PICOXYSTROBIN

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Explanation

Picoxystrobin is the International Organization for Standardization (ISO)–approved name for methyl (*E*)-3-methoxy-2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)phenyl]acrylate (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 117428-22-5). Picoxystrobin is a broad-spectrum, systemic cereal fungicide from the strobilurin group. It is active against plant pathogens from the four major groups of plant pathogenic fungi—namely, Deuteromycetes, Basidiomycetes, Ascomycetes and Oomycetes. Picoxystrobin's mode of fungicidal activity is to block mitochondrial electron transport at the Q_o site of complex III, reducing adenosine triphosphate (ATP) production and inhibiting cellular respiration. Picoxystrobin has been known under the development codes ZA1963 and DPX-YT669. Initial production batches had a purity of approximately 93%; current production batches have a purity of greater than 99%.

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

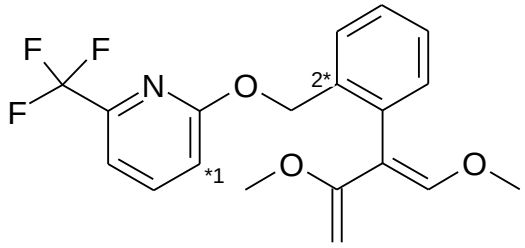
All critical studies contained statements of compliance with good laboratory practice and met the minimum requirements of the applicable Organisation for Economic Co-operation and Development (OECD) or national test guidelines.

Evaluation for acceptable daily intake

1. Biochemical aspects

The structure of and position of radiolabels in picoxystrobin used in the absorption, distribution, excretion and metabolism studies are illustrated in Figure 1.

Figure 1. Structure of picoxystrobin and position of radiolabels



1* (^{14}C-pyridyl)-labelled picoxystrobin

2* (^{14}C-phenyl)-labelled picoxystrobin

1.1 Absorption, distribution and excretion

(a) Oral route

Rats

In an initial study, two groups of Alpk:AP$_f$SD rats, each comprising one male and one female, were administered (^{14}C-pyridyl)- or (^{14}C-phenyl)-labelled picoxystrobin (> 97% radiochemical purity as a single oral dose of 10 mg/kg body weight [bw]) in polyethylene glycol (PEG) 600. The excretion of radioactivity was monitored in urine, faeces and exhaled air. Twenty-four hours after dosing, the rats were killed to investigate the distribution of radioactivity using whole-body autoradiography. Urine and faeces were frozen by collection over solid carbon dioxide, and expired air was monitored for radiolabelled carbon dioxide and other volatile metabolites for 24 hours after dosing. At the end of the collection period, rats were killed and frozen rapidly. The frozen carcasses were processed for autoradiography. The amounts of radioactivity in the dose preparation, urine, plasma, faeces and expired air were measured.

The whole-body autoradiograms showed no marked differences in the distribution of radioactivity between male and female rats dosed with either radiolabelled form of picoxystrobin. In all rats, the greatest intensity of labelling was present in the gastrointestinal tract contents. The absorbed radioactivity was predominant in the liver, with lower intensities apparent in the kidneys, associated with urinary excretion of metabolites. A low intensity of radioactivity was apparent in the blood; consequently, low levels of radiolabel were evident in many tissues, including the heart, lungs and nasal passages. Negligible proportions (< 1%) of the dose were metabolized to volatile exhaled metabolites (Davies, 1997).

The excretion and distribution of picoxystrobin (purity 99.8%; radiochemical purity > 98%; specific activity 2 GBq/mmol) in rats were investigated following a single oral administration of a low dose of 10 mg/kg bw or a high dose of 100 mg/kg bw. Five male and five female Alpk:AP$_f$SD rats

received a single oral dose of (^{14}C-phenyl)-labelled picoxystrobin in PEG 600. Urine was collected 6 hours after dosing, and urine and faeces were collected after 12, 24, 36, 72, 96 and 120 hours. The study was terminated 5 days after dosing, when representative samples of blood, tissues and the gastrointestinal tract contents were removed and submitted to radiochemical analysis.

Following administration of a low dose of 10 mg/kg bw, excretion in both sexes was extensive (Table 1). The majority of urinary excretion occurred within 24 hours. At study termination, all tissue concentrations were low. With the exception of the liver, kidneys, gastrointestinal tract, blood and bone (males only), tissue concentrations of picoxystrobin were less than 0.1 µg equivalents (Eq) per microgram. The amount of administered radioactivity present in the tissue and residual carcass was less than 1%. At the high dose of 100 mg/kg bw, excretion of administered radioactivity was extensive (Table 1) but slightly delayed compared with the low dose. At study termination, the amount of radioactivity present in the tissues and residual carcass of both sexes was low, with the exception of liver, kidneys, gastrointestinal tract, blood and plasma; all tissue concentrations were below 1 µg Eq/g. Following administration of 14 consecutive daily oral doses of unlabelled picoxystrobin at 10 mg/kg bw per day and a 10 mg/kg bw dose of (^{14}C-phenyl)-labelled picoxystrobin, the pattern of distribution and excretion was similar to that seen with a single 10 mg/kg bw dose. With all three dose levels, females excreted a greater proportion of the dose in urine than did males (Table 1).

Table 1. Mean percentage recoveries of administered radioactivity 120 hours after a single oral dose or repeated oral doses of (^{14}C-phenyl)-labelled picoxystrobin (mean of five rats)

	% of administered dose					
	Males			Females		
	10 mg/kg bw	100 mg/kg bw	10 mg/kg bw (repeated)	10 mg/kg bw	100 mg/kg bw	10 mg/kg bw (repeated)
Urine	21	18	19	34	26	32
Faeces	78	74	77	61	65	63
Gastrointestinal tract contents	0.4	0.6	0.3	0.8	0.8	0.5
Cage wash	0.5	0.4	0.5	0.8	1	0.9
Tissues, including carcass	0.8	0.8	0.7	0.9	0.9	0.8
Total	**100**	**94**	**98**	**97**	**94**	**97**

From Brown (1998a,b,c)

The biliary excretion of picoxystrobin (purity 99%) was assessed in Alpk:AP$_f$SD bile duct–cannulated rats receiving a single oral dose of (^{14}C-pyridinyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) or (^{14}C-phenyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) at a dose of 100 mg/kg bw in PEG 600 (two males and two females for each radiolabel). Urine was collected at 6 hours and urine and faeces were collected at 12, 24, 36 and 48 hours after dosing with radiolabelled material; bile was collected at 2, 4, 6, 8, 12, 24, 36 and 48 hours.

Following a single oral dose of (^{14}C-phenyl)-labelled picoxystrobin at 100 mg/kg bw, males excreted 2% of the dose in the urine, 71% via bile and 31% in faeces. Females excreted 24% in urine, 45% via bile and 20% in faeces. Following a single oral dose of (^{14}C-pyridinyl)-labelled picoxystrobin at 100 mg/kg bw, males excreted 4.5%, 72% and 18% and females excreted 17%, 66% and 21% in the urine, bile and faeces, respectively. These data, together with tissue and cage wash residues, indicate that oral absorption of picoxystrobin at a dose of 100 mg/kg bw was 75% or greater, with the greatest proportion being eliminated via the bile and subsequently being excreted in the faeces. The

reduction in urinary excretion indicates that a degree of enterohepatic recirculation occurs in non-cannulated animals (Macpherson, 1999).

The pharmacokinetics of [^{14}C]picoxystrobin (purity 99.9%; lot DPX-YT 669-003) was investigated in the plasma and red blood cells of male and female Sprague-Dawley Crl:CD(SD) rats (four rats of each sex per dose per label). [Phenyl(U)-^{14}C]picoxystrobin or [pyridine-3-^{14}C]picoxystrobin at a dose of 10 or 100 mg/kg bw were administered by gavage in PEG 400 vehicle (4 ml/kg bw). Whole blood was collected predosing and at 15 and 30 minutes and 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours after dose administration. The concentrations of ^{14}C residues were quantified in plasma by liquid scintillation counting (LSC) and in red blood cells by combustion and LSC.

Results (Table 2) were broadly independent of sex and radiolabel position. At the low dose, absorption was relatively rapid (peak concentration in plasma [C_{max}] typically reached before 4 hours [T_{max}]), but delayed at the high dose level (C_{max} reached in approximately 12 hours). Area under the plasma concentration–time curve (AUC) values showed a less than proportionate increase with dose, indicating that absorption was approaching saturation at 100 mg/kg bw relative to the low dose.

Table 2. Mean plasma kinetic parameters in rats administered [^{14}C]picoxystrobin

	10 mg/kg bw		100 mg/kg bw	
	Phenyl[U]-^{14}C	Pyridine-3-^{14}C	Phenyl[U]-^{14}C	Pyridine-3-^{14}C
Males				
C_{max} (µg Eq/g)	4.8	3.4	12.4	14.8
T_{max} (h)	2.2	3.0	12	12
$AUC_{0-\infty}$ (µg·h/g)	110	102	605	579
Elimination $t_{½}$ (h)	47	33	37	46
Females				
C_{max} (µg Eq/g)	2.8	4.5	18	11
T_{max} (h)	7[a]	0.6	9.3	12
$AUC_{0-\infty}$ (µg·h/g)	86	87	710	453
Elimination $t_{½}$ (h)	40	34	31	33

From Himmelstein (2010)
AUC, area under the plasma concentration–time curve; C_{max}, peak concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to C_{max}
[a] Very variable; standard deviation = 5.9 hours.

The tissue distribution of [^{14}C]picoxystrobin (purity 99.9%; lot DPX-YT 669-003) was investigated in male and female Sprague-Dawley Crl:CD(SD) rats (four rats of each sex per dose per label). Doses of 10 or 100 mg/kg bw of a 1:1 mixture of [phenyl(U)-^{14}C]picoxystrobin (radiochemical purity 97%; specific activity 1.9 MBq/mg) and [pyridine-3-^{14}C]picoxystrobin (radiochemical purity 96%; specific activity 1.9 MBq/mg) were administered by gavage in PEG 400 vehicle (4 ml/kg bw). Tissue ^{14}C residues were measured, using combustion and/or LSC, at 1, 24 and 120 hours after administration of the low (10 mg/kg bw) dose and 24, 48 and 120 hours after administration of the high (100 mg/kg bw) dose. The initial time points were chosen to approximate the T_{max}.

The results (Table 3) showed extensive absorption and systemic distribution at the low dose level, with a lower relative total body burden at the high dose level. Females typically had higher concentrations of radiolabel in samples compared with males at the first time point. Tissues with highest concentrations were liver, plasma, pancreas and kidney.

Table 3. Tissue levels of ^{14}C in rats administered [^{14}C]picoxystrobin

	Mean tissue levels (µg Eq/g)			
	10 mg/kg bw		100 mg/kg bw	
	1 h	24 h	24 h	120 h
Males				
Carcass	1.2	0.3	3.0	0.3
Plasma	2.6	0.9	7.9	0.8
Red blood cells	0.8	0.4	3.9	1.0
Fat	0.7	0.4	4.7	0.3
Liver	12	3.5	26	4.1
Pancreas	3.8	0.3	2.9	0.4
Kidney	2.8	0.9	8.6	1.3
Testes	0.3	0.1	1.4	0.2
Females				
Carcass	2.0	0.2	3.2	0.4
Plasma	6.2	0.8	9.1	0.9
Red blood cells	1.7	0.3	4.1	1.4
Fat	2.2	0.4	8.9	0.8
Liver	20	2.4	32	2.7
Pancreas	2.2	0.2	2.9	0.3
Kidney	5.6	0.7	9.3	1.7

From Himmelstein (2010)

(b) Dermal route

The ability of picoxystrobin to penetrate the skin was evaluated using a 250 g/l soluble concentrate formulation. In an in vivo rat study, less than 1% of the dose applied as the concentrated product was absorbed in 24 hours, with less than 1% found at the application site. With a 1:200 aqueous dilution, 5–21% was absorbed in 24 hours. A comparison of the absorption through rat and human skin samples in vitro showed that absorption was approximately 15- to 25-fold greater through rat skin than through human skin for both the concentrated product and the dilution. The absorption of picoxystrobin through intact human skin in vivo is likely to be low (B.K. Jones, 1999; Ward, 1999).

1.2 Biotransformation

Rats

The biotransformation of picoxystrobin was investigated in Alpk:AP$_f$SD rats receiving a single oral dose of (^{14}C-pyridinyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) or (^{14}C-phenyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) at 100 mg/kg bw in PEG 600. Metabolites were characterized by physicochemical, chromatographic and biochemical methods. Urine, bile and extracts of urine, bile and faeces were analysed by liquid chromatography with mass spectrometry and/or liquid chromatography with tandem mass spectrometry. Enzymatic digestion with β-glucuronidase was used to investigate glucuronide conjugation. When available, metabolite standards were used to confirm structural assignments. Many metabolites were isolated to purity to allow mass spectrometric, tandem mass spectrometric and nuclear magnetic resonance spectroscopic analysis. Isolated metabolites and extracts of urine and bile were subjected to methylation to confirm the presence of carboxylic acid functions. Additionally, the metabolites present in samples of excreta from low-, high- and repeated-dose mass balance/excretion studies (see Brown, 1998a,b,c above) were characterized and quantified

using mass spectrometry and/or co-chromatography with reference standards, radio–high-performance liquid chromatography or nuclear magnetic resonance.

Picoxystrobin is extensively metabolized, and 42 metabolites have been found, of which 34 have been structurally identified. All metabolites that represented greater than 5% of the administered dose were identified, with the exception of one faecal metabolite (A), which is considered to originate from bile, in which all significant metabolites were characterized. The majority of the dose (73–83%) is accounted for by identified metabolites in samples collected following single- or repeated-dose mass balance studies. Biotransformation reactions for picoxystrobin include ester hydrolysis, oxidation, *O*-demethylation, cleavage to separate the two ring structures and glucuronide conjugation. The major route of metabolism is ester hydrolysis and glucuronide conjugation and is consistent following both single and repeated daily oral doses at 10 mg/kg bw and following a single oral dose at 100 mg/kg bw. This major route of metabolism was similar in males and females, although there were some minor sex differences in metabolism. Major urinary and bile metabolites are presented in Table 4, and a metabolic pathway is shown in Figure 2.

