↓Alkaline phosphatase 27 %** in males and 33 %** in females ↓Aspartate aminotransferase 34 %** in males and 40 %** in females ↓Alanine aminotransferase 45 %** in males and 33 %** in females ↓Creatine 14 %** in males and 27 %** in females ↓Bilirubin 83 %** in females **<u>1000 ppm (42.9/53.5 mg/kg bw/day):</u>** No toxicologically significant findings NOAEL: 1000 ppm (42.9/53.5 mg/kg bw/day)
1-Year oral dietary study OECD 452 GLP Dogs, Beagle	IKF-309 technical Purity 97.88 % ♂ 0, 500, 3000 or 25000 ppm ♀ 0, 500, 3000 or 15000 ppm	**<u>25000 ppm (701 mg/kg bw/day) (males only):</u>** ***Observations:*** ↓ Body weight 23 % ↓ Body weight gain 71 % Vomiting feed: 3/4 males versus 1/4 in controls Loose stool: 4/4 males versus 2/2 in controls

4/sex/dose DAR: B.6.3.3c (Anon., 2010l)	Equivalent to: ♂ 13.7, 83.5 and 701 mg/kg bw/day ♀ 14.1, 86.2 and 448 mg/kg bw/day Extrapolating from 90 day study cut-off values in rats: STOT-RE 1: ≤ 2.5 mg/kg bw/day STOT-RE 2: > 2.5, ≤ 25 mg/kg bw/day	***Organ weights:*** ↑ Liver 43 %** (abs), 86 %** (rel.) ↑ Kidney 29 %** (rel.) ***Histopathology:*** **Liver** Centrilobular hypertrophy: 3/4 males (versus 0 in controls) Dark colour: 4/4* males (versus 0 in controls) Enlargement: 4/4* males (versus 0 in controls) **Gallbladder** Intraluminal black granules: 3/4 males (versus 1/4 in controls) ***Clinical chemistry:*** ↑Alkaline phosphatase 10 fold** ↑ γ-Glutamyl transpeptidase 2.3 fold* **<u>15000 ppm (448 mg/kg bw/day) (females only):</u>** ***Observations:*** ↓ Body weight 10 % ↓ Body weight gain 40 % Vomiting feed: 2/4 females versus 0 in controls Loose stool: 4/4 females versus 1/4 in controls ***Histopathology:*** **Liver** Dark colour: 3/4 females (versus 0 in controls) Enlargement: 4/4 females (versus 0 in controls) **Gallbladder** Intraluminal black granules: 2/4 males (versus 1/4 in controls) ***Clinical chemistry:*** ↑Alkaline phosphatase 8 fold** **<u>3000 ppm (83.5/86.2mg/kg bw/day):</u>** ***Observations:*** ↓ Body weight gain 49 % in males and 30 % in females Loose stool: 3/4 females versus 1/4 in controls ***Organ weights:*** ↑ Liver 29 % (rel.) in males ↑ Kidney 29 %** (rel.) in males ***Clinical chemistry:*** ↑Alkaline phosphatase 2.5 fold in males and 2.2 fold in females

		500 ppm (13.7/14.1mg/kg bw/day): ***Observations:*** ↓ Body weight gain 44 % in males NOAEL not determined
Dermal studies		
28-Day dermal study OECD GLP Rats, Sprague Dawley 10/sex/dose DAR: B.6.3.4a (Anon., 2010m)	IKF-309 technical Purity 97.88 % 0, 100, 300 or 1000 mg/kg bw/day STOT-RE 1: ≤ 60 mg/kg bw/day STOT-RE 2: > 60, ≤ 600 mg/kg bw/day	**1000 mg/kg bw/day:** No treatment-related findings. NOAEL > 1000 mg/kg bw/day

Further details about these studies and the results are described below. In addition consideration of the information relevant for classification is available in Table 29.

10.12.1 Oral studies in rats

Pyriofenone has been tested in rats by the oral route in a 28 day, a 90-day and a 1-year study. Also available are data from a 2-year carcinogenicity study and a 2-generation reproduction study.

Twenty-eight day study in rats

The doses relevant for classification in this 28-day study were 300 and 3000 ppm (~25 and 255 mg/kg bw/day) (STOT RE 1: C ≤ 30 mg/kg bw/day and STOT RE 2: 30 < C ≤ 300 mg/kg bw/day).

Fischer rats (6/sex/group) were fed dietary concentrations of 0, 300, 3000, 10000 or 20000 ppm of pyriofenone (equivalent to 0, 24.2, 251, 823 and 1657 mg/kg bw/day in males and 0, 26.1, 261, 841 and 1660 mg/kg bw/day in females) for 28 days.

There were no unscheduled deaths in any group of either sex nor were there any effects to body weight or food consumption. Clinical signs were limited to one female of the 10000 ppm group and two females of the 20000 ppm group which were found to have soiled fur in the external genital region. There were no treatment-related adverse findings at concentrations relevant for classification with STOT-RE 1. At 3000 ppm (251/261 mg/kg bw/day) findings were limited to an increase in relative liver weight in males only (10 % greater than controls), with no associated pathology, an increase in hyaline droplet deposition in proximal tubule cells of the kidney in all males (versus none in controls) and distention of the caecum in all males and females (versus none in control groups). There were some changes in clinical chemistry at this dose associated with liver and kidney toxicity. These were a decrease in alkaline phosphatase (10 % lower than controls) in males and a decrease in bilirubin (50 % lower than controls) in females.

At doses greater than 3000 ppm effects to the liver and kidneys described at lower doses became more marked.

Ninety day study in rats

The doses relevant for classification with STOT-RE 2 in this 90-day study were 300 and 1000 ppm (~20 and 65 mg/kg bw/day). There were no doses relevant for classification with STOT-RE 1 (STOT-RE 1: C ≤ 10 mg/kg bw/day and STOT RE 2: 10 < C ≤ 100 mg/kg bw/day).

Fischer rats (10/sex/dose) were administered pyriofenone in the diet at doses of 0, 300, 1000, 2500 or 5000 ppm for 90 days (equivalent to 17.9, 60.5, 150 and 305 mg/kg bw/day in males and 20.6, 69.0, 171 and 350 mg/kg bw/day in females).

At 1000 ppm there were small disturbances to the clinical chemistry, in particular a decrease in levels of alanine aminotransferase in males (10 % lower than controls) and a decrease in total bilirubin in both males and females (25 % lower than controls). The main effects observed at doses ≥ 2500 ppm were increases in absolute and relative weight of the liver, kidney and caecum and associated histopathology. These changes became more apparent with increasing dose. In the liver, diffuse hypertrophy was observed in most males and just over half of the females of the top dose. In the kidney, increased hyaline droplet deposition in proximal cells and tubular basophilic changes were noted in most males. Similar to the 28-day study, the caecum was found to be distended with contents and this occurred in all males of the top dose group and 5/10 females. Clinical chemistry showed various changes to the parameters associated with liver and kidney toxicity.

