TOLFENPYRAD

First draft prepared by
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Explanation

Tolfenpyrad, a pyrazole insecticide, is the International Organization for Standardization–approved name for 4-chloro-3-ethyl-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide (International Union of Pure and Applied Chemistry), which has the Chemical Abstracts Service number 129558-76-5. Tolfenpyrad has broad insecticidal activity against a variety of pests on egg, larval, nymphal and adult stages and is used on a variety of crops. The pesticidal mode of action is thought to be the inhibition of complex I of the respiratory electron transport chain in the mitochondria.

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

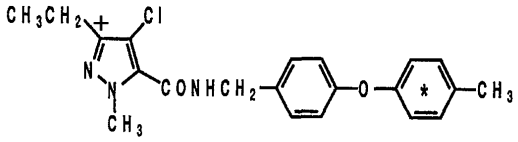
All critical studies contained statements of compliance with good laboratory practice (GLP) unless otherwise specified.

Evaluation for acceptable daily intake

1. Biochemical aspects

The structure of tolfenpyrad and the positions of the labels used in the study of Okada (1998) are shown in Fig. 1.

Fig. 1. Structure of tolfenpyrad



*: position of ^{14}C label in the tolyl ring ([TO-^{14}C]tolfenpyrad) in study of Okada (1998)

+: position of ^{14}C label in the pyrazole group ([PY-^{14}C]tolfenpyrad) in study of Okada (1998)

1.1 Absorption, distribution and excretion

Rats

The absorption, distribution and excretion of [^{14}C]tolfenpyrad, labelled at either the tolyl ring ([TO-^{14}C]tolfenpyrad, radiochemical purity 99.5%) or the 3 position of the pyrazole group ([PY-^{14}C]tolfenpyrad, radiochemical purity 99.7%), were studied in groups of male and female F344 rats dosed orally by gavage at a single dose of 1 or 20 mg/kg body weight (bw). In addition, rats were administered either [PY-^{14}C]tolfenpyrad (five males and five females) or [TO-^{14}C]tolfenpyrad (five males) at a dose of 1 mg/kg bw per day for 14 consecutive days (14 radiolabelled doses). These materials were dissolved in 0.5% weight per volume (w/v) sodium carboxymethyl cellulose and 0.5% w/v Tween 80. The identification of tolfenpyrad and its metabolites in tissues and excreta is described in section 1.2 (Ogawa, 1999a). The experimental designs are presented in Table 1.

After the administration of single doses, similar toxicokinetic parameters for radioactivity in blood were observed with both labels at the two dose levels, indicating that no difference in the disposition of tolfenpyrad was attributable to radiolabel positions (Table 2). The time to reach the maximum concentration in plasma (T_{max}) was higher after a high dose than after a low dose. At least 58% of the 1 and 20 mg/kg bw doses were absorbed. Seven days after administration, 88–93% of the radioactivity was excreted in faeces, and 2–3% of the radioactivity was excreted in urine, independent of dose or sex. No radioactivity was found in expired air. Forty-eight hours after dosing, biliary excretion was 64–70% in males and 51–55% in females. At 48 hours, up to 3% of radioactivity was excreted in urine, 5–11% remained in the carcass and 13–37% remained in the gastrointestinal tract. Radioactivity was widely distributed to the tissues, the highest levels being found in liver, kidney and brown fat 4–12 hours after dosing (Table 3). Tissue levels were similar between males and females, except for brown fat, in which concentrations were about 2 times higher in females than in males. In kidney and brown fat, highest levels were observed 12 hours after dosing. Concentrations of radioactivity decreased rapidly, and by 168 hours, only very low levels of radioactivity were observed in tissues (Okada, 1998).

After the administration of 14 daily doses, the absorption, distribution and excretion of the labelled materials were investigated for up to 168 hours. Different groups of rats were used for determination of radioactivity levels in blood, urine, faeces and tissues. Urine and faeces were collected over 24-hour intervals after the 1st to 13th administrations and at 24, 48, 72, 120 and 168 hours after the 14th administration. Blood was collected at 24 hours after each daily dose, then at the following time points after the 14th dose: 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 120 and 168 hours. Samples of an extensive range of tissues were taken from groups of rats at 4, 12 and 168 hours after the 14th

dose. The identification of parent compound and metabolites in tissues and excreta is described in section 1.2 (Ogawa, 1999b).

The concentration of radioactivity in plasma samples tended to reach a maximum after two or three administrations, at 1.5–3 times the plasma concentration after the first dose. Plasma concentrations in females were about 2 times higher than those in males at all time points. The plasma concentrations reached the maximum level at 8–12 hours after the final administration and thereafter demonstrated biphasic elimination. Calculations based on the second phase showed a relatively slow decrease in plasma concentration, with half-lives of 20–46 hours. Tissue concentrations were highest in liver, kidney, bone marrow and brown fat (Table 4, levels in other tissues are not presented). The decline in radioactivity level in bone marrow at 168 hours appeared to be slower than in other tissues. The tissue distribution after repeated administration tended to be similar to that after a single administration. Urinary and faecal excretions of radioactivity were comparable and almost as complete as with a single administration. Total excretion exceeded 95% 168 hours after the final administration in all treatment groups, suggesting that tolfenpyrad exhibits no residuality in rats.

Table 1. Study designs for absorption, distribution and excretion in rats

Test group	Labelled compound	Dose (mg/kg bw)	Sex	Number of rats	Time post-dosing (h)
Group 1 Measurement of blood concentrations	[PY-^{14}C]Tolfenpyrad	1	Male	5	0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 120 and 168
			Female	5	
		20	Male	5	
			Female	5	
	[TO-^{14}C]Tolfenpyrad	1	Male	5	
			Female	5	
		20	Male	5	
			Female	5	
Group 2 Measurement of excretion rates into urine, faeces and expired air	[PY-^{14}C]Tolfenpyrad	1	Male	5	0–24, 24–48, 48–72, 72–120 and 120–168
			Female	5	
		20	Male	5	
			Female	5	
	[TO-^{14}C]Tolfenpyrad	1	Male	5	
			Female	5	
Group 3 Measurement of excretion rates into bile, urine and faeces	[PY-^{14}C]Tolfenpyrad	1	Male	4	Bile: 3, 6, 12, 24 and 48
			Female	4	
		20	Male	4	Urine and faeces: 0–24 and 24–48
			Female	4	
Group 4 Measurement of tissue concentrations and distribution rates	[PY-^{14}C]Tolfenpyrad	1	Male	5	4, 12 and 168
			Female	5	4, 12 and 168
		20	Male	5	6, 24 and 168
			Female	5	6, 24 and 168
	[TO-^{14}C]Tolfenpyrad	1	Male	5	4, 12 and 168
			Female	5	4, 12 and 168

Source: Okada (1998)

Table 2. Toxicokinetic parameters after a single oral administration of radiolabelled tolfenpyrad to rats

	1 mg/kg bw		20 mg/kg bw	
	Males	Females	Males	Females
[PY-^{14}C]Tolfenpyrad				
C_{max} (µg eq/mL)	0.304	0.253	1.93	2.23
T_{max} (h)	2–4	6–8	8	12
$AUC_{0-\infty}$ (µg·h/mL)	3.1	2.8	44.5	52.4
$t_{½}$ (h)	16.4	27.6	16.3	14.2
[TO-^{14}C]Tolfenpyrad				
C_{max} (µg eq/mL)	0.268	0.284	2.22	2.37
T_{max} (h)	2–4	4	6–8	4
$AUC_{0-\infty}$ (µg·h/mL)	3.0	3.4	62.7	70.8
$t_{½}$ (h)	12.1	11.0	12.6	11.5

AUC: area under the plasma–concentration time curve; C_{max}: maximum concentration; eq: equivalents; $t_{½}$: half-life; T_{max}: time to reach C_{max}
Source: Okada (1998)

Table 3. Tissue concentrations after a single oral dose of tolfenpyrad administered to rats

Organ or tissue	Dose; ^{14}C label	Tissue concentration (mg eq/kg)			
		Males		Females	
		4 h (1 mg/kg bw); 6 h (20 mg/kg bw)	12 h (1 and 20 mg/kg bw)	4 h (1 mg/kg bw); 6 h (20 mg/kg bw)	12 h (1 and 20 mg/kg bw)
Liver	1 mg/kg bw; PY	5.40 ± 0.633	4.72 ± 0.465	5.70 ± 0.656	6.24 ± 0.564
	1 mg/kg bw; TO	5.56 ± 0.314	5.11 ± 0.477	5.74 ± 0.555	6.23 ± 0.835
	20 mg/kg bw; PY	18.6 ± 0.650	19.8 ± 4.95	20.0 ± 1.11	24.1 ± 3.99
Kidney	1 mg/kg bw; PY	1.35 ± 0.192	2.06 ± 0.209	1.38 ± 0.063	2.08 ± 0.486
	1 mg/kg bw; TO	1.65 ± 0.144	2.25 ± 0.163	1.41 ± 0.058	1.83 ± 0.261
	20 mg/kg bw; PY	4.88 ± 0.185	4.26 ± 0.490	4.95 ± 0.207	4.27 ± 0.741
Brown fat	1 mg/kg bw; PY	1.01 ± 0.085	1.73 ± 0.486	1.11 ± 0.089	4.29 ± 0.375
	1 mg/kg bw; TO	0.928 ± 0.099	2.14 ± 0.552	1.39 ± 0.244	5.44 ± 1.10
	20 mg/kg bw; PY	3.12 ± 0.398	20.6 ± 6.46	5.17 ± 1.46	34.6 ± 2.80

Source: Okada (1998)

The toxicokinetics of tolfenpyrad in rats after repeated administration was similar to that after a single administration. Although the declines in organ/tissue concentrations of radioactivity were slightly slower compared with those after a single administration, tolfenpyrad exhibited neither accumulation nor residuality in rats (Okada, 1999).

1.2 Biotransformation

(a) In vivo

Rats

The metabolism of tolfenpyrad ^{14}C-radiolabelled in either the pyrazole or tolyl ring was studied in male and female F344 rats after a single oral gavage administration of 1 or 20 mg/kg bw and after 14 daily oral gavage administrations of 1 mg/kg bw. The levels of tolfenpyrad and its

Table 4. Tissue concentrations after 14 daily oral doses of tolfenpyrad administered to rats

Organ or tissue	Dose; position of label	Tissue concentration (mg eq/kg)					
		Males			Females		
		4 h	12 h	168 h	4 h	12 h	168 h
Liver	1 mg/kg bw; PY	7.75 ± 0.45	7.77 ± 0.61	0.263 ± 0.027	10.6 ± 1.2	11.3 ± 0.3	0.336 ± 0.161
	1 mg/kg bw; TO	10.1 ± 1.3	8.88 ± 0.55	0.275 ± 0.079	–	–	–
Kidney	1 mg/kg bw; PY	2.54 ± 0.13	2.98 ± 0.24	0.187 ± 0.015	2.24 ± 0.19	2.88 ± 0.33	0.162 ± 0.038
	1 mg/kg bw; TO	3.43 ± 0.25	3.55 ± 0.3	0.210 ± 0.059	–	–	–
Brown fat	1 mg/kg bw; PY	3.62 ± 0.94	3.01 ± 0.42	0.278 ± 0.52	6.80 ± 1.81	7.27 ± 1.25	0.178 ± 0.069
	1 mg/kg bw; TO	3.24 ± 0.53	3.02 ± 0.07	0.273 ± 0.067	–	–	–
Bone marrow	1 mg/kg bw; PY	1.18 ± 0.32	1.48 ± 0.24	0.76 ± 0.21	2.94 ± 0.47	3.06 ± 0.45	1.20 ± 0.41
	1 mg/kg bw; TO	1.49 ± 0.23	1.28 ± 0.16	0.63 ± 0.12	–	–	–

Source: Okada (1998)

metabolites were determined in plasma, urine, faeces and bile excreted over 48 hours after dosing. The rats were dosed as part of the study by Okada (1998) described in section 1.1.

Faeces and bile were extracted with methanol, after which samples were digested with glucuronidase and sulfatase preliminary to conjugate analysis. Radioactivity in samples was determined by liquid scintillation counting and radioisotope high-performance liquid chromatography (HPLC), and metabolites were identified by co-chromatography against authentic reference standards. The full chemical names of the metabolites described below can be found in the legend of Fig. 2 below.

Within 48 hours after single administration, 4–15% of the dose was eliminated in faeces as tolfenpyrad, but the largest proportion (24–49%) of the administered dose found in faecal extracts was PT-CA (see Table 5). Smaller fractions found in faecal extracts were Sul-OH-PT-CA (5–12%) and OH-PT-CA (6–13%). Minor amounts of metabolites were not identified. In plasma, liver and kidney, 91–100% of radioactivity represented PT-CA, whereas maximally 3% of radioactivity represented other, unidentified metabolites These data suggest a rapid metabolism of tolfenpyrad in the liver. In bile, 50–67% of the administered dose was excreted within 48 hours (see Table 6), the major part as PT-CA-TA, PT-CA-Gluc and PT-CA (31–43%; these three metabolites could not be further separated). Low levels of Sul-OH-PT-CA (5–8%) and CO-PT (4–6%) and several unidentified metabolites were detected. These data indicate a rapid conjugation of PT-CA in the liver and subsequent excretion into the bile. Less than 0.7% of the administered dose was present as unchanged tolfenpyrad in bile. In bile duct–cannulated rats, only 3–8% of the administered dose was excreted into faeces, predominantly as tolfenpyrad (up to 6%) and PT-CA (up to 1%). The data from the studies in unoperated rats and the bile duct–cannulated rats indicate that following biliary excretion into the gastrointestinal tract, PT-CA is deconjugated, probably by enterobacteria, and excreted in faeces. In urine, no intact tolfenpyrad was detected. Various metabolites (among others, OH-PAM and CA-T-CA) were present at levels less than 0.5% of the administered dose, with the exception of PT-CA and PT-CA-TA (these metabolites could not be further separated), which were present at up to 1.9% of the administered dose. The urinary metabolite profiles were different between the groups of

rats administered [PY-^{14}C]tolfenpyrad and [TO-^{14}C]tolfenpyrad. The complicated metabolite profile in the urine indicates that metabolism of tolfenpyrad includes cleavage of the C–N bond of the benzylamine moiety and oxidation of the side-chains of both of the ^{14}C-labelled rings. In summary, the data indicate that after absorption in the gastrointestinal tract, tolfenpyrad is rapidly metabolized to PT-CA in the liver, which is subsequently polarized by conjugation and then excreted into bile. Once back in the gastrointestinal tract, it is deconjugated and thus appears in faeces as PT-CA. Observed differences in metabolite levels between sexes, doses and position of radiolabel were minor.