Table 4. Main metabolites (> 2%) in urine and bile from cannulated rats administered (^{14}C-phenyl)- or (^{14}C-pyridinyl)-labelled picoxystrobin at 100 mg/kg bw[a]

Metabolite	% of administered radioactivity					
	^{14}C-phenyl		^{14}C-pyridinyl			
	Males Urine	Females Urine	Males Urine	Males Bile	Females Urine	Females Bile
02	—		—	1.02	—	2.12
02/48	—	5.75	—	—	1.72	—
40	—	3.05	—	28.87	2.23	30.14
43	—	Trace	—	6.13	—	6.04
46	—	—	—	—	—	2.14
46/49/49A	—	1.64	—	22.18	1.75	17.96
47	—	Trace	—	3.15	—	1.17
50/54	—	—	—	3.35	—	1.18
52	—	3.13	—	—	3.08	—
59	—	—	—	1.52	—	3.33
Subtotal: characterized	1.68	22.34	4.27	69.46	15.80	65.68
Subtotal: unknown	0.3	0.92	0.27	2.1	0.7	0
Total	1.98	23.26	4.54	71.56	16.50	65.68

From Macpherson (1999)

[a] (^{14}C-pyridinyl)-labelled picoxystrobin in faeces: 15.56% of administered radioactivity for males; 18.81% of administered radioactivity for females.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

In the rat, picoxystrobin is of low acute toxicity by the oral and dermal routes, but of high toxicity by inhalation (Table 5).

Figure 2. Proposed metabolic pathway for picoxystrobin in the rat

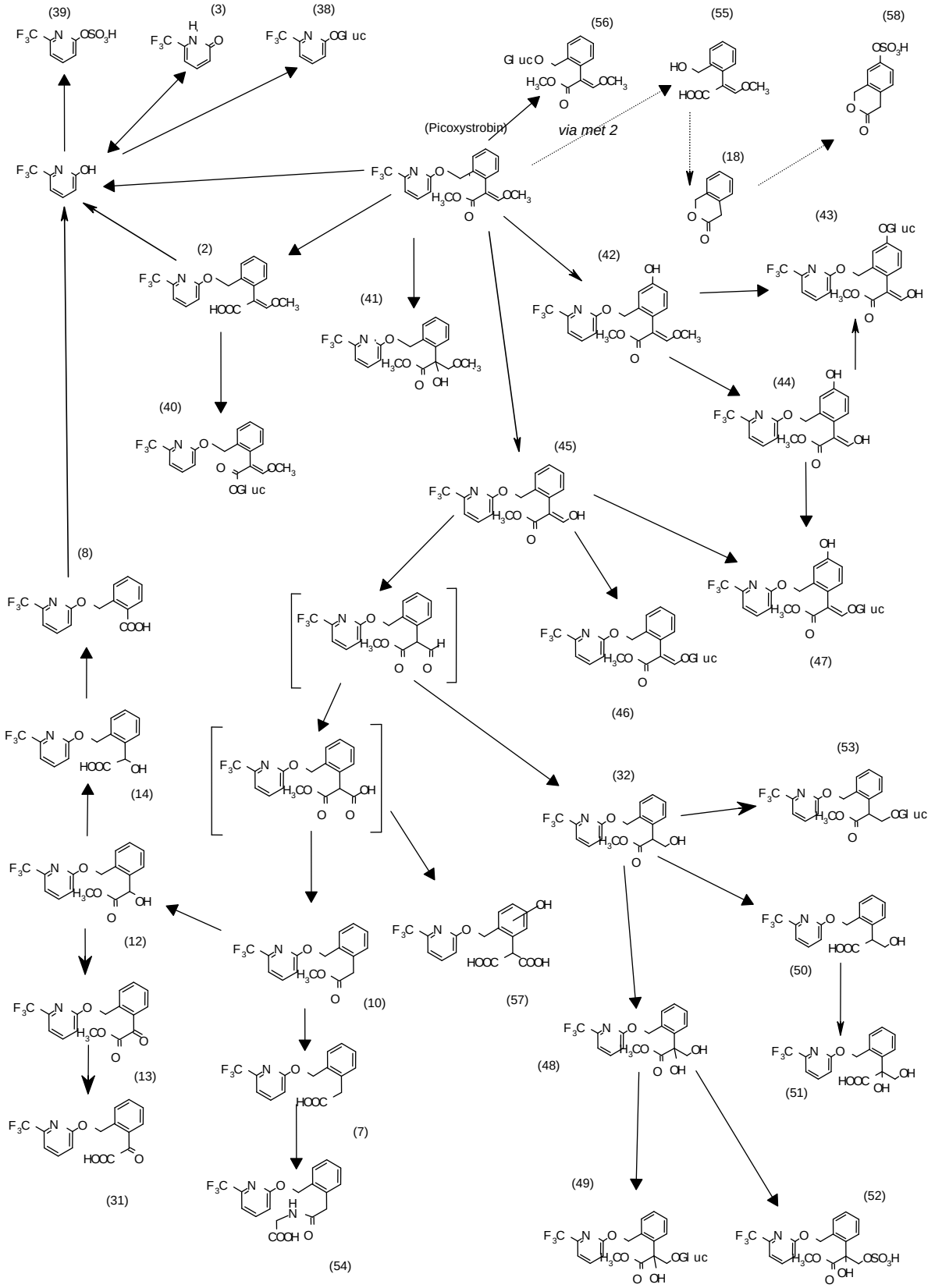


Table 5. Acute toxicity studies with picoxystrobin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity; batch	Vehicle	Reference
Mouse	Crl:CD1	F	Oral	> 5000	—	99.3%; SEP07AS013	0.5% methylcellulose	Kesavan (2010)
Rat	AlPk	M/F	Oral	> 5000	—	93.3%; P25	Corn oil	Lees (1997a)
Rat	Crl:CD (SD)	F	Oral	> 5000	—	99.66%; O21	Water	Carpenter (2007)
Rat	Crl:CD (SD)	F	Oral	> 5000	—	98.7–99.9%; various	0.5% methylcellulose	Bentley (2010)
Rat	Crl:WI (Wistar)	F	Oral	> 2000	—	99.6%; JAN10AS046	Corn oil	Ramu (2010)
Rat	AlPk	M/F	Dermal	> 2000	—	93.3%; P25	Water	Lees (1997b)
Rat	AlPk	M/F	Inhalation (4 h, nose only)	—	M: 2.1–4.6 F: 3.2 (MMAD 6 µm)	93%; P10	None (aerosol)	Kilgour (1998)
Rat	Wistar	M/F	Inhalation (4 h, nose only)	—	0.11 (MMAD 3.4–4.1 µm)	SEP07AS013	None (aerosol)	Rajsekhar (2009)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; MMAD, mass median aerodynamic diameter

(b) Dermal and ocular irritation and dermal sensitization

In the rabbit, picoxystrobin is slightly irritating to skin and moderately irritating to the eye (Lees, 1997c,d). Picoxystrobin was negative in a Magnusson and Kligman maximization assay for skin sensitization in guinea-pigs (Lees, 1997e).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of 10 male and 10 female C57BL/10J$_f$AP/Alpk mice were fed diets containing 0 (control), 200, 800, 1600 or 2400 ppm picoxystrobin (purity 99%; batch P13) for 90 consecutive days. Achieved intakes were 0, 33, 137, 291 and 422 mg/kg bw per day for males and 0, 44, 176, 359 and 535 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were measured routinely. At the end of the scheduled period, the animals were sacrificed and subjected to a full postmortem examination. Selected organs were weighed, and a range of tissues was taken for subsequent histopathological examination.

Analysis of the diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study.

There were no deaths or clinical changes considered to be related to treatment with picoxystrobin. Over the first 4 days of the study, both sexes receiving 2400 ppm and males receiving 1600 ppm lost body weight. There were reductions in feed consumption and body weight (approximately 10%) over the entire study and increases in liver weight, after adjustment for terminal body weight, in animals at dietary concentrations of 800, 1600 or 2400 ppm picoxystrobin (Table 6). Food utilization was less efficient in animals receiving 1600 or 2400 ppm picoxystrobin. There was a treatment-related increase in the incidence of minimal hepatocyte hypertrophy in males given 1600 ppm and above and females given 800 ppm and above (Table 6). The liver and body weight findings at 800 ppm are considered to be not adverse.

Table 6. Findings in mice receiving picoxystrobin for 90 days

	0 ppm	200 ppm	800 ppm	1600 ppm	2400 ppm
Males					
Body weight (g)	29	30	29	27*	27*
Liver weight (g)	1.3	1.4	1.4	1.3	1.3
Relative liver weight (%)	4.4	4.5	4.8*	4.9*	4.9*
Hepatocyte hypertrophy	0/10	0/10	2/10	6*/10	4/10
Females					
Body weight (g)	26	25	24	24*	23*
Liver weight (g)	1.2	1.2	1.2	1.2	1.2
Relative liver weight (%)	4.8	4.7	4.9*	5.1*	5.1*
Hepatocyte hypertrophy	0/10	2/10	5*/10	6*/10	9*/10

From Rattray (1996)
* $P < 0.05$

The no-observed-adverse-effect level (NOAEL) was 800 ppm (equal to 137 mg/kg bw per day), based on reduced body weight gain and increased relative liver weight at 1600 ppm (equal to 291 mg/kg bw per day) (Rattray, 1996).

Rats

Groups of 12 male and 12 female Alpk:AP_fSD rats were fed diets containing 0 (control), 100, 500 or 1250 ppm picoxystrobin (purity 93.3%; batch P25) for 90 consecutive days. Achieved intakes were 0, 8.5, 42 and 105 mg/kg bw per day for males and 0, 10, 48 and 120 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were measured, and the animals were killed at the end of the scheduled period and subjected to a full postmortem examination. Cardiac blood samples were taken and urine samples were collected for clinical pathology, selected organs were weighed and a wide range of tissues was taken from control and top-dose animals for subsequent histopathological examination.

Analysis of the diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study.

One male given 1250 ppm was sacrificed for humane reasons, unrelated to treatment, in week 7 of the study. All other animals survived to scheduled termination. There were no treatment-related clinical signs in any animals. Top-dose animals showed an initial decrease in body weight gain compared with concurrent controls during the 1st week of the study. At the end of the study, body weights in top-dose animals were 10% and 8% below those of concurrent male and female controls, respectively. Body weights in all other treated groups were not different from control values throughout the study (Table 7). Feed consumption was decreased (approximately 10%) throughout the study in top-dose animals compared with concurrent controls. Feed consumption in other dose groups was essentially similar to control values. Feed utilization was less efficient in top-dose females during the first 4 weeks of the study, but thereafter was similar to control values.

Table 7. Body and liver weights of rats exposed to picoxystrobin via the diet

	Mean weight (g)							
	Males				Females			
	0 ppm	100 ppm	500 ppm	1250 ppm	0 ppm	100 ppm	500 ppm	1250 ppm
Body weight								
- week 0	154.4	153.9	154.7	153.7	131.7	132.9	134.4	134.1
- week 1	206.4	208.3	204.7	192.0*	158.8	159.0	158.9	151.6*
- week 13	495.5	484.7	478.2	447.8*	274.2	277.6	268.5	251.8*
Liver weight	20.8	21.0	21.6	20.8	10.5	11.1	11.0	10.8
Liver weight adjusted for terminal body weight	19.8	20.6	21.5*	22.6*	10.3	10.7	10.9	11.5*

From Rattray (1998)
* $P < 0.05$

There were no consistent or notable changes in haematology, clinical chemistry or urine analysis findings, nor were there any treatment-related gross pathological or histopathological changes in any organs.

There was no significant increase in absolute liver weights, but an increase in relative liver weight was seen in both sexes at the top dose level and in males at 500 ppm (Table 7). In the absence of any histopathological changes in the liver, the finding at 500 ppm (< 10% change) was considered to be of no toxicological significance.

The NOAEL was 500 ppm (equal to 42 mg/kg bw per day), based on reduced body weight gain and feed consumption and increased relative liver weight at 1250 ppm (equal to 105 mg/kg bw per day) (Rattray, 1998).

Dogs

Groups of four male and four female Beagle dogs were fed diets containing 0 (control), 125, 250 or 500 ppm picoxystrobin (purity 94.4%; batch P27) for a period of 90 days. Males received 350 g diet per day and females 300 g/day. Dose rates were 0, 4.3, 8.9 and 17 mg/kg bw per day for males and 0, 4.3, 8.5 and 17 mg/kg bw per day for females, respectively. Clinical observations and veterinary examinations (including ophthalmoscopy) were made, and body weights, feed consumption and clinical pathology parameters were measured. Samples for haematology and clinical chemistry were taken pretest and at weeks 4, 8 and 13. At the end of the scheduled period, the animals were killed and subjected to a full gross pathological examination. Selected organs were weighed, and a range of tissues was taken for subsequent histopathological examination.

Analysis of the diets showed that the achieved concentrations, homogeneity and stability of picoxystrobin were satisfactory throughout the study.

There were no deaths. Slightly increased incidences of fluid faeces in males and salivation in females were seen in animals given 500 ppm, but no other clinical signs or ophthalmoscopy findings were observed. Treatment-related effects on body weight were seen for males and females given 500 ppm (Table 8). Throughout most weeks of the study, but particularly at the start, group mean feed consumption for males and females receiving 500 ppm was reduced (10–30%) in comparison with that of concurrent controls (Table 9). Slight reductions in feed consumption and transient effects on body weight at 250 ppm were not considered adverse.