One year study in rats

The doses relevant for classification with STOT-RE 2 in this 1-year study were 200 and 1000 ppm (~45 mg/kg bw/day) (derived by adjusting the standard guidance values for rats, using Haber's rule). There were no doses relevant for classification with STOT-RE 1 (STOT-RE 1: C ≤ 2.5 mg/kg bw/day and STOT RE 2: 2.5 < C ≤ 25 mg/kg bw/day).

Fischer rats (20/sex/dose) were fed concentrations of pyriofenone in their diet for 52 consecutive weeks. The concentrations used were 0, 200, 1000 or 5000 ppm (equivalent to 8.51, 42.9 and 226 mg/kg bw/day in males and 10.6, 53.5 and 275 mg/kg bw/day in females).

At doses ≤ 1000 ppm there were no overt toxicologically significant findings. At 5000 ppm the main organs affected were the liver, kidneys and caecum. Organ weights were increased in both males and females and centrilobular hypertrophy was evident in most males. In the kidney, tubular basophilic change was observed in half of the males and increased deposition of brown pigment in tubular cells was observed in all females. Distention of the caecum was observed in 5/20 males and 10/20 females. Also observed were increased incidences of haematopoiesis in the bone marrow of males. Changes in clinical chemistry were consistent with liver and kidney toxicity.

Two year carcinogenicity study in rats

Only data from animals dosed with 200 ppm (~ 8 mg/kg bw/day) were relevant for classification with STOT-RE 2 (doses were derived by adjustment of the standard guidance values for rats, using Haber's rule). There were no doses relevant for classification with STOT-RE 1 (STOT-RE 1: C ≤ 1.25 mg/kg bw/day and STOT RE 2: 1.25 < C ≤ 12.5 mg/kg bw/day).

Fischer rats (50/sex/dose) were administered pyriofenone in the diet for 104 weeks (Table 21) at doses of 0, 200, 1000 or 5000 ppm [equivalent to 0, 7.25/9.13, 36.4/46.5, 197/254 mg/kg bw/day (males/females)]. There were no toxicologically relevant findings at 200 ppm, the only dose relevant for classification. In brief, there were statistically significant increases in mortality at the top dose (5000 ppm, 197/254 mg/kg bw/day males/females) in the last three weeks of the study. The target organs were the liver (increased incidences of liver spots, fatty changes, hypertrophy in males and females, necrosis in males only and focal congestion in females only), kidneys (increased incidences of coarse surface in males and chronic

nephropathy in females only) and large intestines (increased incidences of distension of the caecum in males and females and mucosal haemorrhage in males).

Two-generation study in rats

The overall length of this study is approximately 90 days and so taking into consideration the relevant guidelines for a 90-day study in rats (STOT-RE 1: C ≤ 10 mg/kg bw/day and STOT RE 2: 10 < C ≤ 100 mg/kg bw/day), only toxicity observed at doses of 1000 ppm and below are is relevant for classification purposes.

Wistar rats (24/sex/dose) received pyriofenone in their diet for a 10 week period prior to mating and then for the duration of the gestation and lactation periods. Concentrations used were 0, 150, 1000 or 5000 ppm [(approximately 10-20, 70-130, 320 – 700 mg/kg bw/day (exact doses in mg/kg bw/day can be found in Table 28, depending on generation, sex and study period)]. The main target organs were liver, kidney and caecum with organ weights increased in males and females of the top dose group and increased incidences of hepatocyte hypertrophy, brown deposition in the Glisson's capsule, darkened liver colour and hyaline droplet deposition in proximal tubule cells of the kidney. At doses relevant for classification with STOT-RE 2 there were small increases in absolute and relative liver weight in parental females of 11 % and 9 % (respectively) compared to the controls, however there were no adverse findings from histopathological examinations pertaining to the liver. There were no adverse findings from the histopathological examination associated with the increase in liver weight.

10.12.2 Oral studies in mice

Pyriofenone has been tested in mice by the oral route in a 90-day study. Data from an 18-month carcinogenicity study is also available.

Ninety day study in mice

The dose relevant for classification with STOT-RE 2 in this 90-day study was 300 ppm ~55 mg/kg bw/day). There were no doses relevant for classification with STOT-RE 1 (STOT-RE 1: C ≤ 10 mg/kg bw/day and STOT RE 2: 10 < C ≤ 100 mg/kg bw/day).

CD-1 mice (10/sex/dose) were fed pyriofenone in the diet at concentrations of 0, 300, 1000, 3000 or 7000 ppm for 13 weeks (equivalent to 53, 176, 515 and 1318 mg/kg bw/day in males and 61, 214, 695 and 1504 mg/kg bw/day in females).

There were no mortalities, clinical signs of significant effects on body weights or food consumption. At 300 ppm there were no toxicologically significant findings. At doses > 300 ppm the liver was the target organ. Liver weight was increased in both males and females and periportal hepatocyte hypertrophy was apparent in both sexes also. Clinical chemistry revealed decreased aspartate aminotransferase in males only. There were some haem effects in males from a dose of 300 ppm in females from a dose of 1000 ppm. However, there was a lot of inter-individual variation and the majority of these effects occurred at doses greater than the guidance values for classification.

Eighteen month carcinogenicity study in mice

All doses used in this study were above the extrapolated guideline cut-off values (as taken from a 90 day rat study), STOT-RE 1: C ≤ 1.6 mg/kg bw/day and STOT RE 2: 1.6 < C ≤ 16 mg/kg bw/day.

CD-1 mice (52/sex/dose) received an oral dose of pyriofenone in their diet at concentrations of 0, 600, 1800 or 5400 ppm for males (equivalent to 0, 77.6, 237 and 716 mg/kg bw/day) or 0, 300, 1000 or 3000 ppm for

females (equivalent to 0, 49.4, 167 and 486 mg/kg bw/day) for 78 weeks (Table 21). The main non-neoplastic findings in this study included an increased incidence of abnormal yellow staining (perigential region) in males, reduced bodyweight gain in females and liver and kidney findings. In males, the main findings in the liver were increased organ weight and masses were found. Also observed was centrilobular hepatocyte hypertrophy and necrosis of individual hepatocytes. In the kidney, increased basophilic and eosinophilic foci with granular kidneys and cortical tubular basophilia was observed. In females, pigment in the liver macrophages was observed and granular kidneys and chronic progressive nephropathy.

10.12.3 Oral studies in dogs

Pyriofenone has been tested in dogs by the oral route in a 28-day, a 90-day and a 1-year study.

