IPCS INCHEM Home

Pesticide residues in food -- 1999

Sponsored jointly by FAO and WHO
with the support of the International Programme
on Chemical Safety (IPCS)

Toxicological evaluations

Joint meeting of the
FAO Panel of Experts on Pesticide Residues
in Food and the Environment
and the
WHO Core Assessment Group

Rome, 20-29 September 1999

PYRIPROXYFEN

First draft prepared by
K. Fujimori
National Institute of Health Sciences, Tokyo, Japan

Explanation
Evaluation for acceptable daily intake
Biochemical aspects
Absorption, distribution, and excretion
Biotransformation
Toxicological studies
Acute toxicity
Short-term studies of toxicity
Long-term studies of toxicity and carcinogenicity
Genotoxicity
Reproductive toxicity
Multigeneration reproductive toxicity
Developmental toxicity
Studies on metabolites
Acute toxicity
Genotoxicity
Comments
Toxicological evaluation
References

Explanation

Pyriproxyfen, 4-phenoxyphenyl *(RS)*-2-(2-pyridyloxy)propyl ether, is a 2-phenoxy phenoxy oxime used as an insecticide which acts as an insect growth regulator. It is intended for use as a spray in the control of arthropods, including cockroaches and fleas, on crops, dumps and indoors. Pyriproxyfen was evaluated for the first time by the Meeting.

Evaluation for Acceptable Daily Intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

In a study carried in compliance with good laboratory practice (GLP), groups of five male and five female Sprague-Dawley rats were given pyriproxyfen labelled uniformly with ^{14}C on the phenoxyphenyl ring (radiochemical purity, > 99%) as a solution in corn oil orally by gavage at doses of 2 or 1000 mg/kg bw, with or without pretreatment with unlabelled pyriproxyfen (2 mg/kg bw). Radiolabel was excreted predominantly in the faeces (90%) at both doses, urinary excretion representing 4-8% of the administered dose over 48 h. The total recovery of radiolabel in the excreta over 168 h was 96-98% of the dose, and the residual radiolabel in the tissues and carcass

represented no more than 0.3% of the dose. The tissue with the highest concentration of radiolabel was fat, which contained 0.01 µg/g as equivalent. Pretreatment with unlabelled pyriproxyfen before administration of 2 mg/kg bw per day for 14 days increased the residual radiolabel in the tissues slightly but did not change the excretion pattern. There was no significant sex- or dose-related difference in excretion rates or in the tissue distribution of radiolabel.

In a further study, groups of three male and three female Sprague-Dawley rats with cannulated bile ducts were given [^{14}C-phenoxyphenyl]pyriproxyfen orally at a single dose of 2 mg/kg bw. Biliary excretion of the radioactive material represented 34-37% of the dose within 48 h of administration. Urinary excretion represented 2-3% of the dose and faecal excretion 38-51% (Isobe et al., 1988a; GLP: Matsunaga et al. 1995). These experiments suggest that as much as 50% of an oral dose of pyriproxyfen is not absorbed.

Groups of five male and five female Sprague-Dawley (Crl-CD) rats were given pyriproxyfen labelled with ^{14}C in the pyridyl ring at a

dose of 2 or 1000 mg/kg bw in a study that complied with GLP. Radiolabel was excreted predominantly in the faeces, representing about 90% of the dose, and urinary excretion comprised 5-11% of the dose over 48 h. Total recovery of radiolabel in the excreta after 168 h represented 92-99% of the dose. Expired air contained < 0.5% of the dose over 48 h. The residual radiolabel in the tissues and carcass represented no more than 0.3% of the dose 168 h after administration. The tissue with the highest concentration of radiolabel was fat, with 0.01-0.02 µg/g as equivalent. No significant differences associated with the position of the label, sex or dose were seen in the excretion rates or tissue distribution (Yoshino, 1993a; Matsunaga et al., 1995)

In another study that complied with GLP, groups of three male and three female Sprague-Dawley rats were given [^{14}C-phenoxyphenyl]pyriproxyfen orally at a single dose of 2 mg/kg bw, and radiolabel in tissues was determined 2, 4, 8, 12, 24, 48, and 72 h after dosing. The peak concentration of radiolabel in blood was observed 8 h after dosing. The concentrations in blood were four times higher in males than in females, with a terminal half-time of 10-14 h. The time to peak concentration in most tissues was 2-8 h after dosing, while that in fat was 12-24 h. At the respective peak time, the concentration in the liver was the highest (2.1-2.4 mg/g at 8 h as equivalent) of the tissues examined; however, 72 h after dosing, the highest concentration was found in fat (0.08-0.09 mg/g tissue as equivalent). The concentration in tissues other than liver (0.02-0.03 mg/g tissue as equivalent) was < 0.01 mg/g as equivalent 72 h after dosing. The half-time of radiolabelled material in the tissues was 8-35 h (Isobe et al., 1988b; Matsunaga et al., 1995).

Groups of three male and three female Sprague-Dawley rats were given [^{14}C-phenoxyphenyl]-pyriproxyfen orally at a single dose of 1000 mg/kg bw, and radiolabel in tissues was determined 2, 4, 8, 12, 24, 48, and 72 h after dosing in a study that complied with GLP. The peak concentration in blood was achieved after 8 h. The concentrations of radiolabel in blood in males were six times higher than in females. The time to peak concentration in all tissues except fat was 4 h in males and 8 h in females. At the respective peak time, the highest concentration was found in liver (160-320 mg/g as equivalent at 8 h, decreasing to 8-12 mg/g at 72 h). The highest residual concentration 72 h after dosing was in fat (45-46 mg/g tissue as equivalent). The concentration of radiolabel in fat peaked 12-24 h after dosing and decreased with a half-time of 23 h in males and 35 h in females. The residual concentrations in other tissues were < 10 mg/g tissue 72 h after dosing, and the half-time was 5-17 h. There was no dose-related difference in the tissue distribution of pyriproxyfen (Yoshino, 1993b; Matsunaga et al., 1995).

(b) Biotransformation

Mice

Groups of three male and three female mice were given [^{14}C-pyridyl]pyriproxyfen orally at a dose of 2 or 1000 mg/kg bw. Faecal excretion represented 78-90% of the low dose and 64-65% of the high dose, and urinary excretion 10-27% and 35-37%, respectively, over 7 days. Complete recovery of radiolabel (100-105% of the dose) was observed over that time. Twelve metabolites were identified by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The major metabolite in faeces was 4'-hydroxypyriproxyfen (36-38% of the low dose and 13-15% of the high dose); minor metabolites were 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether (3%) and *(RS)*-2hydroxypropyl 4-phenoxyphenyl ether (1-3%). The urinary metabolites were 4'-hydroxypyriproxyfen (0-5%), 4'-hydroxypyriproxyfen glucuronide (3-13% of the low dose, 18-28% of the high dose), and *(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether sulfate (3-6% of dose). The percent of the dose represented by glucuronide and sulfate conjugates in urine was higher in mice than in rats, but no other species difference in the metabolic pathways of pyriproxyfen were seen. There was no difference in 4'-hydroxylation by sex (Yoshino et al., 1995).

Rats

The biotransformation of [^{14}C-phenoxyphenyl]pyriproxyfen in rats was investigated in samples from the study of Isobe et al. (1988a,b), described above. After oral administration, more than 26 metabolites were detected in faeces and urine by TLC. The major metabolite was 4'-hydroxypyriproxyfen (25-48% of the dose). Total recovery of radiolabel in faeces and urine represented 93-96% of the dose, and 31-37% was detected as parent compound in the faeces 48 h after the low or high dose, while no parent compound was detected in urine. Ten metabolites, including conjugates, were identified by TLC in excreta, all 10 occurring in faeces at the high dose and two in urine. The major metabolite identified in feces was 4'-hydroxypyriproxyfen, formed by oxidative metabolism of the phenyl ring, and the others were oxidative products (2-hydroxypyriproxyfen and 4',5''-hydroxypyriproxyfen), the products of ether cleavage (*(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether, 4-phenoxyphenol, and their hydroxylated metabolites), and their conjugates (sulfates). The metabolites identified in urine were sulfate conjugates of 4'hydroxypyriproxyfen (0.4-1.0% of the low dose, 0.5-1.0% of the high dose) and 4'-hydroxy-4-phenoxyphenol (0.5-3.0% of the low dose, 0.3-1.6% of the high dose). Less parent compound was found in faeces after repeated oral dosing, but repeated treatment with the vehicle, corn oil, caused a similar decrease. The percentage of the dose recovered as 4'-hydroxypyriproxyfen in faeces was higher in females than in males. The major metabolites identified in fat, liver, kidney, and blood collected 2, 4, 8, 12, 24, 48, and 72 h after administration of 2 mg/kg bw of radiolabelled pyriproxyfen were 4',5''-hydroxypyriproxyfen sulfate in blood and 4-hydroxy- and 4',5''-hydroxypyriproxyfen in kidney and liver. At the time of peak

concentration, the parent compound represented 0% in males and 9% in females of the total radiolabel present. A greater percentage of the dose was recovered as 4'-hydroxypyriproxyfen in the liver of females than males. In the kidney, sulfate conjugates represented a larger percentage of the dose in males than in females. Unmetabolized pyriproxyfen represented 89-93% of the radiolabel in the extractable fraction of fat (90% was extracted) (Isobe et al., 1988a,b).

The biotransformation of [^{14}C-pyridyl]pyriproxyfen in rats was also investigated in samples from the study of Yoshino (1993a), described above. More than 13 metabolites were detected in faeces and urine by TLC and HPLC, with nine, including conjugates, in faeces and four in urine. The major metabolite in faeces was 4'-hydroxypyriproxyfen (23-47% of the dose), and the other metabolites were oxidative products (2-hydroxy- and 4',5''-hydroxypyriproxyfen), the products of ether cleavage (4-hydroxyphenyl *(RS)*-2-(2pyridyloxy)propyl ether and *(RS)*-2-(2-pyridyloxy)-propionic acid), and their sulfate or glucuronide conjugates. The metabolites identified in urine were 4'-hydroxypyriproxyfen (0% of the low dose, 1.0-5.6% of the high dose) and its sulfate conjugate (0.3-0.4% of the low dose, 0% of the high dose), the sulfate conjugate of 4',5''-hydroxypyriproxyfen (0% of the low dose, 0.1-0.2% of the high dose), and *(RS)*-2-(2-pyridyloxy) propionic acid (1-1.7% of the low dose, 0% of the high dose) (Yoshino, 1993a)

These studies indicate that the major route of metabolism of pyriproxyfen is hydroxylation, with cleavage of the ether bonds and conjugation as minor routes. Hydroxylation occurs primarily at the 4' position of the phenyl ring (phenoxy group) and subsequently at the 5'' position of the pyridyl group. Conjugation produces mainly the respective sulfates of the oxidative metabolites. There was no evidence of induction of metabolism by pretreatment with pyriproxyfen. The pattern of metabolites in the excreta and tissues of males and females suggests a considerable sex difference in metabolic activity. There were no significant differences in the metabolic pathway by dose or frequency of dosing. The proposed metabolic pathway for pyriproxyfen in various species is shown in Figure 1.