Table 8. Body weights in dogs receiving picoxystrobin in the diet

Week	Mean body weight (kg)							
	Males				Females			
	0 ppm	125 ppm	250 ppm	500 ppm	0 ppm	125 ppm	250 ppm	500 ppm
1 (start)	9.23	9.18	9.10	9.20	7.95	8.05	7.95	7.83
2	9.32	9.30	9.23	8.95**	8.07	8.00	7.99	7.86*
8	10.42	10.30	10.03	9.90*	8.87	8.70	8.79	8.31**
14	11.14	11.05	10.66	10.42	9.42	9.30	9.34	8.82*

From Horner (1998)
* $P < 0.05$; ** $P < 0.01$

Table 9. Feed consumptions in dogs receiving picoxystrobin in the diet

Week	Mean feed consumption (g/dog)							
	Males				Females			
	0 ppm	125 ppm	250 ppm	500 ppm	0 ppm	125 ppm	250 ppm	500 ppm
1	350	339	318*	256**	300	295	264	221**
2	350	350	348	267**	300	300	294	251**
5	350	350	350	340	300	300	300	281
8	350	350	350	332*	300	300	300	284*
12	350	350	350	332	300	300	300	295
13	350	350	350	331	300	300	300	294

From Horner (1998)
* $P < 0.05$; ** $P < 0.01$

There were no consistent or notable changes in haematology values. Minor decreases in group mean plasma albumin and total protein were seen in top-dose animals, which are probably secondary to feed consumption and body weight deficits. A slight increase in kidney weight relative to body weight (approximately 10%) was seen in males dosed at 500 ppm, but was without any histopathological correlate.

The NOAEL was 250 ppm (equal to 8.5 mg/kg bw per day), based on body weight deficits at 500 ppm (equal to 17 mg/kg bw per day) (Horner, 1998).

Groups of four male and four female Beagle dogs were fed diets containing 0 (control), 50, 150 or 500 ppm picoxystrobin (purity 94.4%; batch P27) for a period of 1 year. Males received 350 g of feed per day and females 300 g/day. Dose rates were 0, 1.6, 4.8 and 16 mg/kg bw per day for males and 0, 1.6, 4.6 and 16 mg/kg bw per day for females, respectively. Clinical observations and veterinary examinations (including ophthalmoscopy) were made, and body weights, feed consumption and clinical pathology parameters were measured periodically before and throughout the study. At the end of the scheduled period, the animals were killed and subjected to a full postmortem examination. Selected organs were weighed, and a range of tissues was taken for subsequent histopathological examination.

Analysis of the diets showed that the concentration, stability and homogeneity of picoxystrobin in the test diets were satisfactory.

None of the animals died before the scheduled termination. Administration of 500 ppm to female dogs resulted in an increased incidence of the observation of "thin appearance", which is related to effects on body weight at this dose level (Table 10). There was an increased incidence of "reddened gums" and of fluid faeces in males receiving 500 ppm.

Table 10. Mean body weights and feed consumption in dogs receiving picoxystrobin in the diet

	Males				Females			
	0 ppm	50 ppm	150 ppm	500 ppm	0 ppm	50 ppm	150 ppm	500 ppm
Body weight (kg)								
- week 1 (start)	9.15	9.25	8.98	8.95	7.75	7.88	7.85	7.88
- week 2	9.20	9.30	9.20	8.60*	7.80	8.08	7.93	7.65*
- week 26	10.98	11.25	10.95	9.58*	9.33	9.88	9.93	8.28*
- week 52	11.25	11.78	11.53	10.48	9.73	10.38	10.53	9.10
Feed consumption (g)								
- week 1	340	350	349	226*	296	300	288	188*

From Lees (1999a)
* $P < 0.05$

There were no clinical or ophthalmoscopic findings that were considered to be related to the administration of picoxystrobin. Dietary administration of 500 ppm to male and female dogs resulted in lower body weight, with a maximal effect in males of 11% at week 26 and in females of 15% at week 36. There were no effects on body weight at 50 or 150 ppm picoxystrobin. Reduced feed consumption was seen in both sexes at 500 ppm (Table 10). There were no treatment-related effects on haematology or clinical chemistry, nor were there any significant findings at gross pathological or histopathological examination.

Absolute and relative to body weight values for thyroid weights were higher than those of concurrent controls for females receiving 500 ppm (Table 11). There were no histopathological

changes in the thyroid, and the values at 500 ppm were reported to be well within the historical control range (0.654–0.938 g); thus, the increase in thyroid weight is considered to be of no toxicological significance.

Table 11. Thyroid weight in female dogs receiving picoxystrobin in the diet

	Mean thyroid weight (g)			
	0 ppm	50 ppm	150 ppm	500 ppm
Absolute weight	0.60	0.60	0.64	0.69
Weight adjusted for terminal body weight	0.61	0.57	0.60	0.74*

From Lees (1999a)
* $P < 0.05$

The NOAEL was 150 ppm (equal to 4.6 mg/kg bw per day), based on reduced body weight and reduced feed consumption at 500 ppm (equal to 16 mg/kg bw per day) (Lees, 1999a).

(b) Dermal application

Groups of five male and five female Alpk:AP$_f$SD rats were administered picoxystrobin (purity 93.3%; batch P25) at 0, 200, 500 or 1000 mg/kg bw per day by dermal application 5 days/week over a 28-day period (20 applications). The test material was moistened with water and applied to clipped skin, which was then covered with an occlusive dressing for 6 hours. Clinical observations, body weights and feed consumption were recorded throughout the study. At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Cardiac blood samples were taken for clinical pathology (haematology and blood clinical chemistry), selected organs were weighed and tissues were taken for histopathological examination.

Other than an apparent increase in platelets that was linked to a low control value, there were no adverse systemic effects. An increase in sloughing at the application site was noted in top-dose males.

The NOAEL was 1000 mg/kg bw per day (equal to 700 mg/kg bw per day, corrected for the non-continuous dosing), the highest dose tested (Lees, 1999b).

Groups of Crl:CD(SD) rats (10 of each sex) were exposed to picoxystrobin (purity 99.3%; batch SEP07AS013) for 6 hours/day for 28 days. Dose levels were 0, 100, 300 and 1000 mg/kg bw per day. Test material was moistened with water and applied to the shaved skin, which was then covered with a semi-occlusive wrapping. An acceptable range of investigations was performed.

There were four deaths across dose groups, attributed to the wrapping being too tight. No adverse systemic or local effects were observed.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Carpenter, 2009).

(c) Exposure by inhalation

Groups of Crl:CD(SD) rats (five of each sex) were exposed to picoxystrobin (purity 99.3%; batch SEP07AS013) for 6 hours/day, 5 days/week, for 4 weeks. Dose levels were 0, 0.001, 0.01 and 0.025 mg/l. The mass median aerodynamic diameters were typically less than 3 µm. An acceptable range of investigations was performed.

No adverse systemic or local effects were observed. The no-observed-adverse-effect concentration (NOAEC) was 0.025 mg/l, the highest concentration tested (Rajsekhar, 2011).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female C57BL/10J$_f$AP Alpk mice were fed diets containing 0, 50, 200 or 800 ppm picoxystrobin (purity 93.3%; batch P25) for 80 weeks. Achieved mean intakes were 0, 6.6, 26 and 109 mg/kg bw per day for males and 0, 8.8, 36 and 145 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were measured throughout the study. At week 53, blood smears were taken for haematology, but were not examined. All animals, including any found dead or killed prematurely, were subjected to a full macroscopic examination. Cardiac blood samples were taken for haematology, selected organs were weighed and a wide range of tissues from the control and top-dose groups was examined by light microscopy. In the low- and intermediate-dose groups, histopathological examinations were performed on preputial gland, salivary gland and sciatic nerves in males and on the ovaries, uterus, stomach and spleen of females.

Homogeneity, stability and content of the diets were acceptable.

Survival was similar in all groups (> 80%), and there were no clinical signs of toxicity. Reduced body weight gains (≤ 10%) were seen in top-dose males for the majority of the study (Table 12) and in top-dose females during the first half of the study. Feed consumption was similar across test and control groups. A slight (approximately 2%), but statistically significant, reduction in mean cell haemoglobin was noted in males receiving 800 ppm picoxystrobin (Table 12). Liver weight was increased by approximately 10% in top-dose males, but was without any associated pathological change. There were no notable findings on histopathological examination other than an increase in erosion and inflammation of the non-glandular stomach in females receiving 800 ppm. The finding of erosion and inflammation of the non-glandular stomach is consistent with a local effect due to the transient irritation seen in irritation studies with picoxystrobin. Total and specific tumour incidences were similar in control and treated animals.

Table 12. Findings in Alpk mice exposed to picoxystrobin for 80 weeks

	0 ppm	50 ppm	200 ppm	800 ppm
Males				
Terminal body weight (mean; g)	35.8	35.6	36.4	34.1*
Liver weight (mean; g)	4.5	4.5	4.5	4.9*
Mean cell haemoglobin (mean; pg)	15.8	15.7	15.9	15.5*
Erosion of non-glandular stomach (n = 50)	3	0	0	2
Inflammation of non-glandular stomach (n = 50)	3	0	0	5
Tumour-bearing animals (n = 50)	12	14	15	16
Females				
Terminal body weight (mean; g)	28.8	29.8*	28.9	28.7
Liver weight (mean; g)	1.3	1.3	1.3	1.3
Erosion of non-glandular stomach (n = 50)	3	1	2	7
Inflammation of non-glandular stomach (n = 50)	4	2	4	8
Tumour-bearing animals (n = 50)	16	16	22	11

From Rattray (1999a)
* $P < 0.05$

The findings seen at 800 ppm are of small magnitude and are not considered to be adverse. The NOAELs for general toxicity and carcinogenicity are both 800 ppm (equal to 109 mg/kg bw per day), the highest dose tested (Rattray, 1999a).

In a second mouse carcinogenicity study, five groups of young adult male and female Crlj:CD-1(ICR) mice (60 of each sex per group) were administered diets that contained 0 (control), 100, 600, 2400 or 4800 ppm picoxystrobin (purity 99.3%; batch SEP07AS013) for approximately 18 months. Mean achieved intakes were 0, 12, 71, 293 and 585 mg/kg bw per day for males and 0, 16, 99, 412 and 799 mg/kg bw per day for females, respectively. Body weights and feed consumption were evaluated weekly for the first 13 weeks, then every other week thereafter. Detailed clinical observations were evaluated weekly. Ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. White blood cell differential counts were evaluated in surviving mice at the end of the exposure period and in mice that were sacrificed in extremis. After approximately 18 months of dietary exposure, mice were sacrificed and given a gross and microscopic pathological examination. A full range of tissues from control and top-dose animals was investigated histopathologically, but only gross lesions, liver, duodenum and stomach were investigated from other groups.

Homogeneity, stability and content of the test diet were confirmed analytically.

No notable changes were observed in clinical observations, body weight, feed intake, ophthalmology, white blood cell differential counts or cause of death in mice exposed to picoxystrobin. Survival was significantly higher in top-dose males than in controls (Table 13). A statistically significant change in the ratio of lymphocytes to neutrophils was seen in top-dose females, but the results were within the normal large variation for this parameter. Histopathological changes of the duodenum consisted of increased incidences and severity of mucosal hyperplasia in males fed dietary concentrations of 4800 ppm, but not in females (Table 13). Increases in liver weights were observed in males and females fed dietary concentrations of 2400 ppm and above (Table 13). In female mice, the increased liver weights correlated with the test article–related microscopic finding of centrilobular hepatocellular hypertrophy (Table 13). Male mice exposed to 600 ppm picoxystrobin and higher had reduced incidences of malignant lymphoma. The incidence of liver nodules and hepatocellular adenoma was higher in the 4800 ppm male group (22%) compared with the concurrent control group (6%), which also resulted in an overall increase in the incidence of combined hepatic adenoma/carcinoma (animals with at least one hepatocellular neoplasm) (Table 13). The higher incidence of hepatocellular neoplasms was considered to be related to the marked increase in survival in the 2400 and 4800 ppm male groups (83% and 93%, respectively) compared with the control group (72%). The increase in liver tumours was statistically significant by the Cochran-Armitage trend test and Fisher's exact test (one tail), but not when adjusted for survival by either the Poly-3 test or Peto analysis. There was no increase in hepatocellular carcinoma and no increase in liver tumours of any type in females (Table 13). The incidence in hepatocellular adenoma in the high-dose males was similar to incidences (1–20%) reported by the animal supplier for a relevant time period (Charles River, 2003–2006), where the average survival at study termination was lower (77%) than that observed in this study (Giknis & Clifford, 2010). The test facility had performed three similar studies that provided historical control values (adenoma 3–15% and carcinoma 5–12%) typical of those for Charles River sourced CD-1 mice (Bentley, 2012). The overall weight of evidence suggests that the liver tumours in male mice are part of the general picture of tumours seen in aged mice.

The NOAEL for non-neoplastic effects was 600 ppm (equal to 71 mg/kg bw per day), based on increased liver weights (> 10%) at 2400 ppm (equal to 293 mg/kg bw per day). The NOAEL for carcinogenicity was 4800 ppm (equal to 585 mg/kg bw per day), the highest dose tested, as the increased incidence of liver tumours in males at 2400 and 4800 ppm is considered to be secondary to increased survival in these groups (Moon, 2011).