Twenty-eight day study in dogs (DAR: B.6.3.3a, Anon., 2010j)

This study was carried out according to OECD guidelines but with a significant deviation of only one dog being used per dose group. Due to this significant deviation, this study was not included in Table 28. The objective of the study was to clarify the toxic characteristics of pyriofenone in dogs and to select dose levels for a 90-day study.

Beagle dogs (1/sex/dose) were administered pyriofenone in their diet at concentrations of 0, 500, 1500, 5000 or 15000 ppm (equivalent to 14.9, 37.9, 144 and 390 mg/kg bw/day in males and 15.9, 47.5, 143 and 485 mg/kg bw/day in females). There was no mortality or treatment-related clinical signs observed and no significant changes in behaviour observations. As this study was intended to be a sighting study it is hard to ascertain the true effects of pyriofenone in dogs, however there were indications of increased liver, kidney, adrenals, thymus, spleen and ovary weight in the top-dosed female. There were no treatment-related gross findings in the male or female.

Ninety day study in dogs

Using the guideline cut-off values for a 90-day rat study, the doses relevant for classification in this 90-day dog study are 500 ppm and 3000 ppm only (15 mg/kg bw/day and 90 mg/kg bw/day). There were no doses relevant for classification with STOT-RE 1 (STOT-RE 1: $C \leq 10$ mg/kg bw/day and STOT RE 2: $10 < C \leq 100$ mg/kg bw/day).

Beagle dogs (4/sex/dose) received pyriofenone in their diet at concentrations of 0, 500, 3000, 15000 (females only) or 25000 ppm (males only) for 90 days (equivalent to 15.0, 90.3 and 776 mg/kg bw/day in males and 15.3, 89.9 and 475 mg/kg bw/day in females). There was no mortality or treatment-related clinical signs observed and no significant changes to behaviour. Body weight and food consumption did not show any treatment-related differences, however body weight gain was reduced in males of the top dose group.

Animals dosed with 500 ppm had no treatment-related adverse effects. Those dosed with 3000 ppm had small changes in clinical chemistry only [increased alkaline phosphatase levels in females only (2.2 fold higher than controls]. At doses higher than 3000 ppm, the liver was the target organ, with increased absolute and relative weight in males and females and increased incidences of centrilobular hypertrophy in males and females. In addition, the mean relative spleen weight was statistically significantly reduced in females of the top dose. Males of the top dose were observed to have decreased testes (19 % absolute and 15 % relative in comparison to controls) and prostate (27 % absolute and 24 % relative in comparison to controls). These findings occurred at a very high dose in the presence of severe effects to body weight gain and the liver. There were no such findings at lower doses or in other studies; therefore they are unlikely to be a specific effect caused by pyriofenone. In females of the top dose, ovary weight was decreased (53 % absolute and 52 % relative, in comparison with controls). Again, these effects occurred in the presence of severe toxicity (increased liver weight and associated histopathology). The study report also described the presence of four sexually immature females, which may have had an impact on the reproductive organ weights.

One year study in dogs

Using the guideline cut-off values for a 90-day rat study and extrapolating to one year as a guide, the dose relevant for classification with STOT-RE 2 in this 1-year dog study was 500 ppm only (~14 mg/kg bw/day). There were no doses relevant for classification with STOT-RE 1 (STOT-RE 1: C ≤ 2.5 mg/kg bw/day and STOT RE 2: 2.5 < C ≤ 25 mg/kg bw/day).

Beagle dogs (4/sex/dose) were administered pyriofenone in their diet at concentrations of 0, 500, 3000, 15000 (females only) or 25000 (males only) ppm for 12-months (equivalent to 13.7, 83.5 and 701 mg/kg bw/day in males and 14.1, 86.2 and 448 mg/kg bw/day in females). There were no unscheduled deaths and at the doses relevant for classification, there were no overt toxicological signs. In animals dosed with 3000 ppm and above, bodyweight gain was affected in a dose-dependent manner. Emesis of feed was noted in males and females and also loose stools. The liver was the primary target organ, with increased weight and centrilobular hypertrophy in males of the top dose and dark colour and enlargement in both sexes at the top dose. Clinical chemistry revealed a dose-dependent increase in alkaline phosphatase in males and females at doses ≥ 3000 ppm. There were small but statistically significant effects to haem parameters in males and females of the top dose. These were not observed at doses of 3000 ppm and less.

10.12.4 Dermal studies in rats

Pyriofenone has been tested for its ability to induce systemic toxicity in rats following dermal administration in a short-term dermal 28-day study.

Twenty-eight day study in rats

In this study, the doses relevant for classification with STOT-RE 2 were 100 and 300 mg/kg bw/day. There were no doses relevant for classification with STOT-RE 1 (STOT-RE 1: C ≤ 60 mg/kg bw/day and STOT RE 2: 60 < C ≤ 600 mg/kg bw/day).

Pyriofenone was applied to the clipped dorsal skin of Sprague Dawley rats (10/sex/dose), under a semi-occlusive dressing daily for 6 hours at doses of 0, 100, 300 or 1000 mg/kg bw/day for four weeks. There were no unscheduled deaths, no treatment-related clinical signs, no treatment-related skin irritation or effects on body weight or food consumption. There were no treatment-related findings at any dose other than an increase in partial thromoplastin time in females of the top dose.

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

The repeated-dose oral toxicity of pyriofenone has been investigated in 28-day, 90-day and 1-year studies in rats and dogs, a 90-day study in mice and in chronic/carcinogenicity studies in rats and mice. Its dermal repeated dose toxicity has been investigated in a 28-day study in rats. No adverse effects were reported in the dermal study when pyriofenone was applied at doses up to 1000 mg/kg bw/day.

Following oral administration, the main target organs were the liver in rats, mice and dogs, the kidney in rats and mice and the caecum in rats.

Effects on the liver comprised of treatment-related increases in absolute and relative weights, clinical chemistry alterations and histopathology findings (hepatocyte hypertrophy and darkening in colour). These findings were observed from doses of 150 mg/kg bw/day in rats (90-day study), 176 mg/kg bw/day in mice (90-day study) and 83.5 mg/kg bw/day in dogs (1-year study). Clear liver toxicity (including liver foci and hepatocellular necrosis) was reported in the carcinogenicity studies in rats and mice from 197 mg/kg bw/day in mice and 486 mg/kg bw/day in rats.

The main effects to the kidney included increased absolute and relative weight and increased hyaline droplet deposition in proximal tubule cells. These occurred in rats from a dose of 226 mg/kg bw/day (1-year study) and in dogs from a dose of 701 mg/kg bw/day (1-year study). In the chronic/carcinogenicity studies, increased incidences of chronic nephropathy were observed in rats from doses of 36 mg/kg bw/day and cortical tubular basophilia was observed in mice from doses of 237 mg/kg bw/day.