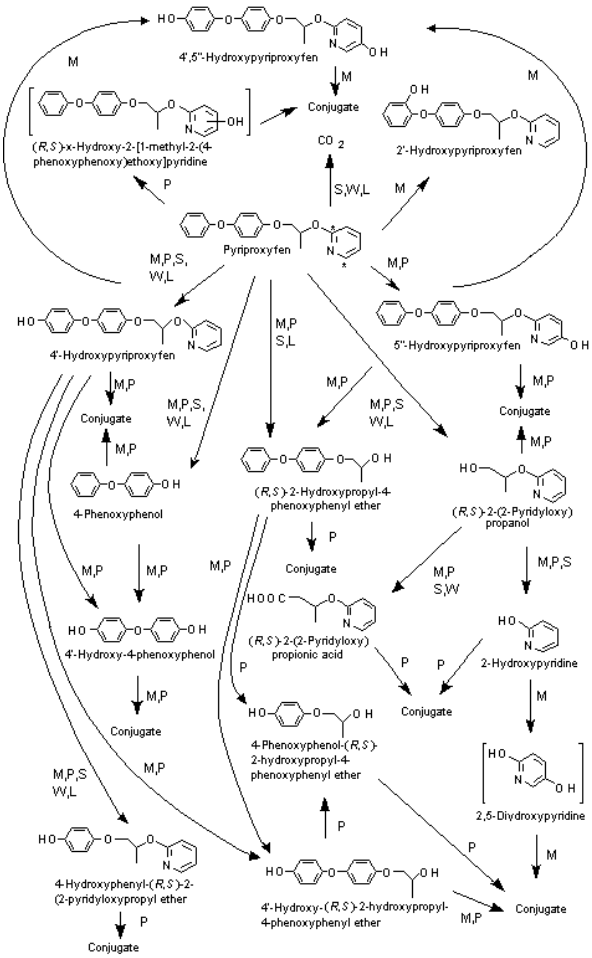

Figure 1. Proposed metabolic and degradation pathways of pyriproxyfen
From Isobe et al. (1988a); Yoshino et al. (1996); M, mammals and chickens; P, plants; S, soil; W, water; L, light * Radiolabelled position

Lactating goats

Lactating goats *(Capra hircus*, weighing 51-57 kg) were given [^{14}C-phenoxyphenyl]-pyriproxyfen (purity, 99.5%) in gelatin capsules at a dose of 1.8-2.0 or 20 mg/animal per day for 5 consecutive days, for a total dose of 100 mg. The animals were killed within 6 h of the last dose. Faeces, urine, and milk were collected twice daily and analysed. Metabolites were purified from extracts of tissues or excreta by HPLC and identified by TLC and/or mass spectral analysis.

The study was carried out according to GLP. Elimination reached a plateau by the third day. The percentages of total radiolabel recovered 1 day after the last dose were 17-18% in urine, 58% in faeces, and 0.3-0.8% in milk. The metabolites identified in milk extracts were 4'-hydroxypyriproxy-fen sulfate (51% of the radiolabel present), 4'-hydroxypyriproxyfen (2%), 4-phenoxyphenol sulfate (10%), 4'-hydroxy-4-phenoxyphenol sulfate (8%), 4'-hydroxy- *(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether sulfate (3%), and 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen (8%). Metabolites found in both liver and kidney were 4'-hydroxypyriproxyfen sulfate, 5''-hydroxypyriproxyfen-sulfate, *(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether, 4'-hydroxy *(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether, and 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen. In addition, 4'-hydroxypyriproxyfen and 5''-hydroxypyri-proxyfen were identified in liver, and 4-phenoxyphenol sulfate and 4'-hydroxy-4-phenoxyphenol in kidney. The metabolites identified in the faecal samples were 4'-hydroxypyriproxyfen (39% of the radiolabel present), 5''-hydroxypyriproxyfen (6%), 4'-hydroxy- *(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether (5%), *(RS)*-2-hydroxypropyl 4phenoxyphenyl ether, and 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen. Parent compound accounted for 11-13% of the dose in faeces, The major metabolites in urine were 4-phenoxyphenol (36% of the radiolabel present), 4-phenoxyphenol sulfate (15%), 4'-hydroxy-4-phenoxyphenol (14%), and 4'-hydroxy-4-phenoxyphenol sulfate (17%) (Panthani et al., 1996a)

In another study that conformed to GLP, lactating goats (*Capra hircus*, weighing 39-46 kg) were given [^{14}C-pyridyl]pyriproxyfen (purity, 97.6%) in gelatin capsules at a dose of 1.8-1.9 or 20 mg/animal per day for 5 consecutive days. The animals were killed within 6 h of the last dose. Faeces, urine, and milk were collected twice daily and analysed by HPLC and TLC and/or mass spectrometry. The total radiolabel recovered represented 17-18% of the dose in urine, 58% in faeces, and 0.4-0.8% in milk. The major metabolites identified in milk extracts were 4'-hydroxypyriproxyfen sulfate (35% of the radiolabel present) and 2,5-dihydroxypyridine conjugate (29%), with smaller quantities of 4'-hydroxypyriproxyfen and 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen. The major metabolites identified in liver and kidney were 4'-hydroxypyriproxyfen sulfate, 2,5-dihydroxypyridine conjugate, and *(RS)*-2-(2-pyridyoxy) propanol conjugates. The major metabolites identified in the urine were 4'-hydroxypyriproxyfen sulfate, *(RS)*-2-(2-pyridyloxy) propionic acid, and 2,5-dihydroxypyridine conjugate. The metabolites identified in the faecal samples were 4'-hydroxypyriproxyfen (43%), 5''-hydroxypyriproxyfen (5%), and 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen (5%). These two studies indicate that the primary routes of metabolism in goats are hydroxylation of the 4' position of the phenoxyphenyl ring and the 5'' position of the pyridyl ring, cleavage of the ether bonds, oxidation of the methylene moiety, and sulfate conjugation of the 4'-hydroxyphenoxyphenyl moiety. There is thus no difference between rats and goats in the metabolic pattern of pyriproxyfen (Panthani et al., 1996b)

Chickens

Laying Leghorn hens *(Gallus domesticus)* were given [^{14}C-phenoxyphenyl]pyriproxyfen (purity, 99.1%) by gelatin capsule at a concentration equivalent to 10 ppm in the feed for 8 consecutive days and were killed within 4 h of the last dose. Excreta were collected once daily and analysed by HPLC and TLC and/or mass spectrometry. The study complied with GLP. The metabolites identified in liver and kidney were free 4'-hydroxypyriproxyfen and its sulfate conjugate, free 4'-hydroxy-4-phenoxyphenol and its sulfate conjugate, free 4'-hydroxy- *(RS)*-2-hydroxypropyl-4-phenoxyphenyl ether and its sulfate conjugate, 4-phenoxyphenol sulfate, 4-hydroxyphenyl- *(RS)*-2-(2-pyridyloxy)propyl ether pyriproxyfen, and *(RS)*-2-hydroxypropyl-4-phenoxyphenyl ether. Metabolites identified in excreta samples were 4'-hydroxypyriproxyfen, free and conjugated 4'-hydroxy-4-phenoxyphenol, free and conjugated 4'-hydroxy- *(RS)*-2-hydroxy-propyl 4-phenoxyphenyl ether, 4-hydroxyphenyl *(RS)*-2-(2pyridyloxy)propyl ether pyriproxyfen, 5''-hydroxypyriproxyfen, *(RS)*-2-hydroxypropyl-4-phenoxyphenyl ether, and 4-phenoxyphenol (Panthani et al., 1996c)

Laying Leghorn hens *(Gallus domesticus)* were given [^{14}C-pyridyl]pyriproxyfen (purity, 98.2%) by gelatin capsule at a concentration equivalent to 10 ppm in the feed for 8 consecutive days and were killed within 4 h of the last dose. Excreta were collected once daily and analysed by HPLC and TLC and/or mass spectrometry. The study complied with GLP. The metabolites identified in liver and kidney were free and conjugated 4'-hydroxypyriproxyfen, 2-hydroxypyridine, free and conjugated 5''-hydroxypyriproxyfen, 4-hydroxyphenyl- *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen, and *(RS)*-2-(2-pyridyloxy)propionic acid. The metabolites identified in excreta were *(RS)*-2-(2-pyridyloxy) propionic acid, 4'-hydroxypyriproxyfen, 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy)propyl ether pyriproxyfen, 5''-hydroxypyriproxyfen, and 2-hydroxypyridine. These two studies indicate that the routes of metabolism in laying hens are hydroxylation at the 4' position of the phenoxyphenyl ring and at the 5'' position of the pyridyl ring, cleavage of the ether linkages, oxidation of the methylene moiety of the side-chain, and sulfation of the 4'-hydroxyphenoxyphenyl moiety (Panthani et al., 1996d)

In vitro

The metabolism of pyriproxyfen in rats and mice was investigated *in vitro* in samples of 10 000 × *g* supernatant (S10) prepared from kidney, lungs, and small intestine of 7-week-old Sprague-Dawley rats and ICR mice. Hepatic microsomal and cytosolic fractions were prepared by an established method (centrifugation of S10 at 105 000 × *g*), and

microsomal, S10, or cytosolic fractions were incubated with [^{14}C-phenoxyphenyl]pyriproxyfen at a concentration of 0, 0.05, 0.1, 0.5, or 1.0 mmol/L with b-NADPH as a cofactor. The reaction mixtures were analysed by TLC. Pyriproxyfen was not metabolized by any of the S10 preparations; it was almost completely metabolized by microsomal fractions from liver but only very slightly by hepatic cytosol. Most of the major metabolites identified in rats *in vivo* were observed *in vitro*. There was no species difference in the major metabolic reactions. The intrinsic clearance calculated from Lineweaver-Burk plots revealed sex-related differences in metabolic reactions in rats but not in mice, and 5''-hydroxylation was observed only in male rats and not in mice of either sex. The intrinsic clearance for microsomal 4'-hydroxylation in female rats was twice that in males, but was not different in male and female mice. Incubation of a hepatic microsomal fraction from male rats with antisera against male-specific forms of cytochrome P450 (CYP2C11 or CYP2C13) revealed that members of the CYP2C family, the expression of which is sex-dependent in rats, are involved in the major hydroxylation reactions of pyriproxyfen. Antiserum against CYP2C11 inhibited all of the major metabolic reactions of the hepatic microsomal fraction from male rats (> 88% inhibition). The antiserum against CYP2C13 also inhibited all reactions (25-64% inhibition) except 4'-hydroxylation (Yoshino et al., 1996).

2. Toxicological studies

(a) Acute toxicity

The results of studies of the acute toxicity of pyriproxyfen are summarized in Table 1. Pyriproxyfen dissolved in corn oil was administered orally in a volume of 10 ml/kg bw to ICR (Crj:CD-1) mice at doses of 1000, 2000, or 5000 mg/kg bw and to Sprague-Dawley (Crj:CD) rats at 1000, 2500, or 5000 mg/kg bw. In mice, pyriproxyfen reduced spontaneous motor activity and caused ataxia, abnormal respiration, and death in males at 2000 mg/kg bw and in animals of each sex at 5000 mg/kg bw. Transiently decreased body weight was observed in males at 5000 mg/kg bw. Deaths occurred in two males at 2000 mg/kg bw and two at 5000 mg/kg bw in males and in one female at 5000 mg/kg bw. In rats, pyriproxyfen caused a decrease in body-weight gain, decreased spontaneous activity, soft stools, and diarrhoea in males at 2500 mg/kg bw and in animals of each sex at 5000 mg/kg bw, but no deaths. Necropsy revealed no abnormal changes in the organs of mice or rats. In dogs, oral administration of pyriproxyfen in capsules caused no deaths at doses up to 5000 mg/kg bw. The only clinical sign, occasional vomiting for the first 24 h, was observed at the highest dose. Dermal application of 2000 mg/kg bw of pyriproxyfen dissolved in corn oil (5 or 10 ml/kg bw) caused no deaths or signs of clinical toxicity in ICR mice or Sprague-Dawley rats. Exposure of ICR mice or Sprague-Dawley rats to a mist aerosol of pyriproxyfen dissolved in corn oil at concentrations of 0.6 or 1.3 mg/L for 4 h caused no deaths or pathological changes. The mass median aerodynamic diameter of the particles was 0.8-0.9 µm. At the high concentration, salivation and urinary incontinence were observed in rats 4 h after the start of inhalation, and irregular respiration was observed in mice. These clinical signs disappeared within 1 h of cessation of exposure.

