PYRIOFENONE

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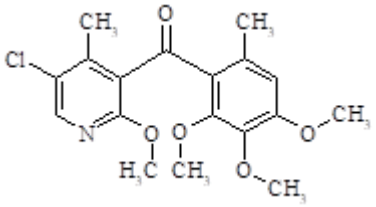
Explanation....651
Evaluation for acceptable intake....652
1. Biochemical aspects....652
1.1 Absorption, distribution and excretion....652
(a) Single-dose and repeated-dose absorption, distribution and excretion of ^{14}C-(phenyl)-pyriofenone....653
(b) Enterohepatic circulation of ^{14}C-(phenyl)-pyriofenone....658
(c) Plasma and whole blood kinetics of ^{14}C-(phenyl)-pyriofenone....659
(d) Single-dose absorption, distribution and excretion of ^{14}C-(pyridyl)-pyriofenone....660
(e) Plasma and whole blood kinetics of ^{14}C-(pyridyl)-pyriofenone....663
(f) Accumulation ratios....663
1.2 Biotransformation....664
2. Toxicological studies....665
2.1 Acute toxicity....665
(a) Lethal doses....665
(b) Dermal irritation....665
(c) Ocular irritation....665
(d) Dermal sensitization....666
2.2 Short-term studies of toxicity....667
(a) Oral administration....667
(b) Dermal application....675
2.3 Long-term studies of toxicity and carcinogenicity....676
2.4 Genotoxicity....690
2.5 Reproductive and developmental toxicity....691
(a) Multigeneration studies....691
(b) Developmental toxicity....695
2.6 Special studies....698
(a) Neurotoxicity....698
(b) Immunotoxicity....699
(c) Mechanistic studies....700
(d) Studies on metabolites....701
(e) Microbiological effects....702
3. Observations in humans....702
Comments....702
Toxicological evaluation....705
References....708

Explanation

Pyriofenone (Fig. 1) is the International Organization for Standardization–proposed common name for (5-chloro-2-methoxy-4-methyl-3-pyridyl)(4,5,6-trimethoxy-*o*-tolyl) methanone, with the Chemical Abstracts Service number 688046-61-9.

Pyriofenone is an aryl phenyl ketone fungicide used to control powdery mildew on grapevines.

Fig. 1. Chemical structure of pyriofenone



Source: Knight (2009)

Pyriofenone has not been previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

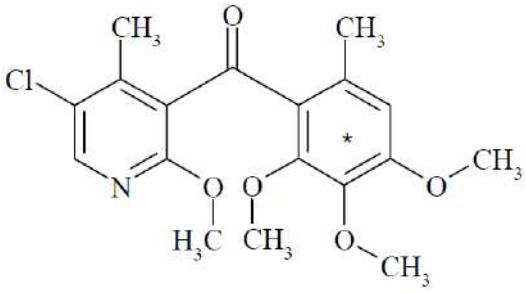
All critical studies contained statements of compliance with good laboratory practice (GLP) and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

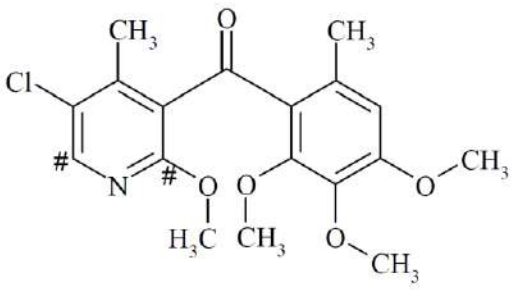
1. Biochemical aspects

The positions of the pyriofenone radiolabels used in the absorption, distribution, metabolism and excretion (ADME) studies described below are shown in Fig. 2.

Fig. 2. Positions of pyriofenone radiolabels in ADME studies



^{14}C-(phenyl)-pyriofenone



^{14}C-(pyridyl)-pyriofenone

* denotes the position of the radiolabel (uniform) in the phenyl ring and # denotes the position of the radiolabel at positions 2 and 6 of the pyridyl ring
Source: Knight (2009)

1.1 Absorption, distribution and excretion

In a toxicokinetics study (Knight, 2009), Fischer rats (four of each sex per dose) received ^{14}C-(phenyl)-pyriofenone (batch no. EPPS-06-045-73-18 [CP-3098]; specific activity 6.22 MBq/mg; radiochemical purity >97%) or ^{14}C-(pyridyl)-pyriofenone (batch no. CP-3097; specific activity 4.75 MBq/mg; radiochemical purity >97%) in a single oral dose of 5 or 200 mg/kg body weight (bw). Urine was collected at 0–6 hours, at 6–24 hours and at 4-hour intervals up to 120 hours post-dosing. Faeces and cage washes were collected at 24-hour intervals up to 120 hours post-dosing. Blood samples were taken by cardiac puncture immediately prior to termination. Animals were terminated by cervical dislocation 120 hours after dosing, and selected tissues and organs were analysed for radioactivity using

liquid scintillation counting. Parent compound and metabolites were identified using high-performance liquid chromatography with radioactivity detection and thin-layer chromatography.

In addition to the above single-dose studies with both radiolabels, investigations included the following:

- *Repeated-dose excretion and distribution:* Four rats of each sex received oral doses of ^{14}C-(phenyl)-pyriofenone at 5 mg/kg bw per day for 14 days. The other radiolabelled compound, ^{14}C-(pyridyl)-pyriofenone, was not tested in repeated-dose experiments. Urine and faeces were collected for 24 hours after the first and seventh daily doses. Cage washes were collected at the end of each excreta collection period. Following the final dose, samples of excreta, tissues, organs, carcasses and cage washes were collected and assessed as described for the single-dose studies.
- *Biliary excretion:* Five rats of each sex per dose were used to assess the role of biliary excretion in pyriofenone metabolism. A flexible cannula was inserted into the common bile duct of each animal, and the other end of the cannula was inserted into the duodenum. The animals were permitted a recovery period of at least 3 days, during which bile flow through the cannula was checked daily. On the day of dosing, the rats received a single oral administration of ^{14}C-(phenyl)-pyriofenone or ^{14}C-(pyridyl)-pyriofenone at 5 or 200 mg/kg bw. Bile was collected from each rat at 0–3, 3–6, 6–9, 9–12, 12–24 and 24–48 hours after dosing. Urine and faeces were collected at 24-hour intervals up to 48 hours post-dosing. The rats were terminated at 48 hours, and the gastrointestinal tract (including contents) and liver were assessed for radioactivity, along with the remaining carcass. The interiors of the cages were rinsed after termination, and the washings were assessed for radioactivity.
- *Enterohepatic circulation:* Cannulated rats (five of each sex) were administered ^{14}C-(phenyl)-pyriofenone or ^{14}C-(pyridyl)-pyriofenone in a single oral dose of 5 or 200 mg/kg bw, and bile was collected at 0–9 hours post-dosing. A portion of this bile pool (0.5 mL) was administered to recipient animals via duodenal cannulas, and samples were collected as described above under biliary excretion.
- *Single-dose plasma and whole blood kinetics:* Twelve rats of each sex per dose received ^{14}C-(phenyl)-pyriofenone or ^{14}C-(pyridyl)-pyriofenone in a single oral dose of 5 or 200 mg/kg bw. Rats in each group were further divided into three subgroups (four of each sex). Blood samples were collected before dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 12, 24, 72, 96 and 120 hours after dosing.
- *Repeated-dose plasma and whole blood kinetics:* Twelve rats of each sex per dose received ^{14}C-(phenyl)-pyriofenone at an oral dose of 5 mg/kg bw per day for 14 days. Rats in each group were further divided into three subgroups (four of each sex). Blood samples were collected before dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 12, 24, 72, 96 and 120 hours after dosing.
- *Tissue distribution experiments:* Nine rats of each sex per dose received ^{14}C-(phenyl)-pyriofenone or ^{14}C-(pyridyl)-pyriofenone in a single oral dose of 5 or 200 mg/kg bw. Groups of six animals (three of each sex) were terminated at times determined by the plasma kinetics.

(a) Single-dose and repeated-dose absorption, distribution and excretion of ^{14}C-(phenyl)-pyriofenone

In bile duct–cannulated rats administered ^{14}C-(phenyl)-pyriofenone at a dose of 5 mg/kg bw, radioactivity was rapidly excreted into bile (65–73% of the dose) and urine (2.8–13% of the dose) 48 hours after dosing (Table 1). In intact rats, radioactivity in faeces accounted for 82–89% of the dose 120 hours after dosing, whereas radioactivity in urine accounted for 11–17% of the dose. No significant

radioactivity was detected in the carcass 120 hours after dosing (Table 2). These data suggest that pyriofenone is rapidly taken up and initially excreted into bile, with final excretion occurring predominately via faeces and minor excretion occurring via urine. No sex-specific differences were observed.

Table 1. Excretion of radioactivity 0–48 hours after administration of single oral doses of ^{14}C-(phenyl)- or ^{14}C-(pyridyl)-pyriofenone to bile duct–cannulated rats

	% of dose							
	^{14}C-(phenyl) label				^{14}C-(pyridyl) label			
	5 mg/kg bw		200 mg/kg bw		5 mg/kg bw		200 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Bile	73	65	33	42	74	81	41	49
Urine	2.8	13	1.8	4.6	7.5	7.6	2.2	3.4
Cage wash	0.15	0.34	0.05	0.14	0.09	0.13	0.05	0.13
Faeces	23	15	59	51	14	6.3	54	45
Liver	0.10	0.04	0.07	0.05	0.10	0.04	0.09	0.04
GI tract + contents	0.13	0.11	1.63	0.92	0.02	0.06	0.39	0.21
Carcass	0.05	0.24	1.72	0.80	0.17	0.16	0.32	0.85
Total	99.5	92.9	97.0	99.3	95.6	95.2	98.2	98.1
Absorption[a]	76.2	77.9	36.1	47.2	81.7	88.8	43.8	53.0

bw: body weight; GI: gastrointestinal
[a] Sum of means for bile, urine, liver and residual carcass.
Source: Knight (2009)

Table 2. Excretion of radioactivity 0–120 hours after administration of single oral doses of ^{14}C-(phenyl)- or ^{14}C-(pyridyl)-pyriofenone to rats

	% of dose							
	^{14}C-(phenyl) label				^{14}C-(pyridyl) label			
	5 mg/kg bw		200 mg/kg bw		5 mg/kg bw		200 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	11	17	6.1	8.1	20	14	8.3	9.1
Cage wash	0.52	1.6	0.45	0.58	2.3	2.2	0.50	0.98
Faeces	89	82	91	85	73	78	89	89
Carcass	0.15	0.09	0.12	0.11	0.20	0.03	0.13	0.11
Total	100	101	97.6	94.6	94.5	94.1	97.6	98.9

bw: body weight
Source: Knight (2009)

The highest concentrations of radioactivity in tissues 120 hours after administration of ^{14}C-(phenyl)-pyriofenone at 5 mg/kg bw were found in liver, kidney and blood cells (Table 3). Tissue:plasma ratios were highest for the liver and kidney in both sexes. Intermediate ratios were

calculated for whole blood in both sexes and for carcass, fat, bone and gastrointestinal tract plus contents in female animals. Tissue:plasma ratios were less than 1 for all other tissues (Table 4).

In rats administered ^{14}C-(phenyl)-pyriofenone at 200 mg/kg bw, excretion followed a similar pattern as was seen with 5 mg/kg bw. However, at the high dose, a higher proportion of radioactivity was found in faeces (51–59% of the dose) than in bile (33–42% of the dose) in bile duct–cannulated rats 48 hours after dosing. The majority of radioactivity was eliminated in faeces 120 hours after dosing (85–91% of the dose) in intact rats. Overall recoveries of radioactivity (excluding tissues) were 97.6% and 94.6% of the dose for males and females, respectively (Tables 1 and 2). The data suggest that absorption at the high dose became saturated and that a lower proportion of the dose was absorbed into bile compared with the low dose. No sex-specific differences were noted.

Similar to animals dosed with 5 mg/kg bw, the highest radioactivity levels in tissues after exposure to 200 mg/kg bw were in liver, followed by kidney, blood cells, whole blood and fat (females) (Table 3). Tissue:plasma ratios were highest for the fat (females), liver and kidney (both sexes) and gastrointestinal tract (females). Intermediate ratios were calculated for whole blood (both sexes), heart (females), lungs (females), spleen (both sexes), ovaries and uterus (females) and residual carcass (females). Tissue:plasma ratios were less than 1 for all other tissues (Table 4).

Table 3. Mean concentrations of radioactivity in tissues 120 hours after administration of single or repeated oral doses of ^{14}C-(phenyl)-pyriofenone to rats

	Mean concentrations of radioactivity (µg equivalents/g)					
	1 × 5 mg/kg bw		**1 × 200 mg/kg bw**		**14 × 5 mg/kg bw**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Sample	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**
Plasma	0.026 ± 0.002	0.006 ± 0.001	0.585 ± 0.096	0.230 ± 0.033	0.131 ± 0.011	0.022 ± 0.003
Whole blood	0.042 ± 0.003	0.006 ± 0.004	1.364 ± 0.137	0.431 ± 0.041	0.411 ± 0.060	0.067 ± 0.004
Blood cells	0.068 ± 0.006	0.010 ± 0.006	2.500 ± 0.450	0.810 ± 0.066	0.819 ± 0.172	0.146 ± 0.010
Brain	nd	nd	nd	nd	0.026 ± 0.003	nd
Heart	0.015 ± 0.002	nd	0.473 ± 0.131	0.245 ± 0.036	0.124 ± 0.033	0.029 ± 0.003
Kidney	0.065 ± 0.006	0.024 ± 0.003	1.925 ± 0.171	1.630 ± 0.176	0.486 ± 0.061	0.208 ± 0.028
Liver	0.163 ± 0.016	0.041 ± 0.006	4.346 ± 0.480	1.703 ± 0.479	0.892 ± 0.027	0.184 ± 0.015
Lungs	0.012 ± 0.001	nd	0.504 ± 0.025	0.248 ± 0.031	0.147 ± 0.021	0.047 ± 0.004
Spleen	0.018 ± 0.003	nd	0.639 ± 0.089	0.301 ± 0.015	0.250 ± 0.024	0.065 ± 0.008
Adrenal glands	nd	nd	nd	nd	0.096 ± 0.007	0.027 ± 0.020
Pituitary gland	nd	nd	nd	nd	nd	nd
Thyroid	nd	nd	nd	nd	0.256 ± 0.052	nd
Epididymis	0.006 ± 0.001	–	0.226 ± 0.021	–	0.046 ± 0.003	–
Testes	0.002 ± 0.003	–	0.041 ± 0.081	–	0.037 ± 0.003	–
Ovaries	–	nd	–	0.315 ± 0.364	–	0.009 ± 0.019
Uterus	–	nd	–	0.394 ± 0.046	–	0.016 ± 0.002
Bone	0.007 ± 0.008	0.004 ± 0.007	0.129 ± 0.257	nd	0.064 ± 0.007	0.032 ± 0.022

	Mean concentrations of radioactivity (µg equivalents/g)					
	1 × 5 mg/kg bw		1 × 200 mg/kg bw		14 × 5 mg/kg bw	
	Males	Females	Males	Females	Males	Females
Sample	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**
Bone marrow	nd	nd	nd	nd	0.081 ± 0.022	nd
Fat (abdominal)	nd	0.002 ± 0.004	nd	2.936 ± 0.760	0.032 ± 0.008	0.005 ± 0.011
Muscle (skeletal)	0.001 ± 0.003	nd	0.055 ± 0.110	nd	0.043 ± 0.003	nd
GI tract + contents	0.020 ± 0.005	0.014 ± 0.004	0.510 ± 0.061	1.331 ± 0.471	0.076 ± 0.041	0.029 ± 0.004
Residual carcass	0.009 ± 0.002	0.006 ± 0.004	0.304 ± 0.051	0.268 ± 0.348	0.065 ± 0.005	0.036 ± 0.011
% of dose recovered in total tissues[a]	0.31 ± 0.05	0.07 ± 0.02	0.21 ± 0.03	0.24 ± 0.02	2.25 ± 0.08	0.40 ± 0.05

bw: body weight; GI: gastrointestinal; nd: not detected; sd: standard deviation

[a] Excluding plasma and carcass. Recoveries calculated assuming the following % body weight values:

Whole blood	7%
Bone	5.46%
Bone marrow	0.35%
Fat (abdominal)	7.1%
Muscle (skeletal)	45.5%

Source: Knight (2009)

Table 4. Tissue:plasma ratios of radioactivity in tissues 120 hours after administration of single oral doses of ^{14}C-(phenyl)-pyriofenone to rats

	Tissue:plasma ratios of radioactivity			
	5 mg/kg bw		200 mg/kg bw	
	Males	Females	Males	Females
Sample	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**
Plasma	1	1	1	1
Whole blood	1.62 ± 0.022	1.46 ± 0.246	2.36 ± 0.211	1.89 ± 0.144
Brain	nd	nd	nd	nd
Heart	0.581 ± 0.120	nd	0.817 ± 0.216	1.09 ± 0.241
Kidney	2.51 ± 0.087	4.21 ± 0.605	3.33 ± 0.286	7.13 ± 0.435
Liver	6.24 ± 0.306	7.16 ± 1.08	7.49 ± 0.559	7.32 ± 0.940
Lungs	0.466 ± 0.032	nd	0.876 ± 0.112	1.09 ± 0.102
Spleen	0.697 ± 0.083	nd	1.10 ± 0.108	1.33 ± 0.185
Adrenal glands	nd	nd	nd	nd
Pituitary gland	nd	nd	nd	nd
Thyroid	nd	nd	nd	nd
Epididymis	0.24 ± 0.008	–	0.393 ± 0.056	–
Testes	0.223 ± na	–	0.230 ± na	–

	Tissue:plasma ratios of radioactivity			
	5 mg/kg bw		200 mg/kg bw	
	Males	Females	Males	Females
Sample	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**
Ovaries	–	nd	–	2.67 ± 0.566
Uterus	–	nd	–	1.75 ± 0.384
Bone	0.515 ± 0.016	2.21 ± na	0.729 ± na	nd
Bone marrow	nd	nd	nd	nd
Fat (abdominal)	nd	1.51 ± na	nd	13.3 ± 4.77
Muscle (skeletal)	0.205 ± na	nd	0.374 ± na	nd
GI tract + contents	0.761 ± 0.155	2.44 ± 0.827	0.900 ± 0.230	5.71 ± 1.59
Residual carcass	0.326 ± 0.043	1.42 ± 0.290	0.532 ± 0.133	2.59 ± 1.37

bw: body weight; GI: gastrointestinal; na: not applicable; nd: not detected; sd: standard deviation
Source: Knight (2009)

In rats administered ^{14}C-(phenyl)-pyriofenone at 5 mg/kg bw per day for 14 days, radioactivity in faeces accounted for the majority of excreted radioactivity, with 99–103% of the daily dose found in faeces 120 hours after the final daily dose (Table 5).

Table 5. Mean excretion and retention of radioactivity after administration of up to 14 consecutive daily oral doses of ^{14}C-(phenyl)-pyriofenone at 5 mg/kg bw to rats

		Mean excretion (% of dose)[a]	
		Males	Females
Sample	**Time point**	**Mean ± sd**	**Mean ± sd**
Urine	Day 1 + 24 h	6.44 ± 0.32	8.03 ± 3.07
Cage wash	Day 1 + 24 h	0.37 ± 0.09	0.71 ± 0.09
Faeces	Day 1 + 24 h	65.24 ± 2.91	58.31 ± 13.78
Total		72.04 ± 2.99	67.04 ± 11.47
Urine	Day 7 + 24 h	9.61 ± 1.16	8.86 ± 2.89
Cage wash	Day 7 + 24 h	0.53 ± 0.19	0.68 ± 0.50
Faeces	Day 7 + 24 h	88.86 ± 9.06	89.67 ± 6.01
Total		98.99 ± 9.62	99.20 ± 3.00
Urine	Day 14 + 6 h	3.47 ± 0.45	2.02 ± 0.39
	Day 14 + 24 h	6.21 ± 0.93	7.93 ± 1.39
	Day 14 + 48 h	1.53 ± 1.03	2.40 ± 0.72
	Day 14 + 72 h	0.50 ± 0.37	0.54 ± 0.18
	Day 14 + 96 h	0.19 ± 0.09	0.21 ± 0.04
	Day 14 + 120 h	0.14 ± 0.05	0.10 ± 0.01

		Mean excretion (% of dose)[a]	
		Males	Females
Sample	**Time point**	**Mean ± sd**	**Mean ± sd**
Total urine		12.04 ± 2.21	13.18 ± 2.36
Cage wash	Day 14 + 120 h	0.88 ± 0.37	1.59 ± 0.25
Faeces	Day 14 + 24 h	84.62 ± 3.89	84.92 ± 5.87
	Day 14 + 48 h	14.50 ± 11.59	12.02 ± 3.83
	Day 14 + 72 h	2.69 ± 2.85	1.31 ± 0.36
	Day 14 + 96 h	0.85 ± 0.73	0.34 ± 0.11
	Day 14 + 120 h	0.41 ± 0.22	0.17 ± 0.02
Total faeces		103.08 ± 11.85	98.75 ± 3.01
Residual carcass	Day 14 + 120 h	1.05 ± 0.09	0.57 ± 0.18
Overall total		117.04 ± 14.35	114.09 ± 4.18

bw: body weight; sd: standard deviation

[a] Results are expressed as % of the dose based on the dose received by an animal on a single day (day 1, 7 or 14), not the cumulative dose received during that period.