Table 13. Findings in CD-1 mice fed diets containing picoxystrobin for 18 months

	0 ppm	100 ppm	600 ppm	2400 ppm	4800 ppm
Males (*n* = 60)					
Survival to termination (%)	72	68	78	83*	93*
Body weight, week 53 (mean; g)	54	52	55	52	52
Terminal body weight (mean; g)	55	52	55	53	53
Feed intake (mean; g/mouse per day; weeks 1–13)	5.1	5.1	5.1	5.0	5.0
Absolute liver weight (mean; g)	2.9	2.9	3.1	3.3*	3.5*
Liver nodules	3	2	9	5	10*
Hepatocellular hypertrophy	2	2	1	2	2
Hepatocellular foci of alteration	1	0	3	1	7*
Hepatocellular adenoma (total / terminal kill)	6 / 6	5 / 3	9 / 8	9 / 7	13* / 13
Hepatocellular carcinoma (total / terminal kill)	6 / 5	6 / 5	6 / 4	12 / 10	9 / 9
Hepatocellular tumours (all types)	12	11	15	20*	19*
Duodenum, dilated mucosal gland	2	2	0	5	7*
Duodenum, mucosal hyperplasia	3	3	1	6	10
Females (*n* = 60)					
Survival to termination (%)	78	85	80	72	75
Body weight, week 53 (mean; g)	43	41	41	42	40
Terminal body weight (mean; g)	45	42	42	42	42
Feed intake (mean; g/mouse per day; weeks 1–13)	5.2	5.1	5.1	5.2	5.1
Lymphocytes (mean; % of total white blood cells)	53	ND	ND	ND	61*
Neutrophils (mean; % of total white blood cells)	44	ND	ND	ND	37*
Absolute liver weight (mean; g)	2.1	2.0	2.2	2.6	2.7*
Liver nodules	0	1	2	2	2
Hepatocellular hypertrophy	2	0	3	5	28*
Hepatocellular adenoma	0	0	1	1	0
Duodenum, dilated mucosal gland	2	0	2	3	5
Duodenum, mucosal hyperplasia	5	0	2	5	7

From Moon (2011)
ND, not determined; * $P < 0.05$

Rats

Groups of 64 male and 64 female Alpk:AP_fSD rats were fed diets containing 0, 50, 200 or 750 ppm picoxystrobin (purity 93.3%; batch 25) for 2 years. Achieved intakes were 0, 3.1, 12 and 46 mg/kg bw per day for males and 0, 3.8, 15 and 58 mg/kg bw per day for females, respectively. Twelve males and 12 females from each group were designated for an interim kill at 53 weeks. The remaining animals continued to termination at 105 weeks. During the study, clinical and ophthalmoscopic observations were made, and body weights and feed consumption were measured. Blood and urine samples were taken at weeks 14 and 27, interim kill, weeks 53 and 79 and termination. Throughout the study, any animals found dead or killed prematurely were subjected to a full gross postmortem examination, and tissues were taken for subsequent histopathological examination. At scheduled termination, all animals were subjected to a full gross postmortem examination, cardiac blood samples were taken, selected organs were weighed and a wide range of tissues from control and top-dose rats plus selected tissues and gross lesions from other rats were evaluated histopathologically.

Homogeneity, stability and content of the diets were confirmed analytically.

Survival was less than 50% in all male groups, but was greater in top-dose males than in controls (Table 14). There were no adverse effects of picoxystrobin on observed clinical signs, ophthalmoscopy, haematology or urine analysis. Reductions in feed consumption (approximately 5%) and body weight gain were seen at 750 ppm during the first half of the study (Table 14). Reductions were seen in serum alanine aminotransferase activities in all male groups at termination and in alkaline phosphatase activities throughout the study in the 750 ppm groups; with no associated histopathological findings, these reductions in marker enzyme activities are not considered as adverse. Liver weight adjusted for body weight was increased by 7% in top-dose males at the interim kill. Kidney weights were reduced in both top-dose groups at termination (Table 14). An unusual finding of ectopic parathyroid in the thymus was noted in top-dose males, together with slight increases in Leydig cell lesions (Table 14). The incidence of large granular lymphocyte leukaemia was increased significantly in top-dose males, the finding being seen in animals dying towards the end of the study as well as at termination, indicating that the finding was not entirely dependent on survival (Table 14; Peto analysis $P = 0.042$). The haematological results provided no indication of any alterations in leukocyte numbers, there were no related changes in other organs, such as the spleen, and a parallel finding was not present in females. Although the incidence of 7 out of 52 is outside the test facility historical control incidence for males, cited as 0–10%, it was not reproduced in a second study that utilized higher dose levels, and the weight of evidence suggests that the leukaemias are incidental findings.

The NOAEL for toxicity was 200 ppm (equal to 12 mg/kg bw per day), based on reductions in body weight gain and kidney weights at 750 ppm (equal to 46 mg/kg bw per day). The NOAEL for carcinogenicity was 750 ppm (equal to 46 mg/kg bw per day), the highest dose tested. The increase in large granular lymphocyte leukaemia in top-dose males is not considered to be an adverse effect of picoxystrobin administration (Rattray, 1999b).

In a second 2-year chronic toxicity and carcinogenicity feeding study, picoxystrobin (purity 99.3%; batch SEP07AS013) was administered to five groups of male and female Crl:CD(SD) rats (approximately 80 rats of each sex per concentration). Concentrations in the diets were 0, 50, 200, 1000 and 3500 ppm. Mean achieved intakes were 0, 2.2, 8.8, 45 and 162 mg/kg bw per day for males and 0, 2.8, 11, 57 and 203 mg/kg bw per day for females, respectively. Ten rats per group were sacrificed after approximately 1 year on study. Parameters evaluated included body weight, body weight gain, feed consumption, feed efficiency, clinical signs, clinical pathology, ophthalmology, organ weights and gross and microscopic pathology. Samples for haematology, clinical chemistry and urine analysis were taken at 3, 6 and 12 months. A full range of tissues from control and top-dose animals and animals dying during the study was examined histopathologically, but only gross lesions, testes and tissue masses with regional lymph nodes were examined from other groups sacrificed at termination.

Homogeneity, stability and content of the test diet were confirmed analytically.

Survival in the 3500 ppm male and female groups and in the 1000 ppm female group was significantly greater than in controls (Table 15). There were no adverse clinical or ophthalmological observations attributed to test substance exposure. Mean body weight and body weight gain were reduced at 1 year and overall during the study in both sexes at 3500 ppm (Table 15). These body weight changes were associated with lower mean feed consumption and feed efficiency over the 1st year, which continued for the duration of the study (Table 15). Feed consumption suffered a marked reduction of approximately 25% in all groups, including controls, at week 88, but subsequently recovered. No consistent, test substance–related effects were noted on any clinical chemistry, haematology or urine analysis parameters, macroscopic findings or incidence of masses. There were no test substance–related microscopic findings following 1 year of treatment.

Table 14. Findings in Alpk rats exposed to picoxystrobin for 105 weeks

	0 ppm	50 ppm	200 ppm	750 ppm
Males				
Survival to termination (%)	23	17	27	40*
Body weight, week 53 (mean; g)	662	665	673	633*
Terminal body weight (mean; g)	546	503	539	535
ALP (mean; IU/l), week 53	240	235	237	170*
ALT (mean; IU/l), week 105	80	36*	45*	49*
Liver weight adjusted for body weight (mean; g; interim kill)	24.6	25.1	25.0	26.4
Liver weight adjusted for body weight (mean; g; terminal kill)	24.1	22.6	23.5	23.4
Kidney weight adjusted for body weight (mean; g; terminal kill)	5.8	5.7	5.6	4.8*
Leydig cell hyperplasia	1	4	4	5
Leydig cell tumour (benign)	5	6	6	9
Ectopic parathyroid	1	1	4	7*
Large granular lymphocyte leukaemia (terminal kill)	0	0	1	3
Large granular lymphocyte leukaemia (total)	2	0	3	7*
Total animals with tumours	40	42	37	37
Females				
Survival to termination (%)	39	46	52	52
Body weight, week 53 (mean; g)	357	356	351	330*
Terminal body weight (mean; g)	370	390	384	343*
ALP (mean; IU/l), week 53	123	103	116	76*
ALT (mean; IU/l), week 105	64	64	53	52
Liver weight adjusted for body weight (mean; g; interim kill)	12.7	12.0	12.1	12.9
Liver weight adjusted for body weight (mean; g; terminal kill)	14.7	15.0	15.2	15.7
Kidney weight adjusted for body weight (mean; g; terminal kill)	3.4	3.1	3.3	3.1*
Ectopic parathyroid	0	0	0	1
Large granular cell lymphocytic leukaemia (total)	5	11	4	2
Total animals with tumours	54	57	54	55

From Rattray (1999b)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; IU, international units; * $P < 0.05$

At the interim kill, testicular weights were increased at 3500 ppm (Table 15), but with no associated histopathological findings. Liver weights relative to body weight were increased (10–20%) in the top-dose groups at both interim and terminal kills (Table 15). Increases in uterine hyperplasia incidence were noted relative to concurrent controls, but either were not statistically significant or showed no dose–response relationship and were within the laboratory's historical control range. A single incidence of large granular lymphocyte leukaemia was seen in a male from the 3500 ppm group. An increased incidence of thyroid gland follicular cell adenoma was seen in males from the 1000 and 3500 ppm groups, but these showed no clear dose–response relationship (Table 15) and were well within the laboratory's historical control range. At the end of the study, statistically significant increases in the incidences of interstitial cell hyperplasia and benign adenoma in the testes

were observed in male rats at 3500 ppm (Table 15). The adenoma incidence was statistically significant by Fisher's exact test (one-tailed, $P = 0.03$) and Cochran Armitage trend test. A Peto analysis for survival correction gave $P = 0.009$, indicating that the increase was not directly associated with greater survival. Although the majority of adenomas and hyperplasia occurred in terminal or near-terminal animals and the per cent incidence of adenomas fell within the historical limits of the laboratory, the study report authors considered it likely that the increases in testicular interstitial cell adenoma and hyperplasia in the 3500 ppm males were related to exposure to the test substance.

Table 15. Findings in rats fed diets containing picoxystrobin for 24 months

	0 ppm	50 ppm	200 ppm	1000 ppm	3500 ppm
Males					
Survival to termination (%)	24	31	26	29	49*
Body weight, week 53 (mean; g)	742	749	757	730	666*
Terminal body weight (mean; g)	750	716	738	724	666
Feed intake (mean; g/rat per day; weeks 1–13)	27	27	27	27	25*
Absolute testes weight (mean; g; interim kill)	3.5	3.9	3.8	3.7	3.9*
Absolute testes weight (mean; g; terminal kill)	3.8	3.3	3.5	3.5	3.7
Liver weight relative to body weight (mean; %; interim kill)	3.0	3.0	3.1	3.1	3.3
Liver weight relative to body weight (mean; %; terminal kill)	2.6	2.9	2.7	2.9	3.2*
Testes, interstitial cell tumour, benign	1	1	0	2	7*
Testes, interstitial cell hyperplasia	1	2	1	1	8*
Thyroid follicular cell adenoma	0	0	0	3	2
Females					
Survival to termination (%)	20	14	21	30*	51*
Body weight, week 53 (mean; g)	427	447	428	438	358*
Terminal body weight (mean; g)	490	493	528	465	406*
Feed intake (mean; g/rat per day; weeks 1–13)	20	20	20	20	18*
Liver weight relative to body weight (mean; %; interim kill)	3.2	3.1	3.1	3.1	3.6*
Liver weight relative to body weight (mean; %; terminal kill)	2.9	3.1	2.9	3.2	3.6*

From Craig (2011)
* $P < 0.05$

The NOAEL was 1000 ppm (equal to 45 mg/kg bw per day), based on testicular interstitial cell hyperplasia and benign adenoma, reduced body weights and increases in relative liver weights at 3500 ppm (equal to 162 mg/kg bw per day). The NOAEL for carcinogenicity was 3500 ppm (equal to 162 mg/kg bw per day), as the testicular tumours were benign adenomas (Craig, 2011).

No studies to investigate the mechanism of the testicular tumours have been provided, but a generic proposal relating to a postulated mechanism for testicular tumour production was submitted. The fungicidal activity of picoxystrobin and other strobilurin fungicides (to block mitochondrial electron transport at the Q_o site of complex III, reducing ATP production and inhibiting cellular respiration) is the same biochemical mechanism that has been demonstrated to inhibit luteinizing

hormone (LH)–induced testosterone production in the rat and in human Leydig cells in vitro (Bartlett et al., 2002; Sudisha et al., 2005; Allen et al., 2006; Midzak et al., 2007). Inhibition of LH-stimulated testosterone production leads to a compensatory increase in LH via homeostatic feedback loops through the hypothalamic–pituitary–gonadal axis, and increased LH levels are known to eventually produce Leydig cell hyperplasia and adenoma in rats if sustained (Walsh, 1977; Neumann, 1991; Bar, 1992; Ewing, 1992; Prentice & Meikle, 1995). Picoxystrobin is not genotoxic (see section 2.4) and did not induce tumours of any other type in rats or in mice. The observed increase in Leydig cell hyperplasia and adenomas in the recent 2-year rat study occurred only at the high dose, where a clear threshold response was evident by a non-linear dose–response relationship at only the very highest level of 3500 ppm. The increase in Leydig cell hyperplasia and adenomas occurred in only one species (in rats and not in mice), consistent with the known response of these species to chronic elevations in LH (Cook et al., 1999). Further, the increases were not evident following 1 year of chronic daily exposure, but only following a lifetime or near-lifetime (2 years) chronic daily exposure. All animals displaying Leydig cell hyperplasia and/or adenoma were those that survived to the end of the study or died late in the study. This delayed onset is consistent with the known pathological timeline for the induction of LH-mediated Leydig cell hyperplasia and adenoma.

There are no data specific to picoxystrobin relating to this proposed mechanism. Other than effects on testes, there were neither pathological nor clinical chemistry findings to support any disruption of the hypothalamic–pituitary–gonadal axis (most test and control rats had pituitary tumours). No data on ATP, LH or testosterone levels in rats administered picoxystrobin were submitted.

2.4 Genotoxicity

Testing of the genotoxicity of picoxystrobin has been performed in a range of assays (Table 16). Positive results were seen at cytotoxic concentrations in a reverse mutation assay with mouse lymphoma L5178Y cells in the presence of metabolic activation. The remaining in vitro and the two in vivo tests were negative. The overall database is considered adequate to conclude that picoxystrobin is not genotoxic.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In an initial reproductive toxicity study, groups of 26 male and 26 female weanling Alpk:AP$_f$SD rats were fed diet containing 0 (control), 50, 200 or 750 ppm picoxystrobin (purity 93.3%; batch P25). After 10 weeks, the animals were mated and allowed to rear the ensuing F_1 litters to weaning. The breeding programme was repeated with the F_1 parents selected from the F_1 offspring to produce the F_2 litters after a 10-week premating period. Test diets were fed continuously throughout the study. Routine assessments were performed of clinical signs, body weight, feed consumption, estrous cycling, mating performance and duration of gestation. A range of pup parameters was investigated, including pup weight and live pup numbers. Litters were not culled. At termination, all parental rats, 5 pups per F_1 group and 10 pups per F_2 group received a necropsy examination, and liver, testes and epididymides were weighed. Microscopic examinations were performed on a range of tissues from all pups that were necropsied and from control and top-dose parents. There were no investigations of sperm parameters or developmental landmarks.