(b) Short-term studies of toxicity

Mice

Groups of 10 male and 10 female ICR (Crj:CD-1) mice were given diets containing technical-grade pyriproxyfen (purity, 95.3%) at concentrations of 0, 200, 1000, 5000, or 10 000 ppm, equal to 0, 28, 150, 840, and 2000 mg/kg bw per day for males and 0, 38, 200, 960, and 2300 mg/kg bw per day for females, for 13 weeks. The observations included clinical signs, mortality, food and water consumption, body weight, clinical chemical parameters including serum enzymes, urinary and haematological parameters, organ weights, and gross and histopathological appearance. The serum enzymes assayed were alanine aminotransferase), aspartate aminotransferase, and gamma-glutamyl transpeptidase. Blood samples were collected shortly before termination of the study. The study conformed to GLP.

Death occurred in two males at 5000 ppm and in seven males and nine females at 10 000 ppm (one of the deaths in males was not treatment-related). The clinical signs observed in the animals that died prematurely were emaciation, hunched appearance, and few or no faeces. There were no treatment-related clinical signs in the mice that survived. Terminal body weights were significantly reduced in males at 5000 ppm (89% of control) and at 10 000 ppm (69% of control); the body weights of females were reduced at 5000 ppm during the first half of the study but were comparable to control values (97% of control) by the end. The water consumption of males at the two higher doses was significantly increased, but food consumption was not affected by treatment. There were significant decreases in erythrocyte parameters, including cell count, haemoglobin concentration, haematocrit, mean cell volume, and mean cell haemoglobin value, in animals at 5000 and 10 000 ppm. Platelet counts were significantly increased in animals of each sex at 5000 ppm and in males at 10 000 ppm. Significant increases in total cholesterol concentration were observed in females at doses $\geq$ 1000 ppm: 130% at 1000 ppm, 210% at 5000 ppm, and 140% of control values at 10 000 ppm. In males, the activities of aspartate and alanine aminotransferases were increased by up to twofold but reached significance only at 5000 ppm. gamma-Glutamyl transpeptidase activity showed slight, nonsignificant changes in some groups. The absolute weight of the liver was significantly increased in females at 5000 ppm (120% at 5000 ppm and 160% of control values at 10 000 ppm) but not in males at any dose. Microscopic examination revealed histomorphological alterations of the

Table 1. Acute toxicity of pyriproxyfen

Species	Strain	Sex	Route	LD_{50} or LC_{50} (mg/kg bw or mg/L air)	Purity (%)	Reference
Mouse	ICR	M&F	Oral	> 5000	97.2	Suzuki et al. (1987a)
Rat	Sprague-Dawley	M&F	Oral	> 5000	97.2	Suzuki et al. (1987b)
Dog	Beagle	M&F	Oral	> 5000	97.2	Nakano et al. (1986)
Mouse	ICR	M&F	Dermal	> 2000	97.2	Suzuki et al. (1987c)
Rat	Sprague-Dawley	M&F	Dermal	> 2000	97.2	Suzuki et al. (1987d)
Mouse	ICR	M&F	Inhalation	> 1.3	97.0	Suzuki et al. (1987e)
Rat	Sprague-Dawley	M&F	Inhalation	> 1.3	97.0	Kawaguchi et al. (1987)

These studies were conducted according to good laboratory practice.

kidney comprising tubular nephrosis with microcytosis and dilatation of the renal tubules and focal mineralization and dilatation of the renal pelvis in males at 5000 ppm and in animals of each sex at 10 000 ppm. There were no treatment-related morphological changes in the liver. The NOAEL was 1000 ppm, equal to 150 mg/kg bw per day, on the basis of effects on erythrocyte parameters, deaths, decreased body weight, histomorphological alterations in the kidney, and increased absolute liver weight at higher doses (Cox et al., 1990).

Rats

Groups of 10 male and 10 female Sprague-Dawley (Crl:CD) rats received diets containing technical-grade pyriproxyfen (purity, 95.3%) at concentrations of 0, 400, 2000, 5000, or 10 000 ppm, equal to 0, 23, 120, 310, and 640 mg/kg bw per day for males and 0, 28, 140, 360, and 780 mg/kg bw per day for females, for 13 weeks. The observations included clinical signs, deaths, body weight, food and water consumption, ophthalmological, clinical chemical, haematological, and urinary parameters, organ weights, and gross and histopathological appearance. The clinical chemical examinations included assays for the serum enzymes alkaline phosphatase, aspartate and alanine aminotransferases, and gamma-glutamyl transpeptidase. Blood samples were collected at the end of the study.

There were no treatment-related deaths, toxic signs, or ophthalmological changes at any dose, and no changes in food or water consumption. The body weights of animals of each sex were significantly decreased at doses $\geq$ 5000 ppm (91% of control at 5000 ppm and 88% at 10 000 ppm at termination). Erythrocyte parameters including cell count, haemoglobin concentration, and haematocrit were significantly decreased in animals at doses $\geq$ 5000 ppm and in males also at 2000 ppm. The mean cell volume was reduced in females at 2000 and 10 000 ppm by < 10%. There was no significant effect on platelet count. The total cholesterol concentration was dose-dependently, significantly increased in males at doses $\geq$ 2000 ppm (150% at 2000 ppm, 200% at 5000 ppm, and 210% of control values at 10 000 ppm) and in females at doses $\geq$ 5000 ppm (130% at 5000 ppm and 180% of control values at 10 000 ppm). The serum phospholipid concentration was also significantly increased in males at doses $\geq$ 2000 ppm (130% at 2000 ppm, 180% at 5000 ppm, and 180% of control values at 10 000 ppm) and in females at 10 000 ppm (160% of control value). Significantly increased gamma-glutamyl transpeptidase activity was observed in animals at 10 000 ppm, but the activities of the other serum enzymes were not significantly affected. Significant increases in the absolute weight of the liver were observed in animals at doses $\geq$ 5000 ppm (110% at 2000 ppm, 130% at 5000 ppm, and 140% of control values at 10 000 ppm in males and 100% at 2000 ppm, 120% at 5000 ppm, and 140% of control values at 10 000 ppm in females), and the relative weight of the liver was significantly increased in males at 2000 ppm and in animals of each sex at doses $\geq$ 5000 ppm (120% at 2000 ppm, 140% at 5000 ppm, and 170% of control values at 10 000 ppm in males and 130% at 5000 ppm and 160% of control values at 10 000 ppm in females). Significant increases in the relative weight of the kidney

were observed in animals at 10 000 ppm, but the absolute weights were comparable to those of controls at all doses. Histopathological examination revealed dose-dependent increases in cytoplasmic changes in the liver in all treated groups (1/10 at 0 ppm, 2/10 at 400 ppm, 6/10 at 2000 ppm, 10/10 at 5000 ppm, and 9/10 at 10 000 ppm in males and 1/10 at 0 ppm, 2/10 at 400 ppm, 6/9 at 2000 ppm, 7/10 at 5000 ppm, and 9/10 at 10 000 ppm in females). The cytoplasmic changes consisted of slight, often equivocal increases in cytoplasmic content reflected in a visibly reduced nucleus:cytoplasm ratio and diminution of sinusoidal spaces. The NOAEL was 400 ppm, equal to 23 mg/kg bw per day, on the basis of mild anaemia, increased incidences of minimal hepatic abnormalities, relative liver weights, and serum concentrations of total cholesterol and phospholipids, indicating effects on lipid metabolism, at higher doses (Cox et al., 1989).

Groups of 21 male and 21 female Sprague-Dawley rats were given diets containing pyriproxyfen (purity, 97.2%) at concentrations of 0, 80, 400, 2000, or 10 000 ppm, equal to 0, 4.8, 24, 120, and 680 mg/kg bw per day for males and 0, 5.4, 28, 140, and 690 mg/kg bw per day for females, for 26 weeks. The observations included clinical signs, deaths, food and water consumption, body weight, ophthalmological, clinical chemical, haematological, and urinary parameters, organ weights, and gross and histological appearance. The serum activities of aspartate and alanine aminotransferases, alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase, creatine phosphokinase, and gamma-glutamyl transpeptidase were measured. Blood samples were taken at the end of the study.

There were no deaths, and the only signs of toxicity were increased incidences of alopecia around the neck and soft stools during the early stage of the study in animals at 10 000 ppm. The body

weights of animals at 10 000 ppm were significantly decreased throughout the study, by 86% in males and 87% in females at the end of study. Marked decreases in body-weight gain were observed at this dose. There were no treatment-related changes in food or water consumption. Proteinuria and increased urinary excretion of potassium ion were observed in animals at 10 000 ppm. Slight but significant decreases in erythrocyte count and haematocrit were observed in males at 2000 and 10 000 ppm and in females at 10 000 ppm. The haemoglobin concentration was also slightly but significantly decreased at this dose. No increase in platelet count was observed. Slight but significant increases in total protein, albumin, and blood urea nitrogen were observed in animals at 10 000 ppm. The albumin and a_{2u}-globulin fractions were slightly but significantly increased in males at 10 000 ppm. Total cholesterol and phospholipid concentrations were significantly increased in males at 2000 and 10 000 ppm and in females at 10 000 ppm. Of the serum enzyme activities studied, only that of gamma-glutamyl transpeptidase was significantly increased in males at 10 000 ppm. Significantly increased absolute weights of the liver were observed in animals at 10 000 ppm (130% of control value in males and in females), and significant increases in the relative weight were observed in males at doses $\geq$ 2000 (110% at 2000 ppm and 160% of control values at 10 000 ppm) and in females at 10 000 ppm

(100% at 2000 ppm and 150% of control values at 10 000 ppm). Significantly increased relative kidney weights were observed in animals at 10 000 ppm, but the absolute weights were not significantly increased. Histopathological examination showed slight hypertrophy of the liver in all animals at 10 000 ppm. The NOAEL was 400 ppm, equal to 24 mg/kg bw per day, on the basis of increased relative liver weight, increased total cholesterol and phospholipid concentrations indicating effects on lipid metabolism, and mild anaemia at higher doses (Koyama et al., 1989).

Groups of five male and five female Sprague-Dawley (Crl:CD) rats received dermal applications of pyriproxyfen (purity, 97.2%) dissolved in corn oil at doses of 0, 100, 300, or 1000 mg/kg bw per day under a semi-occlusive dressing for 6 h/day for 21 days. The observations included clinical signs, deaths, food consumption, body weight, and clinical chemical, haematological, and and histological examinations. Serum was assayed for alanine and aspartate aminotransferases. Blood samples were collected at termination. The study complied with GLP.