Table 5. Amount of tolfenpyrad and its metabolites in faeces of rats

Identification	% of administered dose											
	1 mg/kg bw [PY-^{14}C]				20 mg/kg bw [PY-^{14}C]				1 mg/kg bw [TO-^{14}C]			
	0–24 h		0–48 h		0–24 h		0–48 h		0–24 h		0–48 h	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Extracted	61.5	55.5	77.3	75.3	27.9	24.2	62.7	50.6	56.5	50.0	75	73.3
Sul-OH-PT-CA	7.4	5.0	9.0	8.2	2.4	1.5	10.5	5.3	9.2	8.0	11.5	8.1
OH-PT-CA	6.4	4.8	8.8	8.9	1.5	1.8	7.9	6.4	6.2	7.4	9.0	12.9
PT-CA	36.2	30.0	47.7	41.6	11.1	9.0	29.0	23.9	35.6	28.0	48.9	45.2
Tolfenpyrad	10.6	14.8	10.9	15.1	12.5	11.7	14.3	14.7	4.1	5.6	–	0.3
Total of unidentified	0.8	0.9	0.8	1.4	0.4	0.1	0.6	0.3	1.5	1.0	1.5	1.2
Unextracted	3.2	4.1	2.3	3.5	1.3	1.1	5.1	2.8	6.1	5.8	2.0	3.2

Source: Ogawa (1999a)

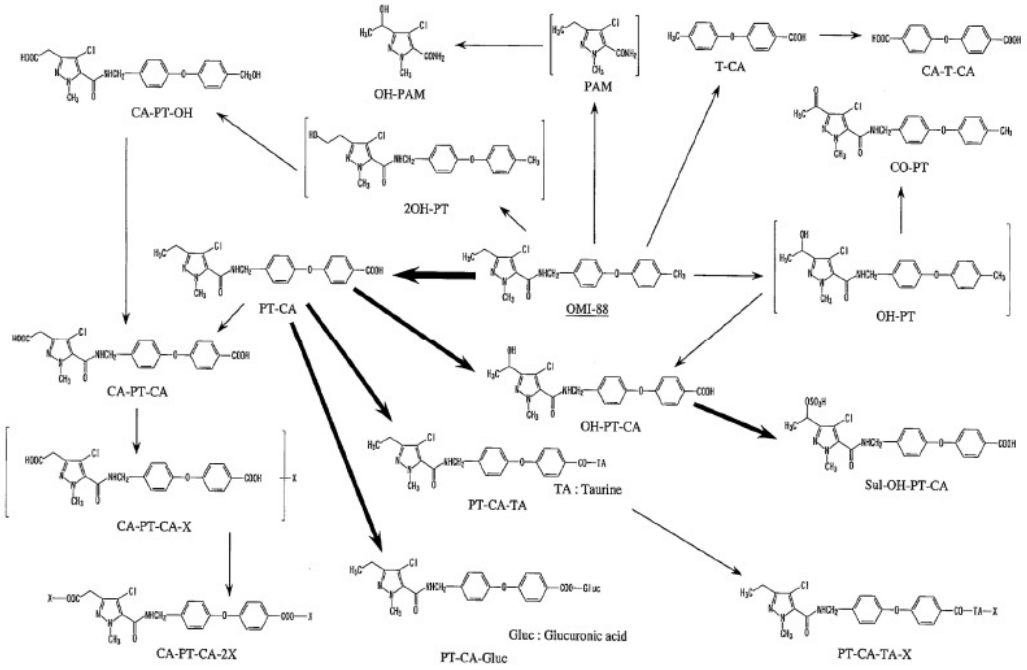
Table 6. Amount of tolfenpyrad and its metabolites in bile of rats

Identification	% of administered dose							
	1 mg/kg [PY-^{14}C]				20 mg/kg [PY-^{14}C]			
	0–24 h		0–48 h		0–24 h		0–48 h	
	Males	Females	Males	Females	Males	Females	Males	Females
Extracted	29.9	26.4	61.6	52.8	35.7	22.6	67.4	50.0
Sul-OH-PT-CA	3.0	2.8	5.9	4.7	4.4	2.6	7.7	5.4
OH-PT-CA	0.2	0.8	0.8	0.8	1.1	0.7	2.0	1.5
PT-CA-TA PT-CA-Gluc PT-CA	20.6	14.3	42.9	34.1	19.0	13.1	41.2	31.3
CO-PT	2.0	4.2	3.7	5.0	5.6	3.1	6.4	5.9
Tolfenpyrad	–	0.3	–	0.3	0.5	0.5	0.5	0.7
Total of unidentified	4.9	3.9	9.0	7.0	5.1	2.1	8.5	5.3
Unextracted	0.8	1.0	2.0	1.9	1.2	0.6	2.1	1.3

Source: Ogawa (1999a)

The proposed metabolic pathway for tolfenpyrad is presented in Fig. 2 (Ogawa, 1999a).

Fig. 2. Proposed metabolic pathway in rats



OMI-88	Tolfenpyrad
OH-PT	4-Chloro-3-(1-hydroxyethyl)-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
CO-PT	3-Acetyl-4-chloro-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
PT-CA	4-[4-[(4-Chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
CA-PT-OH	[4-Chloro-5-[*N*-[4-(4-hydroxymethyl)phenoxy]benzylcarbamoyl]-1-methylpyrazol-3-yl]acetic acid
OH-PT-CA	4-[4-[(4-Chloro-3-(1-hydroxyethyl)-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
CA-PT-CA	4-[4-[(3-Carboxymethyl-4-chloro-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
2OH-PT	4-Chloro-3-(2-hydroxyethyl)-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
T-CA	4-(*p*-Tolyloxy)benzoic acid
CA-T-CA	4,4′-Oxydibenzoic acid
PAM	4-Chloro-3-ethyl-1-methylpyrazole-5-carboxamide
OH-PAM	4-Chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide
Sul-OH-PT-CA	4-[4-[(4-Chloro-1-methyl-3-(1-sulfoxyethyl)pyrazol-5-yl)carbonylarninomethyl]phenoxy]benzoic acid
PT-CA-TA	2-[4-[4-[(4-Chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]phenylcarbonylamino]-ethane-1-sulfonic acid
PT-CA-Gluc	Glucuronide conjugate of 4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]-benzoic acid
X	Biological component

(b) In vitro

In an in vitro metabolism study, unlabelled tolfenpyrad (1.0 mg, purity 99.8%), [TO-^{14}C]tolfenpyrad (0.1 mg, radiochemical purity 99.8%) or [PY-^{14}C]tolfenpyrad (0.1 or 1.0 mg, radiochemical purity 99.3%) was incubated with a 9000 × *g* supernatant fraction from a rat liver homogenate (S9). Metabolites of tolfenpyrad were identified by gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), hydrogen nuclear magnetic resonance (^{1}H-NMR) and HPLC.

Without S9, no significant degradation of tolfenpyrad was observed. After incubation with S9, 5–6% of tolfenpyrad was cleaved between the amide of the pyrazole ring and the methylene bond of the tolyl ring. There was little difference in the metabolite profiles between the labels or substrate amounts (0.1 and l mg). From the metabolite investigation by GC-MS, LC-MS, ^{1}H-NMR and HPLC, unchanged tolfenpyrad made up about 10% of the total substrate; identified or tentatively identified

metabolites made up about 88%, with 2% remaining unidentified. The major metabolic pathways of tolfenpyrad in rat liver S9 were attributed to the ω-1 oxidation (hydroxylation and carbonylation) of the ethyl group of the pyrazole ring and the oxidation of the methyl group of the tolyloxybenzyl group (hydroxylation and carbonylation). Other metabolic pathways included cleavage between the amide and methylene moiety, the demethylation of N-CH_3 and the conversion of the ethyl group of the pyrazole ring to a vinyl group (Ogawa, 1998).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with tolfenpyrad are summarized in Table 7.

Table 7. Results of studies of acute toxicity with tolfenpyrad

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw) / LC_{50} (mg/L)	Reference
Mouse	Crl:CD-1(ICR)BR	M/F	Oral	Aqueous carboxymethyl cellulose	99.33	114 (M) 117 (F)	Glaza (1997a)[c]
Rat	Crl:CD(SD)BR	M/F	Oral	Aqueous carboxymethyl cellulose	99.33	386 (M) 150 (F)	Glaza (1997b)[b]
Rat	Crj:CD(SD) IGS	M/F	Oral	Aqueous carboxymethyl cellulose	99.83	260 (M) 113 (F)	Ishii (2000a)[c]
Rat	Crj:CD(SD) IGS	M/F	Oral	Olive oil	99.83	86 (M) 75 (F)	Ishii (2000b)[d]
Rat	Crl:CD®(SD)BR	M/F	Dermal	Distilled water	99.33	> 2 000 (M) > 3 000 (F)	Glaza (1997c)[e]
Rat	Crl:CD®(SD)BR	M/F	Inhalation	–	99.8	2.21 mg/L (M) 1.50 mg/L (F)	Wesson (2000)[f]

F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male

[a] Lot no. 6D-01-2. Performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 401. The mice were given doses of 25–250 mg/kg bw. Mortality was observed at doses of 100 mg/kg bw and higher. No clinical signs were observed at 25 mg/kg bw. Two males and two females treated at 50 mg/kg bw showed hypoactivity and/or staggered gait. In addition, at higher doses, prostration, clonic convulsions, dyspnoea, thin appearance, hunched posture, hypothermic to touch and tremors were observed.

[b] Lot no. 6D-01-2. Performed according to OECD Test Guideline 401. Males were given doses of 100–750 mg/kg bw, and females were given doses of 50–250 mg/kg bw. Mortality was observed at doses of 75 mg/kg bw and higher. Clinical signs of toxicity were observed at all dose levels and included thin appearance, hypoactivity, staggered gait, hunched posture, rough haircoat, red-stained face, soft stool, few faeces and wet and/or yellow- or dark-stained urogenital area. At dose levels of 150 mg/kg bw and higher, weight losses during the 1st week were noted in the majority of the animals. At necropsy, gastric lesions were observed in three males given 500 mg/kg bw and in one female given 250 mg/kg bw.

[c] Lot no. 9L-03. Performed according to OECD Test Guideline 401. The rats were given doses of 40–640 mg/kg bw. Mortality and clinical signs of toxicity were observed at dose levels of 80 mg/kg bw and higher. Clinical signs included decrease in locomotor activity, diarrhoea, soiled perineal region, emaciation, hunchback position, lateral position, prone position or supine position and irregular respiration or bradypnoea. Decreases in body weights were observed in one male in the 40 mg/kg bw group and in all males and females in the 80 mg/kg bw group and higher on day 4. No abnormalities were observed in necropsy of animals that

survived. In animals that died, necropsy revealed haemorrhage in the glandular stomach, white raised patches in the forestomach, small thymus and small spleen.

[d] Lot no. 9L-03. Performed according to OECD Test Guideline 401. The rats were given doses of 20–320 mg/kg bw. Mortality was observed at doses of 80 mg/kg bw and higher. Diarrhoea and soiled perineal region were observed at all dose levels. At higher doses, decrease in locomotor activity, prone position, hunchback position, lateral position, ataxic gait, irregular respiration or dyspnoea, tonic or clonic convulsion and salivation were observed. Necropsy revealed no abnormalities.

[e] Lot no. 6D-01-2. Performed according to OECD Test Guideline 402. Male rats were given a dose of 2000 mg/kg bw, and female rats were given doses of 1000–3000 mg/kg bw. Two females at 2000 mg/kg bw were killed in moribund condition, one on day 8 and one on day 11. At all three dose levels, clinical signs of toxicity were observed, primarily between days 4 and 10, and included hypoactivity, staggered gait, hunched posture, red-stained face, thin appearance, decreased feed consumption, yellow-stained urogenital area, few faeces, soft stool and hypothermia. Necropsy revealed no visible lesions. No dermal irritation was observed.

[f] Lot no. 9L-03. Performed according to OECD Test Guideline 403. The rats were exposed to concentrations of 0.95–2.07 mg/L. In males, mortality was observed at the highest concentration. In females, mortality was observed at all concentrations. Clinical signs were observed at all concentrations and included increased or decreased respiratory rate, laboured and/or noisy respiration, wet fur, hunched posture, piloerection, pallor of the extremities, ataxia, lethargy, tiptoe gait, ptosis, red/brown staining around the snout or eyes, cyanosis, hypothermia and coma. The mass median aerodynamic diameter was 4.1–4.9 µm.

(b) Dermal irritation

In an acute dermal irritation study, performed in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline 404, the intact skin of six female Hra:(NZW)SPF rabbits was exposed for 4 hours under semi-occlusion to 0.5 g tolfenpyrad (purity 99.33%; lot no. 6D-01-2) moistened with 0.5 mL of distilled water. Dermal irritation was scored according to the Draize system at 0.5, 24, 48 and 72 hours after patch removal.

Very slight erythema was observed in two rabbits after patch removal. No other signs of dermal irritation were observed. All signs of irritation had cleared at 72 hours after patch removal. Tolfenpyrad was considered not irritating to rabbit skin (Glaza, 1996a).

(c) Ocular irritation

In an acute eye irritation study, performed according to OECD Test Guideline 405, 0.041 g (0.1 mL weight equivalent) of tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was instilled into the conjunctival sac of the right eye of nine male Hra:(NZW)SPF rabbits. The untreated left eye served as a control. In three rabbits, the treated eyes were washed 30 seconds after instillation; in the remaining six rabbits, the treated eyes were not washed. The eyes were macroscopically examined for signs of irritation according to the Draize system at 1, 24, 48, 72 and 96 hours and at days 7 and 14 post-instillation.

In all six animals with unwashed eyes, mild irritation of the conjunctivae (redness, chemosis) was observed, which cleared in all animals by day 14. In one of these rabbits, iritis was observed 1 hour after instillation. In the three rabbits with treated eyes receiving a washout, the test material produced slight to moderate conjunctival irritation (redness, chemosis), which cleared by 96 hours after treatment. Tolfenpyrad was slightly irritating to the eye of rabbits (Glaza, 1996b).

(d) Dermal sensitization

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline 406, tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was tested in 20 female Hartley strain SPF guinea-pigs. A preliminary study established 1% and 5% test substance concentrations in olive oil as suitable for the intradermal induction and topical dermal induction phases, respectively. A 1% topical dermal application was used for the challenge phase. The control group consisted of 10 animals. 2,4-Dinitrochlorobenzene was used as a positive control.

After the dermal challenge treatment on day 22, no signs of irritation were observed in the control and the treated groups 24 and 48 hours after the removal of the occlusive bandage. None of the 20 guinea-pigs in the treated group showed a positive skin response after the challenge procedure. Under the conditions of this study, tolfenpyrad was not a skin sensitizer (Shibata, 1997).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 4-week dietary range-finding study, tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was administered to groups of 10 male and 10 female Crl:CD-1 mice at 0, 30, 100, 300 or 600 parts per million (ppm) (equal to 0, 5.3, 17.5, 51 and 104 mg/kg bw per day for males and 0, 6.5, 21.8, 67 and 126 mg/kg bw per day for females, respectively). Mice were observed daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. All mice underwent complete necropsy. Liver and gallbladder were weighed.

In mice treated at 600 ppm, one female was found dead and one male and one female were killed in a moribund condition on day 6. At 600 ppm, rough haircoat, hunched posture, ataxia, hypoactivity, animal cold to the touch, few faeces and/or a thin appearance were observed. No mortality or clinical signs were observed at lower doses. At 600 ppm, males and females showed a body weight loss of 10–12%, whereas control mice gained 4–5% during this period. During the remaining 3 weeks of the study, the high-dose animals did gain body weight. However, terminal body weights of both sexes at 600 ppm were statistically significantly lower (16–17%) than those of controls. Total feed consumption in high-dose mice was reduced by 22–27% compared with controls. Body weight gains and feed consumption of the other dose groups were not affected by treatment. Relative liver weights were statistically significantly higher at 300 ppm (12–16%) and 600 ppm (33–35%) than those in controls. Necropsy revealed no toxicologically relevant gross findings (Trutter, 1999a).

Tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was administered via the diet to groups of 10 male and 10 female CD-1 mice for 13 weeks at a dietary level of 0, 15, 100 or 300 ppm (equal to 0, 2.4, 15.9 and 46.2 mg/kg bw per day for males and 0, 3.0, 20.2 and 57.9 mg/kg bw per day for females, respectively). The mice were examined daily for mortality and clinical signs. A detailed clinical examination was performed weekly. Feed consumption and body weights were recorded weekly. Ophthalmological examinations were performed prior to and during week 13. Haematology, clinical chemistry and urine analysis were performed at the end of the treatment period. All mice were necropsied, and weights of brain with brainstem, heart, liver with gallbladder, kidneys, adrenals and testes with epididymides were recorded. A wide range of tissues of mice of the control and 300 ppm groups and the lung, liver, kidneys and gross lesions from mice of the 15 and 100 ppm groups were examined microscopically.