Effects to the large intestine were observed in rats at doses of 150 mg/kg bw/day and higher. The effects to the caecum were described as distention with contents. The study report suggested that these effects might be a substance-related effect on the intestine microflora.

Other adverse changes that were observed included effects on haem in rats and mice, although these changes tended to be very small (less than 5 %) and in some cases there was a lot of inter-individual variation and always at doses higher than the recommended cut-off values for classification. In rats prolongation of activated partial thromboplastin time was observed in most studies, again, always at doses far higher than those relevant for classification.

The majority of the adverse effects observed occurred at doses much higher than the guidance cut-off values for classification with STOT-RE 2 and there were no studies in which adverse effects occurred at doses relevant for classification with STOT RE 1. Table 29 shows the studies with effects at doses relevant for classification.

Table 29: Adverse effects occurring at doses relevant for classification with STOT RE 2 in rats, mice and dogs following oral administration of pyriofenone

Study	**(Adjusted) guidance value for STOT RE 2** **mg/kg bw/day**	**Effects at doses below guidance cut-off values**
28-Day rat study	300	**<u>251/261 mg/kg bw/day</u>** ↑ Liver 10 %** (rel.) in males Increased hyaline droplet deposition in proximal tubule cells of the kidney: 6/6 males** (versus 0 in controls) Distended caecum with contents: 6/6 males** and 6/6 females** (versus 0 in controls) ↓ Alkaline phosphatase 10 %** in males ↓ Total bilirubin 50 %** in females
28-Day dog study	300	No clear data trends due to only one dog per dose group
90-Day rat study	100	**<u>60.5/69 mg/kg bw/day</u>** ↓ Alanine aminotransferase 10 %** in males ↓ Total bilirubin 25 % in males and 25 %** in females
2-Generation rat study	100	**<u>(64.1/62-138.1 mg/kg bw/day):</u>** ↑ Liver weight: 11 %** (abs.) and 9 %** (rel.) in females
90-Day mouse study	100	No treatment-related findings at doses ≤ 100 mg/kg bw/day
90-Day dog study	100	**<u>90.3/89.9 mg/kg bw/day</u>** ↑Alkaline phosphatase 2.2 fold in females
1-Year rat study	25	No treatment-related findings at doses ≤ 25 mg/kg bw/day
1-Year dog study	25	**<u>13.7/14.1mg/kg bw/day</u>** ↓ Body weight gain 44 % in males (not observed in females)

18-Month mouse carcinogenicity study	16	No doses used that are relevant for classification
2-Year rat carcinogenicity study	12.5	No treatment-related findings at doses ≤ 12.5 mg/kg bw/day

10.12.6 Comparison with the CLP criteria

STOT RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day rat study.

'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.

In several oral repeated-dose toxicity studies in rats, mice and dogs, the clear target organs were the liver, kidney and caecum. The effects occurring at doses relevant for classification are summarised in Table 29.

In the majority of the studies, there were no doses used that were relevant for STOT-RE 1. In the one study that did use a dose relevant for classification in category 1, no toxicologically adverse effects were observed (28-day oral study in rats). All treatment-related findings occurred at doses relevant for classification in Category 2 only. Therefore, classification in Category 1 for specific target organ toxicity following repeated dosing is not required.

In a 28-day oral rat study and a 2-generation reproduction study in rats, increased relative liver weight was observed in one sex only, however, there was no associated histopathology. Changes to the proximal cells of the kidney were observed in males of the 28-day study, however in the absence of any such changes occurring at doses relevant for classification in the 90-day study, the UKCA concludes that this change in rats does not warrant classification. Other findings at doses relevant for classification were changes in clinical chemistry in rats and dogs and a decrease in body weight gain in male dogs following a year of treatment. The changes in clinical chemistry were not consistent or significantly adverse and were more indicative of adaptive changes occurring in the liver and kidney due to exposure to pyriofenone. Therefore, whilst they are considered treatment-related, they are not considered adverse and do not support classification.

Therefore, it is concluded that there is no evidence of significant or severe toxicity at doses below the guidance values for classification for specific target organ toxicity following repeated oral or dermal administration of pyriofenone. No classification for this endpoint is warranted.

10.12.7 Conclusion on classification and labelling for STOT RE

Not classified. Data conclusive but not sufficient for classification.

10.13 Aspiration hazard

There are no data available for this endpoint.

10.13.1 Conclusion on classification and labelling for aspiration hazard

Not classified. Data lacking.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

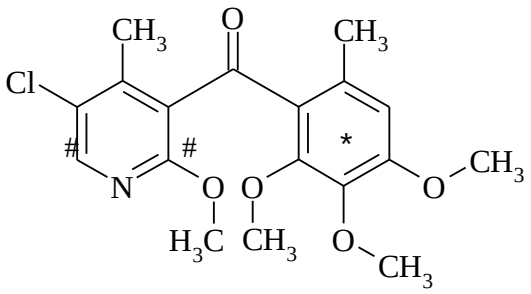
Pyriofenone (often referred to in test reports as IFK-309) is a fungicide intended for use against mildew in cereals and grapes. Available environmental fate and hazard studies have been considered under Regulation (EC) No 1107/2009 and summarised in the Draft Assessment Report (DAR) 2012.

The key information pertinent to determining a classification is presented below.

The water solubility of pyriofenone in pure water has been experimentally determined (OECD 105, column elution method, GLP) to be 1.56 mg/l at 20 ^{o}C and pH 6.6 (Turner, 2007). Based on available data is unclear if solubility is pH dependant.

A dissociation constant is not available and it is unclear if a pKa value for the substance would lie within and environmentally relevant pH range.

All radiolabelled studies used ^{14}C-pyriofene with a purity of ≥ 97% as shown in Figure 2.



*Position of the ^{14}C-(phenyl) uniform radiolabel
position of the ^{14}C-(pyridy) radiolabelled in the 2 and 6 positions of the pyridyl ring

Figure 2: Structure of pyriofenone indicating positions of the ^{14}C labels

A summary of reliable valid information on the aquatic fate of pyriofenone is presented in Table 30 below. Available soil data have not been presented as suitable aquatic data are available.