There were no treatment-related effects on the mortality rate, clinical signs, or haematological or clinical chemical parameters, including serum enzymes. There were no significant changes in body weight or food consumption in the treated group, and no effect of treatment on organ weights was observed. Histopathological examination revealed no treatment-related alterations in the liver or any other tissues examined. The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Moore et al., 1993).

Groups of 10 male and 10 female Sprague-Dawley (Jcl:CD) rats were exposed to a mist aerosol of pyriproxyfen dissolved in corn oil at concentrations of 270, 480, or 1000 mg/m^3 for 4 h/day for 28 days. The mass median aerodynamic diameter of the particles was 0.71–0.88 µm. The observations included clinical signs, deaths, food and water consumption, body weight, and urinary, ophthalmological, clinical chemical, haematological, and histological examinations. Serum was assayed for leucine aminopeptidase, cholinesterase, lactate dehydrogenase, creatine phosphokinase, alanine and aspartate aminotransferase, and alkaline phosphatase activity. Blood samples were collected at termination after a 16-h fast. The study complied with GLP.

There were no deaths. Salivation was observed early in the study in rats at the highest concentration, and the body-weight gain of animals at this dose was sporadically, slightly but significantly lower, although it was normal at the end of the study. There were no treatment-related changes in food consumption or in haematological, urinary, or ophthalmologic parameters. Slightly but significantly increased lactate dehydrogenase activity was observed in males at the highest dose, but the activities of the other serum enzymes showed no treatment-related change. A slight but significant increase (9%) was observed in the relative weight of the liver at 1000 mg/m^3, but the absolute weight was comparable to that of controls. Histopathological examination revealed no treatment-related morphological changes in the

organs of exposed rats. The NOAEL was 480 mg/m^3 on the basis of salivation, sporadically reduced body-weight gain, and increased lactate dehydrogenase activity at 1000 mg/m^3 (Kawaguchi et al., 1988).

Guinea-pigs

The skin sensitizing potential of pyriproxyfen (purity, 97.2%) was tested in a GLP-compliant study in male Hartley guinea-pigs by the maximization method. A volume of 0.05 ml of 1% pyriproxyfen in Freund's complete adjuvant mixed with water or a 0.5% solution of pyriproxyfen in corn oil was injected intradermally for initial sensitization. As a challenge, 0.2 g of pyriproxyfen in 25% petrolatum or 0.2 ml of a positive control was applied dermally in patches to test animals for 24 h, 6 days after the first sensitization. No dermal reaction was observed (Suzuki et al., 1987f).

Rabbits

New Zealand white rabbits of each sex received a single dose of 0.5 g of pyriproxyfen (purity, 97.2%) as a fine powder moistened with physiological saline by dermal application for 4 h. The potential to induce primary skin irritation was examined 4.5, 24, 48, and 72 h after application. The study complied with GLP. No oedema or erythema was observed (Suzuki et al., 1987g).

A single dose of 100 mg of pyriproxyfen (purity, 97.2%) in a volume of 0.1 ml was applied to the right eye of three male and three female New Zealand white rabbits in a study that complied with GLP. The potential to induce primary eye irritation was examined 1, 24, 48, and 72 h after application in unwashed eyes. Slight conjunctival redness (grade 1) and chemosis (grade 1-2) were observed in all treated animals 1 h after application. Conjunctival redness (grade 1), chemosis (grade 1), and discharge (grade 2) were still apparent in one or two animals after 24 h, but these changes had disappeared by 48 h after application. Pyriproxyfen was considered to be a minimal ocular irritant (Suzuki et al., 1987g).

Dogs

Groups of four male and four female beagle dogs, 6 months old, received gelatine capsules containing pyriproxyfen (purity, 97.2%) at doses of 0, 100, 300, or 1000 mg/kg bw per day for 3 months. The observations included clinical signs, deaths, food consumption, body weight, and ophthalmic, electrocardiographic, clinical chemical, haematological, urinary, and histological parameters. The activities of alkaline phosphatase, aspartate and alanine aminotransferases, gamma-glutamyl transpeptidase, creatine phosphokinase, and lactate dehydrogenase were measured in plasma. Ophthalmological examinations were performed during weeks 0, 5, and 12 of treatment, and an electrocardiograph was obtained at the same times. Blood samples were collected during weeks 0, 4, 8, and 12 of treatment; hepatic function was assessed by bromsulphalein retention during weeks 0, 6, and 13 of treatment, and renal function was assessed by retention of *para*-aminohippuric acid during weeks 0, 5, and 11 of treatment.

No deaths were observed. Female dogs at 1000 mg/kg bw per day had a slightly increased incidence of soft stools, but no other treatment-related toxic signs were observed. There were no changes in body weight, body-weight gain, food consumption, or ophthalmological parameters throughout the study, and no treatment-related changes in the electrocardiograph were observed at any dose. Haematological parameters were not significantly changed at any dose, although slight, nonsignificant alterations in the number of platelets (by < 39%) and total cholesterol concentration (by < 67%) were observed, with no obvious dose-dependence. The phospholipid concentration was significantly increased in females at 1000 mg/kg bw per day. No significant changes was found in the activities of the serum enzymes studied, although there were trends to increased alkaline phosphatase activity in males at the high dose, lactate dehydrogenase activity in males at all doses and in females at the high dose, and creatine phosphokinase activity in males in all doses, with no dose-dependence. Aspartate and alanine aminotransferase activities were unaltered. Hepatic function was not significantly affected at any dose. The absolute weights of the liver were significantly increased in males at 300 and 1000 mg/kg bw per day, by 30% and 26%, respectively, and significant increases in relative liver weights were observed in males at 300 mg/kg bw per day, by 24%. Increased incidences of hepatocellular hypertrophy were observed in females at 300 mg/kg bw per day and in animals of each sex at 1000 mg/kg bw (0/4 in controls, 0/4 at 100 mg/kg bw per day, 0/4 at 300 mg/kg bw per day, and 4/4 at 1000 mg/kg bw per day in males, and 0/4, 0/4, 3/4, and 4/4 in females, respectively). At 1000 mg/kg bw per day, increased incidences of eosinophilic bodies in the liver were observed (0/4 in controls, 0/4 at 100 mg/kg bw per day, 0/4 at 300 mg/kg bw per day, and 2/4 at 1000 mg/kg bw per day in males, and 0/4, 0/4, 0/4, and 2/4 in females, respectively). Electron microscopic examination revealed a minimal to slight increase in smooth endoplasmic reticulum with slight dilatation in the livers of all animals at 1000 mg/kg bw per day. These changes are consistent with adaptation of the liver to exposure to the compound through enzyme induction. A slight but significantly prolonged retention ratio in the test for renal function was observed in males at 300 and 1000 mg/kg bw per day after 6 weeks of treatment, but the retention ratio was normal by the end of the study, and no treatment related histopathological changes were observed in the kidney. The NOAEL was 100 mg/kg bw per day on the basis of increased absolute and relative liver weights and an increased incidence of hepatocellular hypertrophy at higher doses (Nakano et al., 1988).

Groups of four male and four female beagle dogs (23-27 weeks old) were given gelatine capsules containing pyriproxyfen (purity, 95.3%) at doses of 0, 30, 100, 300, or 1000 mg/kg bw per day for 52 weeks. The observations included clinical signs, deaths, food consumption, body weight, and ophthalmoscopic, clinical chemical, haematological, urinary, and histological examinations. The activities of alanine and aspartate aminotransferases, alkaline phosphatase, and creatine phosphokinase were measured in plasma. Blood samples were collected 1 week before and on weeks 12, 24, 37, and 50 of treatment. The study conformed to GLP.

Two males at 1000 mg/kg bw per day were killed *in extremis* with acute weight loss and, in one case, liver failure, at weeks 17 and 31 of treatment. Slightly increased frequencies of salivation and diarrhoea were observed in animals at 1000 mg/kg bw per day, and emaciation was observed in males at doses $\geq$ 300 mg/kg bw per day. There were no treatment-related ophthalmological abnormalities. A dose-dependent but nonsignificant reduction in body weight was seen throughout the study, and body-weight gains were significantly reduced in animals at doses $\geq$ 300 mg/kg bw per day. Food consumption was not reduced at any dose. Significant changes were seen in several haematological parameters, including 10-20% decreases in haemoglobin and erythrocyte counts and a slightly but significantly increased mean corpuscular volume in males at doses $\geq$ 300 mg/kg bw per day and in females at doses $\geq$ 100 mg/kg bw per day and a significantly decreased packed cell volume in the latter group. The change in haemoglobin in males at 1000 mg/kg bw per day was not significant. These haematological changes might indicate slight anaemia. There was no abnormality of cellularity or cell composition in the bone marrow.

Statistically significant decreases in the number of lymphocytes were observed in all treated females at weeks 12 and 37, which were attributed to transiently high values for the control group. The platelet count was significantly increased in males at doses ≥ 100 mg/kg bw per day and females at 1000 mg/kg bw per day throughout the study. Slightly but significantly prolonged prothrombin times were observed in males at 300 mg/kg bw per day and in animals of each sex at 1000 mg/kg bw per day. Significantly increased plasma enzyme activities were seen, including those of alkaline phosphatase in animals at doses ≥ 300 mg/kg bw per day throughout the study, alanine aminotransferase in animals at 1000 mg/kg bw per day throughout the study, and aspartate aminotransferase in males at 1000 mg/kg bw per day in weeks 12, 24, and 37 of treatment. The total cholesterol concentration in plasma was significantly increased in animals at doses ≥ 30 mg/kg bw per day (by 24–58% at 30 mg/kg bw per day, 64–160% at 100 mg/kg bw per day, 100–140% at 300 mg/kg bw per day, and 76–100% at 1000 mg/kg bw per day) throughout the study, and the plasma concentrations of triglycerides were significantly increased in animals at doses ≥ 100 mg/kg bw per day. These increases were dose-dependent except at the highest dose in males, but in this group there were only two survivors. No reduction in plasma protein fractions was observed in the treated groups.

A slightly reduced pH and increased volume of urine were observed in males at 1000 mg/kg bw per day throughout the study. The absolute weights of the liver were dose-dependently increased in all treated groups and significantly increased in males at doses ≥ 100 mg/kg bw per day (by 130% at 30 mg/kg bw per day, 150% at 100 mg/kg bw per day, 170% at 300 mg/kg bw per day, and 190% of control values at 1000 mg/kg bw per day) and in females at doses ≥ 300 mg/kg bw per day (110%, 120%, 140%, and 140%, respectively). The relative weights of the liver

were significantly increased in males at doses ≥ 30 mg/kg bw per day (130% of control value) and in females at doses ≥ 300 mg/kg bw per day. The absolute weights of the thyroid were significantly increased in females at doses ≥ 300 mg/kg bw per day, and the relative weights were significantly increased in females at doses ≥ 100 mg/kg bw per day. Significantly increased relative renal weights were observed in males at 300 mg/kg bw per day and in females at doses ≥ 300 mg/kg bw per day; the absolute weights were dose-dependently but not significantly increased.