Homogeneity, stability and content of test diets were confirmed. Achieved doses are given in Table 17.

Table 16. Results of studies of genotoxicity with picoxystrobin

Test substance (vehicle)	End-point	Test object	Concentration	Purity; batch	Result	Reference
In vitro						
Picoxystrobin (DMSO)	Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2*uvrA*	100–5000 µg/plate, with and without S9 mix	93.3%; P-25	Negative	Callander (1996)
Picoxystrobin (DMSO)	Reverse mutation	Mouse lymphoma L5178Y cells	4–75[a] µg/ml with and without S9 mix	93.3%; P-25	Negative −S9 Weak positive +S9	Clay (1996)
Picoxystrobin (DMSO)	Chromosomal aberration	Human lymphocytes	4–60[b] µg/ml, with S9 mix 0.5–5[b] µg/ml, without S9 mix	93.3%; P-25	Negative	Fox & Wildgoose (1996)
In vivo						
Picoxystrobin (corn oil)	Bone marrow micronuclei	Alpk mice (M/F)	0, 2000, 3200 or 5000[c] mg/kg bw (gavage) single	93.3%; P-25	Negative	Fox & Mackay (1996)
Picoxystrobin (corn oil)	Liver unscheduled DNA synthesis	Alpk Rats (M)	0, 3200 or 5000 mg/kg bw (gavage) single	93.3%; P-25	Negative	Mackay (1996)

DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; F, female; M, male; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Solubility limit in treatment medium.

[b] Cytotoxicity.

[c] Marked clinical signs and altered polychromatic erythrocyte:normochromatic erythrocyte ratio.

No test substance–related mortality or clinical signs of toxicity were observed. There were no effects on mating performance, fertility, litter sizes, litter sex ratio, pup birth weights or other reproductive end-points (Table 17). Reductions in parental body weights and pup/litter weights were seen at 750 ppm; although these were statistically significant at some time points, they were small in magnitude (< 10%; Table 17). There were no treatment-related findings in the macroscopic or microscopic examinations. An increase in liver weights corrected for body weights at 750 ppm is not considered adverse, as the magnitude was less than 10% and there were no associated pathological findings.

The NOAEL for reproductive toxicity was 750 ppm (equal to 78 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 200 ppm (equal to 21 mg/kg bw per day), based on reductions in body weight gain at 750 ppm (Milburn, 1998).

Table 17. Findings in a reproductive toxicity study with picoxystrobin

	0 ppm		50 ppm		200 ppm		750 ppm	
	M	F	M	F	M	F	M	F
1st mating								
Mean picoxystrobin intake (mg/kg bw per day)	0	0	5.3	5.8	21	23	78	86
Parental body weight (mean; g; day 70)	436	249	437	252	425	246	403*	234*
Maternal body weight (mean; g; LD 15)	—	351	—	357	—	349	—	332*
Dams with live pups	—	21	—	21	—	26	—	22
Mean live pups/litter (day 1)	11.0		11.2		12.1		10.1	
Mean live pups/litter (day 22)	9.8		9.3		10.9		9.0	
Pup weight (mean; g; day 1)	6.0	5.7	6.0	5.6	6.1	5.8	6.3	5.9
Pup weight (mean; g; day 22)	45	44	47	46	45	43	45	44
Litter weight (mean; g; day 22)	421		405		466		384	
Liver weight adjusted for body weight (mean; g)	20	15	21	14	22*	15	23*	16*
2nd mating								
Mean picoxystrobin intake (mg/kg bw per day)	0	0	5.4	5.8	22	24	82	89
Parental body weight (mean; g; day 70)	437	246	430	255	412*	246	404*	230*
Maternal body weight (mean; g; LD 15)	—	346	—	353	—	350	—	329
Dams with live pups	—	22	—	24	—	23	—	24
Mean live pups/litter (day 1)	10.9		11.0		11.2		12.0	
Mean live pups/litter (day 22)	10.4		10.1		10.3		11.3	
Pup weight (g; day 1)	6.7	6.3	6.3*	6.1	6.5	6.1	6.3	5.9
Pup weight (g; day 22)	45	43	45	44	44	42	38*	37*
Litter weight (g; day 22)	440		437		436		405	
Liver weight adjusted for body weight (g)	21	14	21	14	21	14	23*	15

From Milburn (1998)
F, female; LD, lactation day; M, male; * $P < 0.05$

In a second multigeneration reproductive toxicity study, groups of 30 male and 30 female Crl:CD(SD) rats were fed diet containing 0 (control), 75, 300, 1000 or 2500 ppm picoxystrobin technical (purity 99.3%; batch SEP07AS013). After approximately 70 days, the animals were mated to generate F_1 offspring. F_1 generation rats were given continued access to the same test diet concentrations as their respective P_1 generation sires and dams beginning at weaning and were subsequently mated to produce the F_2 generation. Routine assessments were performed of clinical signs, body weight, feed consumption, estrous cycling, sperm viability, mating performance and duration of gestation. An extensive range of pup parameters was investigated, including pup weight, live pup numbers and time to sexual maturation. Litters were culled to five of each sex on day 5 after birth. Necropsy examinations were performed on all parental animals, one pup of each sex per litter from the day 5 cull and pups discarded on day 22. Major organs and reproductive organs from 10 parental rats of each sex per group were weighed. Histopathological examinations were performed on a wide range of tissues from 10 rats of each sex from the control and top-dose groups. In addition, reproductive organs and thymuses from all first-mating females were examined histopathologically.

Homogeneity, stability and content of test diets were confirmed. Achieved doses are given in Table 18.

Table 18. Findings in a reproductive toxicity study with picoxystrobin

	0 ppm		75 ppm		300 ppm		1000 ppm		2500 ppm	
	M	F	M	F	M	F	M	F	M	F
1st mating										
Mean picoxystrobin intake (mg/kg bw per day)	0	0	4.0	5.4	16	22	52	70	130	173
Parental body weight (mean; g; day 70)	572	304	574	314	569	313	552	297	537*	284*
Maternal body weight (mean; g; LD 15)	—	369	—	377	—	368	—	366	—	344*
Feed consumption (mean; g/rat per day; days 1–70)	27	20	28	20	27	19	26	19	26	19
Feed consumption (mean; g/rat per day; LDs 1–15)	—	132	—	135	—	132	—	127	—	119*
Dams with live pups	—	26	—	23	—	29	—	28	—	27
Mean live pups/litter (day 1)	14.6		14.3		15.0		12.9*		14.1	
Mean live pups/litter (day 22)	7.8		8.0		8.0		8.0		7.9	
Abnormal sperm (mean; %)	6.2	—	8.3	—	4.4	—	4.8	—	5.5	—
Pup weight (mean; g; day 1)	6.8		7.0		6.8		7.0		6.8	
Pup weight (mean; g; day 15)	33.6		36.2		33.9		31.2		25.8*	
Liver weight (mean; g)	22.5	17.4	22.4	16.9	23.0	16.8	23.3	17.0	23.5	18.4*
Thymic atrophy (n = 10)	0	0	NE	0	NE	0	NE	0	0	6*[a]
Relative thymus weight (mean; % body weight)	0.04	0.05	0.04	0.06	0.04	0.05	0.04	0.05	0.04	0.04*
Ratio pup thymus:brain weight (mean; %)	14.5	16.8	15.0	17.5	15.6	16.6	14.9	15.4	10.5*	11.4*
Ratio pup spleen:brain weight (mean; %)	18.1	18.4	18.2	18.9	18.0	17.4	16.3	16.0*	12.8*	13.5*
2nd mating										
Mean picoxystrobin intake (mg/kg bw per day)	0	0	5.3	7.9	21	32	71	106	188	273
Parental body weight (mean; g; day 86)	498	284	505	295	500	290	479	271	446*	256*
Maternal body weight (mean; g; LD 15)	—	364	—	376	—	373	—	357	—	342*
Feed consumption (mean; g/rat per day; days 22–86)	28	21	28	22	28	21	27	20	26*	19*
Feed consumption (mean; g/rat per day; LDs 1–15)	—	48	—	48	—	51	—	47	—	45
Dams with live pups	—	27	—	28	—	24	—	28	—	27
Mean live pups/litter (day 1)	15.2		14.5		15.3		14.5		14.6	
Mean live pups/litter (day 22)	7.9		7.8		8.0		7.9		7.7	
Abnormal sperm (mean; %)	8.5	—	4.1*	—	3.9*	—	3.1*	—	3.7*	—
Pup weight (mean; g; day 1)	6.4		6.6		6.8		6.8		6.8	
Pup weight (mean; g; day 15)	34		35		36		34		30*	
Liver weight (mean; g)	26.7	17.6	27.1	17.2	26.5	18.1	26.7	16.8	27.3	18.5
Thymic atrophy (n = 10)	0	0	NE	NE	NE	NE	NE	NE	0	1

Table 18 (continued)

	0 ppm		75 ppm		300 ppm		1000 ppm		2500 ppm	
	M	F	M	F	M	F	M	F	M	F
Relative thymus weight (mean; % body weight)	0.06	0.07	0.06	0.07	0.06	0.06	0.06	0.06	0.06	0.06
Ratio pup thymus:brain weight (mean; %)	15.4	16.3	14.5	14.4	15.2	15.7	14.9	15.2	11.3*	11.9*
Ratio pup spleen:brain weight (mean; %)	17.1	17.1	16.9	17.1	17.6	18.2	15.7	15.4	13.6*	13.3*

From Barnett (2010)
F, female; LD, lactation day; M, male; NE, not examined; * $P < 0.05$
[a] 11/30 on further examination.

No test substance–related mortality or clinical signs of toxicity were observed. There were no effects on mating performance, fertility, litter sizes, litter sex ratio, pup birth weights or other reproductive end-points (Table 18). Slight extension of time to sexual maturation at 2500 ppm was secondary to the lower body weights in this group. Reductions in body weight, body weight gain and feed consumption were observed in the P_1 and F_1 generation 2500 ppm male and female rats (Table 18). The 1000 ppm F_1 females also had slightly reduced mean body weight and feed consumption during the 1st week of exposure, but this did not persist and is not considered adverse. Pup weights at birth were similar across all groups; subsequently, weights of pups from the 2500 ppm groups were significantly reduced on postnatal day 8 (PND 8) (F_1 pups only), PND 15 and PND 22. F_1 generation pup weights were also significantly reduced (by 8%) at 1000 ppm on PND 22.

Absolute and/or relative liver weights were increased in 1000 ppm P_1 males and 2500 ppm P_1 and F_1 males and females (Table 18), but without any associated histopathological findings. Absolute and relative thymus weights were reduced at 2500 ppm, and this was associated with an increase in thymic atrophy in first-mating dams. There were no other remarkable findings in the macroscopic or microscopic examinations.

The NOAEL for reproductive toxicity was 2500 ppm (equal to 130 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 1000 ppm (equal to 52 mg/kg bw per day), based on body weight deficits and thymic atrophy at 2500 ppm (Barnett, 2010).

(b) Developmental toxicity

Rats

Groups of 24 time-mated female Alpk:AP$_f$SD rats were dosed orally by gavage with 0 (corn oil; 10 ml/kg bw), 10, 30 or 100 mg/kg bw per day (purity 99%; batch P9) on days 7–16 (inclusive) of gestation. Achieved doses were confirmed analytically. Dams were observed regularly for clinical signs, body weight and feed consumption. On day 22 of gestation, dams were killed and the uterine contents examined and removed. Fetuses were assessed for viability and external, visceral and skeletal abnormalities. The study report contained no individual animal data.

One female given picoxystrobin at a dose of 30 mg/kg bw per day was terminated on day 10 of the study for humane reasons, unrelated to treatment, following an accidental injury. The only clinical observations related to treatment with picoxystrobin were an increased incidence of diarrhoea/signs of diarrhoea and urine staining at 100 mg/kg bw per day and a slight increase in the incidence of salivation at 100 and 30 mg/kg bw per day. Administration of 100 mg/kg bw per day was associated with a reduction in body weight gain from the onset of dosing to termination (Table 19). Feed consumption was also reduced in animals given 100 mg kg bw per day throughout the dosing period (Table 19).

Table 19. Findings in rats exposed to picoxystrobin during fetal development

	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg per bw day
Body weight (mean; g)				
- day 7	287.5	290.8	282.3	286.8
- day 8	288.7	290.4	289.2	279.2*
- day 11	306.5	307.7	305.0	297.1*
- day 19	381.3	387.0	382.1	365.3*
- day 22	396.4	392.7	407.4*	387.6
Feed consumption (mean; g/rat per day)				
- days 7–10	22	24	21	15*
- days 13–16	26	27	26	23*
- days 19–22	24	22	28	27
Mean live fetuses/litter	12.7	14.9*	13.0	14.0
3rd centrum not ossified				
- number of pups affected (%)	16 (5.2)	30 (8.4)	11 (3.8)	32* (9.6)
- number of litters affected (%)	8 (33.3)	11 (45.8)	5 (22.7)	12 (50.0)
5th sternebrae misaligned				
- number of pups affected (%)	0 (0)	0 (0)	1 (0.3)	5* (1.5)
- number of litters affected (%)	0 (0)	0 (0)	1 (4.5)	5* (20.8)

From Moxon (1998)
* $P < 0.05$

There were no adverse effects of picoxystrobin on number, weight, growth or viability of fetuses in utero. There was no treatment-related effect on the type or incidence of major fetal defects. The proportion of fetuses with minor external/visceral defects was similar for all groups, including the controls. A statistically significant difference was seen in the number of fetuses in the 100 mg/kg bw per day group with specific skeletal anomalies: 3rd cervical centrum, unossified; and 5th sternebra, misaligned (Table 19). The historical control data for the test facility show that misaligned 5th sternebrae are commonly seen in individual fetuses from litters, although the incidences seen in the picoxystrobin study are outside the historical control range for fetuses (0–1.4%) and litters (0–12.5%). Unossified 3rd centrum is a common finding and, although statistically significant at the top dose level, is considered not to be a treatment-related adverse finding. There was no evidence for a treatment-related effect on the proportion of fetuses with external/visceral variants or on the *manus* and *pes* assessment.