11.1 Rapid degradability of organic substances

Table 30: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Aquatic hydrolysis OECD 111, GLP	Hydrolytically stable at pH 4, 7 and 9 at 50 ^{o}C DT_{50} considered >16 days at environmentally relevant pH and temperature	Valid	Juozenaite, 2009
Ready biodegradation OECD Guideline 310, GLP	0.6% mineralisation day 28 Not readily biodegradable	Valid	Dickinson, 2009
Aquatic photolysis SETAC and EPA	DT_{50} = 33 to 54 days spring sunlight at 35^{o}N	Valid	Kane, 2009

Method	Results	Remarks	Reference
Guidelines (Pesticides Assessment Guidelines, Subdivision N, Series 161-2, GLP			
Freshwater aerobic mineralisation in surface water (simulation biodegradation), OECD Guideline 309, GLP	DT_{50} = 15.9 days at 12 °C based on geometric mean of test systems and primary degradation Maximum 16.8% AR mineralisation as CO_2 by day 100	Valid	Crowe, 2009

11.1.1 Ready biodegradability

Study 1 – Dickinson (2009)

A GLP ready biodegradation study following OECD Test Guideline 310 (CO_2 Evolution) is available using pyriofenone at 10 mgC/l dissolved in acetone (acetone was also included in control and reference samples). A reference substance was included and considered valid. A toxicity control was also included indicating the test item was not inhibitory to the microbial inoculum. The study included 5 replicates although 2 gave anomalous results and were removed from calculations as it was suggested the test vessels may have leaked. During review under Regulation 1107/2009, this was not considered to have affected study results.

By day 28 a mean of 0.6% minerlaisation was observed. On this basis, pyriofenone is not considered readily biodegradable.

11.1.2 BOD_5/COD

No data.

11.1.3 Hydrolysis

Study 1 – Juozenaite (2009)

A preliminary hydrolysis test at 50 °C over 5 days at pH 4, 5, 7 and 9 is available conducted according to OECD Test Guideline 111 with radiolabelled pyriofenone at a concentration approximately half the water solubility. Radioactivity recoveries were 95.1-100%. No hydrolysis of pyriofenone was observed and the test substance was found to be stable in aqueous solution under sterile conditions. Thus, the main test at 25 °C was not performed.

Overall, pyriofenone is considered hydrolytically stable at an environmentally relevant pH and temperature with a half-life greater than 16 days.

11.1.4 Other convincing scientific evidence

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

No data.

11.1.6 Inherent and enhanced ready biodegradability tests

No data.

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

Study 1 – Crowe (2009)

A freshwater aquatic biodegradation simulation study is available following OECD Guideline 309 and GLP. The study used ^{14}C-phenyl and ^{14}C-pyridyl labelled pyriofenone and two natural aquatic systems: Calwich Abbey Lake, England and Swiss Lake, England. Table 31 presents the characteristics of each aquatic system.

Table 31: Physiochemical parameters of the pyriofenone water/sediment systems

Sediment Parameter	Calwich Abbey Lake	Swiss Lake
Geographic Location	Calwich Abbey Lake, Calwich, Ashbourne, Derbyshire, England	Swiss Lake, Chatsworth, Derbyshire, England
Texture Class	Silt Loam	Sand
% Sand	7	98
% Silt	82	2
% Clay	11	0
pH (1:5 soil:water ratio)	7.7	6.0
% Organic Carbon (%)	4.1	0.6
CEC (meq/100 g)	14.2	1.9
Water Parameter	**Calwich Abbey Lake**	**Swiss Lake**
Temperature (°C)	20 °C	20 °C
pH	8.26	5.8
Hardness mg equivalent $CaCO_3$/l (ppm)	250	19
Organic Carbon (mg/l)	4.0	10.8
Total Suspended Solids (ppm)	6	50

Test systems were prepared with filtered water at a ratio of 4:1 water:sediment (w:w). The test item was applied to the water layer at a rate of 0.033 mg/l. The study ran for 100 days in the dark at 20 ^{o}C.

For analysis, water and sediment layers were separated by centrifugation with subsequent decanting of the water layer. The radioactivity was quantified by Liquid Scintillation Counting (LSC) and then analysed by HPLC with UV and radiochemical detection. Overall recoveries were 86.5 to 99.8% Applied Radioactivity (AR).

Pyriofenone was observed to dissipate to sediment. This dissipation was slower in the Swiss Lake system which is considered a function of higher proportion of sand and less organic carbon.

Low levels of mineralisation observed in the Calwich Abbey system (both radiolabels) and in the pyridyl radiolabel in the Swiss Lake system with a maximum of 2.4% AR by day 60 in the Calwich Abbey phenyl radiolabel system. Increased CO_2 levels were observed in the Swiss Lake system using the phenyl radiolabel with 16.8% AR observed on day 100.

Whole systems DT_{50} values (representing primary degradation) at 20 ^{o}C were calculated using SFO kinetics as follows:

- Calwich Abbey: 4.5 to 5.5 days
- Swiss Lake: 13.8 to 14.5 days
- Combined geometric mean: 8.4 days

For the purpose of classification these values have been converted to 12 °C, in line with ECHA guidance and Member State Committee testing protocols, to reflect a more environmentally relevant temperature.

- Calwich Abbey: 8.5 to 10.4 days
- Swiss Lake: 26.4 to 27.5 days
- Combined geometric mean: 15.9 days

The following degradants were observed during the study:

Calwich Abbey:

- 3HDPM: water max. 3.1% AR and sediment max. 5.3% AR
- 2MDPM: water max. 0.3% AR and sediment max. 2.6% AR
- 4MDPM: water max. 1.2% AR and sediment max. 0.6% AR
- 3HDHP: sediment only at max. 2.4% AR

Swiss Lake:

- 3HDPM: water max. 1.8% AR and sediment max. 4.0% AR
- 2MDPM: water max. 4.0% AR and sediment max. 6.3% AR
- 4MDPM: sediment only at max. 4.4% AR
- 3HDHP: sediment only at max. 5.3% AR
- 4HDPM: sediment only at max. 1.4% AR PTBA: sediment only at max. 3.5% AR

The proposed degradation pathway is show in Figure 3.