Macroscopic examination showed enlarged livers and hepatic damage in the two dogs at 1000 mg/kg bw per day which died. Histopathological examination revealed treatment-related hepatic damage in animals of each sex at 1000 mg/kg bw per day, which was characterized by centriacinar fibrosis in 2/2 males and 3/4 females and bile-duct hyperplasia in 2/2 males and 3/4 females; foci of cystic degeneration in 1/2 males and 1/4 females; active chronic inflammation in 2/2 males and 2/4 females; and nodular hyperplasia in 2/2 males and 0/4 females. Submucosal fibrosis in the gall-bladder was observed in all male animals, including those that had died, and in 3/4 females at 1000 mg/kg bw per day, in association with bile-duct hyperplasia. One of four males at 30 mg/kg bw per day had focal bile-duct hyperplasia and focal subcapsular fibrosis, but these effects were not observed at 100 or 300 mg/kg bw per day. There were no preneoplastic or neoplastic alterations. Although no morphological alterations were seen in the liver, the increase in cholesterol concentration and relative liver weight at low doses might be related to treatment. No NOAEL could be identified, as increased total cholesterol concentrations indicating effects on lipid metabolism and increased relative liver weights with a trend towards increased absolute liver weights were seen at all doses (Chapman et al., 1991).

In a complementary study which complied with GLP, groups of four male and four female beagle dogs (19–24 weeks old) were given gelatine capsules containing pyriproxyfen (purity, 95.3%) at doses of 0, 3, or 10 mg/kg bw per day for 52 weeks. The observations included clinical signs, deaths, food consumption, body weight, and ophthalmic, clinical chemical, haematological, urinary, and histological examinations. The activities of alanine and aspartate aminotransferases, alkaline phosphatase, and creatine phosphokinase were assayed in serum. Blood samples were collected after 12, 24, 36, and 50 weeks of treatment.

There were no deaths, signs of clinical toxicity, or changes in body weight, body-weight gain, or food consumption. Significantly increased platelet counts were observed in males at 3 mg/kg bw per day in weeks 24, 36, and 50 of treatment and in those at 10 mg/kg bw per day in week 36, but with no clear dose-dependence. Prothrombin time was not prolonged in males at any dose. Females at 10 mg/kg bw per day also showed significantly increased platelet counts in weeks 36 and 50 (by 8% at 3 mg/kg bw per day and 10% at 10 mg/kg bw per day), and prothrombin time was slightly but significantly prolonged at these doses at the end of study. There were no other treatment-related changes in haematological parameters. The total cholesterol

concentration was unchanged; slight but significant increases in total triglyceride concentrations in males at 10 mg/kg bw per day were seen in weeks 12 and 36 of treatment. There were no treatment-related changes in urinary parameters. The absolute weight of the liver was slightly increased in females at 10 mg/kg bw per day (110% of control), but this was not significant. No histopathological changes were found in any organ, including the liver and kidney. The range of mean total platelet counts in controls in other studies in this laboratory was 273–357 in males and 305–357 in females, whereas those in the present study were 341–367 in controls, 414–462 at the low dose, and 415–456 at the high dose in males, and 321–384 in controls and 430–478 at the high dose in females. The increased numbers of platelets and the prolonged prothrombin time were therefore treatment-related changes although no significant increase in platelet counts was observed in animals at 30 mg/kg bw per day in the previous study (Mitchel et al., 1993).

The NOAEL for the two 52-week studies in dogs was 10 mg/kg bw per

day, on the basis of the absence of treatment-related toxicity at 10 mg/kg bw per day in the second study and changes in lipid metabolism and increased liver weight at 30 mg/kg bw per day in the first study.

(c) Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female ICR(Crj-CD-1) mice were given diets containing pyriproxyfen (purity, 95.3%; 97.6-98.7% dietary concentration) at concentrations of 0, 120, 600, or 3000 ppm for 78 weeks, providing doses equal to 0, 16, 81, and 420 mg/kg bw per day for males and 0, 21, 110, and 530 mg/kg bw per day for females. The observations included clinical signs, deaths, food consumption, body weight, organ weights, and ophthalmological, haematological, and gross and histological examinations. Ten mice from each group were killed during week 52 for interim examination, and the surviving mice were killed during week 78. Blood samples were taken from 10 rats per group during weeks 52 and 78 of treatment. No clinical chemical tests were conducted. The study complied with GLP.

The mortality rate was dose-dependent and significantly increased in males at 600 and 3000 ppm (43% at 0 ppm, 55% at 120 ppm, 72% at 600 ppm, and 82% at 3000 ppm) and in females at 3000 ppm (39% at 0 ppm, 44% at 120 ppm, 55% at 600 ppm, and 64% at 3000 ppm). There were slight, nonsignificant increases in the incidence of clinical signs, including reduced motor activity and hunched position, in animals at 3000 ppm. Statistically significant decreases in body weights, body-weight gains, and/or food consumption were observed in males at 3000 ppm during the study. The absolute and relative weights of the liver were significantly increased in females at 3000 ppm during week 52 of treatment. The haematological parameters showed no treatment-related change. Histopathological examination of animals that died revealed a significantly increased incidence of systemic amyloidosis in the glandular stomach of males at 600 and 3000 ppm and the adrenal, thyroid, heart, liver, kidney, glandular stomach, and duodenum of females at 3000 ppm, and a dose-related relationship was found between the generalized amyloidosis and the mortality rate. Statistical analysis of the incidence of graded amyloidosis revealed a significant positive trend in renal amyloidosis in females and a significant positive trend in hepatic amyloidosis in animals of each sex at 3000 ppm. Females at this dose had a significant increase in the incidence of lymphocytic infiltration in the liver (22/59 at 0 and 34/60 at 3000 ppm) and of tubular mineralization (3/59 at 0 and 46/60 at 3000 ppm). Deposition of amyloid in the kidney causes numerous pathological changes including tubular mineralization and papillary necrosis. In this study, however, the incidences of tubular mineralization and segmental cortical atrophy were increased independently of amyloidosis in female animals at 3000 ppm, suggesting that the chronic nephrosis was directly related to treatment. Histopathological examination revealed no increase in the incidence of neoplastic lesions at any dose. The NOAEL was 120 ppm, equal to 16 mg/kg bw per day, on the basis of increased mortality at higher doses (Osheroff et al., 1991a; Cardy et al., 1994).

Rats

Groups of 50 male and 50 female Sprague-Dawley (Crl:CD) rats were given diets containing pyriproxyfen (purity, 95.3%) at concentrations of 0, 120, 600, or 3000 ppm, equal to 0, 5.4, 27, and 140 mg/kg bw per day for males and 0, 7.0, 35, and 180 mg/kg bw per day for females, for 104 weeks. Satellite groups of 30 males and 30 females were also treated orally. The observations included clinical signs, deaths, food and water consumption, body weight, organ weights, and ophthalmoscopic, clinical chemical, haematological, urinary, and gross and histopathological examination. Assays were performed for the serum enzymes aspartate and alanine aminotransferase, alkaline phosphatase, creatine kinase, and gamma-glutamyl transpeptidase. Blood samples were collected from satellite groups of rats on week 13, 26, 52, 78, and 104 of treatment. The study complied with GLP.

Treatment did not affect mortality (34-46% in males and 32-58% in females), clinical signs, or ophthalmoscopic end-points. The body weights of males were significantly reduced in weeks 13, 26, and 50 of treatment (by 5-7%) and those of females in weeks 13, 26, 50, and 78 of treatment (by 12-14%) at 3000 ppm, but they had returned to the control level by the end of study. The mean body-weight gain was significantly reduced in females at 600 ppm and in animals of each sex at 3000 ppm throughout the study. No treatment-related changes in food consumption were observed. The only alteration in haematological parameters was a transient increase in eosinophils. Alkaline phosphatase activity was significantly increased in males at doses $\geq$ 120 ppm in weeks 26, 52, and 78 of treatment, but the activity (87-104 U/L) remained within the range of historical controls (45-114 U/L), and the changes were not clearly dose-dependent. gamma-Glutamyl transpeptidase activity was significantly increased in males at 3000 ppm in week 104 of treatment and in females at 600 and 3000 ppm in weeks 26 and 52. The activities of other serum enzymes were not significantly affected. Significantly increased total cholesterol concentrations were observed in males at 3000 ppm in weeks 26 and 52 (149% and 147% of control, respectively). Slight, inconsistent, nonsignificant increases in urinary protein concentration were observed in females at 3000 ppm in week 26. At interim necroscopy, the absolute weights of the liver were found to be nonsignificantly increased at week 52 of treatment with 3000 ppm (by 15% in males and 13% in females). A significant increase in relative liver weight was observed only in females at 3000 ppm (120% of control). The only significant or treatment-related increases in the incidence of morphological alterations at 104 weeks seen on gross and histopathological examination were hyperkeratosis of the skin of males at 3000 ppm, which was considered not to be biologically significant, and a significant increase in the incidence of liver necrosis in males

at 3000 ppm that died during the study; however, no liver necrosis was observed in the surviving males at 3000 ppm at the end of the study, indicating nthat it was not related to treatment. Histopathological examination revealed no evidence of neoplastic alterations. The NOAEL was 600 ppm, equal to 27 mg/kg bw per day, on the basis of reductions in body weight and mean body-weight gain and increased absolute and relative liver weights at higher doses (Osheroff et al., 1994a,b).

(d) Genotoxicity

The results of tests for the genotoxicity of pyriproxyfen are summarized in Table 2. All of the positive controls used in the assays produced the expected positive responses. In assays for reverse mutation, no induction of revertant colonies was observed at six doses with or without an exogenous metabolic activation system (S9). In tests for DNA repair, pyriproxyfen was inactive at six doses with or without S9. In tests for gene mutation in mammalian cells, no mutations were observed at four doses ranging from 10 to 300 ppm without S9 or 10 to 100 ppm with S9. In assays for unscheduled DNA repair synthesis, pyriproxyfen was cytotoxic and/or inhibited normal DNA synthesis but it did not induce unscheduled DNA synthesis. In tests for cytogeneticity, Chinese hamster ovary cells (CHOK1) were exposed to pyriproxyfen at a concentration of 10, 30, or 100 µg/ml for 2 h in the presence of S9 and cultured for a further 16 or 22 h or cultured with pyriproxyfen for 18 or 24 h in the absence of S9. Although marked cytotoxicty, characterized by decreased mitotic index and cell cycle delay, were observed at doses $\geq$ 30 mg/ml without S9, and at 100 mg/ml with S9, no increase in the total number of structural aberrations or the frequency of cells with aberrations was observed at any concentration. In the test for micronucleus formation in mice *in vivo*, a single dose of pyriproxyfen at 5000 mg/kg bw slightly but not statistically significantly increased the incidence of micronucleated polychromatic erythrocytes.The Meeting concluded that pyriproxyfen is not genotoxic *in vivo* or *in vitro*.