No mortalities or treatment-related clinical signs were observed. At the end of the study, there was a 7% reduction in body weight gain in males of the 300 ppm group, which was not statistically significant and probably the result of an 8% lower feed consumption. No toxicologically significant effects were observed in ophthalmology or haematology. Aspartate aminotransferase (ASAT) activity in the 300 ppm male mice was statistically significantly increased (86%). In males at 300 ppm, an increase in relative heart weight (16%) was found. Relative liver weight was increased (10–18%) in both sexes at 300 ppm. Necropsy and histological examination showed no treatment-related changes.

The NOAEL was 100 ppm (equal to 15.9 mg/kg bw per day), based on elevated ASAT activity and increased relative heart weight in males and increased relative liver weight in both sexes at 300 ppm (equal to 46.2 mg/kg bw per day) (Trutter, 1999b).

Rats

In a 13-week dietary toxicity study, tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was administered to groups of 10 male and 10 female F344/DuCrj rats at 0, 15, 80 or 160 ppm (equal to 0, 0.906, 4.78 and 9.33 mg/kg bw per day for males and 0, 1.01, 5.17 and 9.32 mg/kg bw per day for females, respectively). In addition, six male and six female F344/DuCrj rats were fed tolfenpyrad at a dietary concentration of 0 or 160 ppm followed by a 4-week recovery period. Animals were checked daily for clinical signs of toxicity. Body weights and feed consumption were measured weekly. Ophthalmological examinations were carried out on all rats before dosing and in control and 160 ppm rats at week 13. Blood was sampled for haematology and clinical biochemistry at termination. Urine was sampled during week 13 for analysis. All rats were necropsied, and a wide range of tissues of rats of the control and 160 ppm groups and the lung, liver and kidneys from rats of the 15 and 80 ppm groups were examined microscopically.

No deaths, clinical signs or ophthalomological changes were observed. Body weight gain was slightly reduced (6–7%) at 80 ppm and markedly reduced (22–24%) at 160 ppm. At 160 ppm, feed consumption was reduced by 12–16%. During the recovery period, feed consumption at 160 ppm was similar to that of controls, whereas body weights of the 160 ppm rats were 11–16% lower. Low white blood cell counts were observed at 80 ppm (−29%) and 160 ppm (−27%) in females. A lower result in males of the 160 ppm group of 14.4% was not statistically significant. After the 4-week recovery period, there was no reduction in white blood cell count in females of the 160 ppm group (+1.9%), whereas in males, the reduction of 14.9%, although essentially unchanged during this period, was statistically significant. Blood chemistry analysis revealed high glucose levels (+31% and +66%, respectively) and a slight reduction in total protein (−8% and −12%, respectively) in females treated at 80 and 160 ppm. In females at 160 ppm, an increase in gamma-glutamyltransferase (GGT, 0 International Units [IU]/L in controls, 0.3 IU/L at 160 ppm) was found. Blood urea nitrogen levels were increased in males at 80 ppm (25%) and in males and females at 160 ppm (39% and 13%, respectively). A reduced triglyceride level (48%) was found in males treated at 160 ppm. Triglyceride levels were slightly reduced (15%) in females at 160 ppm. Furthermore, increased inorganic phosphorus levels (14–19%) were observed in both sexes at 160 ppm, and increased potassium levels were observed in females treated at 80 ppm (26%) and in males and females treated at 160 ppm (27–29%). At necropsy, dark brown change of the liver and brown change of the Harderian gland were observed in males treated at 160 ppm and in females treated at 80 ppm and above. Furthermore, small seminal vesicles and prostate were observed in males treated at 160 ppm, and small ovaries, uteri and vaginas were seen in females treated at 160 ppm. Reduced absolute (−38%) and relative ovary weights (−20% compared with body weight, −35% compared with brain weight) were found in females treated at 160 ppm. At 80 and 160 ppm, compared with body weight, increased relative liver weights (14–15% and 24–25%, respectively) and kidney weights (9–10% and 18–24%, respectively) were observed in both sexes. Compared with brain weight, no changes in liver or kidney weights were observed. Slight, but statistically significant, increases in relative liver weight in males (5%) and relative kidney weight in females (5%) observed at 15 ppm were considered not toxicologically relevant. Histopathological findings included an increase in mast cells in the mesenteric lymph nodes, diffuse hypertrophy of the hepatocytes, hypertrophy of the pancreatic acinar cells and hypersecretion of the Harderian glands in both sexes at doses of 80 ppm and above. Hyaline droplets were observed in the proximal renal tubular epithelium of males treated at 160 ppm; a slightly increased incidence observed in males at 80 ppm was not considered toxicologically relevant. Hypertrophy of the proximal renal tubular epithelium was observed in females treated at 80 ppm and above. Hypertrophy of the acinar cells in the mandibular glands was observed in males treated at 160 ppm and in females treated at 80 ppm and above. Furthermore, a decrease in haematopoietic cells was observed in the marrow of the femur and sternum, as well as atrophy of the ovaries and uteri in females treated at 160 ppm. The observed effects on urea nitrogen, total protein, inorganic phosphorus, potassium and relative liver and kidney weights in females and changes in triglyceride levels and the mesenteric lymph nodes in males and females were still present at the end of the recovery period. The other observed changes partly or completely recovered after discontinuation of treatment.

The NOAEL was 15 ppm (equal to 0.906 mg/kg bw per day), based on increased blood urea nitrogen levels, a reduced white blood cell count, high blood glucose and potassium levels, dark brown change of the liver and brown change of the Harderian gland, hypertrophy of the proximal renal tubular epithelium, hypertrophy of the acinar cells in the mandibular glands in females and increase in mast cells in the mesenteric lymph nodes, diffuse hypertrophy of hepatocytes, hypertrophy of pancreatic acinar cells and hypersecretion of the Harderian glands in both sexes at 80 ppm (equal to 4.78 mg/kg bw per day) (Chida, 1999a).

Dogs

In a 28-day oral (capsule) toxicity study, tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was administered to groups of two male and two female Beagle dogs at 0, 1, 5 or 10 mg/kg bw per day. The dogs were checked daily for clinical signs of toxicity. Feed consumption was measured daily. Body weights were measured weekly. Blood and urine were sampled for haematology, clinical biochemistry and urine analysis 2 weeks before and 2 and 4 weeks after the start of treatment. All animals were necropsied, selected organs were weighed and a wide range of tissues was examined microscopically.

No mortality or morbidity occurred. Vomiting was repeatedly observed in two males and one female treated at 5 mg/kg bw per day and one male and two females treated at 10 mg/kg bw per day, but only once in one control male. Vomiting was already observed on the 1st day of dosing in these dogs, except for the control male and one female at 10 mg/kg bw per day. Increased incidences of mucoid, soft and watery faeces were observed in males and females at 10 mg/kg bw per day. One high-dose male displayed a decrease in body weight of 2.1 kg at the end of the treatment period. This dog showed a reduced feed consumption from week 3 onward, and at week 4, blood urea nitrogen and creatinine and protein levels in urine were increased. Histopathological examination of this dog revealed an increase in cytoplasmic eosinophilia in the hepatocytes, swelling of distal tubular epithelium and vacuolation in collecting tubular epithelium of the kidney. In the other animals, no changes in haematological, clinical chemistry and urine analysis parameters or gross pathology and histopathology were observed.

The NOAEL was 1 mg/kg bw per day, based on the increased incidence of vomiting at 5 mg/kg bw per day in both sexes (Nagashima, 1997a).

In a 90-day toxicity study, four male and four female Beagle dogs per dose group received capsules containing tolfenpyrad (purity 99.33%; lot no. 6D-01-2) at 0, 1, 5 or 10 mg/kg bw per day. Animals were checked daily for clinical signs. Feed consumption and body weights were measured weekly. Ophthalmological examinations were performed pretreatment and in weeks 6 and 12. Haematology, clinical chemistry and urine analysis were performed pretest and in weeks 7 and 13. All dogs were necropsied, and weights of brain, thyroid glands, heart, lungs, liver, adrenals, kidneys, spleen, testes and ovaries were recorded. Histology was performed on a large selection of organs from all dogs.

The incidence of vomiting was increased at 5 and 10 mg/kg bw per day. Soft or mucoid faeces were observed in one male treated at 5 mg/kg bw per day and one female treated at 10 mg/kg bw per day. These signs were already observed on the 1st or 2nd day of treatment. A decrease in urine volume was recorded in females of the 5 and 10 mg/kg bw per day groups in weeks 7 and 13. However, the urine volumes were within the range of background variation and not accompanied by histological or other changes. In addition, no changes in urine volume in males were observed. Therefore, the reduction in urine volume in females was not considered to be toxicologically significant. There were no treatment-related effects on body weight gain, feed consumption, haematological, biochemical, ophthalmological or pathological examinations or organ weights.

The NOAEL was 1 mg/kg bw per day, based on the increased incidence of vomiting at 5 mg/kg bw per day in both sexes (Nagashima, 1997b).

In a 90-day toxicity study, four male and four female Beagle dogs per dose group received capsules containing tolfenpyrad (purity 99.33%; lot no. 6D-01-2) at 0, 10, 30 or 100 mg/kg bw per day. Animals were checked daily for clinical signs. Feed consumption and body weights were measured weekly. Ophthalmological examinations were performed pretreatment and in weeks 6 and 12. Haematology and clinical chemistry were performed pretest and in weeks 4, 7 and 13. Urine analysis was performed pretest and in weeks 7 and 13. All dogs were necropsied, and weights of brain, thyroid glands, heart, lungs, liver, adrenals, kidneys, spleen, testes and ovaries were recorded. Histology was performed on a large selection of organs from all dogs.

Dose-dependent increased incidences of vomiting, soft faeces and mucoid faeces were observed in all treatment groups. One male at 30 mg/kg bw per day and all the dogs in the 100 mg/kg bw per day group died or were killed in extremis. In dogs that died, clinical signs included no faeces, emaciation, a decrease in spontaneous movement, staggering gait, hypothermia, prone position, lateral position and mydriasis. The dogs that were killed in extremis showed decreases in body weight and feed consumption as well as emaciation. In the surviving male dogs at 30 mg/kg bw per day, one dog showed a reduced body weight at the end of the study, but no other dogs at 30 or 10 mg/kg bw per day showed any significant changes in body weight gain. The feed consumption in the dogs reflected the body weight gains. No ophthalmological changes were observed. At 100 mg/kg bw per day, increases in erythrocyte count, haemoglobin levels and haematocrit were observed in one male and one female, and a decrease in leukocytes and an increase in segmented neutrophils were observed in one high-dose male dog. Clinical chemistry showed an increase in alanine aminotransferase (ALAT) in one male in the 30 mg/kg bw per day group and in one female in the 100 mg/kg bw per day group. Blood urea nitrogen was high in males in the 30 mg/kg bw per day group and in both sexes in the 100 mg/kg bw per day group. In the animals that were sacrificed in extremis in the 100 mg/kg bw per day group, an increase in creatinine was observed. Observed decreases in blood cholesterol, triglycerides and phospholipids were attributed to malnutrition.

Microscopic examinations revealed a dose-dependent increase in cytoplasmic eosinophilia and centrilobular vacuolation in hepatocytes at 30 mg/kg bw per day and above. These changes were considered to be treatment related. In the cerebrum and cerebellum, focal haemorrhage with degeneration was observed in one male and one female in the 100 mg/kg bw per day group that died or was killed in extremis. There were no reactive changes in the area of degeneration. Therefore, it was assumed that the lesions were relatively new and similar to focal changes induced by cardiovascular injury. Other changes observed in the 30 and 100 mg/kg bw per day groups included atrophy of seminiferous tubules in the testis, a decrease in sperm numbers and the presence of cell debris in the epididymis, atrophy of the prostate and ileal, mesenteric and submandibular lymph nodes, hypocellularity in the bone marrow (sternum, femur) and atrophy of glandular cells in the parotid and sublingual glands.

A NOAEL could not be established. Mild toxicity (i.e. vomiting, soft and mucoid faeces) was observed at 10 mg/kg bw per day. Severe toxicity, including mortality, was observed at doses of 30 mg/kg bw per day and above (Nagashima, 1999a).

In a 1-year toxicity study, four male and four female Beagle dogs per dose group received capsules containing tolfenpyrad (purity 99.3%; lot no. 6D-01-2) at 0, 1, 5 or 20/10 mg/kg bw per day. The initial top dose of 20 mg/kg bw per day was reduced to 10 mg/kg bw per day at week 5 due to a death on day 26 and decreased feed consumption or body weight loss in the other dogs of this dose group. Animals were examined daily for clinical signs of toxicity. Feed consumption was measured weekly. Body weights were recorded weekly for the first 14 weeks and once every 2 weeks thereafter. Ophthalmological examinations were performed pretreatment and in months 6 and 12. Haematology, clinical chemistry and urine analysis were performed pretest and in months 3, 6, 9 and 12. All dogs were necropsied, and weights of brain, pituitary, thyroid glands, heart, lungs, liver, adrenals, kidneys, spleen, testes, prostate, uterus and ovaries were recorded. Histology was performed on a large selection of organs from all dogs.

One female in the 20 mg/kg bw per day group died on day 26, and one male in the 20/10 mg/kg bw per day group died on day 83. These dogs exhibited body weight loss, low feed intake, salivation, no defecation, decrease in spontaneous movement, emaciation, hypothermia, lateral position, staggering gait and paleness of the mucosa (oral and conjunctival mucosa) prior to death. Dose-dependent increased incidences of vomiting, soft or mucoid stool and salivation were observed at 5 mg/kg bw per day and above. These signs were already observed after the 1st or 2nd day of treatment. Test article–like material was identified in the vomitus of some individuals. Body weight of one male treated with 20/10 mg/kg bw per day decreased from week 38 up to week 42 and was unchanged thereafter. In one female treated with 20/10 mg/kg bw per day, body weight decreased from week 4 up to week 7 and repeatedly increased and decreased from week 24. In another female of the same group, body weight exhibited repeated increases and decreases from week 24. Low feed intake values were recorded simultaneously with variations in body weight. Blood chemistry examination revealed an elevation of ALAT activity in one male treated at 5 mg/kg bw per day in months 9 (850%) and 12 (240%) and in one male treated with 20/10 mg/kg bw per day in month 12 (340%). Microscopic examination revealed increases of cytoplasmic eosinophilia in hepatocytes of two males and three females treated with 20/10 mg/kg bw per day. An increase in pigmentation in hepatocytes and Kupffer cells was observed in females treated at 20/10 mg/kg bw per day. There were no treatment-related effects observed in urine analysis, haematology, ophthalmology, gross pathology or organ weight.

The NOAEL was 1 mg/kg bw per day, based on increased incidences of vomiting, soft or mucoid stool and salivation and increased ALAT levels at 5 mg/kg bw per day (Nagashima, 1999b).

(b) Dermal application

Groups of 10 male and 10 female Sprague-Dawley rats were dermally exposed 6 hours/day for 21 days to tolfenpyrad (purity 99.5%; lot no. 365-65A) at a dose of 0, 10, 50 or 200 mg/kg bw per day. The rats were checked daily for clinical signs. A detailed examination was performed on days 8, 15 and 22. Body weights and feed consumption were recorded weekly. Ophthalmoscopy was performed pretreatment and at termination. Haematology and clinical chemistry were performed at the end of the study. At termination of the study, all animals were killed and necropsied. Weights of heart, spleen, brain, liver, adrenals, kidneys, testes, epididymides, ovaries, thymus and uterus were recorded. Histology was performed on a wide range of organs and tissues of the control and high-dose rats.

Minimal skin flaking was observed in all treatment groups, and slight erythema was observed at 50 and 200 mg/kg bw per day. No other treatment-related clinical signs were noted. Body weight gain was slightly (−7%), but statistically significantly, reduced in females at 50 and 200 mg/kg bw per day. Body weight gain in males was not affected. No effect on feed consumption or ophthalmological examination was observed. Slight reductions in total white blood cell count were observed in males at 50 (−20%) and 200 mg/kg bw per day (−21%) and in females at 200 mg/kg bw per day (−31%). However, the white blood cell counts were at the low end of the historical control range and were considered not toxicologically relevant. Occasional other changes in haematology and clinical chemistry in the treatment groups were not dose related and/or were within the range of normal variation and were considered not to be treatment related. Macroscopic, histopathological and organ weight examinations showed no toxicologically significant effects of tolfenpyrad treatment.