Source: Knight (2009)

Similar to the situation following administration of a single dose of 5 mg/kg bw, radioactivity remaining in the carcass after 14 daily doses of ^{14}C-(phenyl)-pyriofenone at 5 mg/kg bw accounted for 0.57–1.1% of the daily dose (Table 5), and concentrations of radioactivity in tissues were relatively low (Table 3). The highest concentrations in tissues occurred in liver, kidney, whole blood and blood cells (Table 5). Generally, tissue concentrations were higher in males than in females and, except for red blood cells, declined over time. Concentrations of radioactivity in blood cells increased with time for both sexes.

The extent of absorption was estimated by summing the values for bile, urine, liver and carcass after administration of ^{14}C-(phenyl)-pyriofenone to bile duct–cannulated rats. On this basis, it was estimated that the extent of absorption of a low dose (5 mg/kg bw) of ^{14}C-(phenyl)-pyriofenone was 76% of the dose for males and 78% of the dose for females. For the high dose (200 mg/kg bw) of ^{14}C-(phenyl)-pyriofenone, the extent of absorption was 36% of the dose for males and 47% of the dose for females (Table 1).

(b) Enterohepatic circulation of ^{14}C-(phenyl)-pyriofenone

The bile was retained from some of the cannulated rats given ^{14}C-(phenyl)-pyriofenone at a dose of 5 mg/kg bw. This bile was subsequently administered into the duodenum of cannulated recipient animals to determine the extent of enterohepatic circulation. Forty-eight hours after single intraduodenal doses of bile, 66% of the dose was excreted in the bile of recipient male rats. Most of the radioactivity, 65% of the dose, was excreted in bile 24 hours after dosing. Urinary excretion 48 hours after dosing accounted for 11% of the dose, whereas faecal excretion accounted for 20% of the dose. No radioactivity was detected in the carcass at 48 hours. Overall recovery was 96% of the dose. The extent of reabsorption was estimated by summing the values for bile, urine, liver and carcass. On this basis, it was estimated that the extent of reabsorption was 76% of the dose.

(c) Plasma and whole blood kinetics of ^{14}C-(phenyl)-pyriofenone

Following administration of a single oral dose of ^{14}C-(phenyl)-pyriofenone at 5 mg/kg bw, peak mean plasma and whole blood concentrations of radioactivity were reached at 12 hours (Table 6). At 200 mg/kg bw, peak mean plasma concentrations of radioactivity were reached at 6 hours (males) and 12 hours (females), whereas peak mean whole blood concentrations of radioactivity were reached at 6 hours (males) and 2 hours (females). After 14 daily oral doses at 5 mg/kg bw, peak mean plasma and whole blood concentrations of radioactivity were reached at 2 hours (males) and 12 hours (females) (Table 7).

Table 6. Pharmacokinetic parameters derived from mean radioactivity concentrations in plasma and whole blood after administration of single oral doses of ^{14}C-(phenyl)- or ^{14}C-(pyridyl)-pyriofenone to rats

	^{14}C-(phenyl) label				**^{14}C-(pyridyl) label**			
	5 mg/kg bw		**200 mg/kg bw**		**5 mg/kg bw**		**200 mg/kg bw**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Plasma								
C_{max} (µg/g)	0.60	0.58	13	6.2	0.88	0.66	15	7.4
T_{max} (h)	12	12	6	12	4	12	6	24
AUC_{120} (µg·h/g)	26	17	460	230	33	16	620	330
$t_{½}$ (h)	26	17[a]	24	13	46[a]	13[a]	30	20
AR[b]	1.5	1.0	1.4	0.78	2.8	0.77	1.8	1.2
Whole blood								
C_{max} (µg/g)	0.37	0.34	9.4	4.4	0.53	0.40	9.8	5.2
T_{max} (h)	12	12	6	2	12	12	6	24
AUC_{120} (µg·h/g)	19	11	430	170	25	9.9	530	230
$t_{½}$ (h)	36	18[a]	58[a]	18	30	13	54[a]	22
AR[b]	2.2	1.1	3.5	1.1	1.8	0.80	3.2	1.3

AR: accumulation ratio; AUC_{120}: area under the concentration–time curve at 120 hours; bw: body weight; C_{max}: maximum concentration; $t_{½}$: terminal half-life; T_{max}: time at which the maximum concentration is reached

[a] Data did not meet all the acceptance criteria, and therefore this value should be treated cautiously.

[b] Calculated as AR = 1.44*($t_{½}$/dosing interval).

Source: Knight (2009)

Table 7. Summary of pharmacokinetic parameters in rats after administration of 14 consecutive oral daily doses of ^{14}C-(phenyl)-pyriofenone at 5 mg/kg bw

	^{14}C-(phenyl) label, 14 × 5 mg/kg bw	
	Males	**Females**
Plasma		
C_{max} (µg/g)	1.2	0.77
T_{max} (h)	2	12
AUC_{120} (µg·h/g)	54	18
$t_{½}$ (h)	37	26

	^{14}C-(phenyl) label, 14 × 5 mg/kg bw	
	Males	**Females**
AR[b]	2.2	1.6
Whole blood		
C_{max} (µg/g)	1.2	0.55
T_{max} (h)	2	12
AUC_{120} (µg·h/g)	74	20
$t_{½}$ (h)	102[a]	64[a]
AR[b]	6.1	3.8

AR: accumulation ratio; AUC_{120}: area under the concentration–time curve at 120 hours; bw: body weight; C_{max}: maximum concentration; $t_{½}$: terminal half-life; T_{max}: time at which the maximum concentration is reached

[a] Data did not meet all the acceptance criteria, and therefore this value should be treated cautiously.

[b] Calculated as AR = 1.44*($t_{½}$/dosing interval).

Source: Knight (2009)

The extent of exposure, reflected by the area under the concentration–time curve at 120 hours (AUC_{120}), was greater in males than in females, particularly at 200 mg/kg bw. The terminal half-life ($t_{½}$) was longer in males than in females and longer in whole blood than in plasma. Both the maximum concentration (C_{max}) and the extent of exposure (AUC_{120}) increased with increasing dose; however, this increase was less than proportional, indicating non-linear, dose-dependent kinetics.

Following single oral doses, the whole blood:plasma ratio (based on AUC_{120}) was less than 1, was higher in males than in females and increased with increasing dose. After repeated oral doses, the whole blood:plasma ratio was greater than 1 and indicated accumulation of radioactivity in red blood cells during repeated dose administration.

(d) Single-dose absorption, distribution and excretion of ^{14}C-(pyridyl)-pyriofenone

In bile duct–cannulated rats administered ^{14}C-(pyridyl)-pyriofenone at 5 mg/kg bw, radioactivity was rapidly excreted into bile (74–81% of the dose) and urine (7.5–7.6% of the dose) (Table 1). Radioactivity in faeces accounted for 73–78% of the dose, whereas radioactivity in urine accounted for 14–20% of the dose, 120 hours after dosing in intact rats (Table 2). Similar to ^{14}C-(phenyl)-pyriofenone, the data suggest that ^{14}C-(pyridyl)-pyriofenone is rapidly taken up and initially excreted into bile, with final excretion occurring predominately via faeces, with minor excretion occurring via urine. No sex-specific differences were noted.

Radioactivity retained in the tissues accounted for less than 1% of the dose in both male and female rats. The highest concentrations were measured in the liver in both sexes, then kidney (both sexes), plasma (males), whole blood (males) and blood cells (males). Females consistently had lower levels of radioactivity in tissues than males, with the highest value observed in liver (0.046 µg equivalents/g). In males, concentrations of radioactivity in all tissues other than plasma, whole blood, blood cells, kidney and liver were less than or equal to 0.05 µg equivalents/g or below the limit of detection (Table 8). Tissue:plasma ratios were highest for the liver (both sexes), kidney (both sexes), gastrointestinal tract including contents (females) and fat (females). Intermediate ratios were calculated for whole blood in both sexes. Tissue:plasma ratios were less than 1 for all other tissues (Table 9).

In bile duct–cannulated rats administered ^{14}C-(pyridyl)-pyriofenone at 200 mg/kg bw, excretion followed a similar pattern as with 5 mg/kg bw, except that most of the radioactivity was recovered in faeces (45–54% of the dose) rather than bile (41–49% of the dose) 48 hours after dosing (Table 1). The majority of radioactivity was eliminated in faeces (89% of the dose) 120 hours after dosing in intact rats (Table 2). Overall recoveries of radioactivity in intact animals (excluding tissues) 120 hours after dosing were 98% and 99% of the dose for males and females, respectively (Table 2).

Table 8. Mean concentrations of radioactivity in tissues 120 hours after administration of single oral doses of ^{14}C-(pyridyl)-pyriofenone to rats

	Mean concentrations of radioactivity (µg equivalents/g)			
	1 × 5 mg/kg bw		1 × 200 mg/kg bw	
	Males	Females	Males	Females
Sample	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**
Plasma	0.055 ± 0.005	0.003 ± 0.002	0.877 ± 0.112	0.464 ± 0.228
Whole blood	0.084 ± 0.013	0.007 ± 0.001	3.593 ± 1.092	0.954 ± 0.421
Blood cells	0.127 ± 0.019	0.016 ± 0.003	8.016 ± 2.664	1.855 ± 0.914
Brain	0.005 ± 0.001	nd	0.045 ± 0.091	nd
Heart	0.021 ± 0.003	nd	0.587 ± 0.058	0.207 ± 0.140
Kidney	0.118 ± 0.015	0.023 ± 0.003	3.039 ± 0.422	2.937 ± 0.956
Liver	0.356 ± 0.049	0.046 ± 0.010	6.124 ± 0.889	2.857 ± 1.146
Lungs	0.027 ± 0.009	nd	0.658 ± 0.087	0.357 ± 0.093
Spleen	0.026 ± 0.005	nd	0.823 ± 0.108	0.392 ± 0.068
Adrenal glands	0.017 ± 0.012	nd	nd	nd
Pituitary gland	nd	nd	nd	nd
Thyroid	nd	nd	nd	nd
Epididymis	0.011 ± 0.001	–	0.340 ± 0.102	–
Testes	0.008 ± 0.001	–	0.210 ± 0.024	–
Ovaries	–	nd	–	0.219 ± 0.439
Uterus	–	nd	–	0.224 ± 0.162
Bone	nd	nd	nd	nd
Bone marrow	0.005 ± 0.010	nd	nd	nd
Fat (abdominal)	nd	0.011 ± 0.003	0.499 ± 0.144	6.439 ± 0.846
Muscle (skeletal)	0.007 ± 0.001	nd	0.202 ± 0.013	nd
GI tract + contents	0.046 ± 0.001	0.028 ± 0.009	1.166 ± 0.447	6.354 ± 4.107
Residual carcass	0.013 ± 0.003	0.002 ± 0.004	0.346 ± 0.058	0.289 ± 0.336
% of dose recovered in total tissues[a]	0.65 ± 0.05	0.10 ± 0.02	0.39 ± 0.06	0.61 ± 0.19

bw: body weight; GI: gastrointestinal; nd: not detected; sd: standard deviation

[a] Excluding plasma and carcass. Recoveries calculated assuming the following % body weight values:

Whole blood	7%
Bone	5.46%
Bone marrow	0.35%
Fat (abdominal)	7.1%
Muscle (skeletal)	45.5%

Source: Knight (2009)

Table 9. Tissue:plasma ratios of radioactivity in tissues 120 hours after administration of single oral doses of ^{14}C-(pyridyl)-pyriofenone to rats

	Tissue:plasma ratios of radioactivity			
	5 mg/kg bw		200 mg/kg bw	
	Males	Females	Males	Females
Sample	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**
Plasma	1	1	1	1
Whole blood	1.54 ± 0.116	2.17 ± 0.084	4.06 ± 0.984	2.37 ± 1.03
Brain	0.093 ± 0.014	nd	0.174 ± na	nd
Heart	0.390 ± 0.044	nd	0.672 ± 0.042	0.492 ± 0.092
Kidney	2.17 ± 0.215	6.78 ± 0.710	3.47 ± 0.329	7.83 ± 4.23
Liver	6.52 ± 0.498	14.3 ± 2.42	6.98 ± 0.163	6.63 ± 1.39
Lungs	0.481 ± 0.145	nd	0.752 ± 0.063	0.923 ± 0.425
Spleen	0.479 ± 0.054	nd	0.941 ± 0.066	1.09 ± 0.700
Adrenal glands	0.420 ± 0.023	nd	nd	nd
Pituitary gland	nd	nd	nd	nd
Thyroid	nd	nd	nd	nd
Epididymis	0.199 ± 0.015	–	0.387 ± 0.111	–
Testes	0.149 ± 0.020	–	0.241 ± 0.022	–
Ovaries	–	nd	–	1.32 ± na
Uterus	–	nd	–	0.546 ± 0.235
Bone	nd	nd	nd	nd
Bone marrow	0.339 ± na	nd	nd	nd
Fat (abdominal)	nd	3.41 ± 0.611	0.567 ± 0.139	19.7 ± 16.0
Muscle (skeletal)	0.134 ± 0.017	nd	0.232 ± 0.016	nd
GI tract + contents	0.858 ± 0.095	9.08 ± 1.79	1.32 ± 0.428	12.6 ± 3.32
Residual carcass	0.242 ± 0.038	nd	0.397 ± 0.069	0.932 ± 0.025

bw: body weight; GI: gastrointestinal; na: not applicable; nd: not detected; sd: standard deviation
Source: Knight (2009)

Similar to animals dosed with ^{14}C-(pyridyl)-pyriofenone at 5 mg/kg bw, the highest accumulation in tissues after exposure to 200 mg/kg bw was in liver, followed by blood cells, kidney, whole blood, gastrointestinal tract (including contents) and fat (females) (Table 8). Tissue:plasma ratios were highest for the fat (females) and gastrointestinal tract (females), liver (both sexes) and kidney (both sexes). Intermediate ratios were calculated for whole blood, spleen (females), gastrointestinal tract including contents (males) and ovaries (females). Tissue:plasma ratios were less than 1 for all other tissues (Table 9).

Generally, tissue concentrations were higher in males than in females and, except for blood cells, declined over time. Concentrations of radioactivity in blood cells increased with time in both sexes.

The extent of absorption was estimated by summing the radioactivity (expressed as percentage of administered dose) found in bile, urine, liver and carcass after administration of ^{14}C-(pyridyl)-

pyriofenone in bile duct–cannulated rats. On this basis, it was estimated that the extent of absorption of ^{14}C-(pyridyl)-pyriofenone at 5 mg/kg bw was 82–89% of the dose. In contrast, the extent of absorption of ^{14}C-(pyridyl)-pyriofenone at 200 mg/kg bw was estimated to be 44–53% of the dose (Table 1).

(e) Plasma and whole blood kinetics of ^{14}C-(pyridyl)-pyriofenone

Following administration of a single oral dose of ^{14}C-(pyridyl)-pyriofenone at 5 mg/kg bw, peak mean plasma concentrations of radioactivity were reached at 4 hours (males) and 12 hours (females). Peak mean whole blood concentrations of radioactivity were reached at 12 hours (both sexes). At 200 mg/kg bw, peak mean plasma and whole blood concentrations of radioactivity were reached at 6 hours (males) and 24 hours (females) (Table 6).

Similar to the situation with ^{14}C-(phenyl)-pyriofenone, the AUC_{120} was higher in males than in females, although the ratio of AUC_{120} values was greater for the low dose. The time taken to achieve maximum concentrations (T_{max}) was shorter for males than for females at both doses in plasma (but not whole blood), and the terminal half-life ($t_{½}$) in both plasma and whole blood was longer in males than in females at the low dose. The terminal half-life was similar in plasma and whole blood of the female animals at the high dose. Both the maximum concentration (C_{max}) and the extent of exposure (AUC_{120}) increased with increasing dose; however, this increase was less than proportional, indicating non-linear, dose-dependent kinetics.

Following the administration of single oral doses of ^{14}C-(phenyl)-pyriofenone to rats, the whole blood:plasma ratio (based on AUC_{120}) was less than 1, was higher in males than in females and increased with dose.

(f) Accumulation ratios

In plasma, the 24-hour accumulation ratios (AR) based on half-life (AR = 1.44*[$t_{½}$/dosing interval]) were 1.5 in male rats and 1.0 in female rats after a single dose of 5 mg/kg bw ^{14}C-(phenyl)-pyriofenone and were 2.8 in male rats and 0.8 in female rats after a single dose of 5 mg/kg bw ^{14}C-(pyridyl)-pyriofenone. After a single dose of 200 mg/kg bw ^{14}C-(phenyl)-pyriofenone, the accumulation ratios were 1.4 and 0.8 in male and female rats, respectively. After a dose of 200 mg/kg bw ^{14}C-(pyridyl)-pyriofenone, the accumulation ratios were 1.8 and 1.2 in male and female rats, respectively (Table 6).

In whole blood, the 24-hour accumulation ratios were 2.2 in male rats and 1.1 in female rats after a single dose of 5 mg/kg bw ^{14}C-(phenyl)-pyriofenone and were 1.8 in male rats and 0.8 in female rats after a single dose of 5 mg/kg bw ^{14}C-(pyridyl)-pyriofenone. After a single dose of 200 mg/kg bw ^{14}C-(phenyl)-pyriofenone, the accumulation ratios were 3.5 and 1.1 in male and female rats, respectively. After a single dose of 200 mg/kg bw ^{14}C-(pyridyl)-pyriofenone, the accumulation ratios were 3.2 and 1.3 in male and female rats, respectively (Table 6).

After repeated oral doses of ^{14}C-(phenyl)-pyriofenone, additional accumulation of radioactivity was observed. The 24-hour accumulation ratios were 2.2 and 1.6 in plasma of male and female rats, respectively, and 6.1 and 3.8 in whole blood of male and female rats, respectively. The terminal half-life also increased after repeated exposure (Table 7).

Based on these calculations, there is some evidence that pyriofenone and/or its metabolites accumulate in both plasma and whole blood in male rats after single or repeated administration at 24-hour intervals. It may also accumulate in the blood of female rats, but to a lesser extent. However, the Meeting noted that overall radioactivity recovery values 120 hours after single or repeated dosing were approximately 100%, decreasing the concern for potential accumulation.

1.2 Biotransformation

The metabolism of pyriofenone was studied in the toxicokinetics study by Knight (2009).

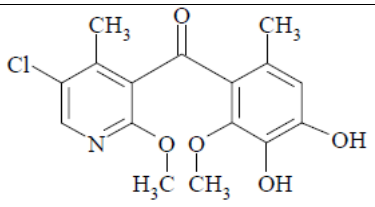
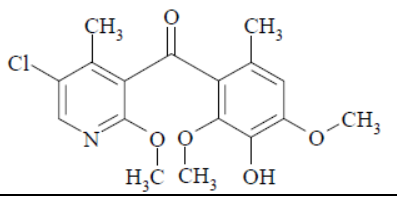
There were no major metabolic differences between ^{14}C-(phenyl)-pyriofenone and ^{14}C-(pyridyl)-pyriofenone. Unchanged pyriofenone was identified as the major component in faecal extracts, accounting for 18–29% of the dose at 5 mg/kg bw and 59–63% of the dose at 200 mg/kg bw (Table 10). Other components identified in faeces were 2MDPM (maximum 21% of the low dose), 3HDPM (maximum 16% of the low dose) and 4HDPM (maximum 11% of the low dose) (Table 11) and accounted for 1.9–7.7% at the high dose. All other components in faecal extracts accounted for less than 5% of the dose.