Table 2. Results of tests for the genotoxicity of pyriproxyfen

End-point	Test system	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a]	S. typhimurium TA98, TA100, TA1537, TA1538, E, coli WP2 uvrA	10-5000 µg/plate	97.2	Negative ± S9	Kogiso et al. (1988a)
DNA repair[b]	B.subtills M45, H17	673-21 500 µg/disc in DMSO	95.3	Negative ± S9	Kogiso et al. (1992)
Gene mutation[c]	Chinese hamster V79 cells, hprt locus	3-300 µg/ ml	95.3	Negative ± S9	Kogiso et al. (1990)
Unscheduled DNA synthesis[d]	Human HeLa S3 epithelioid cells	0.1-205 µg/ ml	95.3	Negative ± S9	Henderson & Proudlock (1989)
Chromosomal aberrations[e]	Chinese hamster ovary cells	10-300 µg/ml	97.2	Negative ± S9	Kogiso et al. (1989)
Chromosomal aberrations[f]	Chinese hamster ovary cells	9.6-321 µg/ml -S9 50-200 µg/ml +S9	97.2	Negative ± S9	Kogiso et al. (1988)
In vivo					
Micronucleus formation[g]	CD-I mice, bone marrow	Single intraperitoneal injections of 5000 mg/kg bw at 24, 48, and 72 h	95.3	Negative	Proudlock et al. (1991)

Table 2. (continued)

All studies were conducted according to good laboratory practice. DMSO, dimethyl sulfoxide

[a] Positive controls were methylmethanesulfonate for TA100, 2-nitrofluorene for TA98 and TA1538, sodium azide for TA1535, ICR-191 for TA1537, *N*-ethyl- *N'*-nitro- *N*-nitrosoguanidine for WP2 *uvr*A, benzo[*a*]pyrene for TA100, TA98, TA1537, and TA1538 and 2-aminoanthracene for TA1535 and WP2 *uvr*A.

[b] Positive controls were mitomycin C for the direct assay and sterigmatocystin for the activation assay. The negative control was kanamycin in both assays.

[c] Positive controls were ethylmethane sulfonate for the direct assay and 9,10-dimethyl-1,2-benzanthracene for the activation assay.

[d] Positive controls were 2-acetylaminofluorene for the activation assay and 4-nitroquinoline-1-oxide for the direct assay.

[e] Positive controls were mitomycin C for the direct assay and cyclophosphamide for the activation assay.

[f] Positive controls were mitomycin C for the direct assay and benzo[*a*]pyrene for the activation assay.

[g] Positive control was mitomycin C.

(e) Reproductive toxicity

(i) Multigeneration reproductive toxicity

Rats

Groups of 26 male and 26 female Sprague-Dawley (Crj) rats were given diets containing technical-grade pyriproxyfen (purity, 95.3%) at concentrations of 0, 200, 1000, or 5000 ppm. The F_0 animals were treated for 70 days before mating and then for 6 subsequent weeks for males and during 3 weeks of gestation and 3 weeks of the lactation period for females. The F_1 generation were treated for 18 weeks from the day of their weaning to the day of weaning of the F_2 generation, including 77-90 days before mating and the mating, gestation, and

lactation periods. The mean daily intakes of pyriproxyfen were 14, 68, and 340 mg/kg bw per day in males and 20, 98, and 500 mg/kg bw per day in females (18, 87, and 440 mg/kg bw per day before mating, 15, 77, and 390 mg/kg bw per day during gestation, and 32, 160, and 830 mg/kg bw per day during lactation) in the F_0 generation, and 17, 83, and 440 mg/kg bw per day in males and 21, 110, and 560 mg/kg bw per day in females (21, 100, and 550 mg/kg bw per day before mating, 14, 72, and 380 mg/kg bw per day during gestation, and 28, 160, and 810 mg/kg bw per day during lactation) in the F_1 generation.

The observations in parental rats included clinical signs, deaths, food consumption, body weight, estrus cycle, and histopathological and reproductive parameters which included mating, fertility, gestation, and live birth indices. All parental animals were killed at the end of weaning, and the reproductive organs, brain, pituitary, liver, and kidney were examined histopathologically. The organs from the F_1 parental animals were weighed. Estrus cycles were examined by a smear assay during the 10 days before mating. The observations in the F_1 and F_2 pups included viability, body weight, and lactation indices. Developmental indices were not examined. Groups of 10 male and 10 female pups were selected randomly from 10 litters for necroscopy. One male and one female were selected randomly from each F_1 litter to provide 26 pairs at each dose to serve as parents for the F_2 generation. Pups were weighed by sex. The study complied with GLP.

The F_0 parent animals showed no treatment-related changes in clinical signs, mortality rate, reproductive parameters, or estrus cycle. Body weight and body-weight gain were significantly reduced in F_0 animals at 5000 ppm during the periods of premating, gestation, and lactation, and food consumption was significantly reduced in F_0 females at this dose during gestation. No gross or histopathological alterations were seen that were related to treatment. The NOAEL for F_0 parental toxicity was 1000 ppm, equal to 68 mg/kg bw per day, on the basis of reductions in body weight and body-weight gain at 5000 ppm. The NOAEL for reproductive toxicity was 5000 ppm, equal to 340 mg/kg bw per day, the highest dose tested.

The F_1 parent animals showed no treatment-related changes in clinical signs, mortality rate, reproductive parameters, or estrus cycles. The body weights of F_1 males and F_1 females at 5000 ppm were significantly reduced, and the terminal body weights of animals at this dose were significantly decreased (to 98% at 1000 ppm and 88% at 5000 ppm in males and 100% at 1000 ppm and 95% at 5000 ppm in females). Body-weight gain was also significantly reduced in F_1 males at 5000 ppm during the premating period. Food consumption was significantly reduced at this dose in F_1 males during treatment and in F_1 females during gestation. The absolute weights of the liver were significantly increased in F_1 adults at 5000 ppm (110% at 1000 ppm and 110% at 5000 ppm in males and 120% at 5000 ppm in females), and the relative weights were significantly increased in males at 1000 ppm (110% of control) and in animals of each sex at 5000 ppm (130% in males and 130% in females). Histopathological examination showed an increased incidence of focal clear cells in the liver in males at 5000 ppm, but this effect is commonly observed in male rats and was considered to be unrelated to treatment. Significant increases in relative kidney weights were observed in males at 1000 ppm (110% of control values) and 5000 ppm (110%), but the absolute weights were not significantly increased at any dose. Histopathological examination showed an increased incidence of chronic interstitial nephrosis (7/26 at 0 ppm, 3/26 at 200 ppm, 7/26 at 1000 ppm, and 15/26 at 5000 ppm) in males at 5000 ppm and in the incidence of hydronephrosis (1/26 at 0 ppm and 4/26 at 5000 ppm) in females at this dose; however, these increases did not reach statistical significance. No other treatment-related morphological lesions were observed. The NOAEL for F_1 parental toxicity was 1000 ppm, equal to 83 mg/kg bw per day, on the basis of decreased body weight, decreased food consumption, and increased absolute and relative liver weights at 5000 ppm. No evidence of reproductive toxicity was observed. The NOAEL for reproductive toxicity was 5000 ppm, equal to 340 mg/kg bw per day, the highest dose tested.

In F_1 pups, there was no treatment-related change in clinical signs, sex ratio, viability index, or lactation index and no significant difference between treated and control groups in body weight at birth. Significant reductions in body weight were observed in male F_1 pups on day 21 and in female F_1 pups on days 14 and 21 *post partum* at 5000 ppm. The total litter weight was also significantly decreased on days 14 and 21 *post partum* at this dose. No treatment-related effects were seen on gross examination. F_2 pups also showed no treatment-related change in clinical signs, sex ratio, viability index, or lactation index. The mean pup weights were significantly decreased on days 14 and 21 *post partum* at 5000 ppm. No gross pathological alteration related to treatment was apparent at any dose. The NOAEL for developmental toxicity was 1000 ppm, equal to 98 mg/kg bw per day, on the basis of reduced body weight in F_1 and F_2 pups at 5000 ppm (Robinson et al., 1991).

In a study of treatment before and during the early stages of gestation (segment 1) conducted according to GLP, groups of 24 male and 24 female Sprague-Dawley rats were given pyriproxyfen (purity, 97.2%) dissolved in corn oil by gavage at doses of 0, 100, 300, 500, or 1000 mg/kg bw per day for 12 weeks comprising 9 weeks before mating and 3 weeks of mating, in males or for at least 3 weeks including 2 weeks before mating and the mating period and on days 0–7 of gestation in females. The observations in parental rats included clinical signs, deaths, food consumption, body weight, organ weights, gross appearance, and reproductive performance including copulation and fertility indices. Clinical examinations were performed twice a day. Males were killed at the end of mating, and female animals on day 21 of gestation.

Treatment-related deaths occurred in two females at the highest

dose. Increased incidences of diarrhoea and erythema and swelling of the anal region were observed in females at 300 mg/kg bw and in animals of each sex at doses ≥ 500 mg/kg bw per day. Dose-dependent increases in the incidence of salivation were frequently observed in all treated groups, with incidences of 0/24 in controls and 18/24, 21/24, 24/24, and 24/24 at the four doses respectively, in males, and 0/24, 1/24, 5/24, 7/24, and 8/24, respectively, in females. Body weight was significantly reduced in females at 100 mg/kg bw per day during gestation and in animals of each sex at doses ≥ 300 mg/kg bw per day throughout the study. Body-weight gain and food consumption were also significantly decreased in males at doses ≥ 300 mg/kg bw per day throughout the study, but significant decreases were observed only during treatment in females. Water consumption was significantly increased in males at doses ≥ 100 mg/kg bw per day and in females at doses ≥ 300 mg/kg bw per day. The absolute weights of the liver, kidney, and adrenal glands were significantly increased and the absolute weight of the thymus was significantly decreased in males at doses ≥ 300 mg/kg bw per day. In females, the absolute weights of the kidney and adrenals were significantly increased at 1000 mg/kg bw per day. Enlarged, dark-red livers, pitted, enlarged kidneys, enlarged adrenals, and thymus atrophy were observed in males at doses ≥ 300 mg/kg bw per day, and congested, enlarged livers, enlarged adrenals, and atrophy of the spleen and thymus were observed in females at 1000 mg/kg bw per day. There was no treatment-related change in reproductive performance. No NOAEL could be identified for maternal toxicity, as reduced body weight during gestation was seen at all doses. The NOAEL for reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested.

Slightly but significantly reduced numbers of corpora lutea (90% of control) and of live fetuses (88% of control) were observed at 1000 mg/kg bw per day, but the values were within the range in historical controls. Slight increases in placental weight and in fetal body weight were observed at 1000 mg/kg bw per day, but the effects were not dose-dependent and were within the range in historical controls. A slight but significant increase in the number of phalanges of the proximal forepaw was observed, but such increases are known to be associated with an altered growth rate. The incidences of external, visceral, and skeletal anomalies were not increased. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Saegusa et al., 1988a).

In a study of treatment during the perinatal and lactation periods (segment 3) conducted according to GLP, groups of 23-24 pregnant Sprague-Dawley rats were given pyriproxyfen (purity, 97.2%) dissolved in corn oil by gavage at doses of 0, 30, 100, 300, or 500 mg/kg bw per day from day 17 of gestation to day 20 *post partum*. The dams were allowed to deliver naturally. On day 4 *post partum*, the offspring were culled to adjust the litter size to eight (four males and four females when possible) for tests of development (F_1I), and the remaining pups (F_1II) were killed for skeletal examination on the day of culling. The dams (F_0) were killed at termination of weaning. The observations in maternal rats included clinical signs, deaths, body weight, food consumption, organ weights, gross appearance, and reproductive indices including delivery rate, birth rate, and body weight of live newborns. The F_1I offspring were weighed on days 0, 4, 7, 14, and 21 *post partum* during lactation; after weaning, one male and one female per litter were weighed once a week. Clinical signs were observed twice a day. The indices of development during lactation included separation of the auricle, emergence of abdominal hair, eruption of incisors, separation of eyelids, and descent of testes or opening of the vagina. On day 20, all F_1 offspring were examined for sensory function in tests for visual placing, righting and mid-air righting reflexes, and response to pain. On day 21 *post partum*, two male and two female offspring from each litter (F_1Ia) were killed for visceral and skeletal examination. After weaning, another male and female from each litter (F_1Ib) were examined for emotionality (behaviour in an open field) at 4 weeks of age, for motor coordination (rotarod performance) at 5 weeks of age, and for learning ability (in a water-filled multiple T maze) at 6 weeks of age. The F_1Ib offspring were killed for necropsy on day 56 *post partum*. Another male and female from each litter (F_1Ic) were tested only for reproductive performance after being paired for mating within the same group (avoiding sibling matings) at 11 weeks of age. The fetuses were removed surgically from mated females on day 21 of gestation. Reproductive performance was assessed from indices of mating, fertility, gestation, and litters. The fetuses (F_2) were weighed and examined for external anomalies.