The NOAEL for systemic effects was 200 mg/kg bw per day, the highest dose tested (Barnett, 2008a).

(c) Exposure by inhalation

Groups of 10 male and 10 female Crl:CD(SD) rats were exposed nose only to dust particulate aerosol atmospheres of tolfenpyrad (purity 99.5%; lot no. 61202) at an actual concentration of 0, 0.5, 2.0 or 10 mg/m^3 air (mass median aerodynamic diameter of 2.8–3.2 µm) 6 hours/day, 5 days/week, over a 4-week period (a total of 20 exposures). The control animals received air only. The behaviour and the general health and condition of the animals were observed daily. Body weights and feed consumption were recorded weekly. Ophthalmoscopy examination was performed pretreatment and

before termination. Haematology, clinical chemistry and urine analysis were performed at the end of the study. One day after the last exposure, the rats were killed and necropsied. Weights of liver, kidneys, heart, lungs, spleen, thymus, adrenals, brain, testes, epididymides, ovaries, oviducts, uterus and cervix were recorded. Histology was performed on a large selection of organs and tissues of the control and high-dose rats and on liver and kidneys of the low- and mid-dose rats.

No treatment-related mortality or clinical signs were observed. Body weights and feed consumption were not affected. Haematology showed a reduction (76% of controls) in white blood cell count in female rats at 10 mg/m^3. Although the decrease was statistically significant, it was within the range of concurrent historical controls. A statistically significant decrease in ALAT levels (74% of control value) was considered not toxicologically relevant. No other effects on haematology, clinical chemistry or urine analysis were observed. Increases in absolute (17%) and relative liver weights (15%) were observed in female rats at 10 mg/m^3. No macroscopic changes were observed. Histological examination revealed hepatocellular hypertrophy in some male and female rats at 10 mg/m^3. In the absence of histopathological damage and relevant blood chemistry changes, the observed hepatocellular hypertrophy is not considered to be adverse. A slight increase in hyaline droplets within the epithelium of the proximal convoluted tubule of the kidneys, observed in two male rats at 10 mg/m^3, was not considered toxicologically relevant.

The no-observed-adverse-effect concentration (NOAEC) was 10 mg/m^3, the highest concentration tested (Kelly, 2008).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 78-week dietary carcinogenicity study, tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was administered to groups of 50 male and 50 female CD-1 mice at 0, 15, 150 or 500 ppm (500 ppm was reduced to 400 ppm at week 13 and from 400 ppm to 300 ppm at week 20 due to suppression in body weight gain, decreased feed consumption and severe clinical signs). Over the entire test period, these doses were equal to 0, 2.2, 20.8 and 60.9 mg/kg bw per day for males and 0, 2.8, 27.1 and 75.9 mg/kg bw per day for females, respectively. The mice were checked daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Feed consumption and body weight were recorded weekly during weeks 1–13 and weeks 20–25, at week 17 or 18 and every 4th week from week 25 onward. Blood samples collected at weeks 52 and 79 were examined for erythrocyte, leukocyte, platelet and differential leukocyte counts. Cell morphology was investigated in all mice at week 52 and in control and high-dose mice at week 79. In addition, blood smears of moribund mice were evaluated. All animals were necropsied, and in 10 mice of each sex per dose, the weights of brain, lung, liver, kidneys, adrenals, spleen, testes with epididymides and ovaries were recorded. A wide range of tissues was examined microscopically from all control and high-dose animals and dead or moribund animals during the treatment period in the 15 and 150 ppm groups. In addition, lung, liver, kidney and gross lesions of all mice in the 15 and 150 ppm groups were examined histologically.

After 4 weeks of treatment, survival in males and females at 500 ppm was 90% compared with 100% in control males and females. No further effects on survival were observed. Hunched and/or thin appearance, hypoactivity, few faeces and pale bodies were observed in the high-dose animals during weeks 0–19. A statistically significant decrease in body weight was observed in males at 150 ppm (−7%) and in high-dose males (−17%) and females (−16%) at termination. The reduction of the dietary concentration to 300 ppm resulted in a significant increase in mean body weight gain during weeks 20–23. Feed consumption was statistically significantly reduced in both sexes at 150 ppm (8–9%) and at the high dose (14–16%). No treatment-related effects on haematology were observed.

At the high dose, a statistically significant reduction in absolute brain weight was observed in females (−13%). Relative brain weight was statistically significantly increased in high-dose males

(+17%) and females (+11%). Statistically significant reductions in absolute (50–64% of control) and relative spleen weights (60–70% of control) were observed in mid- and high-dose males. In high-dose females, absolute spleen weight was significantly reduced (62% of control). Absolute testes/epididymides weight was reduced in high-dose males (80% of control). Relative liver weights were significantly increased in high-dose males (124% of control) and females (120% of control). Macroscopic examination showed atrophy of ovaries (absolute weight 18% of control, relative weight 23% of control), uterus and cervix, and a decreased incidence/severity of cystic endometrial hyperplasia was observed in the high-dose females. No other treatment-related histological changes were reported. Treatment with tolfenpyrad did not result in significantly increased incidences of neoplastic lesions.

The NOAEL was 15 ppm (equal to 2.2 mg/kg bw per day), based on decreased body weight gain and feed consumption and changes in organ weights observed in males and females at 150 ppm (equal to 20.8 mg/kg bw per day). Tolfenpyrad was not carcinogenic in CD-1 mice (Ivett, 1999).

Rats

In a 2-year dietary carcinogenicity study, tolfenpyrad (purity 99.33%; lot no. 6D-01-2) was administered to groups of 60 male and 60 female Fischer F344/DuCrj (SPF) rats at 0, 15, 40 or 80 ppm (equal to 0, 0.56, 1.5 and 3.1 mg/kg bw per day for males and 0, 0.69, 1.9 and 3.8 mg/kg bw per day for females, respectively). Ten rats of each sex per group were designated for interim sacrifice after 52 weeks of treatment. The rats were checked daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Feed consumption and body weight were recorded weekly during months 1–3 and once every 3 or 4 weeks thereafter. Ophthalmoscopy was performed pretest and at scheduled necropsy at week 105. Haematology, clinical chemistry and urine analysis were performed in 10–14 rats of each sex per dose at weeks 14, 27 and 79 and in all animals at scheduled necropsy at weeks 53 and 105. At termination at 52 or 105 weeks of treatment, all animals were necropsied, and weights of brain, lung, heart, liver, kidneys, adrenal gland, spleen, testes and ovaries were recorded. Histological examinations were performed on a wide range of organs and tissues of all rats in the control and 80 ppm groups and of dead or moribund animals during the treatment period in the 15 and 40 ppm groups. In addition, lung, liver, kidney, mesenteric lymph nodes and Harderian glands of all rats of the 15 and 40 ppm groups were examined histologically.

There were no effects of treatment on mortality or clinical signs. At 80 ppm, body weight gain was statistically significantly reduced in males (−7%) and females (−13%). In males at 80 ppm, feed consumption was statistically significantly reduced (7–12%) during the first 5 weeks of treatment. After that, slight reductions in feed consumption (about 5%) were observed in these rats. In males at 40 ppm, slight reductions in feed consumption (3–5%) were observed during the first 5 weeks of treatment. In females at 80 ppm, feed consumption was consistently reduced (5–15%) throughout the treatment period. Statistically significant reductions in feed consumption (up to 9%) were also consistently observed in females at 40 ppm. Occasionally, reductions in reticulocyte counts (6–9%) and blood platelet counts (9%) and prolonged activated partial thromboplastin time (7%) were observed in males at 80 ppm, and reduced white blood cell counts were observed in females at 40 ppm (28%) and in males (14%) and females (29%) at 80 ppm. These changes were relatively small, were within the historical control range, were not consistently found and were therefore not considered to be toxicologically significant. Furthermore, if evaluations are made on the serial time measurements of white blood cell counts, it is clear that in males and females of all dose levels, including controls, the counts were consistently lower in week 53 than in week 79 (in particular) and week 105. This suggests that involvement of factors other than dietary tolfenpyrad may have been responsible for reductions, particularly after about 1 year. Clinical chemistry revealed occasional changes in triglyceride, potassium and magnesium levels. These changes were generally within the historical control range and are not considered toxicologically relevant. At 80 ppm, at the interim and terminal kills, slight to moderate increases in relative liver weight (7–8% in males, 9–16% in females) and relative kidney weight (10–11% in males, 10–18% in females) were observed. Relevant data from the necropsy and histological examination are presented in Table 8. Necropsy at 53 weeks

Table 8. Macroscopic and histological effects of chronic treatment of rats with tolfenpyrad.

	Incidence of finding (n = 60)							
	Males				Females			
	0 ppm	15 ppm	40 ppm	80 ppm	0 ppm	15 ppm	40 ppm	80 ppm
Necropsy (week 105)								
Liver: White patched	2	1	1	6	7	8	9	20**
Kidney: Dark brown change	8	6	12	30**	3	7	7	37**
Harderian gland: Brown change	0	2	6*	29**	2	1	7	24**
Histopathology (week 53)								
Liver: Basophilic focus of altered hepatocytes	0	0	0	0	1	2	2	8**
Mesenteric lymph nodes: Mast cells	0	–	–	2	0	–	–	0
Kidney: Hypertrophy of the proximal tubular epithelial cells	0	0	0	0	0	0	0	0
Kidney: Hyaline droplets in the proximal tubule	0	1	4*	9**	0	0	0	0
Harderian glands: Hypersecretion	4	–	–	6	1	–	–	2
Histopathology (week 105)								
Liver: Basophilic focus of altered hepatocytes								
- Slight	36	38	37	42	32	26	22**	15**
- Moderate	5	0	7	2	11	15	22	25
- Severe	0	0	1	0	0	1	2	8
Mesenteric lymph nodes: Mast cells	0	0	0	8**	2	6	7	4
Mesenteric lymph nodes: Sinus histiocytosis	2	1	7	19**	3	1	17**	33**
Kidney: Hypertrophy of the proximal tubular epithelial cells	0	0	0	18**	0	0	4*	19**
Kidney: Hyaline droplets in the proximal tubule	0	1	0	0	1	2	0	0
Harderian glands: Hypersecretion	6	0*	10	30**	0	2	2	6*

*: $P < 0.05$; **: $P < 0.01$ (chi-squared test)
Source: Chida (1999b)

revealed no treatment-related changes. At necropsy at 105 weeks, an increase in the incidence of white patch on the liver in females treated at 80 ppm, an increase in dark brown changes to the kidney in both sexes treated at 80 ppm and an increase in brown changes to the Harderian gland in males at 40 and 80 ppm and in females at 80 ppm were observed. Histological examination revealed an increase in mast cells in the mesenteric lymph nodes in males treated at 80 ppm, an increase in sinus histiocytosis in the mesenteric lymph nodes in males treated at 80 ppm and in females at 40 and 80 ppm, hypertrophy of the renal proximal tubular epithelial cells in males treated at 80 ppm and in females treated at 40 and 80 ppm and an increase in hypersecretion of the Harderian glands in both sexes treated at 80 ppm. In addition, there was an increase in severity of basophilic foci of altered

hepatocytes of the liver in females at doses of 40 and 80 ppm. There was also an increase in hyaline droplets in the renal proximal tubular epithelial cells in males at doses of 40 and 80 ppm at the interim kill, but not at the end of the study. Treatment with tolfenpyrad did not result in significantly increased incidences of neoplastic lesions in any of the organs and tissues examined.

The NOAEL was 15 ppm (equal to 0.56 mg/kg bw per day), based on reduced feed intake in males and females and increased severity of basophilic foci of altered hepatocytes, sinus histiocytosis of the mesenteric lymph nodes and hypertrophy of the proximal renal tubular epithelia in females at 40 ppm (equal to 1.5 mg/kg bw per day). Tolfenpyrad was not carcinogenic in Fischer F344/DuCrj (SPF) rats (Chida, 1999b).

2.4 Genotoxicity

Tolfenpyrad was tested for genotoxicity in a range of guideline-compliant assays. Tolfenpyrad did not induce any significant responses in mutagenicity tests in bacteria and mouse lymphoma cells in vitro or in a chromosomal aberration test in vivo. In two studies, polyploidy was observed. In an in vitro chromosomal aberration study with Chinese hamster lung (CHL) cells, two experiments were performed. In one experiment, cell cultures were treated with tolfenpyrad for 24 or 48 hours in the absence of S9 mix, resulting in a marked increase in the frequency of cells exhibiting numerical aberrations (predominantly polyploidy), which exceeded the current historical vehicle control range. Such chromosomal aberrations were also noted at the low dose in the other experiment (in one replicate only). The study author noted that the significance of polyploidy in vitro was not completely understood at the time of this experiment. Furthermore, inconsistency of the effect, even within an experiment in this study, does not permit a reasoned interpretation. In all other cultures in the study, the frequencies of numerical aberrations fell within the contemporary historical vehicle control ranges (Riley, 1997).

Subsequently, a study was performed by Murli (2007) to assess the ability of tolfenpyrad to affect the cell cycle kinetics in cultured CHL cells in the absence of S9, which might explain the polyploidy of chromosomes observed in the Riley (1997) CHL chromosomal aberration study. Tolfenpyrad at a concentration of 5.00, 8.50 or 13.0 µg/mL induced severe cell cycle delay in CHL cells in the absence of S9 at all dose levels when cells were treated for 3, 6 and 24 hours, washed and the culture continued in the presence of 5-bromo-2′-deoxyuridine for 24 hours (anticipated to be 1.5 cell cycles), when they were harvested and assessed. Dose-related increases in polyploidy were observed following 6 and 24 hours of treatment. There was also a correlation between the number of cells delayed in M1 phase (a single cell cycle when both chromatids stain darkly) and M1+ phase and the induction of polyploidy and endoreduplicated chromosomes. Cells that had progressed through two cell cycles (M2) had chromatids clearly differentiated between light and dark throughout their length. In M1+ cells, only parts of the chromosomes were differentially stained. It is concluded that the increased polyploidy and endoreduplication observed in the CHL cell chromosomal aberration study of Riley (1997) was a result of a severe cell cycle delay caused by tolfenpyrad. Endoreduplication is normal in many cell types and may involve nothing more than a loss of M-phase cyclin-dependent kinase activity and oscillations in the activity of S-phase cyclin-dependent kinase, resulting in switching from mitotic cell cycles to endocycles (Lee, Davidson & Duronio, 2009; Zielke, Edgar & DePamphilis, 2013). However, normal, programmed endoreduplication should probably be considered as different from this chemically induced change. Consequences may be a general increase in gene expression, but in the absence of any in vivo evidence for a similar response, this in vitro result appears to have little significance.

It is concluded that tolfenpyrad is unlikely to be genotoxic or mutagenic. The results of the genotoxicity tests are summarized in Table 9.