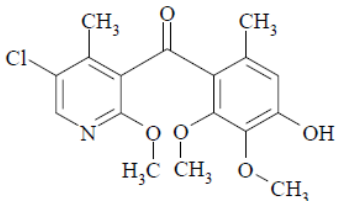
Table 10. Residues after single exposures of rats to 5 or 200 mg/kg bw of pyriofenone with either label[a]

Tissue, fluid or excreta	Principal residue(s)	Secondary residue(s)
Faeces	Pyriofenone (58.6–62.8% of the dose)	2MDPM, 3HDPM, 4HDPM (1.9–7.7% of the dose)
Urine	2MDPM conjugate (females; 9.5% of the dose, but only at 5 mg/kg bw)	Conjugates of 3HDPM and 4HDPM (<1.5% of the dose); pyriofenone (<1% of the dose)
Bile	3HDPM-glucuronide and 4HDPM-glucuronide (10.1–38.6% of the dose)	Pyriofenone (<2% of the dose)
Plasma	2MDPM (22.5–77.5% of plasma radioactivity)	Pyriofenone (<5% of plasma radioactivity)
Liver	3HDPM or 4HDPM (11.4% of liver radioactivity)	Pyriofenone (8.9% of liver radioactivity)
Kidney	Unidentified polar component (25–42% of kidney radioactivity)	Pyriofenone (7.5–39% of kidney radioactivity)
Fat	Pyriofenone (84.3–94.2% of fat radioactivity)	2MDPM, 3HDPM, 4HDPM (≤5.6% of fat radioactivity, each)

[a] Percentages reflect exposure to 200 mg/kg bw except where otherwise indicated.
Source: Data taken from Knight (2009)

Table 11. Structures of the metabolites of pyriofenone

Common name	Chemical name	Structure
2MDPM	(5-Chloro-2-methoxy-4-methyl-3-pyridyl)(3,4-dihydroxy-2-methoxy-6-methylphenyl)ketone	
3HDPM	(5-Chloro-2-methoxy-4-methyl-3-pyridyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)ketone	

Common name	Chemical name	Structure
4HDPM	(5-Chloro-2-methoxy-4-methyl-3-pyridyl)(4-hydroxy-2,3-dimethoxy-6-methylphenyl)methanone	

In the urine of female rats, the major metabolite was an unstable conjugate of 2MDPM (maximum 9.5% of the low dose). There were no metabolites present at greater than 5% of the dose in the urine of male rats at the low dose or of either sex at the high dose. Two major metabolites in bile, each 10–39% of the dose, were identified as glucuronide conjugates of 3HDPM and 4HDPM. Parent pyriofenone accounted for less than 2% of the dose in urine and bile.

Profiles of radioactivity in plasma were similar to those obtained for urine. At the T_{max} time point, the major metabolite in plasma was a glucuronide conjugate of 2MDPM. Pyriofenone and 3HDPM and/or 4HDPM were present in extracts of liver, along with a number of other unidentified metabolites. Pyriofenone was the major identified component in extracts of kidney at 200 mg/kg bw, but was found at a lower concentration than an unidentified polar component. In fat at the high dose, parent pyriofenone was the only major component identified.

The metabolic pathway of pyriofenone in rats is shown in Fig. 3.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Pyriofenone did not cause mortality in acute toxicity tests in rats. The lethal dose of pyriofenone was greater than 2000 mg/kg bw in an oral study, greater than 5.18 mg/L in an inhalation study and greater than 2000 mg/kg bw in a dermal study (Table 12).

Clinical signs in intoxicated animals included abnormal body position (oral), clear nasal discharge (inhalation), very slight erythema (dermal) and low body weight gain (dermal).

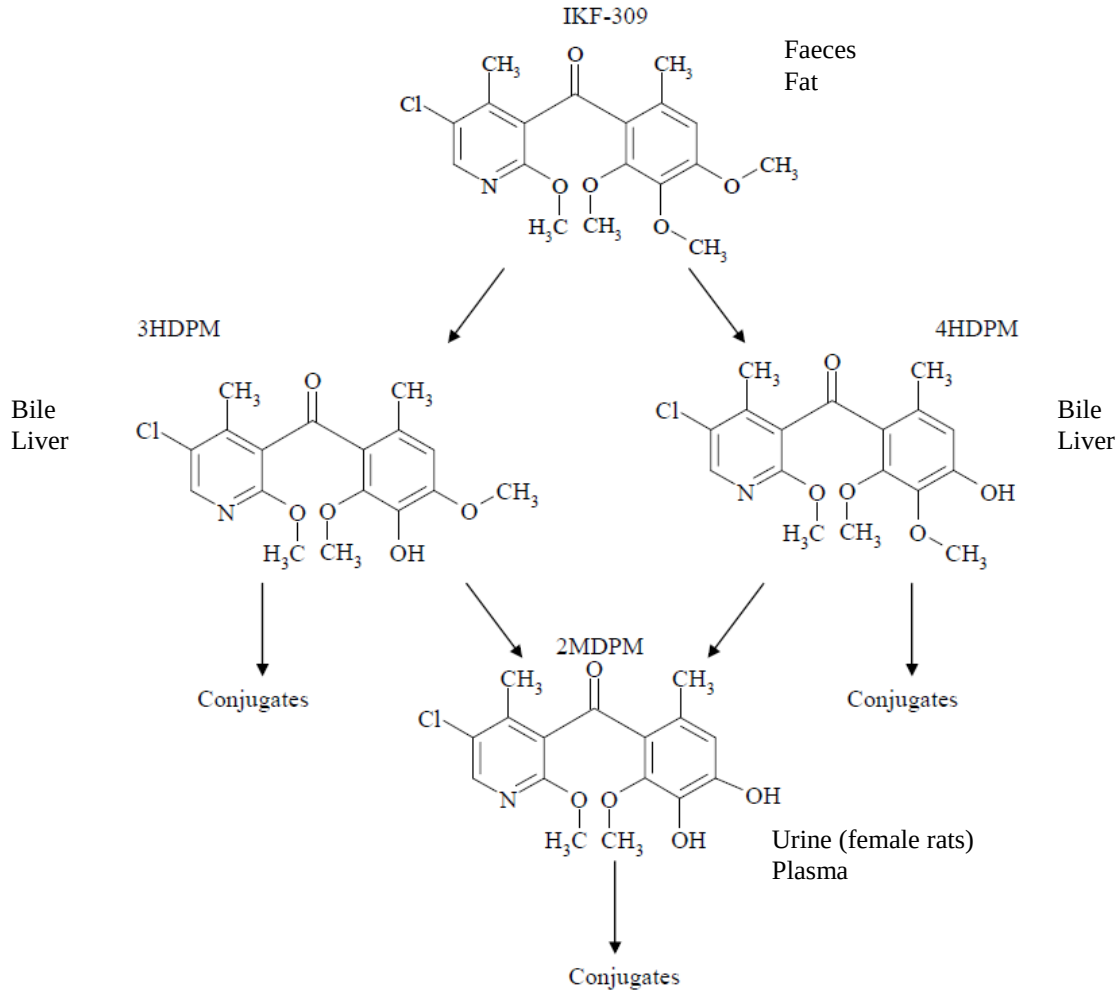
(b) Dermal irritation

Dermal irritation in rabbits was not observed in a study conducted in compliance with Organisation for Economic Co-operation and Development (OECD) Test Guideline 404. No signs of toxicity or ill-health were observed (Rees, 2008a).

(c) Ocular irritation

Ocular irritation was not observed in rabbits in a study conducted in compliance with OECD Test Guideline 405, except for minor conjunctival redness in two animals 1 hour after administration. The redness decreased after 24 hours and was not observed at 48 or 72 hours after administration (Rees, 2008b).

Fig. 3. Metabolic pathway of pyriofenone in rats[a]



[a] Tissue, fluid or excreta where each chemical is the principal component is indicated. IKF-309 = pyriofenone.
Source: Knight (2009)

Table 12. Acute toxicity of pyriofenone

Route	Species	Strain	Sex	Purity (%)	LD_{50}/LC_{50}	Reference
Oral	Rat	SD	F	97.88	>2 000 mg/kg bw	Moore (2008a)
Inhalation	Rat	SD	M + F	97.88	>5.18 mg/L	Hoffman (2008)
Dermal	Rat	SD	M + F	97.88	>2 000 mg/kg bw	Moore (2008b)

bw: body weight; F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male; SD: Sprague Dawley

(d) Dermal sensitization

Dermal sensitization was not observed in mice in a study conducted in compliance with OECD Test Guideline 429. No signs of toxicity or ill-health were observed (Kosaka, 2009).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 13-week toxicity study, pyriofenone (IKF-309; lot no. 0701; purity 97.88%) was administered to CD-1 mice (10 of each sex per group) in the diet at 0, 300, 1000, 3000 or 7000 parts per million (ppm) (equal to 0, 53, 176, 514 and 1318 mg/kg bw per day for males and 0, 61, 214, 695 and 1504 mg/kg bw per day for females, respectively). End-points included clinical observations (daily), body weight (weekly), feed consumption (weekly), haematology (at termination), serum biochemistry (at termination), gross pathology and histopathology.

There was no mortality during this study. There were no treatment-related effects on body weight, feed consumption, haematology or clinical chemistry. Sporadic, low-magnitude effects without evidence of a dose–response relationship were observed in a few haematology and clinical chemistry parameters, but were not considered toxicologically relevant.

Periportal hypertrophy of the liver was observed in mice treated with 7000 ppm (3/10 males; 7/10 females), and higher relative (to body weight) liver weight was observed in male mice administered 3000 or 7000 ppm and females treated with 1000, 3000 or 7000 ppm (up to a 12% increase relative to controls). The Meeting concluded that these effects were adaptive, not adverse, changes.

The no-observed-adverse-effect level (NOAEL) was 7000 ppm (equal to 1318 mg/kg bw per day), the highest dose tested (Moore, 2009).

Rats

In a 28-day dose range–finding toxicity study, Fischer rats (six of each sex per group) were administered pyriofenone (IKF-309 technical; batch no. 0602; purity 98.04%) in the diet at 0, 300, 3000, 10 000 or 20 000 ppm (equal to 0, 24.2, 251, 823 and 1657 mg/kg bw per day for males and 0, 26.1, 261, 841 and 1660 mg/kg bw per day for females, respectively). End-points included clinical signs (daily), body weights (weekly), feed consumption (weekly), feed efficiency (weekly), haematology (termination), clinical chemistry (termination), urine analysis (week 4), organ weights (termination) and histopathological analysis of the liver and kidney (termination). Ophthalmoscopy was not performed.

There was no mortality during this study. There were no significant effects on body weight, feed intake, feed efficiency or urine analysis.

At the highest dose (20 000 ppm), there were treatment-related effects on haematology and clinical chemistry, including decreased haematocrit, decreased haemoglobin concentration, decreased mean corpuscular volume and an increase in platelet count in both sexes. Additionally, the lymphocyte count was increased in males and the mean corpuscular haemoglobin was decreased in females. In clinical chemistry, there was a significant increase in gamma-glutamyl transferase (GGT), total protein, albumin, globulin and total cholesterol and a significant decrease in chloride in both sexes at the high dose. Additional sex-specific effects included increased blood urea nitrogen (males), albumin/globulin ratio (males) and calcium (males) and decreased triglycerides (males), whereas females exhibited an increase in alanine aminotransferase (ALT).

During necropsy, distended caeca were observed in all rats administered dietary concentrations of 3000 ppm or higher. Dark-coloured livers were reported in males at 10 000 ppm and in both sexes at the highest dose. There were dose-dependent increases in absolute and relative kidney and liver weights in both sexes administered dietary concentrations of 3000 ppm or higher. At 20 000 ppm, males also exhibited increased relative (to body weight) thyroid weights, and diffuse hepatocellular hypertrophy was observed in both sexes. Hyaline droplet deposition was observed in the proximal tubular cells in all males at the highest dose, and increased calcification in the corticomedullary junction was seen in 4/6 females at 20 000 ppm. Similar changes were observed in the 10 000 ppm group.

At 3000 ppm, male rats exhibited increased serum alkaline phosphatase (ALP), total protein and albumin in clinical chemistry. Distended caeca with softened contents were observed in all animals of both sexes. Liver weights (absolute and relative) were increased in male rats, whereas females showed increased relative liver weights and increased relative and absolute kidney weights. Diffuse hepatocellular hypertrophy was seen in one female rat.

No treatment-related effects were noted in the 300 ppm group in either sex.

The NOAEL was 300 ppm (equal to 24.2 mg/kg bw per day), based on increased serum ALP, total protein and albumin (males), distended caeca (both sexes), increased liver weights (both sexes) and increased kidney weights (females) at 3000 ppm (equal to 251 mg/kg bw per day) (Ohtsuka, 2010a).

In a 93-day (13-week) oral toxicity study, pyriofenone (IKF-309 technical; lot no. 0701; purity 97.88%) was administered to Fischer rats (10 of each sex per group) in the diet at 0, 300, 1000, 2500 or 5000 ppm (equal to 0, 17.9, 60.5, 150 and 305 mg/kg bw per day for males and 0, 20.6, 69.0, 171 and 350 mg/kg bw per day for females, respectively). End-points included cage-side observations (daily), detailed clinical signs (weekly), body weight, feed consumption, functional observational battery (FOB) (pretest and at 11 weeks), ophthalmology (pretest and at 13 weeks), urine analysis (pretest and at 13 weeks), haematology (13 weeks), serum biochemistry (13 weeks), gross pathology and histopathology.

Treatment-related effects on haematology (Table 13), clinical chemistry (Table 14), organ weights (Table 15) and histopathology (Table 16) were noted.

Table 13. Summary of haematology after 13 weeks of pyriofenone treatment in rats

Parameter	Sex	0 ppm	300 ppm	1 000 ppm	2 500 ppm	5 000 ppm
Mean cell volume (fL)	Males	47.2	47.1	47.1	46.8	46.3**
	Females	49.0	49.7	49.9	49.6	49.7
Mean cell haemoglobin (pg)	Males	16.6	16.6	16.7	16.5	16.4*
	Females	17.4	17.7	17.9	17.8	17.8
Platelet count (10^3/µL)	Males	865	880	865	868	927**
	Females	904	889	897	914	971
Prothrombin time (s)	Males	22.4	23.2	22.8	25.5	25.9**
	Females	17.4	17.3	16.7	16.9	16.6*
APTT (s)	Males	28.7	28.3	28.0	29.5	30.1
	Females	18.1	18.2	19.2*	19.8**	20.0**
Lymphocyte count (10^3/µL)	Males	4.94	5.66	5.86*	5.51	6.10**
	Females	4.25	4.33	4.71	4.74	4.58

APTT: activated partial thromboplastin time; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Ohtsuka (2010b)

Table 14. Summary of clinical chemistry after 13 weeks of pyriofenone treatment in rats

Parameter	Sex	0 ppm	300 ppm	1 000 ppm	2 500 ppm	5 000 ppm
AST (U/L)	Males	78	75	76	67**	63**
	Females	68	65	67	67	65
ALT (U/L)	Males	48	43*	43**	38**	39**
	Females	32	32	31	33	33
GGT (U/L)	Males	0	0	0	0	1**

Parameter	Sex	0 ppm	300 ppm	1 000 ppm	2 500 ppm	5 000 ppm
	Females	1	1	1	1	2**
Creatinine (mg/dL)	Males	0.27	0.28	0.26	0.24	0.24
	Females	0.34	0.33	0.32	0.32	0.25**
Blood urea nitrogen (mg/dL)	Males	16.1	16.6	16.8	17.3	19.3**
	Females	18.2	17.9	16.8	18.1	18.1
Total protein (g/dL)	Males	6.50	6.52	6.52	6.70**	7.02**
	Females	6.04	6.04	6.02	6.20	6.32**
Albumin (g/dL)	Males	4.47	4.43	4.44	4.58*	4.74**
	Females	4.35	4.34	4.29	4.34	4.46
Globulin (g/dL)	Males	2.03	2.09	2.08	2.13	2.28**
	Females	1.69	1.71	1.73	1.86*	1.86*
Total cholesterol (mg/dL)	Males	42	44	46	43	52**
	Females	64	66	59	65	70
Triglyceride (mg/dL)	Males	41	50	57*	44	36
	Females	10	11	9	10	9
Total bilirubin (mg/dL)	Males	0.04	0.04	0.03	0.03**	0.02**
	Females	0.04	0.04	0.03**	0.02**	0.00**
Calcium (mg/dL)	Males	10.1	10.1	10.2	10.3*	10.5**
	Females	9.6	9.5	9.5	9.6	97
Chloride (mEq/L)	Males	109.6	109.4	108.6	108.1**	107.0**
	Females	111.5	111.8	111.2	110.6	110.0

ALT: alanine aminotransferase; AST: aspartate aminotransferase; Eq: equivalents; GGT: gamma-glutamyl transferase; ppm: parts per million; U: units; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Ohtsuka (2010b)

Table 15. Summary of organ weights after 13 weeks of pyriofenone treatment in rats

Organ	Sex	Weight type	0 ppm	300 ppm	1 000 ppm	2 500 ppm	5 000 ppm
Liver	Males	Absolute (g)	6.83	7.01	7.22	7.81**	8.26**
		Relative[a] (%)	2.21	2.23	2.30**	2.44**	2.66*
	Females	Absolute (g)	3.83	3.74	3.83	3.96	4.31**
		Relative[a] (%)	2.21	2.19	2.26	2.34**	2.57**
Kidneys	Males	Absolute (g)	1.95	1.97	1.97	2.11**	2.25**
		Relative[a] (%)	0.63	0.63	0.63	0.66*	0.73**
	Females	Absolute (g)	1.16	1.15	1.13	1.18	1.24**
		Relative[a] (%)	0.67	0.67	0.67	0.70	0.74**
Caecum	Males	Absolute (g)	3.57	3.48	3.33	4.62	9.19**
		Relative[a] (%)	1.16	1.11	1.06	1.44	2.97**
	Females	Absolute (g)	2.72	2.82	2.71	3.78**	5.54**
		Relative[a] (%)	1.58	1.65	1.60	2.23**	3.30**

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
[a] Relative to body weight.
Source: Ohtsuka (2010b)

Table 16. Summary of treatment-related microscopic changes in the liver and kidney after 13 weeks of pyriofenone treatment in rats

	Males					Females				
Finding	**0 ppm**	**300 ppm**	**1 000 ppm**	**2 500 ppm**	**5 000 ppm**	**0 ppm**	**300 ppm**	**1 000 ppm**	**2 500 ppm**	**5 000 ppm**
Liver: Diffuse hepatocellular hypertrophy	0/10	0/10	0/10	0/10	9/10**	0/10	0/10	0/10	0/10	6/10**
Kidney: Increased hyaline droplet deposition in the proximal tubular cells	0/10	0/10	0/10	0/10	9/10**	0/10	0/10	0/10	0/10	0/10
Kidney: Basophilic change of renal tubular cells	1/10	0/10	2/10	4/10	7/10**	0/10	0/10	0/10	0/10	0/10

ppm: parts per million; **: $P < 0.01$ (Fisher's exact probability test)
Source: Ohtsuka (2010b)

At the highest dose (5000 ppm), treatment-related effects included increased motor activity (males), increased urine volume (males), yellow-brown-coloured urine (females), increased platelet (males), lymphocyte (males) and basophil counts (males), prolonged activated partial thromboplastin time (APTT) (females), increased GGT activity, serum total protein and globulin (both sexes) and decreased serum chloride (males). Distended caeca were observed in 10/10 males and 5/10 females. Absolute and relative (to body weight) organ weights were increased for liver, kidneys and caecum (both sexes). Diffuse hepatocellular hypertrophy was observed in 9/10 males and 6/10 females, and males showed an increased incidence of hyaline droplet deposition in the proximal tubular cells (9/10) and basophilic change of renal tubular cells (7/10).