In the F_0 maternal animals, treatment-related deaths were observed at 500 mg/kg bw per day, an increased incidence of diarrhoea at doses ≥ 300 mg/kg bw per day, and dosedependent increases in the incidence of salivation at doses ≥ 100 mg/kg bw per day (0/23 in controls and 0/23, 1/23, 2/24, and 4/24 at the four doses, respectively). During gestation, significantly reduced body weights were observed at 500 mg/kg bw per day, and significantly reduced body-weight gain and food consumption were seen frequently during the study at doses ≥ 300 mg/kg bw per day. The absolute and relative weights of the liver were significantly increased at doses ≥ 300 mg/kg bw per day. Atrophy of the thymus, congestion of the liver and kidneys, enlargement of the adrenals, and atrophy of the spleen were observed at 500 mg/kg bw per day, and an increased number of stillborns was seen at this dose. The body weight of live newborn pups was significantly decreased at doses ≥ 300 mg/kg bw per day. The NOAEL for maternal toxicity was 100 mg/kg bw per day, on the basis of clinical signs, reductions in body weight, body-weight gain, and food consumption, and decreased body weight of delivered newborn at 300 mg/kg bw per day.

At 500 mg/kg bw per day, the survival rate of F_1 offspring was significantly decreased on days 0-4 *post partum*, and the weaning

rate was decreased on days 4-21 *post partum*. No decrease in the viability of F_1I offspring was observed on days 21-77. The body weights of pups of each sex at doses ≥ 300 mg/kg bw per day were significantly reduced on days 0-49 or 56 *post partum*. All of the physical developmental indices were significantly retarded and slight retardation of sexual differentiation was observed at doses ≥ 300 mg/kg bw per day. No treatment-related change in sensory functions was observed in F_1I offspring. Visceral examination of the F_1Ia offspring on day 21 *post partum* revealed a significant increase in the number with anomalies at 30, 300, and 500 mg/kg bw per day (0% in controls and 10%, 1.2%, 17.2%, and 29% at the four doses, respectively).The anomalies consisted mainly of dilatation of the renal pelvis and hyperaemia and/or inflammatory cell infiltration. The incidence of dilatation of the renal pelvis was significantly increased at doses ≥ 300 mg/kg bw per day (0% in controls and 3.8%, 1.2%, 14%, and 18% at the four doses, respectively, with 0-3.2% in historical controls). The incidence of hyperaemia and/or inflammatory cell infiltration was significantly increased at 500 mg/kg bw per day (0% in controls and 3.8%, 0%, 5.8%, and 12% at the four doses, respectively). Although developmental insufficiency of the papilla or stenosis and obstruction of the urethra can cause dilatation of the renal pelvis, as can developmental retardation, the range in historical controls of this strain was 0-2.0% in fetuses and 0-3.2% in 21-day-old offspring, and recovery from dilatation of the renal pelvis usually occurs after birth. No skeletal anomalies were found that were related to treatment. The absolute weights of all organs of F_1 offspring killed on day 21 *post partum* were slightly but significantly decreased, but no treatment-related change was found in the absolute weights of the organs of F_1Ib offspring killed on day 56 *post partum*. The changes in organ weights might have been due to retarded physical development. Ambulation was also slightly but significantly increased at doses ≥ 100 mg/kg bw per day (by 31% at 100 mg/kg bw per day, 50% at 300 mg/kg bw per day, and 37% at 500 mg/kg bw per day). Motor coordination was comparable to that of controls, and no treatment-related changes in learning ability were observed. All the changes observed in the F_1 offspring, including increased ambulation, could have been due to retarded physical development. After maturation, the reproductive perfomance of F_1Ic offspring was comparable to that of controls. The body weight and body-weight gain of pregnant F_1 animals were comparable to those of controls during gestation. The NOAEL for developmental toxicity was 100 mg/kg bw per day, on the basis of decreased body weight,

retardation of physical development, and visceral anomalies associated with the retarded growth rate at 300 mg/kg bw per day.

Significantly reduced numbers of implantations (12 in controls and 10, 12, 11, and 9.2 at the four doses, respectively) and mean numbers of live F_2 fetuses (12 in controls and 9.3, 11, 10, and 8.5 at the four doses, respectively) were observed at 500 mg/kg bw per day. Significantly reduced numbers of corpora lutea, implantations, and live fetuses were found at 30 mg/kg bw per day but not at 100 mg/kg bw per day. These reductions were not dose-dependent. There were no treatment-related differences in fetal body weight or in the incidence of external anomalies between treated and control groups. The NOAEL for F_1 reproductive toxicity was 300 mg/kg bw per day, on the basis of a reduction in the number of implantations and in the mean number of live fetuses at 500 mg/kg bw per day (Saegusa et al., 1988b).

(ii) Developmental toxicity

Rats

In a study that complied with GLP, groups of 36 female Sprague-Dawley rats were given pyriproxyfen (purity, 97.2%) dissolved in corn oil by gavage at doses of 0, 100, 300, or 1000 (42 animals) mg/kg bw per day on days 7-17 of gestation. The fetuses (F_1I) were removed surgically from 23 pregnant dams (F_0I) at 0, 100, or 300 mg/kg bw per day and from 20 at 1000 mg/kg bw per day on day 21 of gestation and examined for developmental toxicity. The remaining 10-13 dams (F_0II) were allowed to deliver normally. On day 4 *post partum*, the offspring were culled to adjust the litter size to eight (four males and four females when possible) for functional testing on day 20 *post partum* (F_1IIa). The remaining pups (F_1IIb) were killed at 21 days of age and examined for skeletal anomalies. During lactation, developmental indices were examined. After weaning, one male and one female from each litter (F_1IIa1) allocated for functional testing were examined for emotionality at 4 weeks of age, for motor coordination at 5 weeks of age, and for learning ability at 6 weeks of age; these offspring were killed for necropsy at 8 weeks of age. Another male and female from each litter (F_1IIa2) were tested for reproductive performance after being paired for mating within the same group (avoiding sibling mating) at 11 weeks of age, and the fetuses were removed surgically from the mated females on day 21 of gestation. The remaining offspring (F_1IIa3) were killed for necroscopy at 21 days of age, after weaning.

The observations in parental rats included clinical signs, deaths, food and water consumption, body weight, estrus cycle, and organ weights. The postnatal developmental indices included separation of the auricle, emergence of abdominal hair, eruption of incisors, separation of eyelids, and descent of testes or opening of the vagina. The sensory function tests included visual placing, righting and mid-air righting reflexes, and response to pain. Emotionality was evaluated by observation of behaviour in an open field, motor

coordination was examined in a rotarod performance test, and learning ability was examined by behaviour in a water-filled multiple T maze test. Reproductive performance included indices of mating, fertility, gestation, and litters.

Of the F_0 dams, 12 of 42 at 1000 mg/kg bw per day died between

day 11 and day 16 of gestation. No deaths occurred in the other groups. Signs of toxicity were observed at doses ≥ 300 mg/kg bw per day. At the highest dose, these included diarrhoea, erythema and swelling of the anal region, hypoactivity, wasting, hypothermia, lachrymation, and piloelection. Signs of toxicity were also observed at 300 mg/kg bw per day but in only one animal. Body weight and body-weight gain were significantly reduced at 300 and 1000 mg/kg bw per day during gestation and at 100 mg/kg bw per day during treatment. Food consumption was significantly reduced at doses ≥ 100 mg/kg bw per day during treatment, and water consumption was dose-dependently and significantly increased at doses ≥ 300 mg/kg bw per day. Thymic atrophy and enlarged adrenals were observed in the dams that died and those killed on day 21 of gestation (F_0I) at 1000 mg/kg bw per day.

In the F_0I dams, from which fetuses were removed surgically on day 21 of gestation, slight but significant increases were found in the relative weights of the liver and kidney at 300 mg/kg bw per day, but no significant increase in the absolute weights was observed at this dose. At 1000 mg/kg bw per day, the absolute weights of the kidney and adrenal were significantly increased and the absolute weight of the thymus was significantly decreased. The relative weight of the liver was significantly increased, but the absolute weight was not significantly affected at 1000 mg/kg bw per day. Higher ratios of resorbed or dead fetuses were observed at 1000 mg/kg bw per day (4.7% in controls, 7.7% at 300 mg/kg bw per day, and 15% at 1000 mg/kg bw per day). None of these differences achieved statistical significance or was outside the historical control range (5.7%; 1.5-20%). There was no difference in the mean numbers of corpora lutea or implantations or the implantation rate. In the F_0II dams, body weight and body-weight gain were significantly reduced at 300 and 1000 mg/kg bw per day during lactation. There were no treatment-related changes in the number of live newborn, the length of gestation, or the delivery rate. No NOAEL could be identified for F_0 maternal toxicity since decreased body-weight gain was seen at all doses. The NOAEL for reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested.

No significant change in F_1I fetal sex ratio, placental weight, or fetal body weight was observed, but the ratio of resorbed or dead fetuses was increased (5% in controls and 15% at 1000 mg/kg bw per day) and the number of live fetuses was decreased at the highest dose, but these changes were not statistically significant. Cyclopia and polydactyly were each observed in one fetus at 300 mg/kg bw per day (0.7%), but not in any other group, and these anomalies were considered to be unrelated to treatment. There were no treatment-related increases in the incidences of external, visceral, or skeletal anomalies. A significant increase in the number of fetuses with a skeletal variation consisting of opening of the foramen transversarium of the seventh cervical vertebra was seen at 300 and 1000 mg/kg bw per day (0% in controls and 1.5%, 5.0%, and 14% at the three doses, respectively). An increased number of fetuses with an extra lumbar rib was observed at 1000 mg/kg bw per day, but this was not significant. The NOAEL for developmental toxicity was 300 mg/kg bw per day, on the basis of increased skeletal variations at 1000 mg/kg bw per day.

The F_1IIa offspring showed no-treatment related effect on survival or weaning rates. The body weights of the treated groups were comparable to those of controls during lactation and before mating. There were no treatment-related effects on postnatal physical development, sexual differentiation, sensory function, emotional behaviour, or motor coordination. A significant retardation in the time taken to reach the goal in the water-filled multiple T maze was observed in females at 1000 mg/kg bw per day, but this slight reduction in learning behaviour was observed only in the second of three consecutive daily trials. There was no impairment of reproductive performance of F_1IIa2 offspring at any dose. No external anomalies were observed in F_2 fetuses. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, if the slight evidence of behavioural teratogenicity in F_1 offspring is ignored.