Table 9. Overview of genotoxicity tests with tolfenpyrad[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Gene mutation	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537; *Escherichia coli* WP2 *uvrA*	62.5–1 000 µg/plate (±S9)	99.33	Negative	Ballantyne (1997)[b,c]
Gene mutation	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537; *E. coli* WP2 *uvrA*	156–5 000 µg/plate; precipitation at all doses; 20 min preincubation (±S9)	99.33	Negative	Ozaki (2000)[b,d]
Structural chromosomal aberrations	CHL cells	8.6–32.8 µg/mL (−S9) 42–85.8 µg/mL (+S9)	99.33	Negative	Riley (1997)[e]
Cell cycle kinetics aberrations	CHL cells	5–15 µg/mL (−S9)	99.5	Polyploidy induction	Murli (2007)[f]
Gene mutation	Mouse lymphoma L5178Y *TK* locus	0.005–75 µg/mL (−S9), 0.01–25 µg/mL (+S9)	99.5	Negative	Cifone (2007)[g]
In vivo					
Micronucleus formation	CD-1 mouse bone marrow	Two gavage doses of 5, 10 or 20 mg/kg bw, separated by 24 h	99.5	Negative	Yong (2007)[h]

CHL: Chinese hamster lung; S9: 9000 × *g* supernatant fraction of rat liver homogenate; TK: thymidine kinase

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

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

[c] Batch 6D-01-2. Performed in accordance with OECD Test Guideline 471. In a range-finding assay (8–5000 µg/plate), no cytotoxicity was observed, but precipitation was observed at and above 1000 µg/plate. In the main test (62.5–1000 µg/plate), the assays in the presence of S9 were performed with a 20-minute preincubation step. In the presence of S9, cytotoxicity was observed at 500 and 1000 µg/plate in strains TA102 and TA1535. Treatment of strain TA102 with 2-aminoanthracene in the presence of S9 failed to provide any increase in revertant numbers. This strain is not very sensitive to this treatment, and 2-aminoanthracene is not a diagnostic agent with which the strain can be identified. The latter function is more closely provided by glutaraldehyde in the absence of S9. However, this did not result in a significant response either; therefore, the use of TA102 in this experiment failed to give valid results. The data showing a lack of mutagenic response with TA102 in the Ozaki (2000) study, however, are valid.

[d] Batch 6D-01-2. Performed in accordance with OECD Test Guideline 471. In a range-finding assay (0.305–5000 µg/plate), no cytotoxicity was observed, but precipitation was observed at and above 19.5 µg/plate. In the main test (156–5000 µg/plate), the assays in the presence of S9 were performed with a 20-minute preincubation step. At all test concentrations, deposition of crystals was observed.

[e] Batch 6D-01-2. Performed in accordance with OECD Test Guideline 473. Two experiments were carried out. Precipitation was observed at concentrations of 85.8 µg/mL and higher. In the absence of S9, 32% cell survival was observed at 13.4 µg/mL. In the presence of S9, 35% cell survival was observed at 80 µg/mL.

[f] Batch 365-65A.

[g] Batch 365-65A. Performed in accordance with OECD Test Guideline 476.

[h] Batch 365-65A. Performed in accordance with OECD Test Guideline 474. Mortality was observed in 6/8 animals at 50 mg/kg bw per day and in 1/5 animals at 20 mg/kg bw per day after the first treatment. After the second treatment, 1/4 animals in the 20 mg/kg bw per day dose group died. The two surviving animals from the 50 mg/kg bw per day dose group were used as replacement animals and were dosed on the 2nd day with tolfenpyrad at 20 mg/kg bw per day. At 20 mg/kg bw per day, hypoactivity, hunched posture, sternal recumbancy, laboured respiration, ataxia and/or irregular respiration were observed. At 20 mg/kg bw per day, a slightly lower polychromatic erythrocyte : normochromatic erythrocyte ratio was observed.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation dietary reproduction study performed according to OECD Test Guideline 416, SD (Crj:CD, SPF) rats (30 of each sex per group for the F_0 generation, 22–24 of each sex per group for the F_1 generation) were fed tolfenpyrad (purity 99.33%; lot no. 6D-01-2). Dietary concentrations were adjusted to maintain the desired dose levels of 0, 0.75, 1.5 and 3 mg/kg bw per day. The mean test substance intake for each treatment group during the premating, gestation and nursing periods was within ±20% of the target values. Parental (F_0) rats were exposed from 6 weeks of age until termination, and F_1 rats were exposed from postnatal week 3 until termination. Clinical examination was performed daily, and body weight and feed consumption were recorded weekly (males, females during premating period) and on gestation days (GDs) 0, 7, 14 and 20 and postnatal days (PNDs) 0, 4, 7, 14, 17 and 21 (females). Rats were mated after 10 weeks of treatment. From GD 21 until completion of delivery, each female was observed for parturition on two occasions per day. Pups were weighed on PNDs 0, 4, 7, 14 and 21. On PND 4, litters were culled to four pups of each sex per litter, and at PND 21, one male and one female were selected per litter from 22–24 litters per dose group. All litters were examined (for number of pups, sex of pups, number of stillbirths, number of live births and gross anomalies). In all F_1 and F_2 pups, the days of pinna unfolding (before culling) and eye opening (after culling) were recorded. In all F_1 pups, the days of cleavage of balanopreputial gland (males) and vaginal opening (females) were recorded. All F_1 animals were examined for the surface righting reflex, midair righting reflex and pupillary reflex. At termination, necropsy was performed on F_0 and F_1 parental rats, and weights of brain, pituitary, thymus, liver, kidney, adrenal gland, spleen, testis, epididymis (whole and caudal), seminal vesicle (including coagulating gland and content), prostate (ventral lobe), ovary and uterus (including cervical region) of all animals were recorded. Histological examination was performed on pituitary, testis, epididymis, seminal vesicle, coagulating gland, prostate (ventral lobe), ovary, fallopian tube, uterus (including cervical region) and vagina of the control and 3 mg/kg bw per day groups and any gross lesions. Pups not selected for breeding were necropsied at PND 21. As treatment-related changes in spleen and thymus weights were observed in these pups, these tissues of the control and 3 mg/kg bw per day groups were examined histologically.

At 3 mg/kg bw per day, three F_0 females died or were killed moribund due to dystocia (difficult parturition). Body weight gain was reduced in high-dose F_0 and F_1 females during gestation (up to 13%) and lactation (up to 17%). Small, but statistically significant, reductions in body weight gain were observed at 3 mg/kg bw per day in F_0 males during the mating period (−5%) and in F_0 females during the premating period (−9%). At 3 mg/kg bw per day, feed consumption was statistically significantly reduced in F_0 and F_1 females during gestation (up to 19%) and lactation (up to 18%). Small, but statistically significant, reductions in body weight gain were observed at 1.5 mg/kg bw per day in females during gestation (up to 6%) and lactation (up to 8%). Feed consumption was only slightly (up to 7%, not statistically significant) reduced in F_0 females at 1.5 mg/kg bw per day. At 3 mg/kg bw per day, birth weights were reduced in male and female F_1 pups (8–9%, $P < 0.01$) and F_2 pups (5–6%, not significant). During the lactation period, body weight gain in high-dose pups was statistically significantly decreased, with a 24% reduction at PND 7 in F_1 pups and a 19–20% reduction at PND 7 in F_2 pups. From PND 7 onward, the differences in body weight between control and high-dose rats became less pronounced. About half of the females of the 3 mg/kg bw per day group in F_0 animals had a prolonged gestation (23–24 days, compared with about 22 days in controls). In four of these dams, a prolonged parturition was observed. One of these dams delivered only dead pups, while the other three proceeded to dystocia and died or were killed in a moribund state. In the F_0 females at 3 mg/kg bw per day, an increased incidence of total litter loss, a low gestation index (number of females delivering live pups/number of pregnant rats) and a low birth index (number of live pups/number of implantations) were noted. At the high dose, the birth index in the F_1 generation was decreased (not statistically significant). In F_1 and F_2 high-dose pups, a slightly increased incidence of postnatal death was observed. No treatment-related changes in estrous cycles, sperm parameters, ovarian follicle counts, copulation index (number of animals that mated/number of animals used for mating), fertility index (number of pregnant females/number of females that mated)

or nursing behaviour were found. Blackish abdominal cavity was observed from PND 0 to PND 7 in a small number of F_1 and F_2 pups at 3 mg/kg bw per day. After PND 4, the incidence of this observation decreased. A significant delay in the completion of surface righting reflex was observed in F_1 male pups at 3 mg/kg bw per day. The incidence of pinna unfolding was significantly lower in both sexes on PND 3 in the high-dose F_1 and F_2 pups and also on PND 2 in F_1 females. At the high dose, there was a significant prolongation of the day when eye opening occurred in F_1 females and in F_2 males. No treatment-related effects were noted in vaginal opening or preputial separation of F_1 animals. Thymus weights at weaning were statistically significantly lower in F_1 males (absolute −30%; relative −19%) and females (absolute −26%; relative −15%) at 3 mg/kg bw per day and in F_2 males of all treated groups (absolute −12%, −13% and −33% at 0.75, 1.5 and 3 mg/kg bw per day, respectively; relative −13%, −15% and −24% at 0.75, 1.5 and 3 mg/kg bw per day, respectively) and in F_2 females at 1.5 (absolute −13%; relative −11%) and 3 mg/kg bw per day (absolute −27%; relative −18%). Similar reductions in thymus weight were observed in a modified two-generation reproductive toxicity study, with a focus on the effects of tolfenpyrad on immune function (Atai, 1999; see below). In the absence of changes in other immunological parameters at 0.75 mg/kg bw per day in the study of Atai (1999), the Meeting considered the small reductions in thymus weight observed at this dose as initial, non-adverse events of a process that results in immunotoxic effects at higher doses. Therefore, these effects were considered not relevant as a basis for a lowest-observed-adverse-effect level (LOAEL). Similar considerations cannot be applied to the same findings at 1.5 mg/kg bw per day, because this dose was not tested in the study of Atai (1999), and relevant immunological parameters were not measured in the present study.

In F_1 adults, slightly, but statistically significantly, lower absolute brain weights were observed at 1.5 mg/kg bw per day in females (3%) and at 3 mg/kg bw per day in both sexes (4–6%). Histological examination of the animals that died in the terminal stage of the gestation period or that had total litter loss showed atrophy of the thymus and spleen, erosion of the forestomach or glandular stomach, erosion/ulcer of the small intestine and hypertrophy of the zona fasciculata of the adrenal cortex. These changes are considered to be secondary to stress. Furthermore, in these females, changes in the reproductive organs related to parturition, such as haemorrhage, inflammatory cell infiltration, necrosis and thrombus in the uterus and inflammatory cell infiltration in the vagina, were observed.

The NOAEL for parental toxicity was 1.5 mg/kg bw per day, based on moribundity, decreased body weight gain and decreased feed consumption at 3 mg/kg bw per day.

The NOAEL for offspring toxicity was 0.75 mg/kg bw per day, based on a reduction in absolute and relative thymus weights in males and females of the F_2 generation at 1.5 mg/kg bw per day.

The NOAEL for reproductive toxicity was 1.5 mg/kg bw per day, based on dystocia, prolonged parturition, prolonged gestation, increased incidence of total litter loss, low gestation index and low birth index at 3 mg/kg bw per day (Matsuura, 1999).

In a two-generation dietary reproduction study performed according to a modified OECD Test Guideline 416 protocol, SD (Crj:CD(SD)IGS, SPF) rats (15 females per dose for the F_0 generation, 36 males and 12 females per dose for the F_1 generation, 12 males per dose for the F_2 generation) were fed tolfenpyrad (purity 99.33%; lot no. 6D-01-2) in order to assess effects on immune function in the F_1 and F_2 rats. Dietary concentrations were adjusted to maintain the desired dose levels of 0, 0.75 and 3 mg/kg bw per day. The mean test substance intake for each treatment group during the premating, gestation and nursing periods was within ±20% of the target values. F_0 rats (females only) were treated with tolfenpyrad during gestation and lactation, F_1 rats were treated from weaning through mating (both sexes), gestation and lactation (females only) and F_2 rats (males only) were treated to maturation. Dietary concentrations were based on the data from the two-generation reproduction study of Matsuura (1999). F_1 clinical examination was performed daily, and body weight and feed consumption were recorded weekly and on GDs 0, 7, 14 and 20 and PNDs 0, 4,

7, 14, 17 and 21 (females). Rats were mated after 10 weeks of treatment. From GD 21 until completion of delivery, each female was observed for parturition on two occasions per day. All litters were examined (number of pups, sex of pups, number of stillbirths, number of live births, presence of gross anomalies). Pups were weighed on PNDs 0, 4, 7, 14 and 21. On PND 4, litters were culled to four pups of each sex per litter. On PND 4, one culled pup of each sex per litter was selected for measurements of organ weight and thymic and splenic lymphocyte subsets by fluorescence-activated cell sorting.

At weaning at PND 21, from each F_1 litter, one rat of each sex was selected to produce the F_2 generation, one male pup was selected for sheep red blood cell (sRBC) antibody production at 10 weeks of age, one male pup was selected for delayed-type hypersensitivity to keyhole limpet haemocyanin responsiveness at 10 weeks of age and one pup of each sex was selected for haematology and measurements of thymic and splenic lymphocyte subsets (male pup only) and organ weight measurements and necropsy (both sexes) at 3 weeks of age. At weaning at PND 21, from each F_2 litter, one male pup was selected for measurements of organ weight, fluorescence-activated cell sorting, haematology and necropsy at 10 weeks of age, one male pup was selected for sRBC antibody production at 10 weeks of age, one male pup was selected for delayed-type hypersensitivity to keyhole limpet hemocyanin responsiveness at 10 weeks of age and one pup of each sex was selected for haematology and measurements of thymic and splenic lymphocyte subsets (male pup only) and organ weight measurements and necropsy (both sexes) at 3 weeks of age. After weaning, the maternal rats were killed and necropsied. No GLP statement was provided, but the study was conducted in accordance with test laboratory standard operating procedures and quality assurance procedures.

At 3 mg/kg bw per day, there were decreases in body weight gain (up to −10% during gestation) and feed consumption (up to −22% during gestation; up to 14% during lactation) in F_0 maternal rats. In F_1 pups, no significant effects on birth weight were observed. However, body weight gain at 3 mg/kg bw per day was reduced (up to 19% in male pups, up to 20% in female pups) during lactation. After weaning, the reduced body weights quickly recovered. No effects on F_1 maternal body weight were observed during gestation or lactation. Body weights of F_2 offspring were not affected by treatment. Feed consumption was not affected in F_1 or F_2 animals. No effects on reproductive outcome were observed. The number of live F_1 pups on PND 4 was significantly lower at 3 mg/kg bw per day. No effect on postnatal survival was observed in F_2 offspring. In F_1 and F_2 pups at 3 mg/kg bw per day, black change in the peritoneal cavity due to an accumulation of dark green contents in the small intestine was observed early after birth. Haematological examination of F_1 and F_2 pups on PND 21 revealed no effects of treatment. A high count of segmented neutrophils in F_2 rats of the high-dose group at postnatal week 10 was considered incidental, as no such effects were observed at PND 21 in F_1 and F_2 pups or in other repeated-dose studies. In F_1 pups necropsied on PND 21, no effects on haematology were observed. Low thymus weights were observed on PND 4 at 0.75 mg/kg bw per day in male F_1 pups (absolute −22%; relative −20%) and at 3 mg/kg bw per day in male F_1 pups (absolute −48%; relative −38%), female F_1 pups (absolute −44%; relative −35%), male F_2 pups (absolute −41%; relative −34%) and female F_2 pups (absolute −30%; relative −22%). Low spleen weights were also observed in male (up to 44%) and female pups (up to 34%) of the F_1 and F_2 generations at 3 mg/kg bw per day. On PND 21, the reduction in thymus weight was smaller, although still statistically significant, at 3 mg/kg bw per day. No statistically significant changes were noted in the spleen weights on PND 21 or in adult thymus or spleen weights. Significantly lower values were noted in thymus and spleen cellularity in F_2 rats in the 3 mg/kg bw per day group on PNDs 4 and 21. F_2 rats in the 3 mg/kg bw per day group showed slightly, but statistically significantly, lower ratios of CD3+/CD45RA− cells and CD4+/CD8− cells in the spleen and consequently relative changes in other cell ratios on PND 21. At 3 mg/kg bw per day, slight, but statistically significant, changes were also noted in CD3−/CD45RA+ cell ratios in the spleen in F_2 rats on PND 4 and CD3+/CD45RA− cell ratios in the spleen in F_2 rats in postnatal week 10. In immune function tests, F_1 and F_2 rats in postweaning week 10 showed normal humoral immunity and cellular immune function, indicating that the observed effects on the immune system on PND 4 have no consequences for the functionality of the immune system in adult rats.