At 2500 ppm, similar, but less severe, effects were seen in serum biochemistry, haematology and gross pathology. Specifically, prolonged APTT (females), increased serum total protein (males), serum albumin (males) and serum calcium (males) and decreased serum chloride (males) were observed. Increased absolute and relative kidney weights (males), absolute liver weight (males) and relative liver weight (both sexes) and absolute and relative caecum weights (females) were observed.

At 1000 ppm, there was a slight increase in relative liver weight (4%) in males and an increase in APTT in females. No other treatment-related effects were observed. There were no treatment-related effects at 300 ppm in either sex.

The NOAEL was 1000 ppm (equal to 60.5 mg/kg bw per day), based on increased liver weights in both sexes, increased caecum weights in females, increased kidney weights in males, prolonged APTT in females, increased serum total protein, albumin and calcium in males and decreased serum chloride in males at 2500 ppm (equal to 150 mg/kg bw per day) (Ohtsuka, 2010b).

Dogs

In a 90-day oral toxicity study, pyriofenone (IKF-309 technical; lot no. 0701; purity 97.88%) was administered to beagle dogs (four of each sex per group) in the diet at 0, 500, 3000 or 25 000 ppm (equal to 0, 15.0, 90.3 and 776 mg/kg bw per day, respectively) for males and at 0, 500, 3000 or 15 000 ppm (equal to 0, 15.3, 89.8 and 475 mg/kg bw per day, respectively) for females. End-points included clinical observations (daily), FOB (pretest and weekly thereafter), feed consumption (daily), water consumption (daily), body weight (weekly), ophthalmoscopy (pretest and week 13), haematology (pretest and weeks 7 and 13), serum biochemistry (pretest and weeks 7 and 13), urine analysis (pretest and weeks 7 and 13), gross pathology and histopathology.

There were no effects on mortality, clinical signs, body weight, urine analysis, haematology or gross pathology in any treated group.

At the high dose, treatment-related effects included prolonged APTT (females), increased ALP and triglycerides (both sexes), increased liver weight (both sexes), decreased spleen weight and increased incidence of centrilobular hepatocellular hypertrophy (both sexes) (Table 17). No effects of toxicological relevance were observed at any other doses.

Table 17. Summary of key findings in a 90-day oral toxicity study in dogs administered pyriofenone

Finding	0 ppm	500 ppm	3 000 ppm	15 000 ppm	25 000 ppm
APTT (s)					
Males					
Pretreatment	10.0	10.5	9.7	–	10.3
Week 7	10.0	10.6	9.2	–	9.4
Week 13	9.9	10.3	9.1*	–	9.3
Females					
Pretreatment	9.9	10.1	10.1	9.9	–
Week 7	10.0	10.0	9.5	9.1*	–
Week 13	9.9	11.1	9.6	8.9*	–
ALP (U/L)					
Males					
Pretreatment	417	353	357	–	332
Week 7	342	283	351	–	1 605
Week 13	287	279	297	–	1 673
Females					
Pretreatment	290	331	374	310	–
Week 7	238	314	486	944**	–
Week 13	226	278	492	1 111**	–
Triglycerides (mg/dL)					
Males					
Pretreatment	20	16	20	–	20
Week 7	21	14	20	–	33*
Week 13	25	20	19	–	46**
Females					
Pretreatment	16	23	22	17	–
Week 7	17	19	19	25	–
Week 13	21	23	21	26	–
Liver weight					
Males					
Absolute (g)	282	280	285	–	373**
Relative[a] (%)	2.69	2.67	2.72	–	3.77**
Females					
Absolute (g)	267	276	305	328	–
Relative[a] (%)	2.66	2.72	3.01	3.34	–

Finding	0 ppm	500 ppm	3 000 ppm	15 000 ppm	25 000 ppm
Spleen weight					
Males					
Absolute (g)	29.0	23.2	20.7**	–	24.2
Relative[a] (%)	0.28	0.22*	0.20**	–	0.25
Females					
Absolute (g)	24.8	27.8	28.1	17.8	–
Relative[a] (%)	0.25	0.27	0.28	0.18*	–
Liver histopathology					
Centrilobular hepatocellular hypertrophy					
Males	0/4	0/4	0/4	–	3/4
Females	0/4	0/4	0/4	3/4	–

–: not applicable; ALP: alkaline phosphatase; APTT: activated partial thromboplastin time; ppm: parts per million; U: units; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test or Fisher's exact probability test)

[a] Relative to body weight.

Source: Nakashima (2010a)

The NOAEL was 3000 ppm (equal to 89.8 mg/kg bw per day), based on markedly increased ALP in females at 15 000 ppm (equal to 475 mg/kg bw per day) (Nakashima, 2010a).

In a 1-year oral toxicity study, pyriofenone (IKF-309 technical; lot no. 0701; purity 97.88%) was administered to beagle dogs (four of each sex per group) at 0, 500, 3000 or 25 000 ppm (equal to 0, 13.7, 83.5 and 701 mg/kg bw per day, respectively) for males and at 0, 500, 3000 or 15 000 ppm (equal to 0, 14.1, 86.2 and 448 mg/kg bw per day, respectively) for females. End-points included clinical observations (daily), FOB (pretest and weekly thereafter), feed consumption (daily), water consumption (daily), body weight (pretest, weekly through week 13 and every 4 weeks from weeks 16 to 52), ophthalmoscopy (pretest and weeks 13, 26 and 52), haematology (pretest and weeks 13, 26 and 52), serum biochemistry (pretest and weeks 13, 26 and 52), urine analysis (pretest and weeks 13, 26 and 52), gross pathology and histopathology.

No treatment-related effects on mortality or ophthalmoscopy were observed.

Treatment-related effects on haematology (Table 18), clinical chemistry (Table 19) and organ weights (Table 20) were observed.

Table 18. Summary of haematological findings in a 52-week oral toxicity study with pyriofenone in dogs

	0 ppm	500 ppm	3 000 ppm	15 000 ppm	25 000 ppm
Haematocrit (%)					
Males					
Pretreatment	43.3	44.9	44.7	–	42.6
Week 13	47.5	49.3	49.6	–	45.4
Week 26	48.5	48.5	50.2	–	43.6**
Week 52	49.2	50.2	50.3	–	45.2
Females					

	0 ppm	500 ppm	3 000 ppm	15 000 ppm	25 000 ppm
Pretreatment	45.2	46.7	42.8	47.2	–
Week 13	47.9	50.5	48.1	52.7**	–
Week 26	47.8	49.6	48.6	51.6	–
Week 52	47.9	50.1	49.3	49.8	–
Haemoglobin concentration (g/dL)					
Males					
Pretreatment	14.4	15.0	15.1	–	14.1
Week 13	16.0	16.7	16.9	–	15.3
Week 26	16.6	16.7	17.6	–	15.1**
Week 52	17.3	17.8	18.2	–	16.1
Females					
Pretreatment	14.9	15.7	14.2	15.7	–
Week 13	16.1	16.9	16.2	17.8*	–
Week 26	16.1	16.8	16.5	17.7*	–
Week 52	16.6	17.5	17.3	17.7	–
Erythrocyte count (10^6/µL)					
Males					
Pretreatment	6.35	6.65	6.68	–	6.34
Week 13	7.30	7.56	7.60	–	6.88
Week 26	7.56	7.59	7.84	–	6.71**
Week 52	7.69	7.89	7.90	–	7.04
Females					
Pretreatment	6.71	6.86	6.14	6.90	–
Week 13	7.26	7.59	7.05	7.71	–
Week 26	7.34	7.63	7.27	7.71	–
Week 52	7.46	7.81	7.45	7.49	–
Mean cell haemoglobin (pg)					
Males					
Pretreatment	22.7	22.6	22.7	–	22.1
Week 13	21.9	22.1	22.3	–	22.3
Week 26	22.0	22.0	22.4	–	22.5
Week 52	22.5	22.6	23.1	–	22.9
Females					
Pretreatment	22.2	22.9	23.1	22.8	–
Week 13	22.2	22.3	22.9	23.1	–
Week 26	22.0	22.0	22.7	23.0*	–
Week 52	22.3	22.5	23.3	23.7*	–
APTT (s)					
Males					
Pretreatment	10.2	10.2	10.3	–	9.9
Week 13	9.9	9.6	9.7	–	8.6**
Week 26	9.8	9.6	9.5	–	8.6*
Week 52	10.0	9.7	9.7	–	8.8**
Females					

	0 ppm	500 ppm	3 000 ppm	15 000 ppm	25 000 ppm
Pretreatment	10.0	9.7	10.0	9.9	–
Week 13	10.2	10.0	9.8	9.7	–
Week 26	9.8	9.7	9.4	8.9*	–
Week 52	9.9	9.9	9.5	9.4	–

–: not applicable; APTT: activated partial thromboplastin time; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Nakashima (2010b)

Table 19. Summary of clinical chemistry findings in a 52-week oral toxicity study with pyriofenone in dogs

	0 ppm	500 ppm	3 000 ppm	15 000 ppm	25 000 ppm
ALP (U/L)					
Males					
Pretreatment	557	438	518	–	430
Week 13	344	330	618	–	1 982*
Week 26	230	265	501	–	2 294**
Week 52	200	229	505	–	2 248**
Females					
Pretreatment	391	454	494	469	–
Week 13	262	299	435	1 636*	–
Week 26	225	305	401	1 749**	–
Week 52	197	307	443	1 746**	–
GGT (U/L)					
Males					
Pretreatment	4	3	4	–	3
Week 13	4	3	4	–	6
Week 26	3	3	5	–	6*
Week 52	3	3	5	–	7*
Females					
Pretreatment	3	3	3	3	–
Week 13	3	3	3	4	–
Week 26	3	3	3	4*	–
Week 52	3	4	3	4	–
Creatinine (mg/dL)					
Males					
Pretreatment	0.51	0.51	0.46	–	0.49
Week 13	0.63	0.66	0.60	–	0.60
Week 26	0.72	0.71	0.65	–	0.60
Week 52	0.74	0.71	0.66	–	0.62*
Females					
Pretreatment	0.47	0.52	0.50	0.47	–
Week 13	0.53	0.62*	0.58	0.59	–
Week 26	0.56	0.63	0.62	0.61	–
Week 52	0.54	0.62	0.58	0.54	–

–: not applicable; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; ppm: parts per million; U: units;
*: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Nakashima (2010b)

Table 20. Summary of organ weight findings in a 52-week oral toxicity study with pyriofenone in dogs

Organ	Sex	Weight type	0 ppm	500 ppm	3 000 ppm	15 000 ppm	25 000 ppm
Liver	Male	Absolute (g)	291	302	313	–	416*
		Relative[a] (%)	2.16	2.63	2.78	–	4.02**
	Female	Absolute (g)	310	300	321	374	–
		Relative[a] (%)	2.65	2.59	2.90	3.52*	–
Kidney	Male	Absolute (g)	45.9	43.9	49.9	–	45.5
		Relative[a] (%)	0.34	0.38	0.44**	–	0.44**
	Female	Absolute (g)	49.3	42.4	44.8	46.8	–
		Relative[a] (%)	0.42	0.37	0.41	0.44	–

–: not applicable; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
[a] Relative to body weight.
Source: Nakashima (2010b)

At the high dose, dogs exhibited vomiting (both sexes), loose stools (both sexes), decreased body weight gain (males), decreased feed consumption (both sexes), low urinary pH (males), decreased haematocrit (males), decreased haemoglobin concentration (males), decreased erythrocyte count (males), increased ALP (both sexes), increased GGT (both sexes), increased relative liver weight (both sexes), increased relative kidney weight (males; 129%), darkened liver colour (both sexes) and centrilobular hepatocellular hypertrophy (males). Of these effects, the haematological findings, clinical chemistry findings and darkened liver colour were considered toxicologically relevant.

At the intermediate dose (3000 ppm), male dogs exhibited increased relative kidney weight (129%). No treatment-related effects were observed in low-dose dogs. In the absence of other renal effects, the change in kidney weight was not considered adverse.

The NOAEL was 3000 ppm (equal to 83.5 mg/kg bw per day), based on markedly increased ALP, increased GGT and darkened liver colour at 15 000 ppm (equal to 448 mg/kg bw per day) (Nakashima, 2010b).

(b) Dermal application

In a 4-week toxicity study, CD rats (10 of each sex per group) were administered pyriofenone (batch no. 0701; purity 97.88%) at 0, 100, 300 or 1000 mg/kg bw per day by dermal occlusion. A control group was sham dosed with purified water and subjected to the same occlusion conditions as the treated animals. The dose preparations were applied for 6 hours per day for 28 days. End-points included clinical observations (daily), examination of application site (before each administration), ophthalmoscopy (pre-exposure and at week 4), body weights (weekly), feed consumption (weekly), haematology and clinical chemistry (week 4), macroscopic pathological assessment (at necropsy) and microscopic histopathological assessment (after necropsy). Urine analysis was not performed.

There were no significant findings in clinical signs, body weight, feed consumption, ophthalmoscopy, haematology, clinical chemistry, organ weights, macroscopic pathological assessment or microscopic histopathological assessment. There were occasional differences in the haematological parameters that reached statistical significance, but these were not considered biologically meaningful.

Pyriofenone did not induce any signs of systemic toxicity after dermal exposure, and it did not induce any signs of skin irritation or sensitivity (Cooper, 2010).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 78-week carcinogenicity study, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered in the diet to Crl:CD-1 mice (52 of each sex per group) at 0, 600, 1800 or 5400 ppm (equal to 0, 77.6, 237 and 716 mg/kg bw per day, respectively) for males and 0, 300, 1000 or 3000 ppm (equal to 0, 49.4, 167 and 486 mg/kg bw per day, respectively) for females. End-points included clinical signs, body weight, feed consumption, haematology (leukocyte counts only), organ weights, gross pathology and histopathology.

No compound-related effects on mortality, palpable masses or leukocyte counts were noted. Survival ranged from 67% to 79%.

Treatment-related effects on liver weights (Table 21) and on neoplastic (Table 22) and non-neoplastic lesions (Table 23) were observed.

Female mice in the high-dose group exhibited reduced body weight (17% reduction). Liver and kidney effects were seen in treated mice. Increases in hepatocellular adenomas and adenocarcinomas were observed, with significance for the combined incidence reached at 5400 ppm (males only). Centrilobular hepatocytic hypertrophy and necrosis of individual hepatocytes were seen in treated male mice. Slight hepatocytic hypertrophy was seen occasionally in female mice.

In the kidney, there was an increased incidence of granular appearance in male mice in the 1800 and 5400 ppm groups and in female mice in the 1000 and 3000 ppm groups. There were an increased incidence and an increased severity of degenerative and regenerative changes (cortical tubular basophilia, cortical scarring and cortical cysts) in mid- and high-dose males. These lesions may have been the cause of increased yellow perigenital staining in male mice. In female mice given 3000 ppm, there was an increase in the incidence of chronic progressive nephropathy (moderate or marked severity), but overall incidence remained low and did not reach statistical significance (maximum of five affected female mice at 3000 ppm). Moderate or marked glomerulonephritis was observed in female mice given 1000 ppm, but occurred in only 3/52 animals and did not occur in any mice at 3000/5400 ppm (Moore, 2010a).

The NOAEL for systemic toxicity was 600 ppm (equal to 77.6 mg/kg bw per day), based on liver and kidney histopathology (hepatocellular hypertrophy and renal cortical tubular basophilia) and an increased incidence of granular appearance of the kidneys in males exposed to 1800 ppm (equal to 237 mg/kg bw per day).

The NOAEL for carcinogenicity was 1800 ppm (equal to 237 mg/kg bw per day), based on an increased incidence of combined hepatocellular adenomas and adenocarcinomas in males at 5400 ppm (equal to 716 mg/kg bw per day) (Moore, 2010a).

Table 21. Summary of liver weights after treatment of mice with pyriofenone for 78 weeks

	Males				**Females**			
	0 ppm	**600 ppm**	**1 800 ppm**	**5 400 ppm**	**0 ppm**	**300 ppm**	**1 000 ppm**	**3 000 ppm**
Number of animals examined	40	37	41	37	35	40	39	40
Terminal body weight (g)	55.6	57.8	56.7	57.4	52.0	50.5	51.1	46.5*
Absolute liver weight (g)	2.682	2.975	2.930	3.141**	2.379	2.364	2.397	2.232
Relative (to body weight) liver weight (%)	4.82	5.15	5.17	5.47**	4.58	4.68	4.69	4.80*

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Fisher's exact probability test)
Source: Moore (2010a)

Table 22. Summary of neoplastic changes in the liver after treatment of mice with pyriofenone for 78 weeks

	Incidence of neoplastic changes									
	Males					Females				
Finding	**0 ppm**	**600 ppm**	**1 800 ppm**	**5 400 ppm**	**HC[a]**	**0 ppm**	**300 ppm**	**1 000 ppm**	**3 000 ppm**	**HC[a]**
Number of animals examined	52	52	52	52	–	52	52	52	52	–
Hepatocellular adenoma	3	7	6	9	15.8% (7.8–26.0%)	1	0	1	2	1.07% (0–3.9%)
Hepatocellular adenocarcinoma	1	2	3	3	3.73% (0–10.0%)	0	0	0	0	0.36% (0–3.9%)
Adenoma/adenocarcinoma combined	4	9	9	12*[#]	18.5% (9.8–32.0%)	1	0	1	2	1.42% (0–5.0%)

HC: historical controls; ppm: parts per million; *: $P < 0.05$ (one-tailed pairwise comparison against controls); [#]: $P < 0.05$ (one-tailed trend test)

[a] Historical control incidence (mean and range provided). Based on 11 78-week studies in mice conducted by the contract laboratory between 1997 and 2007, with 50–60 mice per study.

Source: Moore (2010a)

Table 23. Summary of non-neoplastic changes in the liver and kidney after treatment of mice with pyriofenone for 78 weeks

	Incidence of non-neoplastic changes							
	Males				Females			
Finding	**0 ppm**	**600 ppm**	**1 800 ppm**	**5 400 ppm**	**0 ppm**	**300 ppm**	**1 000 ppm**	**3 000 ppm**
Number of animals examined	52	52	52	52	52	52	52	52
Liver								
Hepatocellular hypertrophy (centrilobular)								
Slight	0	11	13	10	0	1	2	3
Moderate	0	2	3	2	0	0	0	0
Total	0	13**	16**	12**	0	1	2	3
Necrosis of individual hepatocytes								
Minimal	0	4	3	3	0	0	0	2
Slight	1	2	5	4	0	1	0	0
Total	1	6	8*	7*	0	1	0	2
Basophilic foci								
Minimal	0	0	3	1	0	0	0	0
Slight	0	1	0	3	0	0	0	0
Total	0	1	3	4	0	0	0	0
Eosinophilic foci								
Minimal	0	1	0	3	1	0	0	0
Slight	0	0	1	1	0	0	0	0
Total	0	1	1	4	1	0	0	0
Pigment in macrophages	4	0	3	2	8	11	5	18*
Kidney								
Cortical tubular basophilia								
Minimal	32	28	30	17	18	15	16	27
Slight	4	12	15	24	8	2	4	2
Moderate	1	2	2	6	2	0	1	0
Total	37	42	47*	47*	28	17	21	29
Cortical scarring								
Minimal	14	15	11	18	6	10	9	9
Slight	2	2	9	10	5	2	1	1
Moderate	0	0	1	0	0	0	0	0
Total	16	17	21	28*	11	12	10	10
Cortical cysts								
Minimal	3	3	3	1	0	1	0	0
Slight	3	2	9	9	0	1	1	1
Moderate	1	1	0	2	0	0	0	0
Total	7	6	12	12	0	2	1	1

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Fisher's exact probability test)
Source: Moore (2010a)

In a follow-up study, mice were administered pyriofenone (IKF-309; lot no. 0701; purity 97.88%) in the diet to evaluate its effects on enzyme induction and hepatocyte proliferation. This study aimed to provide further mechanistic information regarding the hepatocyte tumour formation observed in Moore (2010a). Two groups of male mice (12 per group) were exposed to pyriofenone in the diet at 5000 or 10 000 ppm (equal to 854 and 1714 mg/kg bw per day, respectively), whereas a control group received untreated diet. End-points included clinical signs, body weight, feed consumption, liver weight, hepatic enzyme assessment (microsomal protein concentration, cytochrome P450 [CYP] concentration, 7-ethoxyresorufin *O*-deethylase activity, testosterone 16α-, 16β- and 6β-hydroxylase activities, testosterone 17β-dehydrogenase activity, and lauric acid 11- and 12-hydroxylase activities), macropathology and histopathology for proliferating cell nuclear antigen (PCNA) as a marker for liver cell proliferation.