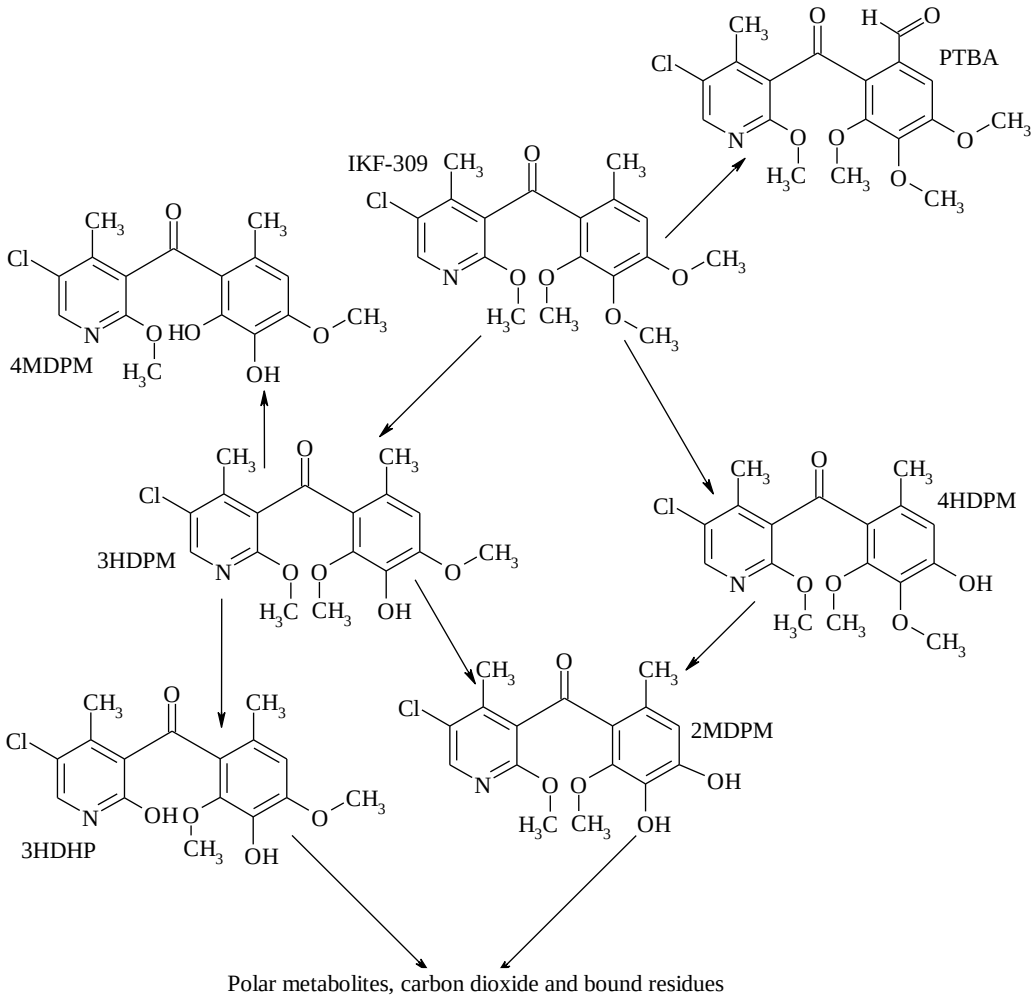


Figure 3 – Proposed degradation pathway for pyriofenone in aquatic sediment systems

11.1.7.1 Photochemical degradation

A GLP aquatic photolysis study is available following SETAC and EPA Guidelines (Pesticides Assessment Guidelines, Subdivision N, Series 161-2) using pyriofenone. Following a preliminary test, the main test used sterile natural river water and purified water to which the radiolabelled test item was added at approximately 0.7 mg/l. Samples were irradiated at 25 ± 2 °C, with stirring for up to 7 days using a xenon arc light source (wavelengths <290 nm removed). Samples were analysed by Liquid Scintillation Counting (LSC) and High Performance Liquid Chromatography (HPLC) with radiodetection. Recoveries for both natural and purified media test systems were 92.6 to 102.7%. Volatile radioactivity reached a maximum of 9.6% CO_2 in both natural and purified systems

In both the natural and purified irradiated media, the test item decreased over the 7 days to 40.5% in natural water and 54.8% in purified water. In non-irradiated media 98.6-98.8% test item was present at study termination. Up to 13 degradants were observed between the two media. None were present at greater than 6.8% Applied Radioactivity (AR).

The study calculated DT_{50} for pyriofenone in natural water was 159 hours of continuous irradiation equivalent to 33 days spring sunlight at latitude 35°N.

The study calculated DT_{50} for pyriofenone in purified water was 261 hours of continuous irradiation equivalent to 54 days spring sunlight at latitude 35°N.

Using GCSOLAR and the calculated quantum yield, the study also calculated theoretical lifetimes at the water surface at latitude 40°N as follows:
- Spring = 29 days

- Summer = 22.7 days
- Autumn = 52.1 days
- Winter = 98 days

Summary

Pyriofenone is not readily biodegradable and is hydrolytically stable. Under experimental conditions, limited photodegradation was observed in pure water. In a water/sediment simulation study pyriofenone underwent primary degradation with low levels of ultimate degradation. Overall, pyriofenone is not considered to be rapidly degradable for the purpose of classification.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.3 Environmental fate and other relevant information

Following OECD 106 and GLP, pyriofenone has experimentally determined log K_{foc} values in the range 705 to 2720 ml/g for sandy clay loam to loamy sand soils (Kane, 2008) indicating it will be of low mobility in soil.

The calculated Henry's Law Constant of 1.9 x 10^{-4} Pa.m^3.mol (Turner, 2009b) indicating it is unlikely to partition from air.

11.4 Bioaccumulation

Table 31: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient, OECD 107, purity 99.19%, GLP	logPow 3.2 at pH 7.2-7.5, 20 °C)	Unclear if pH dependant	Turner, 2009g
Experimental aquatic BCF test in fish to OECD Guideline 305, GLP, purity 97.88%,	Steady state whole fish BCF_{ss}: 142 to 160 l/kg (not lipid normalised) Kinetic whole fish BCF_k: 137 to 176 l/kg (not lipid normalised or growth corrected) Depuration half-life DT_{50}: 0.6 to 0.93 days	Flow through, 28 days exposure, 6 days depuration Valid	Anon., 2009

11.4.1 Estimated bioaccumulation

No data.

11.4.2 Measured partition coefficient and bioaccumulation test data

Study 1 – Turner (2009g)

The octanol:water partition coefficient of pyriofenone was determined following OECD Test Guideline 107 (shake flask method). The study was conducted using pure water at pH 7.2-7.5 and 20 °C. In the absence of measurements at higher and lower pHs, it is unclear if a pH dependence may occur. The $logP_{OW}$ was 3.2.

Study 2 – Anon. (2009)

The study followed GLP and OECD Guideline 305. It used pyriofenone (97.88%), a flow-through system with carp (*Cyprinus carpio*) and two exposure concentrations; 0.01 and 0.001 mg/l with the aid of the solvent dimethylformamide (DMF). A solvent control was included. The exposure period ran for 28 days followed by a 6 day depuration period.

The steady-state fish residue concentration was reached on day 14 with steady state Bioconcentration Factors (BCF_{ss}) determined as 142 to 160 l/kg.

Kinetic Bioconentration Factors (BCF_k) were determined as 137 to 176 l/kg.

The mean lipid content was 4.9% at the start of the exposure period and 5.2% at the end of the depuration phase. While the above steady state BCFs have not been lipid normalised, the mean lipid content at the end of the depuration is only slightly above 5% and lipid normalised would not result in a BCF_{ss} above 500 l/kg.