The NOAEL for maternal toxicity was 0.75 mg/kg bw per day, based on decreased body weight gain and decreased feed consumption at 3 mg/kg bw per day.

The NOAEL for offspring toxicity was 0.75 mg/kg bw per day, based on reduced body weight gain during lactation and a reduced number of live F_1 pups at PND 4, black change in the peritoneal cavity after birth in F_1 and F_2 pups, lower thymus and spleen weights early after birth in F_1 pups and in F_2 male pups, reduced thymus and spleen cellularity, and changes in immune cell ratios in the spleen in F_2 male pups at 3 mg/kg bw per day.

The NOAEL for reproductive toxicity was 3 mg/kg bw per day, the highest dose tested (Atai, 1999).

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 21–24 pregnant female Crj:CD, SPF rats were treated orally, by gavage, with tolfenpyrad (purity 99.33%; lot no. 6D-01-2) in distilled water at a dose level of 0, 1, 3 or 4.5 mg/kg bw per day from days 6 through 15 of gestation (day 0 = day on which sperm were detected in the vaginal smear). The doses were based on a range-finding study. Clinical signs and feed consumption were recorded daily. Body weight and feed consumption were measured on GDs 0, 6, 9, 12, 15 and 20. Feed consumption was measured over 2-day periods. All females were killed on day 20 of gestation. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea, implantations and early and late resorptions were counted. Body weight and sex of the fetuses were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations.

No clinical signs were noted. At 4.5 mg/kg bw per day, body weight was reduced at GD 9 (−2 g compared with GD 6). During this period, dams at 3 mg/kg bw per day gained 7 g, whereas control dams gained 16 g. Feed consumption was reduced at GD 9 and GD 12 at 3 mg/kg bw per day (up to 12%) and at 4.5 mg/kg bw per day (up to 28%). Necropsy of the dams revealed no treatment-related effects. Fetal weights were reduced at 4.5 mg/kg bw per day in males (−7%) and females (−9%). An increased incidence of fetuses with 14th ribs and reduced ossification of the metacarpal bones were observed at 4.5 mg/kg bw per day.

The NOAEL for maternal toxicity was 1 mg/kg bw per day, based on reduced body weight gain and feed consumption during the first days of treatment at 3 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 3 mg/kg bw per day, based on decreased fetal weight, increased incidence of fetuses with 14th ribs and reduced ossification of the metacarpal bones observed at 4.5 mg/kg bw per day. No evidence of a teratogenic effect was observed (Hoshino, 1995).

Rabbits

In a developmental toxicity study, groups of 12–15 pregnant Kbl:JW, SPF Japanese White rabbits were treated orally, by gavage, with tolfenpyrad (purity 99.33%; lot no. 6D-01-2) in aqueous sodium carboxymethyl cellulose at a dose level of 0, 1, 3 or 6 mg/kg bw per day from days 6 through 18 of gestation (the day after artificial insemination was designated as GD 0). The doses were based on a range-finding study. Clinical signs were recorded daily. Body weight and feed consumption were measured on GDs 0, 6, 9, 12, 15, 18, 21, 25 and 28. All females were killed on day 28 of gestation. All does were examined macroscopically for abnormalities. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea and implantations were counted. Body weight and sex of the fetuses were recorded. All fetuses were subjected to skeletal and visceral examinations.

One doe in the 3 mg/kg bw per day group was found dead at GD 28, and one doe at 6 mg/kg bw per day delivered prematurely on GD 27. These findings were considered to be treatment related, as both does had shown body weight loss and reduced feed consumption from GD 9 onward. At the high dose, a mild body weight loss (61 g) was observed in the does at GD 9. Body weight gain was

slightly reduced (not statistically significant) at 3 mg/kg bw per day from GD 15 to termination and at 6 mg/kg bw per day from GD 9 to termination. Feed consumption was statistically significantly reduced at 6 mg/kg bw per day at GD 9. No treatment-related clinical signs or effects on numbers of corpora lutea, implantations, or live and dead fetuses, implantation loss, live fetal weight or sex ratio were observed. However, total litter loss (nine implanted embryos) in one doe in the 6 mg/kg bw per day group was considered treatment related, as the rabbit showed body weight loss and reduced feed consumption during the treatment period. The study author reported that similar effects were observed at 6 and 9 mg/kg bw per day in a preliminary study in rabbits (data not shown). Necropsy of the other surviving does showed no treatment-related changes. No treatment-related changes were observed in individual findings after external, visceral or skeletal examinations of fetuses showed that the incidences of 13 ribs at 1 and 6 mg/kg bw per day (27.8% and 38.1%, respectively) were higher than in controls (15.9%) and the 3 mg/kg bw per day group (18.5%). The increases were not statistically significant, not dependent on dose and within the historical control range of this laboratory (33.6% [29.7–40.5] in data from the past 5 years) and were therefore not considered toxicologically significant. An increased incidence of accessory sternebrae at 1, 3 and 6 mg/kg bw per day (17.0%, 11.5% and 9.1%, respectively) compared with controls (3.3%) was also not considered toxicologically significant, as the increases were not statistically significant and lacked dose dependency. The total number of skeletal variations was significantly increased at 1 and 6 mg/kg bw per day, but not at 3 mg/kg bw per day (17.6%, 43.7%, 31.4% and 48.2% in the 0, 1, 3 and 6 mg/kg bw per day groups, respectively). However, as the values of the treatment groups were within the range of contemporary historical control values (range 34.8–51.2% in 10 studies conducted during 1991–1996, data provided by the sponsor), there was a lack of dose dependency and the control for this experiment was clearly lower than could have been expected, the finding is considered incidental and not toxicologically relevant.

The NOAEL for maternal toxicity was 1 mg/kg bw per day, based on one mortality observed at 3 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 6 mg/kg bw per day, the highest dose tested (Hoshino, 1997).

2.6 Special studies

(a) Neurotoxicity

Rats

In an acute oral neurotoxicity study, Crl:CD(SD) rats (10 of each sex per dose) were treated by gavage with tolfenpyrad (purity 99.5%; lot no. 365-65A) in corn oil at a dose of 0, 20, 40 or 60 mg/kg bw (males) or 0, 10, 20 or 40 mg/kg bw (females). The doses were based on a range-finding study in which groups of five male and five female rats were treated by gavage with tolfenpyrad at 0, 20, 40, 60, 80 or 100 mg/kg bw and subsequently observed in an open-field arena for 1 minute at 1, 2, 3, 4, 5, 6, 7 and 8 hours post-dosing and again at 7 and 14 days after dosing. All rats were necropsied. The range-finding study indicated that the time of peak effect was 6 hours after dosing. The rats were observed daily for clinical signs. The rats were tested in a functional observational battery, including detailed clinical observations, pretest and at 6 hours and 7 and 14 days after application. Motor activity was measured pretest, on the day of dosing and at 7 and 14 days after dosing. Body weights and feed consumption were measured daily. All animals were killed on test day 15 and examined macroscopically. Five rats of each sex per dose were selected for neurohistological examination.

In the range-finding test, one male at 80 mg/kg bw and all males at 100 mg/kg bw died. All females dosed at 60 mg/kg bw or higher were found dead or were killed in a moribund condition. Clinical signs were observed at all doses, with the peak effect at 6 hours after dosing.

In the main test, one female rat at 40 mg/kg bw died on day 4. Males and females of all treatment groups lost weight after dosing (5%, 7% and 11% in males at 20, 40 and 60 mg/kg bw, respectively, and 4%, 10% and 15% in females at 10, 20 and 40 mg/kg bw, respectively). Compared with controls, reductions in body weight gain were observed in the male rats at 40 (up to 9%) and 60

mg/kg bw (up to 16%) and in female rats at 20 (up to 12%) and 40 mg/kg bw (up to 21%). A slight (6%), but statistically significant, reduction in body weight was observed on the day of dosing in males at 20 mg/kg bw. At the highest doses in males and females, body weights remained significantly reduced up to 11–13 days after dosing. At the middle doses, body weights remained decreased for 3–6 days. On the day of dosing, feed consumption was reduced by 64%, 76% and 85% in males at 20, 40 and 60 mg/kg bw, respectively, and by 30%, 44% and 63% in females at 10, 20 and 40 mg/kg bw, respectively. Feed consumption quickly recovered in males, but remained decreased for 5 and 7 days after dosing in mid- and high-dose females, respectively. Increased incidences of adverse clinical signs (including dehydration, scant faeces, soft or liquid faeces, chromorhinorrhoea and urine-stained abdominal fur) were observed in females at 40 mg/kg bw and in males at 60 mg/kg bw. Functional observational battery testing revealed no treatment-related effects, except for urine staining in high-dose females on the day of dosing. Motor activity was not affected by treatment. No test substance–related microscopic lesions were revealed by the neurohistological examination.

The LOAEL was 10 mg/kg bw, based on reductions in body weight and feed consumption observed in females on the day of dosing. No evidence of neurotoxicity was observed in rats (Barnett, 2008b).

In a 90-day neurotoxicity study, groups of 10 male and 10 female Crl:CD (SD)IGS BR rats were given tolfenpyrad (purity 99.3%; lot no. 6D-01-2) at a dietary level of 0, 15, 40 or 80 ppm (equal to 0, 1.0, 2.7 and 5.4 mg/kg bw per day for males and 0, 1.2, 3.2 and 6.0 mg/kg bw per day for females, respectively). The animals were observed daily for clinical signs. A detailed physical examination and body weight and feed consumption measurements were performed weekly. The rats were subjected to a functional observational battery and a locomotor activity test before the start of treatment and during weeks 2, 4, 8 and 12. Ophthalmological examination was perfomed pretest and in week 13. At termination, rats were killed and examined macroscopically. The brain was measured and weighed. Five rats of each sex in the control and the high-dose groups were selected for neurohistological examination of the brain, spinal cord, dorsal root ganglia, dorsal root fibres, ventral root fibres, eyes, optic nerves, skeletal muscle and sciatic and tibial nerves.

No treatment-related mortality or clinical signs were observed. Females at 80 ppm showed a reduction in body weight gain (14%) and feed consumption (17%) throughout the treatment period. There were no treatment-related effects observed in functional observational battery parameters, locomotor activity testing, ophthalmology or macroscopic and histological examination.

The NOAEL was 40 ppm (equal to 3.2 mg/kg bw per day), based on reductions in body weight gain and feed consumption in females at 80 ppm (equal to 6.0 mg/kg bw per day). No evidence of neurotoxicity was observed in rats (Kilpatrick, 2003).

(b) Studies with metabolites

Acute toxicity and genotoxicity studies with metabolites of tolfenpyrad were available.

Acute toxicity

The results of studies of acute toxicity with metabolites of tolfenpyrad are summarized in Table 10. In all of these studies, the test substance was dissolved in aqueous 0.5% carboxymethyl cellulose.

Table 10. Results of studies of acute toxicity with metabolites of tolfenpyrad[a]

Species	Strain	Sex	Route	Metabolite	Purity (%)	LD_{50} (mg/kg bw)	Reference
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	OH-PT[b]	99.9	70.8 (M) 35.5 (F)	Ikeya (1999a)
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	PT-CA[c]	99.5	27.4 (M) 15.4 (F)	Ikeya (1999b)
Rat	Sprague-Dawley (Crj:CD(SD))	F	Oral	PT(A)-4OH[d]	99.9	> 2 000 (F)	Oda (2012a)
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	T-CA[e]	98.5	600–2 000 (M) > 2 000 (F)	Ikeya (1999c)
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	T-AM[f]	99.9	> 2 000 (M/F)	Ikeya (1999d)
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	CA-T-CA[g]	99.7	> 2 000 (M/F)	Ikeya (1999e)
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	OH-T-CA[h]	94.6	2 024 (M) > 2 000 (F)	Ikeya (1999f)
Rat	Sprague-Dawley (Crj:CD(SD))	F	Oral	PAM[i]	100.0	300–2 000 (F)	Oda (2012b)
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	OH-PAM[j]	99.1	1 095 (M/F)	Ikeya (1999g)
Rat	Sprague-Dawley (Crj:CD(SD))	M/F	Oral	PCA[k]	99.9	> 2 000 (M/F)	Ikeya (1999h)

F: female; LD_{50}: median lethal dose; M: male

[a] Statements of adherence to quality assurance and GLP were included in all studies. See Table 12 (below) for names of all metabolites included here.

[b] Lot no. Y980423. Performed according to OECD Test Guideline 401. Doses of 0, 10, 30, 100, 300 and 1000 mg/kg bw were used. Mortality and clinical signs were observed at doses of 30 mg/kg bw and higher.

[c] Lot no. Y980626. Performed according to OECD Test Guideline 401. Doses of 0, 6.25, 12.5, 25, 50 and 100 mg/kg bw were used. Mortality and clinical signs were observed at doses of 12.5 mg/kg bw and higher.

[d] Lot no. 2HO0301S. Performed according to OECD Test Guideline 423. A dose of 2000 mg/kg bw was administered to female rats. No mortality or clinical signs were observed.

[e] Lot no. Y980415. Performed according to OECD Test Guideline 401. Doses of 0, 60, 200, 600 and 2000 mg/kg bw were used. At 2000 mg/kg bw, 4/5 males died. No mortality was observed in the other groups. Clinical signs were observed at doses of 600 mg/kg bw and higher.

[f] Lot no. Y980420. Performed according to OECD Test Guideline 401. Doses of 0 and 2000 mg/kg bw were used. No mortality or clinical signs were observed.

[g] Lot no. FGC01. Performed according to OECD Test Guideline 401. Doses of 0 and 2000 mg/kg bw were used. No mortality was observed. At 2000 mg/kg bw, some rats showed diarrhoea.

[h] Lot no. Y980629. Performed according to OECD Test Guideline 401. Doses of 0, 1000, 2000, 3000 and 4000 mg/kg bw were used for males, and doses of 0, 1000 and 2000 mg/kg bw were used for females. In males, mortality was observed at 2000 mg/kg bw and higher. No mortality was observed in females. Clinical signs were noted at doses of 2000 mg/kg bw and higher. Body weight loss or reduced body weight gain was observed in males at all doses and in females at 2000 mg/kg bw.

[i] Lot no. 2HO4602S. Performed according to OECD Test Guideline 423. Doses of 300 and 2000 mg/kg bw were applied to female rats (three per dose step). At 2000 mg/kg bw, all rats died. No mortality was observed at 300 mg/kg bw. Decreased body weight of the surviving rats and clinical signs were observed at both doses.

[j] Lot no. Y981207. Performed according to OECD Test Guideline 401. Doses of 0, 60, 200, 600 and 2000 mg/kg bw were used. No mortality occurred at doses up to 600 mg/kg bw. At 2000 mg/kg bw, all rats died. At doses of 600 mg/kg bw and higher, clinical signs were observed.

[k] Lot no. OL22MC01. Performed according to OECD Test Guideline 401. Doses of 0, 1000 and 2000 mg/kg bw were used. No mortality or clinical signs were observed at 1000 mg/kg bw. At 2000 mg/kg bw, 2/5 males and 1/5 females died. Surviving rats at 2000 mg/kg bw showed diarrhoea and decreased spontaneous movements.