There were no effects on mortality, clinical signs, body weight gain or feed consumption.

There was a dose-dependent increase in relative liver weight (up to 180%), but no gross lesions were observed in the liver. There was an increase in cytochrome P450 concentration (150%) at both doses, increased activity (120%) of 7-ethoxyresorufin *O*-deethylase (CYP1A marker) at both doses, decreased activity (40%) of testosterone 17β-dehydrogenase at both doses and increased activity (120%) of testosterone 16α-hydroxylase (CYP2 marker) at the high dose.[1] There was no evidence that pyriofenone induced or suppressed hepatic cytochrome P450s. There was no evidence that pyriofenone increased the rate of hepatocyte proliferation (Table 24).

Table 24. Summary of hepatocyte liver enzymes after 28 days of treatment of mice with pyriofenone[a]

Parameter	5 000 ppm	10 000 ppm
Microsomal protein (mg/g liver)	0.9	1.0
Cytochrome P450		
nmol/mg protein per minute	1.4**	1.6**
nmol/g liver per minute	1.3**	1.5**
7-Ethoxyresorufin *O*-deethylase (CYP1A marker)		
nmol/mg protein	1.5**	1.4**
nmol/g liver	1.4*	1.4*
Testosterone 6β-hydroxylase (CYP3A marker)		
nmol/mg protein per minute	1.0	1.1
nmol/g liver per minute	0.9	1.1
Testosterone 16α-hydroxylase (CYP2 marker)		
nmol/mg protein per minute	1.1	1.2*
nmol/g liver per minute	1.0	1.2
Testosterone 16β-hydroxylase (CYP2 marker)		
nmol/mg protein per minute	b	b*
nmol/g liver per minute	b	b

[1] Of the four subgroups of pooled livers from the vehicle control group examined, only one gave a testosterone 16β-hydroxylase (CYP2 marker activity) above the level of quantification (0.175 nmol/mg protein per minute) for the assay. All four subgroups of both treated groups showed quantifiable activity. When analysed statistically, the activity expressed per gram of liver in the 10 000 ppm group was significantly greater ($P < 0.05$) than the control group activity. Owing to the prevalence of non-detects in the control group, it is not possible to accurately determine the fold increase of activity in the treated groups.

Parameter	5 000 ppm	10 000 ppm
Testosterone 17β-dehydrogenase (CYP2 marker)		
nmol/mg protein per minute	0.6**	0.6**
nmol/g liver per minute	0.5**	0.6**
Lauric acid 11-hydroxylase (CYP2E marker)		
nmol/mg protein per minute	1.1	1.0
nmol/g liver per minute	1.0	1.0
Lauric acid 12-hydroxylase (CYP4A marker)		
nmol/mg protein per minute	1.1	0.9
nmol/g liver per minute	1.0	0.9

CYP: cytochrome P450; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

[a] Values shown are normalized to control.

[b] CYP2B activity was quantifiable in all subgroups of the pyriofenone-treated groups, but in only one subgroup of the control group. Values below the limit of quantification were taken as 0 in the calculation of the control group mean, which was then below the limit of quantification.

Source: Arrowsmith (2010a)

The author (Arrowsmith, 2010a) concluded that pyriofenone caused only a minor induction of some cytochrome P450 isoforms (CYP1A and CYP2) and had no effect on other isoforms (CYP3A, CYP2E and CYP4A). There was no evidence that pyriofenone increased the rate of cell proliferation in the liver. The minor increases in CYP1A and CYP2 activities were not considered relevant to the increased hepatic tumours seen in Moore (2010a).

Rats

In a 1-year (52-week) oral toxicity study, pyriofenone (IKF-309 technical; lot no. 0701; purity 97.88%) was administered to Fischer rats (20 of each sex per group) in the diet at 0, 200, 1000 or 5000 ppm (equal to 0, 8.51, 42.9 and 226 mg/kg bw per day for males and 0, 10.6, 53.5 and 275 mg/kg bw per day for females, respectively). End-points included cage-side observations (daily), detailed clinical signs (weekly), body weight, feed consumption, FOB (pretest and at 49 weeks), ophthalmology (pretest and at 52 weeks), urine analysis (pretest and at 13, 25 and 51 weeks), haematology (pretest and at 14, 26 and 52 weeks), serum biochemistry (pretest and at 14, 26 and 52 weeks), gross pathology and histopathology.

There were no treatment-related effects on mortality. One high-dose male and one low-dose male were found dead at weeks 40 and 46, respectively. There were no unscheduled deaths in females at any dose. There were no effects on feed efficiency, ophthalmoscopy or functional observations.

Treatment-related effects on haematology (Table 25), clinical chemistry (Table 26), organ weights (Table 27) and histopathology (Table 28) were observed.

Female rats at the high dose (5000 ppm) exhibited increased incidences of soiled fur and decreased body weight (89% of control). At the high dose, rats of both sexes exhibited decreased haematocrit, decreased haemoglobin concentration and decreased red blood cell count, accompanied by occasional decreases in mean corpuscular volume (males), mean corpuscular haemoglobin concentration (males), haemoglobin width (males) and red cell distribution width (both sexes). APTT was increased in both sexes. In clinical chemistry, there were increases in GGT, ALP, AST and ALT (both sexes) and increased total cholesterol (both sexes). In addition, sex-specific effects at the high dose included increased urine volume (males), increased urinary ketones (females) and increased blood urea nitrogen (males).

Table 25. Summary of haematology in a 52-week toxicity study with pyriofenone in rats

	Males				Females			
Parameter	**0 ppm**	**200 ppm**	**1 000 ppm**	**5 000 ppm**	**0 ppm**	**200 ppm**	**1 000 ppm**	**5 000 ppm**
Haematocrit (%)								
Week 14	46.7	46.1	46.5	45.2**	45.9	45.9	45.8	44.5*
Week 26	45.1	44.8	45.6	43.5**	45.4	45.1	45.1	43.6**
Week 52	46.3	46.3	46.1	41.7**	47.2	46.5	46.9	45.6**
Haemoglobin conc. (g/dL)								
Week 14	16.4	16.2	16.4	15.8**	16.4	16.5	16.4	15.9*
Week 26	16.2	16.1	16.4	15.6**	16.5	16.3	16.3	15.9**
Week 52	15.4	15.4	15.4	13.3**	16.2	15.9**	16.0	15.7**
Haemoglobin width (g/dL)								
Week 14	2.80	2.81	2.80	2.80	2.42	2.43	2.43	2.45
Week 26	2.65	2.60	2.65	2.63	2.19	2.15	2.18	2.22
Week 52	2.52	2.49	2.51	2.43**	2.21	2.22	2.21	2.21
RBC count (10^6/µL)								
Week 14	9.55	9.47	9.51	9.42	8.97	8.98	8.93	8.65**
Week 26	9.52	9.35	9.55	9.28*	8.84	8.75	8.82	8.57**
Week 52	9.34	9.27	9.26	8.72**	8.80	8.64	8.74	8.58**
Red cell distribution width (g/dL)								
Week 14	13.9	13.9	14.0	13.7	12.4	12.3	12.3	12.3
Week 26	14.1	14.0	13.8*	14.2	12.4	12.3	12.3	12.3
Week 52	12.9	12.7	12.9	13.3	11.7	11.7	11.7	11.3**
MCV (pg)								
Week 14	48.9	48.7	48.8	48.0*	51.1	51.1	51.3	51.4
Week 26	47.4	47.9	47.8	46.9	51.3	51.6	51.1	50.8
Week 52	49.6	49.9	49.8	47.8	53.6	53.8	53.7	53.2
MCH (pg)								
Week 14	17.1	17.1	17.3	16.8**	18.3	18.4	18.4	18.4
Week 26	17.0	17.2	17.2	16.8*	18.7	18.6	18.6	18.6
Week 52	16.5	16.6	16.6	15.2	18.5	18.4	18.4	18.3
MCHC (g/dL)								
Week 14	35.1	35.3	35.4	35.0	35.8	36.0	35.8	35.8
Week 26	35.9	36.0	35.9	35.7	36.5	36.2	36.3	36.5
Week 52	33.2	33.3	33.3	31.6*	34.4	34.2	34.2	34.3
Reticulocytes (10^9/L)								
Week 14	168.8	156.3	154.4	158.5	154.6	135.9	139.6	132.0
Week 26	201.7	190.6	182.1	200.7	178.6	172.6	176.8	159.3
Week 52	162	158	163	210	167	164	171	139**
Platelet count (10^3/µL)								
Week 14	774	755	762	824**	829	775	794	849
Week 26	776	750	741	837	722	717	723	790**

Parameter	Males				Females			
	0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
Week 52	907	876	877	1 128	860	840	848	897
Prothrombin time (s)								
Week 52	19.1	19.0	19.6	21.9**	18.4	18.1	17.8*	17.0**
APTT (s)								
Week 52	22.9	23.0	23.5	24.6**	16.5	17.0	17.0	18.3**

APTT: activated partial thromboplastin time; conc.: concentration; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; ppm: parts per million; RBC: red blood cell (erythrocyte); *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Ohtsuka (2010c)

Table 26. Summary of clinical chemistry in a 52-week toxicity study with pyriofenone in rats

Parameter	Males				Females			
	0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
ALP (U/L)								
Week 14	448	424	452	393**	332	306	299	267**
Week 26	393	378	373	354**	264	257	269	208**
Week 52	387	347*	321**	275**	214	194	191	143**
AST (U/L)								
Week 14	93	82	76	65**	73	72	72	66*
Week 26	113	87**	91*	68**	88	93	79	63**
Week 52	99	98	94	65**	97	115	83	58**
ALT (U/L)								
Week 14	50	46	43	44	31	31	32	34*
Week 26	63	51	51*	46**	46	49	42	36
Week 52	74	70	63	41**	54	66	48	36**
GGT (U/L)								
Week 14	1	1	1	2*	1	1	1	2**
Week 26	1	1	0	1	1	2	2*	3**
Week 52	3	3	2	2**	2	3	3	2
Creatinine (mg/dL)								
Week 14	0.42	0.40	0.39	0.36**	0.38	0.36	0.37	0.31**
Week 26	0.42	0.41	0.40	0.39	0.42	0.43	0.38	0.31**
Week 52	0.34	0.33	0.33	0.29**	0.33	0.32	0.31	0.24**
Blood urea nitrogen (mg/dL)								
Week 14	16.6	15.7	16.9	20.8**	16.7	16.7	16.8	17.3
Week 26	18.6	18.4	18.6	23.2**	20.4	20.3	19.5	20.7
Week 52	15.7	15.0	15.1	16.7	18.8	17.7	18.2	19.6
Total protein (g/L)								
Week 14	6.46	6.38	6.55	7.22**	6.45	6.51	6.46	6.87**
Week 26	6.83	6.78	6.79	7.24**	6.68	6.67	6.73	7.21**

Parameter	Males				Females			
	0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
Week 52	6.78	6.68	6.72	6.48	6.87	7.00	7.02	7.45**
Albumin (g/L)								
Week 14	4.28	4.20	4.36	4.83**	4.48	4.57	4.53	4.71**
Week 26	4.44	4.36	4.43	4.78**	4.72	4.71	4.71	4.93**
Week 52	4.26	4.18	4.22	4.17	4.83	4.90	4.92	5.17**
Globulin (g/L)								
Week 14	2.19	2.18	2.19	2.39**	1.98	1.94	1.93	2.16**
Week 26	2.39	2.42	2.35	2.46	1.96	1.96	2.02	2.28**
Week 52	2.51	2.50	2.50	2.31*	2.04	2.10	2.10	2.29**
Albumin/globulin ratio								
Week 14	1.96	1.93	1.99	2.02	2.28	2.36	2.35	2.18
Week 26	1.86	1.81	1.89	1.95	2.41	2.41	2.34	2.17**
Week 52	1.70	1.67	1.70	1.81	2.04	2.10	2.35	2.27
Total cholesterol (mg/dL)								
Week 14	48	48	47	62**	58	63	64	86**
Week 26	65	66	61	75**	88	90	91	111**
Week 52	77	77	69	63*	107	108	108	133**
Triglycerides (mg/dL)								
Week 14	66	60	56	24**	29	26	25	20
Week 26	81	80	79	59*	43	34	32*	30*
Week 52	57	55	48	45	58	59	51	43
Total bilirubin (mg/dL)								
Week 14	0.05	0.04	0.04*	0.01**	0.06	0.06	0.04*	0.02**
Week 26	0.06	0.05	0.05*	0.01**	0.07	0.07	0.05*	0.01**
Week 52	0.06	0.06	0.05**	0.01**	0.06	0.06	0.05*	0.01**
Calcium (mg/dL)								
Week 14	9.8	9.8	9.9	10.5**	9.6	9.6	9.5	9.9**
Week 26	10.1	10.1	10.1	10.5**	9.8	9.7	9.7	10.1**
Week 52	10.0	10.0	10.1	10.1	10.1	10.1	10.0	10.6**
Phosphorus (mg/dL)								
Week 14	5.4	5.8	5.3	5.9*	4.7	5.0	4.8	5.1
Week 26	4.5	4.4	4.4	4.9	3.8	3.9	3.4	4.0
Week 52	4.0	4.2	4.2	4.6**	4.0	3.9	3.6	4.6
Chloride (mEq/L)								
Week 14	107.7	107.3	107.2	106.4**	108.4	108.2	108.5	106.9**
Week 26	106.6	106.8	106.5	105.3**	107.0	106.8	107.5	105.5*
Week 52	108.6	109.0	108.6	107.9	109.8	110.1	110.3	107.7**

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Eq: equivalents; GGT: gamma-glutamyl transferase; ppm: parts per million; U: units; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Ohtsuka (2010c)

Table 27. Summary of organ weights in a 52-week toxicity study with pyriofenone in rats

Organ	Weight type	Males				Females			
		0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
Brain	A (mg)	2 058	2 027	2 059	2 062	1 849	1 890*	1 855	1 861
	R-b (%)	0.50	0.51	0.50	0.52	0.87	0.87	0.89	0.95**
Heart	A (mg)	1 069	1 018	1 106	1 072	659	696*	673	691*
	R-b (%)	0.26	0.26	0.27	0.27	0.31	0.32	0.32	0.35**
Liver	A (g)	8.19	7.88	8.54	9.46**	4.34	4.51	4.48	5.06**
	R-b (%)	1.99	1.98	2.05	2.38**	2.03	2.08	2.15	2.58**
Kidneys	A (g)	2.227	2.217	2.370	2.570**	1.339	1.397	1.365	1.494**
	R-b (%)	0.54	0.56	0.57	0.65**	0.63	0.65	0.66	0.76**
Adrenals	A (mg)	41.1	40.8	40.0	45.7	50.6	51.2	50.9	52.5
	R-b (%)	0.010	0.010	0.010	0.012**	0.024	0.023	0.025	0.027**
Testes	A (mg)	3 210	3 085	3 299	3 279			–	
	R-b (%)	0.78	0.77	0.80	0.82*				
Epididy-mides	A (mg)	950	896	991	1 008*			–	
	R-b (%)	0.23	0.23	0.24	0.25**				
Caecum	A (g)	4.176	4.206	4.383	8.787**	3.657	3.487	3.619	6.447**
	R-b (%)	1.02	1.06	1.06	2.21**	1.71	1.61	1.74	3.29**
Spleen	A (mg)	705	678	691	647*	437	445	435	404**
	R-b (%)	0.17	0.17	0.17	0.16	0.20	0.21	0.21	0.21

A: absolute weight; ppm: parts per million; R-b: relative to body weight; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Ohtsuka (2010c)

Table 28. Summary of histopathology in a 52-week toxicity study with pyriofenone in rats

Site and lesion		Incidence of lesions observed							
		Males				Females			
		0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
Bone marrow (sternum)	[*N* =]	[20]	[20]	[20]	[20]	[20]	[0]	[0]	[20]
Granuloma		0	0	0	0	10	–	–	2**
Haematopoiesis, increased		0	0	0	9**	0	–	–	0
Bone marrow (femur)	[*N* =]	[20]	[20]	[20]	[20]	[20]	[0]	[0]	[20]
Granuloma		0	0	0	0	15	–	–	6**
Haematopoiesis, increased		0	0	0	9**	0	–	–	0
Liver	[*N* =]	[20]	[20]	[20]	[20]	[20]	[20]	[20]	[20]
Hypertrophy, hepatocyte, centrilobular		0	0	0	18**	0	0	0	0
Hyperplasia, bile duct		20	19	20	0**	2	2	3	2

Site and lesion		Incidence of lesions observed							
		Males				Females			
		0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
Foci of cellular alteration (basophilic cell type)		8	11	11	7	11	9	7	0**
Hepatodiaphragmatic nodule		2	5	2	0	2	5	8*	4
Microgranuloma		1	0	0	0	7	6	6	1*
Kidney	[*N* =]	[20]	[20]	[20]	[20]	[20]	[20]	[20]	[20]
Tubular basophilic change		1	1	2	10**	0	0	0	1
Increased deposition, brown pigment, renal tubular cell		0	0	0	0	0	0	0	20**
Pituitary	[*N* =]	[20]	[1]+	[0]+	[20]	[20]	[2]+	[3]+	[20]
Cyst, anterior lobe		1	0	–	0	6	0	0	0*

+: not subjected to statistical evaluation because only animals with macroscopic lesions were histopathologically examined; [*N*]: number of animals examined; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Fisher's exact probability test)
Source: Ohtsuka (2010c)

At necropsy, high-dose rats of both sexes showed distended caecum with contents (five males, 10 females) and increased caecum weight. Absolute and relative (to body weight) liver weights were increased (both sexes) and were accompanied by centrilobular hepatocellular hypertrophy in males (18/20). Absolute and relative (to body weight) kidney weights were increased in both sexes and were accompanied by tubular basophilic changes (10/20 males) and increased brown pigment deposit in renal tubular cells (20/20 females). Additionally, slightly increased haematopoiesis in the bone marrow was seen in 9/20 males.

At the intermediate dose (1000 ppm), male and female rats exhibited some low-magnitude and/or sporadic (occurring at one time point but not others) effects, but these were not considered toxicologically relevant.

There were no treatment-related effects observed at the low dose (200 ppm).

The NOAEL was 1000 ppm (equal to 42.9 mg/kg bw per day), based on increased liver weight and centrilobular hypertrophy, increased APTT, decreased haemoglobin and red blood cell parameters (haematocrit, haemoglobin concentration, haemoglobin width, red blood cell count), increased GGT, ALP, AST and ALT, and increased total cholesterol in male and female rats at 5000 ppm (equal to 226 mg/kg bw per day). At 5000 ppm, nephrotoxicity was observed in rats of both sexes, including increased kidney weights, altered clinical chemistry suggestive of renal dysfunction, and renal histopathology (tubular basophilic change in 10/20 males and increased brown pigment deposition in renal tubular cells in 20/20 females) (Ohtsuka, 2010c).

In a 2-year (104-week) carcinogenicity study, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered in the diet to Fischer rats (50 of each sex per group) at 0, 200, 1000 or 5000 ppm (equal to 0, 7.25, 36.4 and 197 mg/kg bw per day for males and 0, 9.13, 46.5 and 254 mg/kg bw per day for females, respectively). End-points included clinical signs, body weight, feed consumption, haematology (leukocyte counts; week 104), necropsy and histopathology. No clinical chemistry measurements were included.

There were no effects on total leukocyte count or differential leukocyte count.

At the high dose, there was a significant increase in mortality in males during the last 4 weeks of treatment, corresponding to a cumulative mortality rate of 34%. There was an increase in the incidence of soiled fur in both sexes, and females showed an increased incidence of fur loss. Feed consumption was increased in males, whereas body weights were decreased from weeks 11 to 104 in males and from weeks 16 to 104 in females.