During the study the degradants 3HDPM and 4HDPM were detected in fish indicating pyriofenone parent underwent metabolism as neither degradant were detected in test water. The maximum concentrations were as follows:

4HDPM: 0.3145 mg/kg (high concentration group) and 0.04108 mg/kg (low concentration group)

3HDPM: 0.0363 mg/kg (high concentration group) and <0.01 mg/kg (low concentration group)

During the depuration phase, pyriofenone, 4HDPM and 3HDPM were rapidly depurated from the fish with the following half-lives:

- Pyriofenone: 0.6 to 0.93 days
- 4HDPM: 1.32 to 1.73 days
- 3HDPM: 5.65 days

Summary

Pyriofenone has a logPow below the CLP threshold of 4 and experimental BCFs below the CLP threshold of 500. It is therefore considered to have a low potential for bioaccumulation.

11.5 Acute aquatic hazard

A summary of available valid information on the aquatic toxicity of pyriofenone is presented in Table 32. Where available, a summary of valid information for degradants is also included in Annex I, Table A1. Based on the limited available data, degradants are not considered more toxic than the parent substance and not considered further for classification.

Table 32: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Acute toxicity to fish, OECD 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Pyriofenone (97.88%)	96-h LC_{50} >1.44 mg a.s./l (mm)	Valid	Anon., 2007
Acute toxicity to fish, OECD 203, GLP	Common Carop (*Cyprinus carpio*)	Pyriofenone (97.88%)	96-h LC_{50} >1.41 mg a.s./l (mm)	Test fish were longer length than test guideline recommendation	Anon., 2008
Daphnia sp Acute Immobilisation OECD 202,	*Daphnia magna*	Pyriofenone (97.88%)	48-h EC_{50} >1.55 mg a.s./l (mm)	Valid	Burke, Manson and Scholey, 2008b

GLP					
Freshwater Algal Growth Inhibition OECD 201, GLP	*Pseudokirchneriella subcapitata*	Pyriofenone (97.88%)	72-h E_rC_{50} 1.77 mg a.s./l (mm)	Valid	Burke, Manson and Scholey, 2008d

Notes:
mm refers to mean measured concentrations

11.5.1 Acute (short-term) toxicity to fish

Two valid semi-static, acute toxicity to fish studies using pyriofenone following GLP and OECD Test Guideline 203 are discussed below.

Study 1 - Anon., (2007)
Using Rainbow Trout (*Oncorhynchus mykiss*) a nominal exposure range of 0.02, 0.2 and 2 mg a.s./l was employed with the aid of acetone as a solvent (a solvent control was included). Study conditions were acceptable and validity criteria were met. Analytical concentrations by HPLC-UV were 72 to 79% of nominal. No mortality was observed. The study 96-h LC_{50} was >1.44 mg a.s./l based on mean measured concentrations.

Study 2 – Anon., (2008)
Using Common Carp (*Cyprinus carpio*) a single test concentration of 2 mg a.s./l nominal was employed prepared with the aid of acetone as solvent (a solvent control was included). Study conditions were acceptable and validity criteria were met. However, it is noted that at the start of the study, the test fish were 5.5 to 6.6 cm which is longer than the guideline recommendation of 3 cm ± 1 cm any may have influenced sensitivity. Analytical concentrations by HPLC UV were 70 to 72% of nominal. No mortality was observed. The study 96-h LC_{50} was >1.41 mg a.s./l based on mean measured concentrations.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Study 1 – Burke, Manson and Scholey (2008b)

A semi-static acute toxicity to *Daphnia magna* study is available following GLP and OECD Test Guideline 202. Study conditions were acceptable and validity criteria were met. The exposure range was nominally 0.002, 0.02, 0.2 and 2 mg a.s./l prepared with the aid of acetone as a solvent (a solvent control was included). Analytical measurement by HPLC-UV was 78 to 91% of nominal with mean measured concentrations 0.00164, 0.0181, 0.181 and 1.55 mg a.s./l. There was no effect on immobilisation at any concentration. Based on mean measured concentrations, the 48-h EC_{50} was >1.55 mg a.s./l.

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

Study 1 – Burke, Manson and Scholey (2008b)

A 72-hour static algal growth inhibition test using the freshwater algae *Pseudokirchneriella subcapitata* is available following GLP and OECD Test Guideline 201 (2006). Study conditions were acceptable and validity criteria were met. The nominal exposure range was 0.023, 0.052, 0.114, 0.250, 0.550, 1.21 and 2.66 mg a.s./l. Exposure concentrations were prepared with the aid of acetone as a solvent and a solvent control was included. Analytical measurement by HPLC-UV was 75 to 124% of nominal with mean measured concentrations 0.0274, 0.0591, 0.141, 0.249, 0.575, 1.00 and 1.98 mg a.s./l.

In three highest treatments, suspended particulate test material was observed.

The 72-h E_rC_{50} was calculated to be 1.77 mg a.s./l based on mean measured concentrations. The 72-h NOE_rC was determined to be 0.249 mg a.s./l based on mean measured concentrations.

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

No data.

11.6 Long-term aquatic hazard

Table 33: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish Early-Life Stage toxicity, OECD 210, GLP	Fathead Minnow (*Pimephales promelas*)	Pyriofenone (97.88%)	28-d NOEC 1.27 mg a.s./l (mm)	Valid	Anon. , 2008a
Daphnia magna Reproduction OECD 211, GLP	*Daphnia magna*	Pyriofenone (97.88%)	21-d NOEC 0.0899 mg a.s./l (mm)	Valid	Burke, Manson and Scholey, 2008c
Freshwater Algal Growth Inhibition OECD 201, GLP	*Pseudokirchneriella subcapitata*	Pyriofenone (97.88%)	72-h NOE_rC 0.249 mg a.s./l (mm)	Valid	Burke, Manson and Scholey, 2008d

Notes:
mm refers to mean measured concentrations
n refers to nominal concentrations

11.6.1 Chronic toxicity to fish

Study 1 – Anon. (2008a)

A semi-static chronic toxicity to fish study using pyriofenone following GLP and OECD Test Guideline 210 is available. The study used Fathead Minnow (*Pimephales promelas*) and the following endpoints: hatching success, survival and growth (length and dry weight). General observations were also recorded.

Study conditions were acceptable and validity criteria were met. It is noted that although test eggs were >24 hours past fertilisation they were considered acceptable as they were in the 'tail bud' stage of development. The nominal exposure range was 0.019, 0.061, 0.195, 0.625 and 2 mg a.s./l. Exposure solutions were prepared with the aid of a solvent (DMF at 0.1 ml/l) and a solvent control was included.

Analytical verification was by HPLC-UV with measured values 63.5 to 73.3% of nominal with mean measured concentrations 0.0126, 0.0416, 0.143, 0.435 and 1.27 mg a.s./l.