Short-term studies of toxicity

In acute toxicity studies, the metabolites OH-PT and PT-CA were more potent than tolfenpyrad in causing mortality. Therefore, in a comparative 4-week toxicity study, the effect of treatment with tolfenpyrad (purity 99.33%; lot no. 6D-01-2), OH-PT (purity 99.9%; lot no. Y980423) and PT-CA (purity 99.5%; lot no. Y980626) was investigated. Groups of male and female F344/DuCrj (Fischer) strain SPF rats, consisting of five rats of each sex per dose, received diets containing tolfenpyrad at 0, 10, 30 or 100 ppm (equal to 0, 0.9, 2.5 and 8.0 mg/kg bw per day for males and 0, 0.9, 2.6 and 8.2 mg/kg bw per day for females, respectively), OH-PT at 0, 3, 10, 30 or 100 ppm (equal to 0, 0.2, 0.9, 2.5 and 8.4 mg/kg bw per day for males and 0, 0.3, 0.9, 2.7 and 8.8 mg/kg bw per day for females, respectively) or PT-CA at 0, 3, 10, 30 or 100 ppm (equal to 0, 0.3, 0.8, 2.5 and 8.1 mg/kg bw per day for males and 0, 0.3, 0.9, 2.7 and 8.5 mg/kg bw per day for females, respectively). Animals were checked daily for clinical signs of toxicity. Body weights and feed consumption were measured weekly. Ophthalmological examinations were carried out before dosing and at termination. Blood and urine samples were taken at termination for haematology, clinical biochemistry and urine analysis. At termination, all rats were necropsied, and brain, adrenals, thymus, spleen, lung, liver, kidney and testis/ovary were weighed. An extensive range of organs and tissues from control and high-dose rats was examined histologically. In addition, in the other dose groups, pancreas (females only), heart, lung, liver, kidney, spleen, prostate and any macroscopic lesions were examined histologically.

No treatment-related mortality or clinical signs were seen in any treatment group. For tolfenpyrad, a mild reduction in body weight gain in males at 100 ppm (up to 9%) and feed consumption in males and females at 100 ppm (about 10%) were observed. Slight reductions in body weight gain (6%) and feed consumption (7%) of high-dose females treated with PT-CA were considered not toxicologically relevant. No toxicologically relevant changes in ophthalmoscopy, urine analysis, haematology or clinical chemistry were observed. In rats treated with tolfenpyrad, there was an increase in relative weight of liver in males at 30 ppm (7%) and 100 ppm (11%) and in females at 30 ppm (5%) and 100 ppm (16%), and also an increase in relative weight of kidney in males at 30 ppm (5%) and 100 ppm (11%). In rats treated with PT-CA, an increase in absolute and relative liver weights in females at 30 ppm (absolute 3%; relative 6%) and 100 ppm (absolute 6%; relative 12%) and an increase in relative kidney weight in males at 30 ppm (7%) and 100 ppm (9%) and in females at 100 ppm (10%) were observed. In rats treated with OH-PT, there was a slight increase of relative kidney weight in males at 100 ppm (5%). Histopathological examination revealed that the increased liver weights observed in the groups treated with tolfenpyrad and PT-CA were associated with mild hepatocyte hypertrophy. The mild increases in liver weights and hepatocellular hypertrophy were considered not to be adverse. In the absence of histological changes, the mild changes in kidney weight observed in PT-CA- and OH-PT-treated rats were not considered adverse. In rats treated with tolfenpyrad, increased incidences and severity of hypertrophy of acinar cells in pancreas were found in 100 ppm females. The observation of hyaline droplets (graded as slight) in tubular epithelium in kidney observed in 3/5 males at 100 ppm was not considered toxicologically relevant.

For tolfenpyrad, the NOAEL was 30 ppm (equal to 2.5 mg/kg bw per day), based on a mild reduction in feed consumption in males and females, a mild reduction in body weight gain observed in males and increased incidences and severity of hypertrophy of acinar cells in pancreas in females at 100 ppm (equal to 8 mg/kg bw per day).

For PT-CA, the NOAEL was 100 ppm (equal to 8.1 mg/kg bw per day), the highest dose tested.

For OH-PT, the NOAEL was 100 ppm (equal to 8.4 mg/kg bw per day), the highest dose tested (Nishimura, 2001).

Genotoxicity

The results of genotoxicity studies with metabolites of tolfenpyrad are summarized in Table 11.

Table 11. Results of studies on the genotoxicity of metabolites of tolfenpyrad[a]

Metabolite	End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro						
OH-PT[b]	Gene mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2 *uvrA*	2.44–1 250 µg/plate (−S9) 2.44–5 000 µg/plate (+S9)	99.9	Negative	Ozaki (1999a)
PT-CA[c]	Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvrA*	39.1–5 000 µg/plate (±S9)	99.5	Negative	Ozaki (1999b)
T-CA[d]	Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvrA*	39.1–1 250 µg/plate (±S9), except for *E. coli*: 156–5 000 µg/plate (±S9)	98.5	Negative	Ozaki (1999c)
T-AM[e]	Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvrA*	39.1–5 000 µg/plate (±S9)	99.9	Negative	Ozaki (1999d)
CA-T-CA[f]	Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvrA*	156–5 000 µg/plate (±S9)	99.7	Negative	Ozaki (1999e)
OH-T-CA[g]	Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvrA*	39.1–5 000 µg/plate (±S9)	94.6	Negative	Ozaki (1999f)
OH-PAM[h]	Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvrA*	156–5 000 µg/plate (±S9)	99.1	Negative	Ozaki (1999g)
PCA[i]	Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvrA*	313–5 000 µg/plate (±S9)	99	Negative	Machigaki (1988)
OH-PT[j]	Chromosomal aberration	CHL cells	40–200 µg/mL (±S9), experiment 1 10–130 µg/mL (−S9), experiment 2	99.9	Negative	Ozaki (2001a)
PT-CA[k]	Chromosomal aberration	CHL cells	78.1–1 250 µg/mL (±S9), experiment 1 4.89–313 µg/mL (−S9), experiment 2	99.5	Negative	Ozaki (2001b)

Metabolite	End-point	Test object	Concentration	Purity (%)	Results	Reference
In vivo						
OH-PT[l]	Micronucleus formation	Crj:CD(SD)IGS rat bone marrow	Two gavage doses of 5, 10 or 20 mg/kg bw, separated by 24 h	99.9	Negative	Saigo (2000a)
PT-CA[m]	Micronucleus formation	Crj:CD(SD)IGS rat bone marrow	Two gavage doses of 5, 10 or 20 mg/kg bw, separated by 24 h	99.7	Negative	Saigo (2000b)

CHL: Chinese hamster lung; S9: 9000 × *g* supernatant fraction of rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies. In all studies, statements of adherence to GLP and quality assurance were included. See Table 12 (below) for names of all metabolites included here.

[b] Lot no. Y980423. Performed in accordance with OECD Test Guideline 471. Cytotoxicity in strains TA98, TA100, TA1535 and WP2 *uvrA* was observed at 1250 µg/plate and in strain TA1537 at 313 µg/plate with S9 mix. Without S9 mix, cytotoxicity was observed in strains TA98 and WP2 *uvrA* at 1250 µg/plate and in strains TA100, TA1535 and TA1537 at 313 µg/plate. Precipitation of the test compound was observed at and above 313 µg/plate with and without S9 mix.

[c] Lot no. Y980626. Performed in accordance with OECD Test Guideline 471. Bacterial growth inhibition was observed in the test group at 5000 µg/plate for *S. typhimurium* TA98 and TA1537 and *E. coli* WP2 *uvrA* and in the test group at 5000 µg/plate for TA1535 in the treatment without metabolic activation.

[d] Lot no. Y980415. Performed in accordance with OECD Test Guideline 471. Bacterial growth inhibition was observed at 625 and 1250 µg/plate for *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and at 2500 and 5000 µg/plate for *E. coli* WP2 *uvrA*.

[e] Lot no. Y980420. Performed in accordance with OECD Test Guideline 471. Bacterial growth inhibition was observed in the test group at 5000 µg/plate for *S. typhimurium* TA98 and TA1537 and *E. coli* WP2 *uvrA* in the treatment with and without metabolic activation and in the test group at 5000 µg/plate for TA100 in the treatment with metabolic activation.

[f] Lot no. FGC01. Performed in accordance with OECD Test Guideline 471.

[g] Lot no. Y980629. Performed in accordance with OECD Test Guideline 471. Bacterial growth inhibition was observed in the test group only at 5000 µg/plate for *S. typhimurium* TA98, TA100, TA1535 and TA1537, but not for *E. coli* WP2 *uvrA*.

[h] Lot no. Y981207. Study design resembles OECD Test Guideline 471.

[i] Lot no. 88002. Performed in accordance with OECD Test Guideline 471.

[j] Lot no. Y980423. Performed in accordance with OECD Test Guideline 473. In preliminary assays, a concentration-related increase in cytotoxicity was observed in the dose range between 39.1 and 313 µg/mL following 6 hours of culture, and a concentration-related increase in cytotoxicity was observed in the dose range between 39.1 and 156 µg/mL following both 24 and 48 hours of culture. Experiment 1: Incubation with OH-PT ±S9 mix for 6 hours, followed by an 18-hour recovery period. Experiment 2: Continuous incubation with OH-PT −S9 for 24 or 48 hours.

[k] Lot no. Y980626. Performed in accordance with OECD Test Guideline 473. In preliminary assays, a concentration-related increase in cytotoxicity was observed in the dose range between 156 and 1250 µg/mL following 6 hours of culture, and a concentration-related increase in cytotoxicity was observed in the dose range between 39.1 and 313 µg/mL following 24 hours of culture and between 39.1 and 78.1 µg/mL following 48 hours of culture. Experiment 1: Incubation with PT-CA ±S9 mix for 6 hours, followed by an 18-hour recovery period. Experiment 2: Continuous incubation with PT-CA −S9 for 24 or 48 hours.

[l] Lot no. Y000328. Performed in accordance with OECD Test Guideline 474. Mortality was observed in 1/10 animals at 20 mg/kg bw per day. It was judged that the exposure of the rat bone marrow to the test article was sufficient, as the percentage of immature erythrocytes in all erythrocytes in all the test article groups was significantly lower than in the negative control group.

[m] Lot no. 0000228-1. Performed in accordance with OECD Test Guideline 474. Mortality was observed in 4/10 animals at 20 mg/kg bw per day. It was judged that the exposure of the rat bone marrow to the test article was sufficient, as the percentage of immature erythrocytes in all erythrocytes in all the test article groups was significantly lower than in the negative control group.

3. Observations in humans

No information on adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to tolfenpyrad was available.

Comments

Biochemical aspects

After administration of a single oral dose of radiolabelled tolfenpyrad to rats, maximum concentrations in blood and plasma were reached 2–8 hours after a low dose (1 mg/kg bw) and 4–12 hours after a high dose (20 mg/kg bw). Excretion of radioactivity in urine (2–3%) and bile (51–70%) and residual radioactivity in the carcass (5–11%) 48 hours after dosing indicate that absorption was at least 58% of the dose. Plasma half-lives were 11–28 hours. Radioactivity was widely distributed to the tissues, higher concentrations being found in liver, kidney, bone marrow and brown fat. Seven days after dosing, 88–93% and 2–3% of the radioactivity were excreted in faeces and urine, respectively. In faeces, 4–15% of the radioactivity represented tolfenpyrad, and 24–49% of the radioactivity represented the metabolite PT-CA (see Table 12 for names of metabolites). In plasma, liver and kidney, 91–100% of the radioactivity represented PT-CA, indicating extensive metabolism of tolfenpyrad. In bile, 50–67% of the administered dose was excreted within 48 hours, the major part as PT-CA-TA, PT-CA-Gluc and PT-CA, whereas low levels of Sul-OH-PT-CA and CO-PT and other, unidentified metabolites were also detected. These data indicate extensive conjugation of PT-CA in the liver and subsequent excretion into the bile. Less than 0.7% of the administered dose was present in bile as unchanged tolfenpyrad. In bile duct–cannulated rats, only 3–8% of the administered dose was excreted into faeces, predominantly as tolfenpyrad (up to 6%) and PT-CA (up to 1%). PT-CA is deconjugated following its biliary excretion and excreted in faeces. In urine, no intact tolfenpyrad was detected. Various individual metabolites (including OH-PAM and CA-T-CA) were present in urine at less than 0.5% of the administered dose, with the exception of PT-CA and PT-CA-TA (these metabolites could not be further separated), which were present at up to 1.9% of the administered dose. Observed differences in metabolite levels between sexes, doses and positions of radiolabel were minor. Following repeated dosing of [^{14}C]tolfenpyrad, plasma concentrations of radioactivity stabilized after two or three administrations at 1.5–3 times the plasma concentration found after the first dose. Tissue distribution, excretion and metabolism were similar following the single low and high doses and following single and repeated dosing.

Toxicological data

The oral LD_{50} values for tolfenpyrad dissolved in aqueous carboxymethyl cellulose were greater than or equal to 113 mg/kg bw in two rat studies, and the oral LD_{50} for tolfenpyrad dissolved in olive oil was greater than or equal to 75 mg/kg bw in one rat study. The LD_{50} for dermal toxicity was greater than 2000 mg/kg bw in rats. The acute inhalation LC_{50} was 1.50–2.21 mg/L. Tolfenpyrad was not irritating to the skin and slightly irritating to the eye of rabbits. Tolfenpyrad was not a skin sensitizer in a Magnusson and Kligman test in guinea-pigs.

In repeated-dose toxicity studies with tolfenpyrad, multiple adverse effects were observed. In a 90-day toxicity study in mice using dietary concentrations of 0, 15, 100 and 300 ppm (equal to 0, 2.4, 15.9 and 46.2 mg/kg bw per day for males and 0, 3.0, 20.2 and 57.9 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 15.9 mg/kg bw per day), based on increased ASAT activity and increased relative heart weight in males and increased relative liver weight in both sexes at 300 ppm (equal to 46.2 mg/kg bw per day). In a 90-day toxicity study in rats using dietary concentrations of 0, 15, 80 and 160 ppm (equal to 0, 0.906, 4.78 and 9.33 mg/kg bw per day for males and 0, 1.01, 5.17 and 9.32 mg/kg bw per day for females, respectively), the NOAEL was 15 ppm (equal to 0.906 mg/kg bw per day), based on changes in clinical chemistry, a reduced white blood cell count, dark brown change of the liver, hypertrophy of the proximal renal tubular epithelium and the acinar cells in the mandibular glands in females and an increase in mast cells in the

Table 12. List of abbreviations and chemical names of metabolites used in the report

Abbreviation	Chemical name
OH-PT	4-Chloro-3-(1-hydroxyethyl)-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
CO-PT	3-Acetyl-4-chloro-1-methyl-*N*-[4-(*p*-tolyloxy)benzyl]pyrazole-5-carboxamide
PT-CA	4-[4-[(4-Chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid
OH-PT-CA	4-[4-[(4-Chloro-3-(1-hydroxyethyl)-1-methylpyrazol-5-yl)carbonylaminomethyl]-phenoxy]benzoic acid
T-CA	4-(*p*-Tolyloxy)benzoic acid
CA-T-CA	4,4′-Oxydibenzoic acid
PAM	4-Chloro-3-ethyl-1-methylpyrazole-5-carboxamide
OH-PAM	4-Chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide
Sul-OH-PT-CA	4-[4-[(4-Chloro-1-methyl-3-(1-sulfoxyethyl)pyrazol-5-yl)carbonylaminomethyl]-phenoxy]benzoic acid
PT-CA-TA	4-[4-[(4-Chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]phenyl-carbonylamino]ethane sulfonic acid
PT(A)-4OH	4-Chloro-3-ethyl-*N*-(4-hydroxybenzyl)-1-methylpyrazole-5-carboxamide
T-AM	4-(4-Tolyloxy)benzamide
PCA	4-Chloro-3-ethyl-1-methylpyrazole-5-carboxylic acid
OH-T-CA	4-[4-(Hydroxymethyl) phenoxy] benzoic acid
PT-CA-Gluc	Glucuronide conjugate of 4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylamino-methyl]phenoxy]benzoic acid

mesenteric lymph nodes, diffuse hypertrophy of hepatocytes and hypertrophy of the pancreatic acinar cells in both sexes at 80 ppm (equal to 4.78 mg/kg bw per day).