Macropathological and micropathological observations at the high dose included an increased incidence of distention of the caecum (with contents) in both sexes, accompanied by soiled fur in the genital region and general loss of fur. The integumentary areas that showed a loss of fur also showed increased atrophy of hair follicles and perifolliculitis. The kidney had a coarse appearance in males. Liver, kidney and caecum weights were increased in both sexes (Table 29). Male rats also showed an increased incidence of mesenteric lymph node sinus dilatation (Table 30). Both sexes showed an increased incidence of centrilobular hepatocellular hypertrophy and centrilobular hepatocellular fatty changes. Male rats also showed an increased incidence of centrilobular hepatocellular necrosis, whereas female rats showed an increased incidence of focal hepatic congestion. Female rats also showed an increased incidence of chronic progressive nephropathy (CPN). Although the incidence of CPN was not statistically significant with respect to treatment in male rats (Table 30), there was a significant increase in the severity of CPN lesion grade in males (Table 31).

Table 29. Summary of organ weights in a carcinogenicity study in rats administered pyriofenone

		Males				Females			
Organ	**Weight type**	**0 ppm**	**200 ppm**	**1 000 ppm**	**5 000 ppm**	**0 ppm**	**200 ppm**	**1 000 ppm**	**5 000 ppm**
Body weight (final)	A (g)	449	441	436	377**	267	271	276	228**
Brain	A (g)	2.12	2.14	2.12	2.14	1.96	1.94	1.96	1.94
	R-b (%)	0.47	0.48	0.49	0.57**	0.74	0.72	0.71	0.85**
Heart	A (g)	1.16	1.19	1.15	1.23	0.835	0.803	0.896	0.892
	R-b (%)	0.26	0.27	0.27	0.33**	0.32	0.30	0.33	0.38**
Liver	A (g)	9.84	11.2	10.3	10.6	6.14	5.81	6.85	6.96*
	R-b (%)	2.19	2.54	2.36	2.85**	2.31	2.16	2.49	3.07**
Kidneys	A (g)	2.50	2.60	2.54	2.93**	1.75	1.67	1.81	1.89*
	R-b (%)	0.56	0.59	0.58	0.78**	0.66	0.62	0.66	0.83**
Caecum	A (g)	4.75	4.70	4.25	7.17**	3.59	3.70	3.41	7.63**
	R-b (%)	1.06	1.06	0.98	1.92**	1.35	1.37	1.25	3.36**
Adrenals	A (g)	0.058	0.056 7	0.056 3	0.070 8	0.066 4	0.062 1	0.069 0	0.064 3
	R-b (%)	0.013	0.013	0.013	0.019*	0.025	0.023	0.025	0.029
Testes	A (g)	2.65	3.60	3.43	3.57				
	R-b (%)	0.59	0.82	0.79	0.96*			–	
Epididymides	A (g)	0.696	0.528**	0.682	0.586				
	R-b (%)	0.16	0.12**	0.16	0.16			–	

A: absolute weight; ppm: parts per million; R-b: relative to body weight; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Ohtsuka (2010d)

Table 30. Summary of non-neoplastic lesions in a rat carcinogenicity study

		Incidence[a] of non-neoplastic lesions							
		Males				Females			
Findings	**Fate**	**0 ppm**	**200 ppm[b]**	**1 000 ppm[b]**	**5 000 ppm**	**0 ppm**	**200 ppm[b]**	**1 000 ppm[b]**	**5 000 ppm**
Atrophy of hair follicle (lumbo-dorsal)	tk	0/43	1/2	2/4	4*/33	0/35	0/2	0/6	1/38
	ke/fd	0/7	0/11	0/7	2/17	2/15	0/16	0/14	2/12
	all	0/50	1/13	2/11	6*/50	2/50	0/18	0/20	3/50
Perifolliculitis (lumbo-dorsal)	tk	0/43	0/2	0/4	2/33	2/35	2/2	5/6	8/38
	ke/fd	0/7	0/11	0/7	0/17	0/15	0/16	0/14	0/12
	all	0/50	0/13	0/11	2/50	2/50	2/18	5/20	8*/50
Increased extramedullary haematopoiesis in the spleen	tk	2/43	1/4	1/3	5/33	4/35	0/4	0/3	3/38
	ke/fd	4/7	4/11	0/7	7/17	2/15	5/16	5/14	1/12
	all	6/50	5/15	1/10	12/50	6/50	5/20	5/17	4/50
Dilatation of sinus in mesenteric lymph nodes	tk	0/43	0/39	1/43	12**/33	0/35	–	–	1/38
	ke/fd	0/7	1/11	0/7	5/16	0/15	0/16	0/14	0/12
	all	0/50	1/50	1/50	17**/49	0/50	0/16	0/14	1/50
Hyperplasia of alveolar epithelial cells	tk	10/43	1/5	1/1	3/33	0/35	1/1	1/1	3/38
	ke/fd	0/7	1/11	1/7	1/17	0/15	1/16	0/14	1/12
	all	10/50	2/16	2/8	3*/50	0/50	2/17	1/15	4/50
Focal hepatic congestion	tk	0/43	0/39	0/43	0/33	1/35	1/34	2/36	12**/38
	ke/fd	0/7	0/11	0/7	0/17	0/15	0/16	0/14	1/12
	all	0/50	0/50	0/50	0/50	1/50	1/50	2/50	13**/50
Centrilobular hepatocyte fatty change	tk	0/43	0/39	0/43	19**/33	0/35	0/34	0/36	27**/38
	ke/fd	2/7	1/11	0/7	4/17	7/15	5/16	4/14	6/12
	all	2/50	1/50	0/50	23**/50	7/50	5/50	4/50	33**/50
Centrilobular hepatocyte necrosis	tk	0/43	0/39	0/43	0/33	0/35	0/34	0/36	0/38
	ke/fd	0/7	1/11	0/7	8*/17	1/15	2/16	0/14	0/12
	all	0/50	1/50	0/50	8**/50	1/50	2/50	0/50	0/50
Centrilobular hepatocyte hypertrophy	tk	0/43	0/39	0/43	33**/33	0/35	0/34	0/36	36**/38
	ke/fd	0/7	0/11	0/7	1/17	0/15	0/16	0/14	1/12
	all	0/50	0/50	0/50	34**/50	0/50	0/50	0/50	37**/50
Hyperplasia of bile duct	tk	43/43	39/39	4*/43	21**/33	14/35	13/34	17/36	5**/38
	ke/fd	6/7	10/11	7/7	6*/17	7/15	5/16	5/14	1*/12
	all	49/50	49/50	50/50	27**/50	21/50	18/50	22/50	6**/50
Hepato-diaphragmatic nodule	tk	11/43	6/39	4*/43	4/33	7/35	5/34	7/36	6/38
	ke/fd	2/7	2/11	1/7	1/17	4/15	4/16	5/14	3/12
	all	13/50	8/50	5*/50	5*/50	11/50	9/50	12/50	9/50
	tk	6/43	3/39	1/43	0*/33	16/35	21/34	20/36	15/38

Findings	Fate	Incidence[a] of non-neoplastic lesions							
		Males				Females			
		0 ppm	200 ppm[b]	1 000 ppm[b]	5 000 ppm	0 ppm	200 ppm[b]	1 000 ppm[b]	5 000 ppm
Hepatic microgranuloma	ke/fd	0/7	0/11	1/7	0/17	2/15	3/16	5/14	2/12
	all	6/50	3/50	2/50	0*/50	18/50	24/50	25/50	17/50
Oedema of renal papilla	tk	22/43	24/39	24/43	8*/33	13/35	15/34	16/36	19/38
	ke/fd	1/7	5/11	4/7	2/17	3/15	5/16	6/14	5/12
	all	23/50	29/50	28/50	10*/50	16/50	20/50	22/50	24/50
Chronic progressive nephropathy	tk	38/43	34/39	42/43	31/33	16/35	20/34	30**/36	36**/38
	ke/fd	3/7	6/11	4/7	13/17	1/15	5/16	5/14	9**/12
	all	41/50	40/50	46/50	44/50	17/50	25/50	35**/50	45**/50
Luminal dilatation of the uterine horn	tk					8/35	0/6	1/11	2*/38
	ke/fd			–		0/15	3/16	2/14	0/12
	all					8/50	3/22	3/25	2*/50
Hyperplasia of anterior cell in the pituitary	tk	17/43	2/15	3/13	8/33	13/35	7/24	1/23	15/38
	ke/fd	1/7	3/11	0/7	3/17	7/15	1*/16	1*/14	2/12
	all	18/50	5/26	3/20	11/50	20/50	8/40	2/37	17/50
Hyperplasia of adrenal medullary cells	tk	10/43	1/3	0/3	16/33	4/35	–	0/1	2/38
	ke/fd	3/7	0*/11	0/7	5/17	0/15	0/16	0/14	0/12
	all	13/50	1/14	0/10	21/50	4/50	0/16	0/15	2/50

–: no data or not applicable; all: all animals examined; ke/fd: killed in extremis or found dead during treatment period; ppm: parts per million; tk: terminal kill after 104-week treatment; *: $P < 0.05$; **: $P < 0.01$ (Fisher's exact probability test)

[a] Figures show the incidence of findings observed/number of animals examined.

[b] For the 200 and 1000 ppm groups, with the exception of liver and kidneys, results were not subject to statistical analysis, as not all animals were examined.

Source: Ohtsuka (2010d)

Table 31. Statistical analysis of grade of CPN in rats in a carcinogenicity study

Fate	Grade	Incidence[a] of CPN, by grade							
		Males				Females			
		0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
tk	−	5	5	1	2	19	14	6	2
	+	32	28	32	11	11	16	23	22
	++	5	4	10	9	5	3	5	14
	+++	1	2	0	11	0	1	2	0
	Incidence	38/43	34/39	42/43	31/33	16/35	20/34	30/36	36/38
	Significance				**			*	**
ke/fd	−	4	5	3	4	14	11	9	3
	+	1	6	3	8	1	4	2	5
	++	0	0	1	2	0	1	3	4

		Incidence[a] of CPN, by grade							
		Males				Females			
Fate	Grade	0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
	+++	2	0	0	3	0	0	0	0
	Incidence	3/7	6/11	4/7	13/17	1/15	5/16	5/14	9/12
	Significance								**
all	−	9	10	4	6	33	25	15	5
	+	33	34	35	19	12	20	25	27
	++	5	4	11	11	5	4	8	18
	+++	3	2	0	14	0	1	2	0
	Incidence	41/50	40/50	46/50	44/50	17/50	25/50	35/50	45/50
	Significance				**			**	**

all: all animals examined; ke/fd: killed in extremis or found dead during treatment period; tk: terminal kill after 104-week treatment; *: $P < 0.05$; **: $P < 0.01$ (Dunnett type mean rank test)
[a] Figures show the incidence of findings observed/number of animals examined.
Source: Ohtsuka (2010d)

There were no toxicologically relevant effects on the incidence of neoplastic lesions in treated rats (Table 32). The combined incidence of hepatocellular adenomas and carcinomas in treated rats did not reach statistical significance.

Table 32. Summary of neoplastic lesions in a rat carcinogenicity study

		Incidence[a] of neoplastic lesions							
		Males				Females			
Findings	Fate	0 ppm	200 ppm	1 000 ppm	5 000 ppm	0 ppm	200 ppm	1 000 ppm	5 000 ppm
Mononuclear cell leukaemia	tk	3/43	3/39	1/43	0/33	3/35	5/34	3/36	3/38
	ke/fd	1/7	3/11	1/7	3/17	9/15	6/16	3*/14	6/12
	all	4/50	6/50	2/50	3/50	12/50	11/50	6/50	9/50
Interstitial testicular cell tumour	tk	38/43	32/39	31/43	25/33				
	ke/fd	3/6	7/11	1/7	4/16			–	
	all	41/49	39/50	32/50	29*/49				
Hepatocellular adenoma/ carcinoma	tk	4/43	2/39	3/43	4/33	0/35	0/34	0/36	1/38
	ke/fd	0/7	0/11	0/7	4/17	0/15	0/16	0/14	0/12
	all	4/50	2/50	3/50	8/50	0/50	0/50	0/50	1/50

all: all animals examined; ke/fd: killed in extremis or found dead during treatment period; tk: terminal kill after 104-week treatment; *: $P < 0.05$ (Fisher's exact probability test)
[a] Figures show the incidence of findings observed/number of animals examined.
Source: Ohtsuka (2010d)

Peto's test was applied to analyse the occurrence of neoplastic lesions in males with an incidence of four or more in at least one dose group (Table 33). This analysis was undertaken because the male 5000 ppm group showed a statistically significant increase in mortality during the final stage of the study. Peto's test for the combined incidence of hepatocellular adenoma and carcinoma and the incidence of systemic mononuclear cell leukaemia was performed on the data from all male dose groups. Peto's test for other neoplastic lesions was performed on the data only from the control and 5000 ppm groups, as all animals in the 200 and 1000 ppm groups were not examined histopathologically for the organs/tissues concerned. No dose-related increasing trends were observed in the neoplastic lesions analysed by Peto's test.

Table 33. Summary of Peto's test in a rat carcinogenicity study with pyriofenone

Sex	Site	Neoplastic lesion	*P*-value	Judgement
Male	Systemic	Mononuclear cell leukaemia	0.56	Not significant
	Skin	Fibroma	1.00	Not significant
	Pancreas	Islet cell adenoma/carcinoma	0.13	Not significant
	Liver	Hepatocellular adenoma/carcinoma	0.14	Not significant
	Pituitary	Anterior adenoma/carcinoma	0.26	Not significant
	Thyroid	C-cell adenoma/carcinoma	1.00	Not significant
	Adrenal	Phaeochromocytoma (benign/malignant)	1.00	Not significant

Source: Ohtsuka (2010d)

At the intermediate dose (1000 ppm), the only test article–related effect was an increased incidence of CPN in female rats. No adverse effects were noted in male rats.

There were no effects related to treatment observed at the low dose.

The NOAEL was 200 ppm (equal to 9.13 mg/kg bw per day), based on CPN in female rats at 1000 ppm (equal to 46.5 mg/kg bw per day). The nephropathy in female rats at 1000 ppm and in both sexes at 5000 ppm (Ohtsuka, 2010d) was considered related to the nephrotoxicity observed in both sexes at 5000 ppm in the 52-week rat study (Ohtsuka, 2010c). There was no evidence of increased neoplasia or tumour incidence after exposure to pyriofenone (Ohtsuka, 2010d).

2.4 Genotoxicity

Pyriofenone was tested for genotoxicity in an adequate range of in vitro and in vivo assays. It did not produce evidence of genotoxicity in any assay (Table 34).

Table 34. Summary of genotoxicity studies with pyriofenone[a,b,c]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* and *Escherichia coli*	5, 15, 50, 150, 500, 1 500 or 5 000 µg/plate ±S9	97.88	Negative	May (2007)[d]
Mammalian cell gene mutation	Mouse lymphoma L5178Y cells (*tk* locus)	5, 9.93, 19.86, 20, 39.72, 40, 50, 60, 70, 79.44, 80, 90, 100, 125,	97.88	Negative	Hynes (2008a)[e]

End-point	Test object	Concentration	Purity (%)	Results	Reference
		158.88, 317.75, 635.5 or 1 271 µg/mL ±S9			
Chromosomal aberration	CHL cells	0, 20, 30, 60, 65, 70, 90, 100, 110, 120 or 130 µg/mL ±S9	97.88	Negative	Pritchard (2008)[f]
In vivo					
Mouse micronucleus test (oral gavage)	CD-1 mice, 5/sex per dose	0, 500, 1 000 or 2 000 mg/kg bw	97.88	Negative	Hynes (2008b)[g]
Comet assay	F344 CrlCrlj rats, male, 5/dose	0, 500, 1 000 or 2 000 mg/kg bw	97.88	Negative	Wada (2017)[h]

bw: body weight; CHL: Chinese hamster lung; GLP: good laboratory practice; OECD: Organisation for Economic Co-operation and Development; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *tk*: thymidine kinase

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to quality assurance and GLP were included in all studies.

[c] All studies used pyriofenone batch no. 0701.

[d] Performed in accordance with OECD Test Guideline No. 471.

[e] Performed in accordance with OECD Test Guideline No. 476.

[f] Performed in accordance with OECD Test Guideline No. 473.

[g] Performed in accordance with OECD Test Guideline No. 474.

[h] Performed in accordance with OECD Test Guideline No. 489.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a multigeneration reproductive toxicity study, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered in the diet to Wistar Hannover rats (24 of each sex per group) at 0, 150, 1000 or 5000 ppm for two generations. These dietary concentrations were equal to 0, 7.29, 47.8 and 257 mg/kg bw per day for parental (F_0) males, 0, 9.5, 58.1 and 301 mg/kg bw per day for F_0 females, 0, 7.73, 52.2 and 267 mg/kg bw per day for first filial generation (F_1) males and 0, 9.3, 59.5 and 311 mg/kg bw per day for F_1 females, respectively. Rats were exposed for 18 weeks from premating through mating, gestation and postnatal growth of offspring. Parental animals of both sexes were exposed to the test article for 10 weeks prior to mating and during mating, gestation and lactation, and offspring were exposed from weaning for 10 weeks until mating to produce the second generation. F_0 adults were terminated after weaning of the F_1 offspring. Adult F_1 rats and second filial generation (F_2) pups were terminated after weaning of the F_2 generation.

Measurements in parental animals (F_0 and adult F_1 rats) included clinical signs, urine analysis, haematology, feed and water consumption, body weight, estrous cyclicity, reproductive performance, pregnancy rate, gestation duration, sperm evaluation and necropsy. Histopathology was performed on reproductive organs, pituitary, adrenals, liver, kidney, thyroid and caecum.

Litter measurements included clinical observation of pups, number of pups per litter, sex ratio of pups, external abnormalities and survival to postnatal day 21. F_1 rats were evaluated for physical development and sexual maturation (preputial separation and vaginal opening).

There were no treatment-related effects on reproductive performance, sexual development, estrous cyclicity, gestation index, number of implantation sites or sperm parameters in either generation.

No adverse effects related to treatment were observed in either sex of F_0 or F_1 parental animals at 150 or 1000 ppm. At the high dose (5000 ppm), treatment-related effects included decreased haematocrit (F_0 and F_1 females; F_1 males), decreased haemoglobin concentration (F_0 and F_1 females; F_1 males) and decreased erythrocyte count (F_1 females). There was also a significant increase in haemoglobin distribution width and platelet count in F_1 females. High-dose rats also showed increased

kidney, thyroid and caecum weights (F_0 and F_1) and distention of the caecum (F_1) (Table 35). F_2 pups at the high dose showed decreased spleen weights (14.4% decrease in males; 13.3% decrease in females), but no histopathology in the spleen.