No statistically significant effects were observed for any parameter.

The 28-d NOEC for all parameters was considered to be 1.27 mg a.s./l based on the highest treatment and mean measured concentrations.

11.6.2 Chronic toxicity to aquatic invertebrates

Study 1 – Burke, Manson and Scholey (2008c)

A semi-static chronic toxicity to *Daphnia magna* study is available following GLP and OECD Test Guideline 211. The nominal exposure range was 0.0519, 0.104, 0.208, 0.415, 0.830 and 1.66 mg a.s./l. Exposure solutions were prepared with the aid of a solvent (DMF 0.1 ml/l) and a solvent control was included Analytical measurement by HPLC-UV and time-weight mean measured concentrations were calculated to be 0.0442, 0.0899, 0.188, 0.359, 0.670 and 1.16 mg a.s./l. Study conditions were acceptable and the study is considered valid.

The 21-day NOEC for reproduction was 0.0899 mg a.s./l based on time-weight mean measured concentrations.

The 21-day NOEC for weight was 0.670 mg a.s./l and the 21-day for length was 0.359 mg a.s./l, both based on time-weight mean measured concentrations.

11.6.3 Chronic toxicity to algae or other aquatic plants

A toxicity to algae study is available using pyriofenone. Study details are presented in section 11.5.3 above with the chronic endpoint detailed below.

Study 1 – Burke, Manson and Scholey (2008b)

The 72-h NOE_rC was determined to be 0.249 mg a.s./l based on mean measured concentrations.

11.6.4 Chronic toxicity to other aquatic organisms

Study 1 – Burke and Scholey (2009)

A GLP, 28-day toxicity to *Chironomus riparius* study using pyriofenone is available following OECD Test Guideline 219. The study employed a static water-sediment system and spiked overlying water (nominally 0.2, 0.4, 0.8, 1.6 and 3.2 mg a.s./l).

Significant losses were observed in the aqueous phase as the test item partitioned to the sediment phase and potentially underwent primary degradation. By day 28, concentrations in the water phase were 5.03 to 9.38% of the applied nominal dose.

The study reported a 28-day NOEC of 1.6 mg a.s./l for emergence and a 28-day NOEC of 3.2 mg a.s./l for development. Both NOECs are based on nominal applied water phase concentrations.

The above information is presented for completeness although the nominal endpoints are not considered for hazard classification due to difficulties interpreting data from water-sediment test systems.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Pyriofenone acute toxicity data are available for fish, invertebrates, algae and aquatic plants.

All acute ecotoxicity endpoints are >1 mg/l. Algae are the most acutely sensitive trophic level with an E_rC_{50} of 1.77 mg a.s./l.

Based on available data, degradation products are not considered more acutely toxic than the parent substance (see Annex I) and are not considered further for classification.

Based on these data, pyriofenone does not require an Aquatic Acute classification.

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

Pyriofenone is not readily biodegradable and is hydrolytically stable.

Under experimental conditions, photodegradation was observed in pure water. The study calculated a DT_{50} of 54 spring days sunshine at 35°N. However, information on photochemical degradation in the aquatic environment is difficult to use for classification purposes because the degree of degradation is dependent on local conditions.

In a water/sediment simulation study pyriofenone underwent primary degradation with low levels of ultimate degradation (maximum of 16.8% AR as CO_2 after 100 days). Whole system DT_{50} values at 12 °C based on primary degradation were 8.5 to 27.5 days with a geometric mean of 15.9 days for the two systems.

Multiple aquatic degradants were observed although none at >10% AR.

Overall, pyriofenone is not considered to be rapidly degradable for the purpose of classification.

Pyriofenone has a logPow below the CLP threshold of 4 and experimental BCFs below the CLP threshold of 500, therefore it is considered to have a low potential for bioaccumulation.

Chronic toxicity data for fish, invertebrates and algae are available. The fish 28-day NOEC is >1 mg/l and the algal 72-hour NOE_rC is 0.249 mg/l. However, invertebrates (*Daphnia magna*) are the most chronically sensitive trophic level with a 21-day NOEC of 0.0899 mg/l.

Based on available data, degradation products are not considered more chronically toxic than the parent substance (see Annex I) and are not considered further for classification.

Given the chronic ecotoxicity data and that pyriofenone is not rapidly degradable, it should be classified for the environment as Aquatic Chronic 1. Based on the *Daphnia magna* endpoint, a chronic M-factor of 1 is appropriate (0.01 mg/l < NOEC ≤ 0.1 mg/l).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

No Aquatic Acute classification.

Aquatic Chronic 1, Chronic M-Factor: 1

Data conclusive and sufficient for classification

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

Physchem. references:

Study Author	Year	Reference
Turner, B.	2009a	IKF-309 (PAI): Physico-chemical properties Huntingdon Life Sciences Ltd Laboratory no. ISK0399 Ishihara Sangyo Kaisha Ltd. GLP, unpublished
Turner, B.	2009b	IKF-309 (PAI): Vapour Pressure and Calculation of Volatility (Henry's Law Constant) Huntingdon Life Sciences Ltd Laboratory no. ISK0396 Ishihara Sangyo Kaisha Ltd. GLP, unpublished

Turner, B.	2009c	IKF-309 (TGAI): Physico-chemical properties Huntingdon Life Sciences Ltd Laboratory no. ISK0392 Ishihara Sangyo Kaisha Ltd. GLP, unpublished
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15 ANNEXES

ANNEX I – Aquatic toxicity data for pyriofenone degradants.

Ecotoxicity studies using pyriofenone degradants are described in the DAR (2012). The majority of studies were considered unreliable and endpoints not valid due to study deficiencies including lack of analytical verification and GLP status (refer to DAR, 2012). However, the studies are a useful indicator that the degradants 2MDPM and 3HDPM are unlikely to be more ecotox than the parent pyriofenone.

The remaining valid ecotoxicity data is presented below.

Table A1: Summary of relevant information on aquatic toxicity for pyriofenone degradants

Degradant / Guideline / GLP	Species	Exposure		Results		Notes / observations	Reference
		Design	Duration	Endpoint	Toxicity (mg/l)		
2MDPM Freshwater Algal Growth Inhibition OECD 201, GLP, pyriofenone (98.17%)	*Pseudokirchneriella subcapitata*	Static	72 hours	E_rC_{50} NOE_rC	>0.418 (mm) 0.418 (mm)	Single treatment of nominal 2mg/l 0 h concentrations were 67-100% nominal 72 h concentrations were < LoD of 0.05 mg/l No microscopic abnormalities were observed.	Wilby, 2010

Notes:
mm refers to mean measured concentrations

ANNEX II – Confidential reference list (separate document).