The NOAEL for teratogenicity was 100 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 30 mg/kg bw per day, based on an increase in misaligned 5th sternebrae at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on reduced body weight and feed consumption during the dosing period at 100 mg/kg bw per day (Moxon, 1998).

Rabbits

Groups of 20 time-mated, female New Zealand White rabbits were dosed orally by gavage with picoxystrobin (purity 93.3%; batch P25) at doses of 0 (1% carboxymethylcellulose, 1 ml/kg bw), 8, 25 or 100 mg/kg bw per day on days 8–20 (inclusive) of gestation. Achieved doses were confirmed analytically. Dams were observed regularly for clinical signs, body weight and feed consumption. On day 30 of gestation, dams were killed and the uterine contents examined and removed. Fetuses were

assessed for viability and external, visceral and skeletal abnormalities. The study report contained no individual animal data.

All animals survived the duration of the study. Administration of 100 mg/kg bw per day was associated with reduced body weight and feed consumption during the early phases of dosing (Table 20). Incidences of "signs of diarrhoea" and "reduced faecal output" were also increased at 100 mg/kg bw per day. Transient body weight loss and reduced feed consumption were also seen at 25 mg/kg bw per day (Table 20). Macroscopic examination of the dams post mortem revealed no treatment-related findings.

Table 20. Findings in rabbits exposed to picoxystrobin during fetal development

	0 mg/kg bw per day	8 mg/kg bw per day	25 mg/kg bw per day	100 mg/kg bw per day	Historical control
Body weight (mean; kg)					
- day 8	3.73	3.68	3.78	3.82	
- day 9	3.73	3.70	3.74	3.73*	
- day 11	3.73	3.72	3.74	3.68*	
- day 20	3.86	3.88	3.90	3.90	
- day 30	4.08	4.13	4.14	4.07	
Feed consumption (mean; g/rabbit per day)					
- days 8–11	134	142	96*	49*	
- days 17–20	167	162	147	146	
- days 23–26	141	144	132	161*	
Dams with live litters	17	16	18	16	
Corpora lutea (mean/dam)	11.1	11.3	11.6	10.2	
Implantations (mean/dam)	9.8	9.6	108	8.1*	
Preimplantation loss (total/litters affected) (%)	21/10 (12)	26/11 (14)	13/8 (6)	33*/14 (21*)	
Post-implantation loss (%)	14	7*	12	15	
Mean litter weight (g)	359	374	387	305	
Live fetuses (total/litter mean)	142/8.4	144/9.0	172/9.6	111/6.9*	
Major skeletal anomalies (fetuses/litters)	6/4	2/2	1/1	0/0	
Skeletal variants (fetuses/% fetuses)	108/74	109/74	144/84	96/84*	
Odontoid incompletely ossified (% fetuses) (fetuses/litters)	0.7 (1/1)	1.4 (2/1)	3.5 (6/4)	9.0* (10*/3)	1–8%
27 pre-pelvic vertebrae, bilateral (% fetuses) (fetuses/litters)	16.9 (24/10)	20.8 (30/9)	18.6 (32/13)	39.6* (44*/15*)	15–37%
Rib 13 long length (% fetuses) (fetuses/litters)	32.4 (46/15)	36.1 (52/11)	50* (86*/18)	53.2* (59*/14)	17–55%

From Moxon (1999)
* $P < 0.05$

Picoxystrobin did not alter the weight, growth or viability of the fetuses in utero, no dams aborted, there were no dams with total litter loss and there was no evidence of teratogenicity. A reduction in the number of live fetuses and litter weight at 100 mg/kg bw per day (Table 20) appeared to be secondary to a reduction in numbers of corpora lutea and implantations, both of which occurred before the start of the dosing period; post-implantation loss was unaffected. Evaluation of the major and minor external/visceral fetal defects and variants provided no evidence of an adverse effect of

picoxystrobin on development. Evaluation of the minor skeletal defects and variants revealed three findings (Table 20). Odontoid incompletely ossified is normally a low-incidence finding in the New Zealand White rabbit. The incidence of fetuses with an incompletely ossified odontoid in the 100 mg/kg bw per day group was slightly, but statistically significantly, increased compared with concurrent controls. The findings were concentrated in three litters, but as no individual litter data are available, it is not possible to determine whether the affected fetuses came from dams exhibiting maternal toxicity. The two other skeletal findings, 27th pre-pelvic vertebrae and 13th thoracolumbar ribs of long length, are relatively common findings in the New Zealand White rabbit. The incidence of fetuses with 27th pre-pelvic vertebrae (combined to include unilateral and bilateral) was increased in the 100 mg/kg bw per day group only (fetal incidence 44.1%, litter incidence 93.8%) and was of a slightly higher incidence than seen in control rabbits in the testing laboratory (fetal incidence 14.6–36.5%, litter incidence 52.9–89.5%). The incidence of fetuses with 13th thoracolumbar ribs of long length was apparently increased in the 100 mg/kg bw per day group and also in the 25 mg/kg bw per day group. The incidence of this finding in both groups was within the range seen previously in control rabbits in the laboratory (fetal incidence 17.1–55.2%, litter incidence 50–100%). The available historical control data come from nine studies performed from 1992 to 1998; however, only one of these studies used rabbits from the same supplier as used for the picoxystrobin study. At 100 mg/kg bw per day, the incidence of 13th thoracolumbar ribs of long length, although within the range of historical control values, coincided with a slightly increased incidence of 27th pre-pelvic vertebrae; for this reason, an association with treatment at 100 mg/kg bw per day cannot entirely be dismissed. At 25 mg/kg bw per day, the statistically significant increase in incidence of 13th thoracolumbar ribs of long length was within the test facility historical control values, was not associated with any other skeletal findings at this dose level and is considered not to be an adverse effect of treatment with picoxystrobin.

The NOAEL for teratogenicity was 100 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 25 mg/kg bw per day, based on an increase in skeletal anomalies at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduced body weight and feed consumption during the dosing period at 100 mg/kg bw per day (Moxon, 1999).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity

Crl:CD(SD) rats (12 of each sex per group) received picoxystrobin (purity 99.3%; batch SEP07AS013) in a single oral gavage dose of 0, 200, 1000 or 2000 mg/kg bw, using 0.5% methylcellulose as vehicle. Achieved doses were confirmed by analysis. Regular, routine observations included clinical signs and appearance, body weight and feed consumption. An extensive functional observational battery was performed pretest and on days 1 (2 hours), 8 and 15; motor activity was monitored over a 90-minute session on each observation day. At sacrifice on day 16, six animals of each sex were anaesthetized and perfused; brain and spinal cord were removed. Peripheral nerve samples were prepared from six rats of each sex per group.

Three females dosed with 2000 mg/kg bw died. Clinical signs of toxicity, including reduced body temperature, reduced levels of arousal, palpebral closure and abnormal posture, were seen in animals from the 1000 and 2000 mg/kg bw groups. Transient reductions in body weight, body weight gain and feed consumption were seen in all treated groups (Table 21). Decreased motor activity was recorded in the first half of the 90-minute observation period on day 1 in all test groups (Table 21), but not on day 8 or 15. There were no abnormal findings in the neuropathological examinations.

Table 21. Body weight, feed consumption and motor activity in rats receiving picoxystrobin

	0 mg/kg bw		200 mg/kg bw		1000 mg/kg bw		2000 mg/kg bw	
	M	F	M	F	M	F	M	F
Body weight (g)								
- day 1	290	189	290	188	289	181	292	187
- day 2	292	190	284	185	280	178	276	181
- day 8	340	214	340	209	326	203	327	207
- day 15	384	232	386	229	373	223	379	231
Body weight gain (g)								
- days 1–2	1.8	1.1	−6.2*	−2.6*	−9.0*	−3.1*	−15.3*	−5.8*
- days 2–8	49	25	56	24	46	25	51	27
Feed consumption (g/rat per day)								
- days 1–2	26	20	18*	13*	12*	9*	10*	8*
- days 2–8	29	22	31	21	29	21	29	20
Movement duration index (mean (SD))								
- day 1, total	1009 (317)	819 (330)	641* (234)	752 (254)	428* (147)	511 (240)	303* (146)	335* (192)
- day 1, 0–10 min	395 (49)	355 (58)	326* (83)	328 (77)	235* (87)	241* (102)	144* (59)	135* (70)
- day 1, 10–20 min	286 (85)	221 (105)	138* (66)	133* (84)	56* (76)	81* (99)	16* (25)	58* (77)
- day 1, 20–30 min	193 (64)	113 (91)	64* (84)	72 (70)	28* (56)	42* (63)	17* (27)	16* (32)

From Malley (2010)
F, female; M, male; SD, standard deviation; * $P < 0.05$

The NOAEL for neuropathy was 2000 mg/kg bw, the highest dose tested. For general toxicity, a NOAEL could not be identified, based on decreases in motor activity and body weight at 200 mg/kg bw, the lowest dose tested (Malley, 2010).

To obtain an indication of where the NOAEL for the acute neurotoxicity study might lie, the Meeting performed basic benchmark dose modelling using the United States Environmental Protection Agency's (USEPA) benchmark dose software (BMDS version 2.2). The modelled data were the mean and standard deviation for the movement duration index in males for each of the first three time periods on day 1, a sensitive, relevant end-point with a clear dose–response relationship. Individual animal values were not used, and no correction was made for pretest values (there were no notable differences in pretest values between test and control groups). A suite of continuous models was run with a benchmark response of a 5% relative variation and a 95% confidence level for the BMD_L. The model that gave the lowest values for benchmark dose (BMD) and lower-bound confidence limit on the benchmark dose (BMD_L), which could not be dismissed, was a Hill model. The obtained values (mg/kg bw) were: session 1, BMD 79.7, BMD_L 35.6; session 2, BMD 10.4, BMD_L 5.7; and session 3, BMD 4.6, BMD_L 0.8. The BMD values (particularly for session 3) that were generated involved modelling over an order of magnitude outside the limits of the dose–response data, and the true shape of the dose–response curve is unknown; therefore, the BMD and BMD_L values should not be taken as anything more than indicating that the NOAEL in the acute neurotoxicity study of Malley (2010) is much lower than 200 mg/kg bw.

Repeated-dose neurotoxicity

Crl:CD(SD) rats (12 of each sex per dose group) received picoxystrobin (purity 99.3%, batch SEP07AS013) in the diet at a concentration of 0, 100, 600 or 3500 ppm for 14 weeks. The overall mean daily intakes were 0, 6, 36 and 207 mg/kg bw per day for males and 0, 8, 46 and 246 mg/kg bw per day for females, respectively. Regular, routine observations included clinical signs and appearance, body weight and feed consumption. An extensive neurobehavioural test battery, consisting of motor activity and functional observational battery assessments, was conducted on all study rats prior to test substance administration in order to obtain baseline measurements. This neurobehavioural test battery was conducted again during weeks 4, 8 and 13. On test days 97 and 98, six rats of each sex per group were anaesthetized and subjected to whole-body in situ perfusion for neuropathology examination. A wide range of brain, spinal cord, peripheral nerve and skeletal muscle sections was prepared and stained using haematoxylin and eosin, periodic acid Schiff or luxol fast blue, singly or in combination. Sections from control and top-dose animals were examined microscopically.

Achieved doses and homogeneity were confirmed by analysis.

Body weights in animals from the 3500 ppm group were approximately 15–25% lower than those of controls at week 13. Overall feed consumption and feed efficiency in male and female rats were reduced approximately 15–20% at 3500 ppm. There were no test substance–related adverse effects on survival, clinical signs of toxicity, motor activity, temperature, behavioural parameters, or gross pathological or neuropathological examination in males or females administered any concentration of the test substance. Males administered 3500 ppm of the test substance exhibited statistically significant reductions in forelimb and hindlimb grip strength during week 8 and forelimb grip strength during week 13. These changes are probably secondary to decreases in body weight, because the reductions were small (6–9%) relative to the body weight effects, were reported to be within the typical background values and were not associated with any significant indication of neurotoxicity.

The NOAEL for neuropathy and neurotoxicity was 3500 ppm (equal to 207 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity was 600 ppm (equal to 36 mg/kg bw per day), based on body weight effects at 3500 ppm (Anand, 2010).

(b) Immunotoxicity

The potential of picoxystrobin (purity 99.3%; batch SEP07AS013) to suppress the primary humoral response to sheep red blood cells (sRBCs) was assessed in Crl:CD1 (ICR) mice and in Crl:CD (SD) rats. Groups of 10 animals of each sex were administered the test substance for 4 weeks at a dietary level of 0 (control), 100, 600, 2400 or 4800 ppm for mice and 0, 50, 200, 1000 or 3500 ppm for rats. In mice, the overall mean daily intakes in the 0, 100, 600, 2400 and 4800 ppm groups were calculated to be 0, 16, 95, 358 and 727 mg/kg bw per day for males and 0, 20, 127, 449 and 931 mg/kg bw per day for females, respectively. In rats, the overall mean daily intakes in the 0, 50, 200, 1000 and 3500 ppm groups were calculated to be 0, 3.5, 15, 68 and 231 mg/kg bw per day for males and 0, 3.9, 16, 75 and 229 mg/kg bw per day for females, respectively. Body weights, feed consumption measurements and clinical observations were recorded during the in-life period. Prior to sacrifice, the immune system was stimulated on test day 23 by injecting sRBCs into the tail vein. Blood samples were collected from each rat on test day 28, and serum samples were assayed for their concentrations of sRBC-specific immunoglobulin M (IgM) antibodies. Contemporary data from a positive control immunosuppressive agent (cyclophosphamide, 25 mg/kg bw per day) provide confirmation that the assay performance was acceptable for detection of immunosuppression. At sacrifice, each animal was examined grossly, and selected organs were weighed (brain, spleen and thymus). No histopathological examinations were performed.