Table 35. Summary of dietary two-generation reproductive toxicity study in rats administered pyriofenone

	Males				Females			
Parameter	**0 ppm**	**150 ppm**	**1 000 ppm**	**5 000 ppm**	**0 ppm**	**150 ppm**	**1 000 ppm**	**5 000 ppm**
F_0 generation								
Number of animals/group	24	24	24	24	24	24	24	24
Mortality	0	0	1	0	0	0	1	0
Body weight (g)								
At pairing								
Week 10	394	395	389	397	231	233	232	238
Gain: weeks 0–10	238	239	233	242	109	111	110	116
Gestation								
Day 0	–	–	–	–	232	234	234	238
Gain, days 0–20	–	–	–	–	102	106	100	103
Lactation								
Day 0	–	–	–	–	246	251	254	254
Day 21	–	–	–	–	280	284	286	296**
Feed consumption (g/rat per day)								
Pre-pairing								
Weeks 1–10	19.5	19.8	19.7	20.9	15.2	15.6	15.5	15.8
Gestation								
Days 0–7	–	–	–	–	15.3	15.8	15.7	15.3
Days 7–14	–	–	–	–	17.7	18.4	18.2	18.0
Days 14–20	–	–	–	–	18.7	19.3	19.4	20.5**
Lactation								
Days 0–7	–	–	–	–	31.7	30.6	31.8	32.0
Days 7–14	–	–	–	–	49.2	51.0	48.1	47.4
Days 14–20[a]	–	–	–	–	59.7	61.1	56.5	60.2
Litter data (F_1 litters)								
Live litters born	–	–	–	–	22	20	23	23
Live litter size, day 0	–	–	–	–	12.0	12.7	11.3	11.8
Fertility index (%)	–	–	–	–	91.7	83.3	100	95.8
Gestation index (%)	–	–	–	–	100	100	100	100
Offspring body weight (g)								
Day 0	5.9	5.7	6.0	5.8	5.6	5.4	5.6	5.5
Day 4	10.1	9.6	10.4	10.0	9.8	9.4	9.8	9.6
Day 21 (weaning)	54.9	54.4	54.3	51.7*	52.9	53.1	51.8	49.6*

Parameter	Males				Females			
	0 ppm	150 ppm	1 000 ppm	5 000 ppm	0 ppm	150 ppm	1 000 ppm	5 000 ppm
% of control	–	99	99	94	–	100	98	94
Terminal observations: F_0 adults[b]								
Number examined	24	24	23	24	24	24	23	24
Terminal body weight (g)	447	439	439	440	261	266	265	268
Organ weights								
Relative liver weight (%)	2.94	3.03	3.13*	3.78**	3.90	4.33**	4.26**	5.40**
Relative kidney weight (%)	0.266	0.276	0.281	0.330**	0.350	0.360	0.363	0.386**
Relative thyroid weight (% × 1 000)	5.32	5.99	5.82	6.54**	8.87	8.64	7.21	9.39*
Relative caecum weight (%)	1.72	1.80	1.87	3.18**	2.39	2.42	2.59	3.56**
Gross pathology								
Dark liver	0	0	0	13**	0	0	0	19**
Histopathology								
Number examined	22	20	23	23	21	20	23	23
Liver								
Centrilobular hypertrophy	0	0	0	0	0	0	0	20**
Diffuse hypertrophy	0	0	0	22**	0	0	0	0
Lipofuscin in Glisson's capsule	0	0	0	13**	0	0	0	0
Thyroid								
Follicular cell hypertrophy	13	–	–	15	3	5	5	14**
Kidney								
Hyaline droplets in proximal tubule	0	0	0	11**	0	0	0	0
Haematology								
Haematocrit (%)	44.6	44.1	44.1	43	47.6	48.7	47.0	43.4*
Haemoglobin concentration (g/dL)	15.0	14.9	14.9	14.5	16.2	16.4	16.0	14.5*
RBC count (10^6/µL)	8.37	8.25	8.27	8.02	8.26	8.23	8.16	7.56
Sperm morphology								
Sperm head count (× 10^6/g testis)	127	127	131	131	–	–	–	–
Sperm count (× 10^6/g epididymis)	544	556	586	568	–	–	–	–
% motile sperm	91.0	92.3	93.0	91.4	–	–	–	–
% normal sperm	97.0	98.3	98.7	97.8	–	–	–	–
F_1 generation								
Number of animals/group	24	24	24	24	24	24	24	24
Mortality	0	0	0	0	0	0	0	0
Body weight (g)								
At pairing								
Week 10	383	386	381	393	230	225	228	229
Gain, weeks 0–10	320	324	320	330	171	167	170	170

Parameter	Males				Females			
	0 ppm	150 ppm	1 000 ppm	5 000 ppm	0 ppm	150 ppm	1 000 ppm	5 000 ppm
Gestation								
Day 0	–	–	–	–	232	229	232	232
Gain, days 0–20	–	–	–	–	98	95	95	102
Lactation								
Day 0	–	–	–	–	253	256	258	256
Day 21	–	–	–	–	281	281	289	296**
Feed consumption (g/rat per day)								
Pre-pairing								
Weeks 1–10	18.8	19.0	17.9	19.9	14.5	14.6	14.2	14.7
Gestation								
Days 0–7	–	–	–	–	14.8	15.4	14.7	15.6
Days 7–14	–	–	–	–	17.6	18.3	17.7	18.5
Days 14–20	–	–	–	–	18.9	20.1	19.7	21.1**
Lactation								
Days 0–7	–	–	–	–	32.1	30.2	29.6	33.9
Days 7–14	–	–	–	–	48.7	48.7	46.0	49.1
Days 14–20[a]	–	–	–	–	58.0	57.3	54.5	61.5
Sexual maturation								
Preputial separation								
Age	42.3	41.9	42.4	42.0	–	–	–	–
Body weight (g)	182	182	182	181	–	–	–	–
Vaginal opening								
Age	–	–	–	–	31.9	31.6	31.2	31.6
Body weight (g)	–	–	–	–	101	100	97	100
Litter data (F_2 litters)								
Live litters born	–	–	–	–	22	24	22	24
Live litter size, day 0	–	–	–	–	11.6	10.5	9.9	10.7
Fertility index (%)	–	–	–	–	95.7	100	91.7	100
Gestation index (%)	–	–	–	–	100	100	100	100
Offspring body weight (g)								
Day 0	6.0	6.1	6.0	6.0	5.7	5.8	5.7	5.9
Day 4	10.3	10.7	10.7	10.6	10.1	10.5	10.3	10.4
Day 21 (weaning)	55.0	55.3	54.6	53.1	52.7	53.2	52.8	51.8
% of control	–	101	99	97	–	101	100	98
Terminal observations: F_1 adults[c]								
Number examined	24	24	24	24	24	24	24	24
Terminal body weight (g)	446	448	449	459	265	266	270	277
Organ weights								

Parameter	Males				Females			
	0 ppm	150 ppm	1 000 ppm	5 000 ppm	0 ppm	150 ppm	1 000 ppm	5 000 ppm
Relative liver weight (%)	3.06	3.22	3.16	3.90**	4.32	4.46	4.63	6.41**
Relative kidney weight (%)	0.268	0.282	0.282	0.325**	0.357	0.366	0.367	0.403**
Relative thyroid weight (% × 1 000)	5.57	6.77	5.38	6.45*	7.64	7.61	7.03	8.89*
Relative caecum weight (%)	1.68	1.75	1.79	3.17**	2.20	2.42	2.39	3.38**
Histopathology								
Number examined	22	24	22	24	22	24	23	23
Liver								
Centrilobular hepatocellular hypertrophy	0	0	0	0	0	0	0	21**
Diffuse hepatocellular hypertrophy	0	0	0	22**	0	0	0	0
Lipofuscin in Glisson's capsule	0	0	0	8**	0	0	0	0
Thyroid								
Follicular cell hypertrophy	5	7	5	19**	5	7	4	16**
Kidney								
Hyaline droplets in proximal tubule	0	0	0	11**	0	0	0	0
Haematology								
Haematocrit (%)	43.9	43.6	44.4	41.6**	47.5	46.4	46.7	44.4*
Haemoglobin concentration (g/dL)	14.9	14.7	14.9	14.1*	16.2	15.7	15.9	15.3*
RBC count (10^6/µL)	8.59	8.45	8.58	8.19	8.52	8.31	8.47	8.05*

–: no data or not applicable; F_0: parental generation; F_1: first filial generation; ppm: parts per million; RBC: red blood cells;
*: $P < 0.05$; **: $P < 0.01$

[a] Dam + litter.

[b] For F_0 males after 18 weeks of treatment; for F_0 females after weaning of litters (day 26 postpartum).

[c] For F_1 males after 18 weeks of treatment; for F_1 females after weaning of litters (day 26 postpartum).

Source: Hojo (2009a)

The NOAEL for parental toxicity was 1000 ppm (equal to 47.8 mg/kg bw per day), based on altered haematological parameters and changes in organ weights in F_0 and F_1 rats at 5000 ppm (equal to 257 mg/kg bw per day).

The NOAEL for reproductive toxicity was 5000 ppm (equal to 257 mg/kg bw per day), the highest dose tested.

The NOAEL for offspring toxicity was 5000 ppm (equal to 257 mg/kg bw per day), the highest dose tested (Hojo, 2009a).

(b) Developmental toxicity

Rats

In a developmental toxicity study, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered by gavage to pregnant Wistar Hannover rats (24 per group) from gestation day (GD) 6 to GD 19 at 0, 30, 300 or 1000 mg/kg bw per day. The vehicle was 1% carboxymethylcellulose. End-points included clinical signs, maternal body weight (GDs 0, 6, 9, 12, 15, 18 and 20), feed consumption, necropsy (maternal animals), histopathology of liver and kidney, gravid

uterine weight, numbers of corpora lutea and implantations, resorptions (early/late), placental weights, and number and position of live and dead fetuses in the uterus. Live fetuses were sexed, weighed, terminated and examined for external and orifice abnormalities. Half of the fetuses were used to evaluate visceral abnormalities, and the other half were used to evaluate skeletal abnormalities after appropriate fixation techniques.

Rats were observed daily throughout the dosing period for mortality and clinical signs. Body weight was determined on GDs 1, 3, 6, 10, 14, 17 and 20. All rats were terminated on GD 20; macroscopic examination was performed, with emphasis on ovaries and uteri.

No deaths occurred on study. There were no treatment-related effects on clinical signs, gravid uterine weights, numbers of corpora lutea or implantations, or preimplantation loss. There were no treatment-related effects on the number of live fetuses, resorptions, fetal weight, placental weight, sex ratio or external, visceral or skeletal malformations or variations.

Although there were increases in skeletal variations (supernumerary rib, discontinuous rib cartilage, presacral vertebrae) in fetuses of the 300 and 1000 mg/kg bw per day groups when considering fetal incidence (Table 36), after comparing the litter incidence of skeletal variations across dose groups, there were no significant trends related to dose.

Table 36. Summary of rat developmental toxicity study with pyriofenone: malformations and variations

	Incidence of finding			
Parameter	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Number of fetuses examined	149	150	148	157
External malformations	No treatment-related effect			
Visceral examinations	No treatment-related effect			
Skeletal malformations	No treatment-related effect			
Number of fetuses with skeletal variation	84	83	105*	121**
Supernumerary ribs	64	51	85*	98**
Discontinuous rib cartilage	38	45	47	62*
27 presacral vertebrae	6	3	19	14
Number of litters per group	24	24	24	24
Skeletal variation (litters affected)	22	22	23	23
Supernumerary ribs (litters affected)	21	18	23	22
Discontinuous rib cartilage (litters affected)	15	17	20	23*
27 presacral vertebrae (litters affected)	6	2	10	7
Skeletal variation (% litters affected)	91.6	91.6	95.8	95.8
Supernumerary ribs (% litters affected)	87.5	75.0	95.8	91.6
Discontinuous rib cartilage (% litters affected)	62.5	70.8	83.3	95.8
27 presacral vertebrae (% litters affected)	25.0	8.3	41.6	29.1

bw: body weight; *: $P < 0.05$; **: $P < 0.01$
Source: Hojo (2009b)

The only treatment-related effects in maternal animals were increased absolute and relative weights of liver and caecum at 300 and 1000 mg/kg bw per day and decreased feed consumption during GDs 6–9 and 9–12 at 1000 mg/kg bw per day (Table 37).

Table 37. Summary of rat developmental toxicity study with pyriofenone: effects in maternal animals

Parameter	0 mg/kg bw per day	30 mg/kg bw per day	300 mg/kg bw per day	1 000 mg/kg bw per day
Number of females mated	24	24	24	24
Number of females pregnant	24	24	24	24
Number of females that aborted	0	0	0	0
Number of females that were killed prematurely	0	0	0	0
Number of females with live young at termination	24	24	24	24
Number of litters examined	24	24	24	24
Clinical signs	No treatment-related effect			
Body weight (g)				
Day 0	210	210	210	210
Day 6	229	231	231	229
Day 20	319	329	324	324
Body weight change (g)				
Days 6–20	90	98	93	95
Feed consumption (g/day)				
Days 6–9	16.6	16.4	15.3	12.8**
Days 9–12	17.7	17.9	17.2	15.6*
Days 12–15	18.0	19.1	18.6	18.0
Days 15–18	20.5	21.7	20.7	21.1
Days 18–20	19.1	20.2	20.0	20.4
Absolute gravid uterine weight (g)	64	66	64	66
Adjusted body weight, day 20 (g)	255	263	260	258
Organ weights				
Liver				
Absolute (g)	12.3	13.0	13.1*	14.3**
Relative to body weight (%)	4.84	4.94	5.03*	5.54**
Kidney				
Absolute (g)	0.778	0.818	0.799	0.806
Relative to body weight (%)	0.305	0.312	0.308	0.313
Caecum				
Absolute (g)	4.83	4.99	5.61*	6.52**
Relative to body weight (%)	1.90	1.90	2.17	2.53**
Number of litters	24	24	24	24
Number of corpora lutea	13.5	14.4	13.8	14.1
Number of implantations	12.8	13.0	12.8	13.3
% resorptions/fetal deaths	7.5	6.4	7.8	5.9

Parameter	0 mg/kg bw per day	30 mg/kg bw per day	300 mg/kg bw per day	1 000 mg/kg bw per day
Number of live fetuses	11.8	12.1	11.8	12.5
Sex ratio (% male)	49.6	45.9	46.1	49.5
Preimplantation loss (%)	5.4	8.9	6.7	5.3
Fetal weight (g)				
Males	3.61	3.63	3.56	3.52
Females	3.43	3.47	3.40	3.34
Placental weight (g)	0.431	0.441	0.435	0.419

bw: body weight; *: $P < 0.05$; **: $P < 0.01$
Source: Hojo (2009b)

The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on increased liver and caecum weights in dams at 300 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Hojo, 2009b).

Rabbits

In a developmental toxicity study, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered by gavage to pregnant Japanese white rabbits (24 per group) from GDs 6 to 27 at 0, 30, 300 or 1000 mg/kg bw per day. The vehicle was 1% carboxymethylcellulose. End-points included clinical signs, maternal body weight (GDs 0, 6, 9, 12, 15, 18, 21, 24, 27 and 28), feed consumption, necropsy (maternal animals), gravid uterine weight, numbers of corpora lutea and implantations, placental weights, and number and position of live and dead fetuses in the uterus. Live fetuses were sexed, weighed, scarified and examined for external and orifice abnormalities. Half of the fetuses were used to evaluate visceral abnormalities, and the other half were used to evaluate skeletal abnormalities after appropriate fixation techniques.

There were no treatment-related effects on gravid uterine weight, numbers of corpora lutea or implantations, preimplantation loss, number of live fetuses, resorptions or fetal deaths, sex ratio, fetal weight or placental weight. There were no treatment-related trends in external, visceral or skeletal malformations or variations.

Two high-dose females aborted. There was a reduction in feed intake from GD 12 to GD 15 at 300 mg/kg bw per day. There were no other treatment-related effects observed in the study.

In a preliminary study, enlarged livers and elevated relative (to body weight) liver weights were observed in gravid female rabbits dosed at 1000 mg/kg bw per day, but no histopathology was performed. Despite the liver being identified as a potential target organ, the comprehensive developmental toxicity study in rabbits did not include an assessment of the liver.

The NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Takahashi, 2009).

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study in rats, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered once by oral gavage to Sprague Dawley rats (10 of each sex per group) at 0, 125, 500 or 2000 mg/kg bw. The vehicle was 1% carboxymethylcellulose. The rats were observed for

14 days after dosing. End-points included clinical signs, home cage observations, feed consumption, brain weight and anatomical measurement, macropathology and neurohistopathology.

There was no mortality on study. There were no effects of treatment on feed consumption, neurobehaviour, brain anatomical measurements, brain weight, or macroscopic or microscopic investigations.

Reduced body tone (three males, two females) and piloerection (two males, seven females) were observed at the time of peak effect on the day of administration in rats given 2000 mg/kg bw. These signs were not observed on day 8 or 15 of the observation period.

Pyriofenone was not neurotoxic in this study. The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested (Powell, 2010).

In a 13-week subchronic neurotoxicity study in rats, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered in the diet to Sprague Dawley rats (10 of each sex per group) at 0, 1000, 4000 or 15 000 ppm (equal to 0, 62, 310 and 927 mg/kg bw per day for males and 0, 77, 378 and 1147 mg/kg bw per day for females, respectively). End-points included clinical signs, home cage observations, feed consumption, brain weight and anatomical measurement, macropathology and neurohistopathology.

There was no mortality reported on study. There were no effects of treatment on clinical signs, feed consumption, neurobehaviour, brain anatomical measurements, brain weight, or macroscopic or microscopic investigations.

The only treatment-related effects were decreased body weight gain in males at 4000 ppm (11%) and in both sexes at 15 000 ppm (11–19%).

Pyriofenone was not neurotoxic in this study. The NOAEL for neurotoxicity was 15 000 ppm (equal to 927 mg/kg bw per day), the highest dose tested (Arrowsmith, 2010b).

(b) Immunotoxicity

Mice

In a 4-week immunotoxicity study in mice, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered through the diet to female CD-1 mice (10 per group) at 0, 1000, 3000 or 7000 ppm (equal to 0, 192, 553 and 1270 mg/kg bw per day, respectively). A separate group of eight female mice was set aside as the cyclophosphamide (positive control) group. Four days prior to necropsy, all treatment groups were administered a single intravenous dose of sheep red blood cells (2×10^8 cells/mL) in 0.9% saline. The positive control group was given a single intraperitoneal dose of cyclophosphamide at 20 mg/kg bw for 5 days (study days 22–26). The positive control group was administered sheep red blood cells after the fourth consecutive dose of cyclophosphamide and terminated 4 days later. Study end-points included clinical condition, body weight, feed and water consumption, spleen and thymus weights, macropathology and plaque-forming cell assays. Spleen samples were taken for immunotoxicology investigations, but no other tissues were retained.

There were no unscheduled deaths on study. There were no treatment-related effects on body weight, feed intake, water consumption, spleen or thymus weights, or macropathology findings.

There were no treatment-related effects on the number of cells per spleen or plaque-forming cells (per million cells or per spleen) in the pyriofenone treatment groups. The cyclophosphamide positive control group showed significant decreases in plaque-forming cells, demonstrating the sensitivity of the assay.

Pyriofenone did not affect immune function in this study, as assessed by the measurement of antigen-specific, T-cell-dependent antibody formation. The NOAEL for immunotoxicity was 7000 ppm (equal to 1270 mg/kg bw per day), the highest dose tested (Laurent, 2010).

Rats

In a 4-week immunotoxicity study in rats, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered through the diet to female Sprague Dawley rats (10 per group) at 0, 2000, 6000 or 20 000 ppm (equal to 0, 179, 505 and 1690 mg/kg bw per day, respectively). A separate group of eight female rats was set aside as the cyclophosphamide (positive control) group. Four days prior to necropsy, all treatment groups were administered a single intravenous dose of sheep red blood cells (2×10^8 cells/mL) in 0.9% saline. The positive control group was given a single intraperitoneal dose of cyclophosphamide at 50 mg/kg bw (5 mg/mL solution at 10 mL/kg bw) 2 days prior to necropsy. Study end-points included clinical condition, body weight, feed and water consumption, spleen and thymus weights, macropathology and plaque-forming cell assays. Spleen samples were taken for immunotoxicology investigations, but no other tissues were retained.

Spleen samples were used to create single-cell suspensions for each animal. The cells were mixed with an agar matrix containing a suspension of sheep red blood cells and guinea-pig serum complement. The mixture was poured into petri dishes, allowed to set and incubated for up to 3 hours. The number of lytic plaques for each animal was determined, and the number of plaque-forming cells per one million splenocytes, the number of plaque-forming cells per spleen and total number of white blood cells per spleen were calculated for each rat.

There were no unscheduled deaths on study. There were no treatment-related effects on clinical signs, water intake, spleen or thymus weights, or necropsy findings.

There was a significant decrease in body weight gain (72% of control) in high-dose rats. Feed consumption was decreased relative to controls for these animals. Transiently lower body weights were observed in rats exposed to 2000 or 6000 ppm during study days 1–4, but no general trend was observed.

There were no treatment-related effects on the number of white blood cells per spleen or the number of plaque-forming cells (per million cells or per spleen) in the pyriofenone treatment groups. The cyclophosphamide positive control group showed significant decreases in all parameters, demonstrating the sensitivity of the assay.

Pyriofenone did not affect immune function in this study, as assessed by the measurement of antigen-specific, T-cell-dependent antibody formation. The NOAEL for immunotoxicity was 20 000 ppm (equal to 1690 mg/kg bw per day), the highest dose tested (Chambers, 2010).

(c) Mechanistic studies

In a non-GLP-compliant 7-day mechanistic study, pyriofenone (IKF-309 technical; batch no. 0701; purity 97.88%) was administered to wild-type constitutive androstane receptor (CAR) or CAR knockout rats (five males per group) in the diet at 0 or 5000 ppm (equal to 0 and 363.56 mg/kg bw per day in wild-type CAR rats and 0 and 357.17 mg/kg bw per day in CAR knockout rats, respectively). End-points included clinical signs, feed and water intake, body weight, clinical chemistry (at termination), liver weight and liver histopathology. Additional end-points included liver Ki-67 antibody staining, CYP2B1 gene expression measured through quantitative reverse transcription polymerase chain reaction, and CYP2B1 (testosterone 16β-hydroxylase) activity.