In a 28-day as well as a 90-day capsule study in dogs using doses of 0, 1, 5 and 10 mg/kg bw per day, the NOAEL was 1 mg/kg bw per day, based on the increased incidence of vomiting at 5 mg/kg bw per day. In a second 90-day capsule study in dogs with administration of tolfenpyrad at doses of 0, 10, 30 and 100 mg/kg bw per day, mild toxicity (i.e. vomiting, soft and mucoid faeces) was observed at 10 mg/kg bw per day, the lowest dose tested. Severe toxicity including mortality was observed at doses of 30 and 100 mg/kg bw per day. In a 1-year capsule study in dogs using doses of 0, 1, 5 and 20/10 mg/kg bw per day, the NOAEL was 1 mg/kg bw per day, based on increased incidences of vomiting, soft or mucoid stool and salivation and increased ALAT levels at 5 mg/kg bw per day. In all the studies in dogs, vomiting and soft stool were observed as early as the 1st day of dosing. Therefore, the overall NOAEL for these effects was 1 mg/kg bw per day, with an overall LOAEL of 5 mg/kg bw per day.

In a 78-week toxicity study in mice using dietary concentrations of 0, 15, 150 and 500/400/300 ppm (equal to 0, 2.2, 20.8 and 60.9 mg/kg bw per day for males and 0, 2.8, 27.1 and 75.9 mg/kg bw per day for females, respectively), the NOAEL was 15 ppm (equal to 2.2 mg/kg bw per day), based on decreased body weight gain and feed consumption and changes in organ weights observed in males and females at 150 ppm (equal to 20.8 mg/kg bw per day). No increased incidences of tumours were observed at doses up to 500/400/300 ppm (equal to 60.9 mg/kg bw per day), the highest concentration tested.

In a 2-year toxicity and carcinogenicity study in rats using dietary concentrations of 0, 15, 40 and 80 ppm (equal to 0, 0.56, 1.5 and 3.1 mg/kg bw per day for males and 0, 0.69, 1.9 and 3.8 mg/kg bw per day for females, respectively), the NOAEL was 15 ppm (equal to 0.56 mg/kg bw per day), based on reduced feed intake in males and females and increased severity of basophilic foci of altered hepatocytes, sinus histiocytosis of the mesenteric lymph nodes and hypertrophy of the proximal renal

tubule epithelia in females at 40 ppm (equal to 1.5 mg/kg bw per day). There was no compound-related increase in the incidence of tumours.

The Meeting concluded that tolfenpyrad is not carcinogenic in mice or rats.

Tolfenpyrad was tested for genotoxicity in an adequate range of in vitro and in vivo assays. These assays provided no evidence of genotoxic potential.

The Meeting concluded that tolfenpyrad is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that tolfenpyrad is unlikely to pose a carcinogenic risk to humans.

A standard two-generation reproductive toxicity study in rats was performed in which dietary concentrations were adjusted to maintain the desired dose levels of 0, 0.75, 1.5 and 3 mg/kg bw per day. This was followed by a modified non-GLP two-generation reproductive toxicity study focusing on the effects of tolfenpyrad on immune function, in which the dietary concentrations were adjusted to maintain target dose levels of 0, 0.75 and 3 mg/kg bw per day. The overall NOAEL for parental toxicity from these two studies was 1.5 mg/kg bw per day, based on moribundity, decreased body weight gain and decreased feed consumption at 3 mg/kg bw per day observed in the first study. The overall NOAEL for offspring toxicity was 0.75 mg/kg bw per day, based on a reduction in absolute and relative thymus weights in males and females of the F_2 generation at 1.5 mg/kg bw per day, observed in the first study, and on reduced body weight gain during lactation and a reduced number of live F_1 pups at PND 4, black change in the peritoneal cavity after birth in F_1 and F_2 pups, lower thymus and spleen weights soon after birth in F_1 pups and in F_2 male pups, reduced thymus and spleen cellularity, and changes in immune cell ratios in the spleen in F_2 male pups at 3 mg/kg bw per day, observed in the second study. The Meeting concluded that the small reductions in absolute and relative thymus weights observed at 0.75 mg/kg bw per day, in the absence of other relevant effects, were not toxicologically significant. In the second study, humoral immunity and cellular immune function were normal in adult F_1 and F_2 rats. The overall NOAEL for reproductive toxicity was 1.5 mg/kg bw per day, based on a range of effects occurring late in gestation resulting in a reduced number of live offspring, observed in the first but not in the second study, at 3 mg/kg bw per day.

In a developmental toxicity study in rats using doses of 0, 1, 3 and 4.5 mg/kg bw per day, the NOAEL for maternal toxicity was 1 mg/kg bw per day, based on reduced body weight gain and feed consumption at 3 mg/kg bw per day, observed during the first days of treatment. The NOAEL for embryo and fetal toxicity was 3 mg/kg bw per day, based on decreased fetal weight, increased incidence of skeletal variations and delayed ossification observed at 4.5 mg/kg bw per day. No evidence of a teratogenic effect was observed.

In a developmental toxicity study in rabbits using doses of 0, 1, 3 and 6 mg/kg bw per day, the NOAEL for maternal toxicity was 1 mg/kg bw per day, based on one mortality observed at 3 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 6 mg/kg bw per day, the highest dose tested. No evidence of a teratogenic effect was observed.

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

In an acute oral (gavage) neurotoxicity study in rats using doses of 0, 20, 40 and 60 mg/kg bw in males and 0, 10, 20 and 40 mg/kg bw in females, the LOAEL was 10 mg/kg bw, the lowest dose tested, based on reductions in body weight and feed consumption observed in females on the day of dosing. Clinical signs were observed at 40 mg/kg bw in females and at 60 mg/kg bw in males. No neurotoxicity was observed.

In a 90-day neurotoxicity study in rats using dietary concentrations of 0, 15, 40 and 80 ppm (equal to 0, 1.0, 2.7 and 5.4 mg/kg bw per day for males and 0, 1.2, 3.2 and 6.0 mg/kg bw per day for females, respectively), the NOAEL was 40 ppm (equal to 3.2 mg/kg bw per day), based on reductions in body weight gain and feed consumption in females at 80 ppm (equal to 6.0 mg/kg bw per day). No clinical, functional or histological signs of neurotoxicity were observed at doses up to 80 ppm (equal to 5.4 mg/kg bw per day), the highest dose tested.

The Meeting concluded that tolfenpyrad is not neurotoxic.

In the modified two-generation reproductive toxicity study focusing on effects of tolfenpyrad on immune function (see above), tolfenpyrad caused changes in the immune system in rat pups but did not affect normal humoral immunity or cellular immune function in adult rats.

Toxicological data on metabolites and/or degradates

Studies of acute oral toxicity were performed with the tolfenpyrad metabolites OH-PT, PT-CA, PT(A)-4OH, T-CA, T-AM, CA-T-CA, OH-T-CA, PAM, OH-PAM and PCA dissolved in aqueous carboxymethyl cellulose. In general, the metabolites had low acute toxicity, except for OH-PT ($LD_{50} \geq 35.5$ mg/kg bw) and PT-CA ($LD_{50} \geq 15.4$ mg/kg bw), which were slightly more toxic than tolfenpyrad. All these metabolites showed negative results in tests for reverse mutation induction in bacteria (Note: PT(A)-4OH and PAM were not tested). OH-PT and PT-CA were also tested in a chromosomal aberration test in vitro and a micronucleus test in vivo. No genotoxicity was observed. In a 4-week dietary study in rats, the toxicity of tolfenpyrad at dietary concentrations of 0, 10, 30 and 100 ppm was compared with the toxicities of PT-CA and OH-PT at dietary concentrations of 0, 3, 10, 30 and 100 ppm. The NOAEL for tolfenpyrad was 30 ppm (equal to 2.5 mg/kg bw per day), based on a mild reduction in feed consumption in males and females, a mild reduction in body weight gain observed in males and increased incidences and severity of hypertrophy of acinar cells in pancreas in females at 100 ppm (equal to 8 mg/kg bw per day). The NOAEL for PT-CA was 100 ppm (equal to 8.1 mg/kg bw per day), the highest dose tested, and the NOAEL for OH-PT was 100 ppm (equal to 8.4 mg/kg bw per day), the highest dose tested. In view of the lower LD_{50} values for the metabolites PT-CA and OH-PT compared with tolfenpyrad, the Meeting considered these two compounds to be toxicologically relevant.

In the absence of any toxicological information on the livestock metabolite OH-PT-CA, but on consideration of the similarity of its structure to that of tolfenpyrad, it was assumed that OH-PT-CA was of similar toxic potency to tolfenpyrad. No data were available on the toxicity of the livestock metabolites, PT-CA conjugates and OH-PT-CA conjugates. However, as these are likely to be hydrolysed to PT-CA and OH-PT-CA, respectively, the Meeting concluded that their toxicities should be considered equivalent to those of PT-CA and OH-PT-CA.

Human data

No information on adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to tolfenpyrad was available.

The Meeting concluded that the existing database on tolfenpyrad is sufficient to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for tolfenpyrad of 0–0.006 mg/kg bw on the basis of a NOAEL of 0.56 mg/kg bw per day in a 2-year rat study with tolfenpyrad, for reduced feed intake, increased severity of basophilic foci of altered hepatocytes, sinus histiocytosis of the mesenteric lymph nodes and hypertrophy of the proximal tubule epithelia in females at 1.5 mg/kg bw per day, using a safety factor of 100.

The Meeting established an acute reference dose (ARfD) of 0.01 mg/kg bw for tolfenpyrad based on a NOAEL of 1 mg/kg bw per day for reduced body weight and feed consumption observed during the first days of treatment in a developmental toxicity study with tolfenpyrad in rats at 3 mg/kg bw and an overall NOAEL of 1 mg/kg bw per day for vomiting and soft stool observed on the 1st day of treatment in 28-day, 90-day and 1-year studies with tolfenpyrad in dogs at 5 mg/kg bw per day. A

safety factor of 100 was applied. The ARfD provides a margin of exposure of 1000 over the LOAEL in the acute neurotoxicity study in rats. The Meeting considered it unlikely that the acute effects observed in rats and dogs are the result of the unpalatability of tolfenpyrad, as the effects were observed after gavage or capsule administration. The Meeting also considered it unlikely that the acute effects were secondary to local gastrointestinal irritation, as no such effects were reported in any of the studies.

The Meeting considered that the ADI and ARfD are also applicable to the metabolites PT-CA and OH-PT, which showed similar toxicity to tolfenpyrad in LD_{50} studies but lower toxicity in a 4-week dietary study. In addition, the Meeting considered the ADI and ARfD applicable to all the livestock metabolites: OH-PT-CA, PT-CA conjugates and OH-PT-CA conjugates. The Meeting noted that in the absence of data on the effects of these metabolites in long-term and developmental toxicity studies in rats and capsule studies in dogs (i.e. studies that formed the basis of the ADI and ARfD), it would not be possible to establish the relative potency of the metabolites to tolfenpyrad in order to refine the dietary exposure assessment.

Levels relevant for risk assessment of tolfenpyrad

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	15 ppm, equal to 2.2 mg/kg bw per day	150 ppm, equal to 20.8 mg/kg bw per day
		Carcinogenicity	500/400/300 ppm, equal to 60.9 mg/kg bw per day[b]	–
Rat	Thirteen-week study of toxicity[a]	Toxicity	15 ppm, equal to 0.906 mg/kg bw per day	80 ppm, equal to 4.78 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	15 ppm, equal to 0.56 mg/kg bw per day	40 ppm, equal to 1.5 mg/kg bw per day
		Carcinogenicity	80 ppm, equal to 3.1 mg/kg bw per day[b]	–
	Two-generation studies of reproductive toxicity[a,c,d]	Parental toxicity	1.5 mg/kg bw per day	3 mg/kg bw per day
		Offspring toxicity	0.75 mg/kg bw per day	1.5 mg/kg bw per day
		Reproductive toxicity	1.5 mg/kg bw per day	3 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Embryo and fetal toxicity	3 mg/kg bw per day	4.5 mg/kg bw per day
	Acute neurotoxicity study[e]	Neurotoxicity	40 mg/kg bw[b]	–
		Toxicity	–	10 mg/kg bw[f]
	Ninety-day neurotoxicity study[a]	Neurotoxicity	80 ppm, equal to 5.4 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[e]	Maternal toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Embryo and fetal toxicity	6 mg/kg bw per day[b]	–
Dog	Four-week, 13-week and 1-year studies of toxicity[c,g,h]	Toxicity	1 mg/kg bw per day	5 mg/kg bw per day

LOAEL: lowest-observed-adverse-effect level; NOAEL:no-observed-adverse-effect level

[a] Dietary administration.

[b] Highest dose tested.

[c] Two or more studies combined.

[d] Dietary concentrations of tolfenpyrad were adjusted over the course of the study in order to obtain the required daily doses (mg/kg bw per day) for the different dose groups and sexes. Therefore, ppm values are not presented.
[e] Gavage administration.
[f] Lowest dose tested.
[g] Identical NOAELs and LOAELs were observed in all three dog studies. In all studies, vomiting and soft stool were observed on the 1st day of testing.
[h] Capsule administration.

Estimate of acceptable daily intake

0–0.006 mg/kg bw

Estimate of acute reference dose

0.01 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid (T_{max} 2–8 h); ≥ 58% (rats)
Dermal absorption	No data available (probably relatively low in view of data from LD_{50} and short-term studies with oral and dermal dosing)
Distribution	Widely distributed (rats)
Potential for accumulation	Low
Rate and extent of excretion	88–93% in faeces and 2–3% in urine; plasma half-lives at 1 and 20 mg/kg bw: 11–28 h
Metabolism in animals	Extensive, rapidly metabolized in the liver and subsequently conjugated and then excreted into bile
Toxicologically significant compounds in animals, plants and the environment	Tolfenpyrad, PT-CA, PT-CA conjugates, OH-PT, OH-PT-CA, OH-PT-CA conjugates
Acute toxicity	
Rat, LD_{50}, oral	Dissolved in aqueous carboxymethyl cellulose: ≥ 113 mg/kg bw Dissolved in olive oil: ≥ 75 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	≥ 1.50 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating
Dermal sensitization	Not sensitizing (Magnusson and Kligman guinea-pig test)
Short-term studies of toxicity	
Target/critical effect	Many end-points affected
Lowest relevant oral NOAEL	0.906 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	200 mg/kg bw per day, the highest dose tested (rat)

Lowest relevant inhalatory NOAEC	2 mg/m^3 (rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Feed intake, liver, kidneys, mesenteric lymph nodes (rats)
Lowest relevant NOAEL	0.56 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	Reduced number of live offspring at parentally toxic doses
Lowest relevant parental NOAEL	1.5 mg/kg bw per day
Lowest relevant offspring NOAEL	0.75 mg/kg bw per day
Lowest relevant reproductive NOAEL	1.5 mg/kg bw per day
Developmental toxicity	
Target/critical effect	Decreased fetal weight, increased skeletal variations at maternally toxic doses
Lowest relevant maternal NOAEL	1 mg/kg bw per day (rat, rabbit)
Lowest relevant embryo/fetal NOAEL	3 mg/kg bw per day (rat)
Neurotoxicity	
Acute and subchronic neurotoxicity	Not neurotoxic
Other toxicological studies	
Immunotoxicity	Increased thymus and spleen weights and changed immune cell ratios in pups, but not in adults
Studies with PT-CA	
- Acute toxicity	$LD_{50} \geq 15.4$ mg/kg bw
- Four-week dietary toxicity	8.1 mg/kg bw per day, the highest dose tested
- Genotoxicity	Not genotoxic
Studies with OH-PT	
- Acute toxicity	$LD_{50} \geq 35.5$ mg/kg bw
- Four-week dietary toxicity	8.4 mg/kg bw per day, the highest dose tested
- Genotoxicity	Not genotoxic
Medical data	
	No data

LC_{50}: median lethal concentration; LD_{50}: median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level; T_{max}: time to reach peak concentration

Summary

	Value	Study	Safety factor
ADI	0–0.006 mg/kg bw	Two-year study of toxicity in rats	100
ARfD	0.01 mg/kg bw	Developmental toxicity study in rats; 28-day, 90-day and 1-year toxicity studies in dogs	100

ADI: acceptable daily intake; ARfD: acute reference dose

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