Homogeneity, stability and content of the diet were confirmed by analysis.

No clinical signs of systemic toxicity were observed. There were no statistically significant or adverse effects on mean body weight, mean body weight gain or nutritional parameters in male or

female mice at any dose level or in male or female rats fed 0, 50, 200 or 1000 ppm picoxystrobin. Lower mean body weight (approximately 6–10%), body weight gain, feed consumption and feed efficiency were observed in male and female rats fed 3500 ppm (Table 22) and are considered to be adverse. No significant adverse effects were observed on 1) gross pathology; 2) absolute and relative brain, spleen and thymus weights; or 3) humoral immune response (Table 22).

Table 22. Body weight and anti-sRBC IgM titres in mice and rats treated with picoxystrobin

	M	F	M	F	M	F	M	F	M	F
Mice										
Dietary concentration (ppm)	0		100		600		2400		4800	
Body weight (g)										
- day 0	31	23	30	23	30	22	30	23	30	23
- day 7	32	25	32	25	32	24	32	25	31	24
- day 28	35	27	35	28	36	27	35	27	33	27
IgM[a]	10.7	11.0	10.1	11.2	11.0	10.4	10.7	10.9	9.7	10.4
Rats										
Dietary concentration (ppm)	0		50		200		1000		3500	
Body weight (g)										
- day 0	245	189	244	189	246	189	246	190	245	190
- day 7	296	207	301	207	304	212	293	207	269*	191*
- day 28	400	245	415	242	413	248	397	243	366*	224*
IgM[a]	13.7	14.0	14.4	15.5	14.5	15.0	13.4	14.7	14.5	14.7

From Hoban (2010a,b)
F, female; M, male; * $P < 0.05$
[a] Mean $\log_2$ of the sRBC-specific serum IgM titre data for pooled samples.

The NOAELs for immunotoxicity (IgM response) were 4800 ppm (equal to 727 mg/kg bw per day) in mice and 3500 ppm (equal to 229 mg/kg bw per day) in rats, the highest doses tested. The NOAELs for general toxicity were 4800 ppm (equal to 727 mg/kg bw per day) in mice, the highest dose tested, and 1000 ppm (equal to 68 mg/kg bw per day) in rats, based on body weight effects at 3500 ppm (Hoban, 2010a,b).

(c) Studies on metabolites

Metabolite IN-H8612 (ZA1963 metabolite 24 / R135305; 1,3-dihydro-3-oxoisobenzofuran-1-carboxylic acid)

IN-H8612 is formed in cereal grain treated with picoxystrobin; it has not been identified as a metabolite in rats.

IN-H8612 (purity 99%; batch P4) was of low acute oral toxicity to male and female Alpk:AP$_f$SD rats (LD_{50} > 2000 mg/kg bw) when administered in 1% carboxymethylcellulose. There were no signs of systemic toxicity (Twomey, 1999a).

In a 28-day toxicity study, IN-H8612 (purity 99%; batch P4) was administered to male and female Alpk:AP$_f$SD rats (five of each sex) at 0, 30, 500 or 1600 ppm in the diet. Overall mean intakes were 0, 3.5, 58 and 186 mg/kg bw per day for males and 0, 3.4, 58 and 182 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were determined routinely. A functional observational battery and motor activity analyses were performed during week 4. At the end of the scheduled period, blood and urine samples were obtained, and the animals were

sacrificed and subjected to a full postmortem examination. Selected organs were weighed, and a wide range of tissues from control and top-dose animals and all tissues showing gross lesions from other groups were examined histopathologically. Individual animal data were not presented.

Achieved doses and homogeneity were confirmed by analyses.

All animals survived. There were no adverse effects on clinical signs of toxicity, body weight, feed consumption, ophthalmoscopy, functional observational battery, motor activity or haematology. Serum alanine aminotransferase activity was decreased significantly in 500 ppm (30%) and 1600 ppm (50%) females. Urine volume was increased in all treated groups, with a related reduction in specific gravity, but with no clear dose–response relationship. Increases in absolute testes weights, although statistically significant, were without a dose–response relationship and were secondary to slightly higher body weights. Reduced (15%) mean spleen weight in top-dose females persisted even when an outlier was removed, but were without any histopathological or haematological correlate. There were no adverse effects seen on macroscopic or microscopic examination of tissues. The findings at 1600 ppm are considered to be minimal in nature.

The NOAEL was 1600 ppm (equal to 182 mg/kg bw per day), the highest dose tested (Twomey, 1999b). (For comparison, the NOAEL in a 90-day rat toxicity study with picoxystrobin was 42 mg/kg bw per day, based on findings seen early on in the study.)

IN-H8612 (purity 99%; batch P4) was negative when tested for gene mutation at concentrations up to 5000 µg/plate in the presence and absence of metabolic activation in an Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2P *uvrA* (Callander, 1999a).

IN-H8612 (purity 99%; batch P4) produced positive results in a chromosomal aberration assay in human lymphocytes. The positive results were seen at a high concentration that produced a decrease in culture medium pH (by 1.1–2.2 pH units) and mild to moderate cytotoxicity. When the test material was neutralized to pH 7.4 with sodium hydroxide, cytotoxicity was reduced, and higher concentrations could be tested. In neutralized assays, a consistent pattern of increases in aberrations, particularly fragments, was seen at concentrations producing mild cytotoxicity. It is considered that the potential clastogenicity of IN-H8612 needs to be evaluated further (E. Jones, 1999).

Metabolite IN-QDY63 (ZA1963 metabolite 8 / R408509; 2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)] benzoic acid)

IN-QDY63 is a major soil metabolite of picoxystrobin that has been found at low levels (< 5%) in rats and very occasionally in studies on plants.

IN-QDY63 (purity 97%; batch P5) was of moderate acute oral toxicity to male and female Alpk:AP_fSD rats (LD_{50} = 387 mg/kg bw) when administered in 1% carboxymethylcellulose (Twomey, 1999c).

In a 90-day toxicity study, IN-QDY63 (purity 97%; batch P5) was administered to male and female Alpk:AP_fSD rats (12 of each sex) at 0, 60, 180 or 600 ppm in the diet. Overall mean intakes were 0, 4.8, 14 and 48 mg/kg bw per day for males and 0, 5.2, 16 and 53 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were determined routinely. A functional observational battery and motor activity analyses were performed during week 12. At the end of the scheduled period, blood and urine samples were obtained, and the animals were sacrificed and subjected to a full postmortem examination. Selected organs were weighed, and a wide range of tissues from control and top-dose animals and kidneys from all males were examined histopathologically. Individual animal data were not presented.

Achieved doses and homogeneity were confirmed by analyses.

All animals survived. There were no adverse effects on clinical signs of toxicity, feed consumption, ophthalmoscopy, functional observational battery, motor activity or haematology. Body weights from the top- and intermediate-dose male groups were lower (5%) than those of controls in the second half of the study. White blood cell count was reduced in the 60 and 600 ppm male groups, but not the 180 ppm group. Serum cholesterol levels were increased in all males, dose relatedly, but by a small amount (2.5, 2.7*, 2.8* and 2.9* mmol/l; * $P < 0.05$, *t*-test); top-dose groups had increases in plasma albumin levels. Relative liver weights were increased in top-dose males (7%) and females (9%), with absolute kidney weights increased in males (10%) and females (5%) from the 600 ppm groups. Renal tubular basophilia and interstitial cell infiltration were increased in top-dose males.

The NOAEL was 180 ppm (equal to 14 mg/kg bw per day), based on a number of findings at 600 ppm (equal to 48 mg/kg bw per day) (Twomey, 2000).

IN-QDY63 (purity 97%; batch P5) was negative when tested for gene mutation at concentrations up to 5000 µg/plate in the presence and absence of metabolic activation in an Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2P *uvrA* (Callander, 1999b). No mammalian cell genotoxicity studies were available on IN-QDY63.

IN-QDY63 was negative in an Ames test and has no structural alerts for genotoxicity (OECD QSAR [Quantitative Structure–Activity Relationship] Toolbox, version 2.1.2; Benigni/Bossa rules) and could be assigned to Cramer class 3, equating to a threshold of toxicological concern (TTC) of 90 µg/person per day (Kroes et al., 2004).

Phthalic acid

Phthalic acid is a widely used industrial chemical that has been identified as a plant metabolite of picoxystrobin. The toxicity of phthalic acid has been reviewed in a number of fora (e.g. USEPA, 1988; Integral, 2007). Phthalic acid is of low acute toxicity and is negative in an Ames test for mutagenicity (Zeiger et al., 1992; NTP, 2012) . It is not a teratogen and was not maternally toxic in rats at dose levels up to 5% in the diet (equal to 2981 mg/kg bw per day); the NOAEL for developmental toxicity was 2.5% (equal to 1763 mg/kg bw per day), based on decreased fetal weight and ossification at 5% (Ema et al., 1997). Phthalic anhydride, which hydrolyses to phthalic acid, is not carcinogenic in B6C3F1 mice or F344 rats at dose levels up to 1.5% (equal to 748 mg/kg bw per day) and 1.25% (equal to 3000 mg/kg bw per day), respectively (NCI, 1979). In the carcinogenicity studies, the NOAEL for toxicity was 1.5% (equal to 748 mg/kg bw per day) in rats, and the lowest-observed-adverse-effect level (LOAEL) was 6250 ppm (equal to 1562 mg/kg bw per day) in mice, based on lung and kidney pathology (NCI, 1979). The USEPA has set a chronic oral exposure reference dose of 2 mg/kg bw per day for phthalic anhydride, based on a LOAEL of 1562 mg/kg bw per day in a mouse carcinogenicity study, with the application of a nominal 1000-fold uncertainty factor (USEPA, 1988). The data indicate that phthalic acid is not a toxicologically relevant metabolite of picoxystrobin.

2-(2-Formylphenyl)-2-oxoacetic acid

2-(2-Formylphenyl)-2-oxoacetic acid is a plant metabolite of picoxystrobin that is not supported by any toxicological studies. It has not been identified in studies of metabolism in rats. An analysis using the profiling model in the OECD QSAR Toolbox (version 2.1.2) indicated that the aldehyde group on the molecule triggered an alert for potential "genotoxic carcinogenicity" (Benigni/Bossa rules). Until the genotoxicity of 2-(2-formylphenyl)-2-oxoacetic acid is investigated, the TTC-based exposure would be the lowest threshold of 0.15 µg/person per day (Kroes et al., 2004).

PAG3 (2-(2-Hydroxymethylphenyl)2-oxoacetic acid)

PAG3 is a plant metabolite of picoxystrobin that is not supported by any toxicological studies. It has not been identified in studies of metabolism in rats. An analysis using the profiling model in the

OECD QSAR Toolbox (version 2.1.2) indicated that PAG3 had no structural alerts for genotoxicity (Benigni/Bossa rules) and could be assigned to Cramer class III. Using a TTC approach, a compound in Cramer class III equates to an acceptable exposure of 90 µg/person per day (Kroes et al., 2004).

3. Observations in humans

No poisoning cases have been identified. No adverse findings have been reported in production plant workers (Calero, 2010; Bentley, 2012).

Comments

Biochemical aspects

Radiolabelled picoxystrobin administered by oral gavage is rapidly absorbed, with peak plasma ^{14}C levels seen at approximately 2 or 12 hours in rats administered 10 or 100 mg/kg bw, respectively. Picoxystrobin is well absorbed, with approximately 70% of the radioactivity from an oral dose of 100 mg/kg bw detected in bile and urine. Distribution is extensive, with peak radioactivity levels being detected in liver, pancreas, kidney and blood plasma. Excretion is predominantly via the bile and thence into faeces and is essentially complete within 120 hours for a dose of 100 mg/kg bw. Excretion in urine was greater in females (approximately 30%) than in males (approximately 20%). Picoxystrobin is extensively metabolized, with over 30 identified metabolites. Significant biotransformation reactions include ester hydrolysis, oxidation, *O*-demethylation and glucuronide conjugation.

Toxicological data

In the rat, picoxystrobin is of low acute oral and dermal toxicity (LD_{50}s > 2000 mg/kg bw), but is of high acute toxicity by inhalation (LC_{50} = 0.11 mg/l). In the rabbit, picoxystrobin is slightly irritating to the skin and moderately irritating to the eye, and it was not a skin sensitizer in a maximization test in guinea-pigs.

A consistent finding in animals exposed to picoxystrobin is reduced body weight gain, frequently associated with reduced feed consumption. Given the mode of pesticidal action on ATP production, the body weight effects might not be entirely secondary to the reduced feed consumption. Another common finding is increased liver weights.

In a 90-day dietary toxicity study in mice, the NOAEL was 800 ppm (equal to 137 mg/kg bw per day), based on increased relative liver weight (> 10%) and reduced body weight gains at 1600 ppm (equal to 291 mg/kg bw per day).

In a 90-day dietary toxicity study in rats, body weight gain was reduced from the first observation period (week 1), and terminal body weights were approximately 10% lower in the 1250 ppm (equal to 105 mg/kg bw per day) groups than in controls. Liver weight corrected for body weight was increased by more than 10% at 1250 ppm and by 5–8% at 500 ppm, but there were no associated pathological findings. The NOAEL was 500 ppm (equal to 42 mg/kg bw per day), based on the reductions in body weight gain and increased liver weights (> 10%) at 1250 ppm.

In a 90-day dietary toxicity study in dogs, reductions in body weights and feed consumption were seen from the first observation period (week 1) at 500 ppm (equal to 17 mg/kg bw per day), with a NOAEL of 250 ppm (equal to 8.5 mg/kg bw per day). In a 1-year dietary toxicity study in dogs, reddened gums, fluid faeces and thin appearance were seen, together with reductions in body weight and feed consumption, at 500 ppm (equal to 16 mg/kg bw per day). The NOAEL was 150 ppm (equal to 4.6 mg/kg bw per day). The Meeting concluded that an overall NOAEL for the dog studies was 8.5 mg/kg bw per day.