There were no treatment-related effects on clinical signs, body weight or necropsy findings.

Treatment-related effects included higher serum total cholesterol (wild-type CAR), lower serum triglycerides (wild-type CAR), higher total serum protein (CAR knockout) and lower serum albumin/globulin ratio (CAR knockout). Liver weights were increased in both wild-type CAR and CAR knockout treated rats. Diffuse hepatocellular hypertrophy was observed in both wild-type CAR and

CAR knockout treated rats. Additionally, both wild-type CAR and CAR knockout rats showed a decreased ki-67 ratio, suggesting a decrease in hepatocyte proliferation.

CYP2B1 gene expression and activity were increased in treated wild-type CAR rats. In contrast, in CAR knockout rats, CYP2B1 gene expression increased, but there was no increase in CYP2B1 activity. The authors concluded that pyriofenone induced CYP2B1 activity through CAR activation (Yamamoto, 2017).

(d) Studies on metabolites

Acute toxicity

The acute oral toxicity of 2MDPM, 3HDPM and 4HDPM was studied by the acute toxic class method (Table 38). All three metabolites showed low oral toxicity. No deaths or treatment-related clinical signs were reported in any of the studies.

Table 38. Acute oral toxicity of 2MDPM, 3HDPM and 4HDPM in rats

Test article	Strain	Sex	Purity (%)	LD_{50}	Reference
2MDPM	SD	F	99.2–99.8	>2 000 mg/kg bw	Moore (2010b)
3HDPM	SD	F	96.1	>2 000 mg/kg bw	Moore (2010c)
4HDPM	SD	F	99.28	>2 000 mg/kg bw	Fukuyama (2010)

bw: body weight; F: female; LD_{50}: median lethal dose; SD: Sprague Dawley

Genotoxicity

2MDPM, 3HDPM and 4HDPM were tested in bacterial reverse mutation assays (Table 39). None of the studies indicated a potential for mutagenicity.

Table 39. Summary of genotoxicity of pyriofenone metabolites[a,b]

Test substance	End-point	Test object	Concentration	Purity (%)	Results	Reference
2MDPM	Reverse mutation	*Salmonella typhimurium* and *Escherichia coli*	Up to 5 000 µg/plate ±S9	99.4	Negative	May (2010a)[c]
3HDPM			Up to 5 000 µg/plate ±S9	94.8	Negative	May (2010b)[d]
4HDPM			Up to 1 250 µg/plate ±S9 in *S. typhimurium*	99.28	Negative	Wada (2010)[e]
			Up to 5 000 µg/plate ±S9 in *E. coli*			

GLP: good laboratory practice; OECD: Organisation for Economic Co-operation and Development; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to quality assurance and GLP were included in all studies.

[c] Batch no. 155-100318-1; performed in accordance with OECD Test Guideline No. 471.

[d] Batch no. 155-100316-4; performed in accordance with OECD Test Guideline No. 471.

[e] Batch no. 155-100324-5; performed in accordance with OECD Test Guideline No. 471.

(e) Microbiological effects

The fungicide pyriofenone was evaluated at the 2018 JMPR to determine its impact on microbiota in the gastrointestinal tract. As no data were submitted by the sponsors, a literature search was performed using a number of search engines. These included BioOne (http://www.bioone.org/), Google (https://www.google.com/), Google Scholar (http://scholar.google.com/), PubMed (http://www.ncbi.nlm.nih.gov/pubmed), ScienceDirect (http://www.sciencedirect.com/) and Web of Science (https://apps.webofknowledge.com).

The search strategy used the input keywords of the fungicide chemical name (pyriofenone), chemical structure, antimicrobial mode of action, antimicrobial spectrum of activity, antimicrobial resistance, resistance mechanisms and genetics, microbiome, microbiota, gut microbiota, gut microbiome, gastrointestinal microbiota, gastrointestinal microbiome, etc., as well as the Boolean operators AND, OR and NOT.

The extensive search and review of the scientific literature did not find any reports on the effects of pyriofenone on the intestinal microbiome to include in the toxicological risk assessment.

3. Observations in humans

The sponsor provided some workplace monitoring information. Annual medical examinations (laboratory tests, chest X-rays, clinical examinations, lung function, skin and eye examinations) have been carried out by the Occupational Medical Advisor for Ishihara Sangyo Kaisha on manufacturing plant personnel since the start of manufacture of pyriofenone. In these reports on manufacturing plant personnel, no adverse health effects have been noted (Ishihara Sangyo Kaisha, Ltd, 2017). No information on accidental or intentional poisoning in humans is available.

Comments

Biochemical aspects

Following oral administration of ^{14}C-(phenyl)-pyriofenone or ^{14}C-(pyridyl)-pyriofenone to bile duct–cannulated rats at 5 or 200 mg/kg bw, the compound was rapidly absorbed; absorption 48 hours after dosing ranged from 76% to 89% of the dose at 5 mg/kg bw and from 36% to 53% of the dose at 200 mg/kg bw for the two radiolabels. The compound was largely excreted into bile (at the low dose, 65–81%; at the high dose, 33–49%) and urine (<13%, regardless of dose, sex or label) within 48 hours. In intact animals, most of the radiolabel was excreted in the faeces, probably via the bile, within 120 hours. Based on half-life considerations, there was some evidence of accumulation of radioactivity in plasma and whole blood. Pharmacokinetic parameters after single oral doses indicated that the rate and extent of exposure were higher in males than in females and that this difference was greater at 200 mg/kg bw than at 5 mg/kg bw (Knight, 2009).

In a metabolic study, unchanged pyriofenone represented maxima of 29% of the low dose and 63% of the high dose in faeces. Other components identified in faeces were 2MDPM (maximum 21% of the low dose), 3HDPM (maximum 16% of the low dose) and 4HDPM (maximum 11% of the low dose). In bile, two major metabolites were identified as glucuronide conjugates of 3HDPM and 4HDPM, each accounting for a maximum of 39% of the low dose. All other metabolites in bile accounted for 2% of the dose or less. In the urine of female rats at the low dose, there was one major metabolite at a maximum of 9.5% of the dose, which was identified as an unstable conjugate of 2MDPM. In male and female rats, no other metabolites accounted for more than 2% of the dose in urine (Knight, 2009).

At the T_{max}, the major metabolite in plasma was identified as a glucuronide conjugate of 2MDPM. Parent pyriofenone, 3HDPM and/or 4HDPM were present in extracts of livers from treated rats. Parent pyriofenone was the major identified component in extracts of kidney at the high dose (Knight, 2009).

Toxicological data

The acute toxicity of pyriofenone was studied by oral (LD_{50} > 2000 mg/kg bw), inhalation (LC_{50} > 5.18 mg/L) and dermal (LD_{50} > 2000 mg/kg bw) administration in rats (Hoffman, 2008; Moore, 2008a,b). Pyriofenone was not irritating to the skin of rabbits (Rees, 2008a), but it induced slight ocular irritation in rabbits (Rees, 2008b). Pyriofenone was not sensitizing in mice (Kosaka, 2009).

In a 13-week study in mice using dietary concentrations of pyriofenone of 0, 300, 1000, 3000 and 7000 ppm (equal to 0, 53, 176, 514 and 1318 mg/kg bw per day for males and 0, 61, 214, 695 and 1504 mg/kg bw per day for females, respectively), the NOAEL was 7000 ppm (equal to 1318 mg/kg bw per day), the highest dose tested (Moore, 2009).

In a 28-day dose range–finding study in rats using dietary concentrations of pyriofenone of 0, 300, 3000, 10 000 and 20 000 ppm (equal to 0, 24.2, 251, 823 and 1657 mg/kg bw per day for males and 0, 26.1, 261, 841 and 1660 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 24.2 mg/kg bw per day), based on increased serum ALP, total protein and albumin (males), distended caeca (both sexes), increased liver weights (both sexes) and increased kidney weights (females) at 3000 ppm (equal to 251 mg/kg bw per day) (Ohtsuka, 2010a).

In a 93-day study in which rats were administered a dietary concentration of pyriofenone of 0, 300, 1000, 2500 or 5000 ppm (equal to 0, 17.9, 60.5, 150 and 305 mg/kg bw per day for males and 0, 20.6, 69.0, 171 and 350 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 60.5 mg/kg bw per day), based on increased liver weights in both sexes, increased kidney weights in males, increased caecum weights in females, altered clinical chemistry in both sexes and prolonged APTT in females at 2500 ppm (equal to 150 mg/kg bw per day) (Ohtsuka, 2010b).

In a 90-day study in dogs administered a dietary concentration of pyriofenone of 0, 500, 3000 or 25 000 ppm (equal to 0, 15.0, 90.3 and 776 mg/kg bw per day, respectively) for males and 0, 500, 3000 or 15 000 ppm (equal to 0, 15.3, 89.8 and 475 mg/kg bw per day, respectively) for females, the NOAEL was 3000 ppm (equal to 89.8 mg/kg bw per day), based on a marked increase in ALP in females at 15 000 ppm (equal to 475 mg/kg bw per day) (Nakashima, 2010a).

In a 1-year study in dogs administered a dietary concentration of pyriofenone of 0, 500, 3000 or 25 000 ppm (equal to 0, 13.7, 83.5 and 701 mg/kg bw per day, respectively) for males and 0, 500, 3000 or 15 000 ppm (equal to 0, 14.1, 86.2 and 448 mg/kg bw per day, respectively) for females, the NOAEL was 3000 ppm (equal to 83.5 mg/kg bw per day), based on markedly increased ALP, increased GGT and darkened liver colour at 15 000 ppm (equal to 448 mg/kg bw per day) (Nakashima, 2010b).

The overall NOAEL for pyriofenone in dogs was 3000 ppm (equal to 89.8 mg/kg bw per day), and the overall lowest-observed-adverse-effect level (LOAEL) was 15 000 ppm (equal to 448 mg/kg bw per day).

In a 78-week carcinogenicity study in mice administered a dietary concentration of pyriofenone of 0, 600, 1800 or 5400 ppm (equal to 0, 77.6, 237 and 716 mg/kg bw per day, respectively) for males and 0, 300, 1000 or 3000 ppm (equal to 0, 49.4, 167 and 486 mg/kg bw per day, respectively) for females, the NOAEL for systemic toxicity was 600 ppm (equal to 77.6 mg/kg bw per day), based on liver and kidney histopathology (hepatocellular hypertrophy and single-cell necrosis and renal cortical tubular basophilia) in males at 1800 ppm (equal to 237 mg/kg bw per day). The NOAEL for carcinogenicity was 1800 ppm (equal to 237 mg/kg bw per day), based on an increase in the incidence of combined hepatocellular adenomas and adenocarcinomas in males at 5400 ppm (equal to 716 mg/kg bw per day) (Moore, 2010a). The Meeting noted that the incidence, while significant in comparison with concurrent controls, was within the historical control range.

In a 52-week study in rats using dietary administration of pyriofenone at 0, 200, 1000 and 5000 ppm (equal to 0, 8.51, 42.9 and 226 mg/kg bw per day for males and 0, 10.6, 53.5 and 275 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 42.9 mg/kg bw per day), based on increased liver weight and centrilobular hypertrophy, increased APTT, decreased haemoglobin and red blood cell parameters (haematocrit, haemoglobin concentration, haemoglobin width, red blood cell

count), increased GGT, ALP, AST and ALT, and increased total cholesterol in rats of both sexes at 5000 ppm (equal to 226 mg/kg bw per day). Additionally, at 5000 ppm, nephrotoxicity was observed in rats of both sexes, including increased kidney weights, altered clinical chemistry suggestive of renal dysfunction, and renal histopathology (tubular basophilic change in 10/20 males and increased brown pigment deposition in renal tubular cells in 20/20 females) (Ohtsuka, 2010c).

In a 2-year carcinogenicity study in rats administered a dietary concentration of pyriofenone of 0, 200, 1000 or 5000 ppm (equal to 0, 7.25, 36.4 and 197 mg/kg bw per day for males and 0, 9.13, 46.5 and 254 mg/kg bw per day for females, respectively), the NOAEL was 200 ppm (equal to 9.13 mg/kg bw per day), based on CPN in female rats at 1000 ppm (equal to 46.5 mg/kg bw per day). The nephropathy in female rats at 1000 ppm and in both sexes at 5000 ppm was considered related to the nephrotoxicity observed in the 52-week rat study in both sexes at 5000 ppm (Ohtsuka, 2010c). There was no evidence of increased neoplasia or tumour incidence after exposure to pyriofenone (Ohtsuka, 2010d).

The Meeting concluded that there was limited evidence of carcinogenicity in the livers of male mice and that pyriofenone was not carcinogenic in female mice or rats.

Pyriofenone was tested for genotoxicity in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was found.

The Meeting concluded that pyriofenone is unlikely to be genotoxic.

In view of the lack of genotoxicity, the absence of carcinogenicity in rats and the fact that hepatocellular adenomas and adenocarcinomas were increased only in male mice at the highest dose tested, the Meeting concluded that pyriofenone is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation study of reproductive toxicity in rats, pyriofenone was administered in the diet to rats at 0, 150, 1000 or 5000 ppm (equal to 0, 7.29, 47.8 and 257 mg/kg bw per day for F_0 males and 0, 9.5, 58.1 and 301 mg/kg bw per day for F_0 females, respectively). The NOAEL for parental toxicity was 1000 ppm (equal to 47.8 mg/kg bw per day), based on altered haematological parameters and changes in liver, kidney, thyroid and caecum weights in F_0 and F_1 rats at 5000 ppm (equal to 257 mg/kg bw per day). The NOAEL for reproductive toxicity and offspring toxicity was 5000 ppm (equal to 257 mg/kg bw per day), the highest dose tested (Hojo, 2009a).

In a study of developmental toxicity, rats were dosed with pyriofenone by oral gavage from GD 6 to GD 19 at 0, 30, 300 or 1000 mg/kg bw per day. The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on increased liver and caecum weights in dams at 300 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Hojo, 2009b).

In a study of developmental toxicity, rabbits were dosed with pyriofenone by gavage from GDs 6 to 27 at 0, 30, 300 or 1000 mg/kg bw per day. The NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Takahashi, 2009).

The Meeting concluded that pyriofenone is not teratogenic in rats or rabbits.

Pyriofenone did not produce neurotoxicity in acute studies in rats at doses up to 2000 mg/kg bw or in subchronic studies in rats at doses up to 927 mg/kg bw per day (Arrowsmith, 2010b; Powell, 2010).

The Meeting concluded that pyriofenone is not neurotoxic.

Pyriofenone did not produce immunotoxicity in 4-week studies in female mice at doses up to 1270 mg/kg bw per day or in female rats at doses up to 1690 mg/kg bw per day (Chambers, 2010; Laurent, 2010).

The Meeting concluded that pyriofenone is not immunotoxic.

No information on the potential effects of pyriofenone on the microbiome of the human gastrointestinal tract is available.

Toxicological data on metabolites and/or degradates

The main metabolites of pyriofenone were tested in acute toxicity studies and genotoxicity studies. The acute oral toxicity of 2MDPM, 3HDPM and 4HDPM was studied by the acute toxic class method in rats (all LD_{50}s > 2000 mg/kg bw) (Fukuyama, 2010; Moore, 2010b,c). All three metabolites tested negative for mutagenicity in bacterial reverse mutation assays (May, 2010a,b; Wada, 2010).

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted. No information on accidental or intentional poisonings in humans was identified.

The Meeting concluded that the existing database on pyriofenone was adequate to characterize the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.09 mg/kg bw on the basis of the NOAEL of 9.13 mg/kg bw per day from the 2-year carcinogenicity study in rats for CPN in females at 46.5 mg/kg bw per day. A safety factor of 100 was applied. The upper bound of the ADI provides a margin of about 8000 relative to the LOAEL for carcinogenicity in male mice.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for pyriofenone in view of its low acute oral toxicity and the absence of any other toxicological effects, including developmental toxicity, that would be likely to be elicited by a single dose.

Levels relevant to risk assessment of pyriofenone

Species	Study	Effect	NOAEL	LOAEL
Mouse	Seventy-eight-week study of carcinogenicity[a]	Toxicity	600 ppm, equal to 77.6 mg/kg bw per day	1 800 ppm, equal to 237 mg/kg bw per day
		Carcinogenicity	1 800 ppm, equal to 237 mg/kg bw per day	5 400 ppm, equal to 716 mg/kg bw per day
Rat	Two-year study of carcinogenicity[a]	Toxicity	200 ppm, equal to 9.13 mg/kg bw per day	1 000 ppm, equal to 46.5 mg/kg bw per day
		Carcinogenicity	5 000 ppm, equal to 197 mg/kg bw per day[b]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	5 000 ppm, equal to 257 mg/kg bw per day[b]	–
		Parental toxicity	1 000 ppm, equal to 47.8 mg/kg bw per day	5 000 ppm, equal to 257 mg/kg bw per day
		Offspring toxicity	5 000 ppm, equal to 257 mg/kg bw per day[b]	–
	Developmental toxicity study[c]	Maternal toxicity	30 mg/kg bw per day	300 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	1 000 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Dog	Three-month and 1-year studies of toxicity[a,d]	Toxicity	3 000 ppm, equal to 89.8 mg/kg bw per day	15 000 ppm, equal to 448 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two or more studies combined.

Acceptable daily intake (ADI)

0–0.09 mg/kg bw per day

Acute reference dose (ARfD)

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to pyriofenone

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; 76–89% of the dose at 5 mg/kg bw and 36–53% of the dose at 200 mg/kg bw in bile duct–cannulated rats
Dermal absorption	No data
Distribution	Widespread: highest concentrations in liver, kidney, plasma, whole blood and blood cells
Potential for accumulation	Some evidence of accumulation in plasma and whole blood
Rate and extent of excretion	Rapid: Largely excreted into bile and urine by 48 hours
Metabolism in animals	Converted to 2MDPM, 3HDPM and 4HDPM
Toxicologically significant compounds in animals and plants	Pyriofenone
Acute toxicity	
Rat, LD_{50}, oral	>2 000 mg/kg bw
Rat, LD_{50}, dermal	>2 000 mg/kg bw
Rat, LC_{50}, inhalation	>5.18 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating

Mouse, dermal sensitization	Not sensitizing
Short-term studies of toxicity	
Target/critical effect	Liver, kidney, caecum / increased APTT, increased serum protein, albumin and calcium, and decreased serum chloride
Lowest relevant oral NOAEL	60.5 mg/kg bw per day (rat)[a]
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney/CPN in female rats
Lowest relevant NOAEL	9.13 mg/kg bw per day (rat)
Carcinogenicity	Limited evidence of carcinogenicity in the liver of male mice, not carcinogenic in female mice or rats[b]
Genotoxicity	
	No evidence of genotoxicity[b]
Reproductive toxicity	
Target/critical effect	No reproductive effects / increased liver and caecum weights, anaemia in parental animals
Lowest relevant reproductive NOAEL	257 mg/kg bw per day, highest dose tested (rat)
Lowest relevant parental NOAEL	47.8 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	257 mg/kg bw per day, highest dose tested (rat)
Developmental toxicity	
Target/critical effect	No embryo/fetal effects / increased liver and caecum weights in maternal rats
Lowest relevant maternal NOAEL	30 mg/kg bw per day (rat)
Lowest relevant developmental NOAEL	1 000 mg/kg bw per day, highest dose tested (rat and rabbit)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	2 000 mg/kg bw, highest dose tested (rat)
Subchronic neurotoxicity	927 mg/kg bw, highest dose tested (rat)
Other toxicological studies	
Immunotoxicity	Negative
Studies with metabolites 2MDPM, 3HDPM and 4HDPM	Oral LD_{50} > 2 000 mg/kg bw (rats) Negative in bacterial mutagenesis assays
Human data	
	None identified

[a] A NOAEL of 24.2 mg/kg bw per day was noted in a 28-day dose range–finding study in rats.
[b] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

Summary

	Value	Study	Safety factor
ADI	0–0.09 mg/kg bw per day	Two-year carcinogenicity study in rats	100
ARfD	Unnecessary	–	–

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