The chronic toxicity and carcinogenicity of picoxystrobin have been investigated in two studies in mice at dose levels up to 800 ppm or 4800 ppm and in two studies in rats at dose levels up to 750 ppm or 3500 ppm. In the first mouse carcinogenicity study, slight, but not adverse, reductions in body weight (approximately 5%) and increases in liver weights (approximately 10%) were seen at

800 ppm (equal to 109 mg/kg bw per day), the highest dose tested. In the second mouse carcinogenicity study, survival was significantly increased in males receiving 2400 or 4800 ppm. Pathological changes seen in the liver in the 4800 ppm groups were macroscopic nodules, microscopic foci of alteration and a significant increase in total hepatocellular tumours in males and centrilobular hepatocyte hypertrophy in females. The tumours were seen predominantly in males surviving to the end of the study, and additional statistical analyses indicated that the increases were related to the increased survival in these mice. The NOAEL for toxicity in mice was 600 ppm (equal to 71 mg/kg bw per day), based on increased liver weights (> 10%) in both sexes at 2400 ppm (equal to 293 mg/kg bw per day). The NOAEL for carcinogenicity was 4800 ppm (equal to 585 mg/kg bw per day), the highest dose tested, as the increase in liver tumours in males at 2400 and 4800 ppm is considered to be secondary to increased survival in these groups and therefore not relevant to the risk assessment of picoxystrobin.

In the first rat chronic toxicity and carcinogenicity study, Alpk (Wistar-derived) male rats receiving 750 ppm had increased survival and increased incidences of large granular lymphocyte leukaemia. The incidence of leukaemia was outside the test facility's historical control range and was still statistically significant when corrected for the increased survival. The leukaemia incidence was of marginal statistical significance, it is a spontaneous finding in this strain of rat, there were no associated pathological changes in other organs (e.g. the spleen) and the finding was not duplicated in a second study that employed higher dose levels; therefore, the Meeting concluded that these leukaemias were incidental findings. In the first study, the NOAEL for toxicity was 200 ppm (equal to 12 mg/kg bw per day), based on reduced body weight gain and kidney weights at 750 ppm (equal to 46 mg/kg bw per day). In the second rat chronic toxicity and carcinogenicity study, in Crl:CD(SD) rats (Sprague-Dawley derived), survival was increased in the picoxystrobin groups, with over twice as many rats from the 3500 ppm groups surviving to the end of the study. Reductions in body weight gain, feed consumption and feed conversion efficiency were seen during the 1st year of the study in the 3500 ppm groups. Liver weight relative to body weight was increased by more than 10% at both interim and terminal kills in the 3500 ppm groups. Testes weights were increased in top-dose rats at the interim kill. Statistically significant increases in the incidences of interstitial cell hyperplasia and benign adenoma in the testes were observed in male rats at 3500 ppm. Although the majority of adenomas and hyperplasia occurred in terminal or near-terminal animals, the results were still statistically significant when corrected for the increased survival, and the Meeting considered it likely that the increases in testicular interstitial cell adenoma and hyperplasia in the 3500 ppm males were related to exposure to the test substance. The NOAEL for toxicity in rats was 1000 ppm (equal to 45 mg/kg bw per day), based on testicular interstitial cell hyperplasia and benign adenoma, reduced body weights and increased relative liver weights at 3500 ppm (equal to 162 mg/kg bw per day).

The Meeting concluded that picoxystrobin is not carcinogenic to mice or rats.

Picoxystrobin has been tested in an adequate range of genotoxicity studies. No evidence of genotoxicity was seen, other than a weak response in a mouse lymphoma mammalian cell gene mutation assay with metabolic activation.

The Meeting concluded that picoxystrobin is unlikely to be genotoxic.

Based on the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that picoxystrobin is unlikely to pose a carcinogenic risk to humans.

Two multigeneration reproductive toxicity studies in rats have been performed with picoxystrobin at dose levels up to 750 ppm or 2500 ppm. In the first study, the NOAEL for reproductive toxicity was 750 ppm (equal to 78 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 200 ppm (equal to 21 mg/kg bw per day), based on reductions in body weight gain at 750 ppm. In the second study, the NOAEL for reproductive toxicity was 2500 ppm (equal to 130 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 1000 ppm (equal to 52 mg/kg bw per day), based on body weight deficits and thymic atrophy at 2500 ppm in parental animals and pups.

The developmental toxicity of picoxystrobin has been assessed in rats and rabbits. In rats, misaligned 5th sternebrae were present in 1.5% of fetuses in the 100 mg/kg bw per day group, above the cited historical control range of 0–1.4%. The NOAEL for developmental toxicity was 30 mg/kg bw per day, based on an increased incidence of misaligned 5th sternebrae at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on body weight deficits and reduced feed consumption during the dosing period at 100 mg/kg bw per day.

In rabbits, the mean number of fetuses per litter was reduced at 100 mg/kg bw per day, but this appeared to be related to increased preimplantation losses, which occurred before the start of administration of picoxystrobin. Increased incidences of skeletal anomalies were seen in the 100 mg/kg bw per day group. The NOAEL for developmental toxicity was 25 mg/kg bw per day, based on an increase in skeletal anomalies at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on body weight deficits and reduced feed consumption during the dosing period at 100 mg/kg bw per day.

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

In an acute neurotoxicity study in rats, there was no evidence of neuropathy at 2000 mg/kg bw, the highest dose tested. The NOAEL for toxicity was less than 200 mg/kg bw, based on dose-related, transient decreases in motor activity and in body weight at all dose levels. A BMD evaluation indicated that a derived NOAEL in the acute neurotoxicity study was likely to be much lower than 200 mg/kg bw. There was no evidence of neurotoxicity in a 90-day neurotoxicity study in rats at dose levels up to 3500 ppm (equal to 207 mg/kg bw per day), the highest dose tested.

In a 28-day dietary immunotoxicity study in rats and mice, no effects on IgM response to sRBCs were observed at the highest doses tested, 4800 ppm (equal to 727 mg/kg bw per day) in mice or 3500 ppm (equal to 229 mg/kg bw per day) in rats.

The plant metabolite IN-H8612 (1,3-dihydro-3-oxoisobenzofuran-1-carboxylic acid) was of low acute oral toxicity to rats (LD_{50} > 2000 mg/kg bw). In a 28-day toxicity study in rats, the NOAEL was 1600 ppm (equal to 182 mg/kg bw per day), the highest dose tested. IN-H8612 was negative in an Ames test for bacterial gene mutation and produced equivocal results at high concentrations in a chromosomal aberration assay in human lymphocytes. On the basis of the limited in vivo data available, the toxicological potency of IN-H8612 is similar to or lower than that of picoxystrobin, but additional data are required to resolve its potential to induce chromosomal aberrations in vivo. The Meeting was unable to conclude on the genotoxic potential of IN-H8612. The international estimated daily intake (IEDI) was above 0.15 µg/person per day, the TTC for a compound with evidence of genotoxicity. The Meeting was unable to conclude on the toxicological relevance of estimated intakes of IN-H8612.

The soil metabolite IN-QDY63 (2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)] benzoic acid) was of moderate acute oral toxicity to rats (LD_{50} = 387 mg/kg bw). In a 90-day dietary toxicity study in rats, the NOAEL was 180 ppm (equal to 14 mg/kg bw per day), based on increased kidney weights and renal tubule pathological changes at 600 ppm (equal to 48 mg/kg bw per day). IN-QDY63 was negative in an Ames test for bacterial gene mutation. The limited data available indicate that the toxicity of IN-QDY63 is quantitatively similar to or greater than that of picoxystrobin. The estimated IEDI for IN-QDY63 was 9.7 µg/person per day, below the applicable TTC of 90 µg/person per day. The Meeting concluded that IN-QDY63 was not of toxicological concern at the estimated dietary intake levels.

Three other plant metabolites of picoxystrobin were considered:

- Phthalic acid is a widely used industrial chemical. It is not genotoxic and is not a developmental toxicant in rats (NOAEL = 1763 mg/kg bw per day). Phthalic anhydride, which hydrolyses to phthalic acid, is not carcinogenic in mice or rats, with NOAELs equal to or above 748 mg/kg bw per day. The Meeting concluded that phthalic acid is not a toxicologically relevant metabolite of picoxystrobin.

- 2-(2-Formylphenyl)-2-oxoacetic acid is not supported by any toxicological studies, but a structural alert for genotoxicity was identified. The IEDI was above 0.15 µg/person per day, the TTC for a compound with a structural alert for genotoxicity. The Meeting was unable to conclude on the toxicological relevance of estimated intakes of 2-(2-formylphenyl)-2-oxoacetic acid.
- 2-(2-Hydroxymethylphenyl)-2-oxoacetic acid (PAG3) is not supported by any toxicological studies, and no structural alerts for genotoxicity were identified. The estimated IEDI for PAG3 was 16.4 µg/person per day, below the applicable TTC of 90 µg/person per day. The Meeting concluded that PAG3 was not of toxicological concern at the estimated dietary intake levels.

Medical surveillance of production plant workers has not identified any cases of occupational illness related to picoxystrobin. There are no reports of poisoning cases with picoxystrobin.

The Meeting concluded that the existing database on picoxystrobin was adequate to characterize the potential hazards to 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 overall NOAEL of 8.5 mg/kg bw per day in the 90-day and 1-year dog studies, based on body weight loss, reduced feed consumption and clinical signs at 16 mg/kg bw per day. A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) of 0.09 mg/kg bw on the basis of the overall NOAEL of 8.5 mg/kg bw per day in the 90-day and 1-year dog studies, based on body weight loss and reduced feed consumption at the beginning of the study at 16 mg/kg bw per day. A safety factor of 100 was applied. This value is supported by a BMD analysis of the motor activity changes seen at the lowest dose in the acute neurotoxicity study. The Meeting noted that this ARfD is possibly conservative and that it might be possible to refine it.

Picoxystrobin's mode of fungicidal activity is to block mitochondrial electron transport, reducing ATP production and inhibiting cellular respiration; this could result in impaired body weight gains. In the absence of any information on the mode of toxicological action for reductions in body weight and body weight gain, these effects were considered as adverse and relevant for the setting of guidance values.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month studies of toxicity and carcinogenicity[a,b]	Toxicity	600 ppm, equal to 71 mg/kg bw per day	2400 ppm, equal to 293 mg/kg bw per day
		Carcinogenicity	4800 ppm, equal to 585 mg/kg bw per day[c]	—

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity study[d]	Toxicity	—	200 mg/kg bw per day[e]
	Ninety-day study of toxicity[a]	Toxicity	500 ppm, equal to 42 mg/kg bw per day	1250 ppm, equal to 105 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	1000 ppm, equal to 45 mg/kg bw per day	3500 ppm, equal to 162 mg/kg bw per day
		Carcinogenicity	3500 ppm, equal to 162 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	2500 ppm, equal to 130 mg/kg bw per day[c]	—
		Parental toxicity	1000 ppm, equal to 52 mg/kg bw per day	2500 ppm, equal to 130 mg/kg bw per day
		Offspring toxicity	1000 ppm, equal to 52 mg/kg bw per day	2500 ppm, equal to 130 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[a,b]	Toxicity	250 ppm, equal to 8.5 mg/kg bw per day	500 ppm, equal to 16 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.09 mg/kg bw

Estimate of acute reference dose

0.09 mg/kg bw

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

Additional data on the genotoxicity of the plant metabolites IN-H8612 and 2-(2-formylphenyl)-2-oxoacetic acid

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	75% in 48 h, based on urinary and biliary excretion
Distribution	Extensive; highest levels in liver and kidneys
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	> 95% within 5 days (low dose); mainly bile and faeces
Metabolism in animals	Extensive, with over 30 identified metabolites
Toxicologically significant compounds in animals, plants and the environment	Picoxystrobin, IN-H8612 and 2-(2-formylphenyl)-2-oxoacetic acid (plant metabolites) and IN-QDY63 (soil metabolite)
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	0.11 mg/l (4 h, nose-only)
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Moderately irritating
Dermal sensitization	Not a sensitizer (Magnusson & Kligman test in guinea-pigs)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight and feed consumption; clinical signs (dog)
Lowest relevant oral NOAEL	8.5 mg/kg bw per day (90-day and 1-year dog combined)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day rat)
Lowest relevant inhalation NOAEC	0.025 mg/l (28-day rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight and feed consumption; liver (mouse); testes, interstitial cell hyperplasia and adenoma (rat)
Lowest relevant NOAEL	45 mg/kg bw per day (2-year rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	
Reproduction target/critical effect	Reduced body weight gain in parents and pups
Lowest relevant reproductive NOAEL	130 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	52 mg/kg bw per day
Lowest relevant offspring NOAEL	52 mg/kg bw per day
Developmental toxicity	
Developmental target/critical effect	Reduced maternal and fetal body weight; skeletal anomalies
Lowest relevant maternal NOAEL	25 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	25 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity	Reduced motor activity, reduced body weight: LOAEL 200 mg/kg bw (rat)

Subchronic (90-day) neurotoxicity	Not neurotoxic: NOAEL 207 mg/kg bw per day (highest dose tested) (rat)
Immunotoxicity	
Twenty-eight-day study	Not immunotoxic in mice or rats: NOAEL 229 mg/kg bw per day (highest dose tested) (rats)
Other toxicological studies	
Metabolite IN-H8612	Oral LD_{50} > 2000 mg/kg bw (rat) 28-day toxicity study in rats: NOAEL 182 mg/kg bw per day Not mutagenic in bacteria; equivocal results for chromosomal aberrations in vitro
Metabolite IN-QDY63	Oral LD_{50} 387 mg/kg bw (rat) 90-day toxicity study in rats: NOAEL 14 mg/kg bw per day Not mutagenic in bacteria
Medical data	
	No reports of poisonings or adverse effects in production plant workers

Summary

	Value	Study	Safety factor
ADI	0–0.09 mg/kg bw	90-day and 1-year studies (dog)	100
ARfD	0.09 mg/kg bw	90-day and 1-year studies (dog)	100

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