In the offspring killed at 21 days of age (F_1IIb), an increased incidence of skeletal variations was observed at 1000 mg/kg bw per day (9% in controls and 18% at 1000 mg/kg bw per day), but this was not statistically significant. No external or skeletal anomalies were observed. The incidence of visceral anomalies was increased significantly in F_1IIaI offspring at 1000 mg/kg bw per day killed at 56 days of age (0/46 in controls and 7/39 at 1000 mg/kg bw per day). F_1IIb and F_1IIaI offspring at 1000 mg/kg bw per day also showed an increased incidence of dilatation of the renal pelvis. The total incidence of dilatation of the renal pelvis observed in F_1I offspring (killed at 21 and 56 days of age and at the end of the fertility test) was increased dose-dependently (0/98 in controls and 1/95, 3/85, and 9/79 at the three doses, respectively). The incidences in controls from 15 previous studies were 0-2.0% in fetuses, 0-3.2% in offspring 21 days old, 0-4.3% at 56 days of age, and 0-4.5% at the end of the fertility test. The incidence in the controls in the present study was 0% at all times. The incidence of protrusion or partial adhesion of the liver parenchyma on the diaphragmatic side was also increased at 1000 mg/kg bw per day (1/98 in controls and 0/95, 2/85, and 6/79 at the three doses, respectively). These anomalies were not observed in fetuses removed surgically. The overall NOAEL for developmental toxicity was 300 mg/kg bw per day, on the basis of an increased incidence of skeletal variations in fetuses and an increased incidence of visceral anomalies in offspring at 1000 mg/kg bw per day (Saegusa et al., 1988c).

Rabbits

Groups of 15-18 female JW-NIBS rabbits were treated by gavage with pyriproxyfen (purity, 97.2%) at doses of 0, 100, 300, or 1000 mg/kg bw per day on days 6-18 of gestation and were killed on day 28

of gestation. The study complied with GLP.

In the maternal animals, abortion or premature delivery occurred at doses ≥ 300 mg/kg bw per day, in one control, none at 100 mg/kg bw per day, three at 300 mg/kg bw per day, and six at 1000 mg/kg bw per day. Dead and moribund animals were found at 1000 mg/kg bw per day (none at 0, 100, or 300 mg/kg bw per day and three at 1000 mg/kg bw per day). Several signs of toxicity, including soft stools, emaciation, decreased spontaneous activity, and bradypnoea were observed in aborted, prematurely delivered, prematurely dying, and moribund dams at doses ≥ 300 mg/kg bw per day. Body weight, body-weight gain, and food consumption were significantly reduced in dams at 1000 mg/kg bw per day. There were no significant effects on the mean number of corpora lutea or implantations or the number, sex ratio, or body weight of live fetuses.

The live fetuses showed no treatment-related external anomalies. Skeletal or visceral malformations were observed in fetuses at 300 mg/kg bw per day, comprising a defect of the third distal phalanx of the hind leg in one; cystic lung, ventricular septal defect, hypoplasia of the left atrial auricle, and persistent truncus arteriosus in one; and a defect of the gall-bladder in one. None was observed in the other groups. External malformations were observed in three fetuses, comprising local oedema, a visceral malformation, and microphthalmia in one fetus at 300 mg/kg bw per day; persistent truncus arteriosus in one fetus at 1000 mg/kg bw per day; and a ventricular septal defect in another at this high dose. The total incidence of malformations was 1% in controls, 1% at 100 mg/kg bw per day, 6% at 300 mg/kg bw per day, and 2% at 1000 mg/kg bw per day. The authors concluded that pyriproxyfen did not cause treatment-related changes in the incidences of skeletal anomalies, skeletal variations, ossification, visceral anomalies, or visceral variations. The NOAEL for reproductive toxicity was 100 mg/kg bw per day on the basis of abortion or premature delivery and a number of signs of toxicity at 300 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Hirohashi et al., 1988).

(f) Studies on metabolites

(i) Acute toxicity

The metabolites of pyriproxyfen were administered orally to mice in a 0.5% solution of methylcellulose at 1000 or 2000 mg/kg bw. One out of five males given 5''-hydroxypyriproxyfen (purity, 97.5%) at 2000 mg/kg bw died, but no deaths occurred with the other metabolites. 4'-Hydroxypyriproxyfen (purity, 98.3%) caused no abnormal clinical signs; 5''-hydroxypyriproxyfen caused decreased spontaneous activity in animals at both doses and ataxic gait at 2000 mg/kg bw;

4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen (purity, 90.1%) produced decreased spontaneous activity, ataxic gait, and prone position in animals at 2000 mg/kg bw; *(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether (purity, 99.0%) produced decreased spontaneous activity, ataxic gait, prone position, lateral position, and irregular respiration in animals at 2000 mg/kg bw; and *(RS)*-2-(2-pyridyloxy) propionic acid (purity, 100%) produced decreased spontaneous activity at 2000 mg/kg bw (Misaki, 1993a).

(ii) Genotoxicity

4'-Hydroxypyriproxyfen (purity, 98.3%), 5''-hydroxypyriproxyfen (purity, 97.5%), 4-hydroxyphenyl *(RS)*-2-(2-pyridyloxy) propyl ether pyriproxyfen (purity, 90.2%), *(RS)*-2-hydroxypropyl 4-phenoxyphenyl ether (purity, 99.0%), and *(RS)*-2-(2-pyridyloxy) propionic acid (purity, 100%) were tested for mutagenicity in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and in *Escherichia coli* WP2 *uvrA*, with and without exogenous metabolic activation at concentrations of 15-5000 µg/plate. None of the metabolites caused reverse mutation, whereas the positive controls used in these assays produced the anticipated responses (Hara et al., 1993).

No data were available on the effects of the metabolites of pyriproxyfen on chromosomal integrity, but as these metabolites are formed *in vivo* and pyriproxyfen had no effect on this end-point, this is not considered a major gap in the data.

Comments

After oral administration to rats, [^{14}C]pyriproxyfen is slowly (time to peak concentration in plasma, 8 h) and incompletely (≤ 50% of the dose) absorbed but is then rapidly eliminated, predominantly in the faeces (90%), with only 4-11% in the urine, after 48 h. Absorbed pyriproxyfen is excreted mainly via the bile (34-37% of the administered dose in 48 h). The metabolism of pyriproxyfen is qualitatively similar in rats, mice, lactating goats, and laying hens. A large number of metabolites have been detected, the main route of biotransformation being 4'-hydroxylation. Other pathways include hydroxylation of the pyridyl ring, ether cleavage and conjugation. Mice conjugate a much greater proportion of the dose than rats. The concentration of pyriproxyfen in tissues other than fat was very low (generally < 0.01 µg equivalent per g after 72 h; fat < 0.1µg equivalent per g). The half-times of the radiolabel in tissues, including blood and fat, were 8-36 h. The dermal absorption of pyriproxyfen has not been studied.

The acute oral toxicity of pyriproxyfen is low, with LD_{50} values > 5000 mg/kg bw in mice, rats, and dogs. The acute dermal toxicity is also low, with LD_{50} values > 2000 mg/kg bw in mice and rats, and after exposure by inhalation, with an LC_{50} value > 1.3 mg/l air in mice and rats. WHO (1999) has classified pyriproxyfen as 'unlikely to present acute hazard in normal use'. Pyriproxyfen was

mildly irritating to the eye but not to the skin of rabbits. It did not sensitize the skin of Hartley guinea-pigs in a maximization test.

In short- and long-term studies of the effects of pyriproxyfen in mice, rats, and dogs, the liver was the main toxicological target, with increases in liver weight and changes in plasma lipid concentrations, particularly cholesterol, at doses of 120 mg/kg bw per day and above in rats. There was some evidence that the compound might cause modest anaemia in mice, rats, and dogs at high doses. In mice treated with pyriproxyfen in the diet for three months, additional effects seen included increased mortality rates, histopathological changes in the kidney, and decreased body weight. The NOAEL was 150 mg/kg bw per day in mice, 23 mg/kg bw per day (two studies) in rats, and 100 mg/kg bw per day in dogs fed pyriproxyfen in the diet for 3 months. In long-term studies of toxicity in mice, pyriproxyfen also caused a dose-dependent increase in the occurrence of systemic amyloidosis, which was associated with increased mortality rates. The NOAEL was 120 ppm, equal to 16 mg/kg bw per day. In rats, the only additional effect was reduced body-weight gain, and the NOAEL was 600 ppm, equal to 27 mg/kg bw per day. In two 1-year studies in dogs, pyriproxyfen was administered in capsules. The overall NOAEL was 10 mg/kg bw per day on the basis of increased relative liver weight and increased total plasma cholesterol concentration in males. There was some evidence that pyriproxyfen can act as a hepatic enzyme inducer, at least in dogs. Pyriproxyfen was not toxic when administered dermally to rats for 21 days at doses of up to 1000 mg/kg bw per day. Inhalation of pyriproxyfen for 4 h per day for 28 days caused only minor effects in rats (initial salivation, sporadically reduced body-weight gain, slightly increased serum lactate dehydrogenase activity) at 10 000 mg/m^3. The NOAEL was 480 mg/m^3.

Pyriproxyfen was not carcinogenic when given in the diet at doses up to 420 mg/kg bw per day in a study in mice or at doses up to 140 mg/kg bw per day in rats. Pyriproxyfen showed no evidence of carcinogenicity in a 1-year study in dogs at doses up to 1000 mg/kg bw per day. The Meeting concluded that pyriproxyfen does not pose a carcinogenic risk to humans.

Pyriproxyfen was not genotoxic in an adequate range of tests for mutagenicity and cytogenicity *in vitro* and *in vivo*. The Meeting concluded that pyriproxyfen is not genotoxic.

The reproductive toxicity of pyriproxyfen in rats has been investigated in a two-generation study of reproductive toxicity, a study involving treatment of males and females before and in the early stages of gestation (segment 1), and a study of treatment during the prenatal and lactation periods (segment 3). The NOAEL for maternal toxicity was 1000 ppm, equal to 98 mg/kg bw per day, in the two-generation study and 100 mg/kg bw per day in the segment 3 study. Reproductive toxicity was observed only in the segment 3 study, in which there was an increased number of stillbirths in the F_0 generation and a reduction in the number of implantations and in the mean number of live fetuses in the F_1 generation at 500 mg/kg bw per day. The NOAEL for reproductive toxicity was 300 mg/kg bw per day. No reproductive toxicity was observed in the two-generation study, the NOAEL being 5000 ppm, equal to 340 mg/kg bw per day, the highest dose tested, or in the segment 1 study, the NOAEL being 1000 mg/kg bw per day, the highest dose tested.

The developmental toxicity of pyriproxyfen has been studied in rats and rabbits. In rats, a NOAEL for maternal toxicity was not identified, as decreased body-weight gain was observed at 100 mg/kg bw per day, the lowest dose tested. Pyriproxyfen caused little developmental toxicity and was not teratogenic. In a segment 3 study, the F_1 offspring were subjected to a series of developmental tests for possible neurotoxicity, including physical indices, tests of behaviour, motor and sensory function, and learning ability. Although there were some effects on growth at doses $\geq$ 300 mg/kg bw per day, there was no developmental neurotoxicity at 500 mg/kg bw per day, the highest dose tested. Visceral anomalies (dilatation of the renal pelvis) were found at doses $\geq$ 300 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day, on the basis of retarded physical development and visceral anomalies at higher doses. In a more conventional study of developmental toxicity in rats, no evidence of growth retardation or of developmental neurotoxicity was found at doses up to and including 1000 mg/kg bw per day, the highest dose tested. There was an increased frequency of skeletal variations (opening of the foramen transversalium of the seventh cervical vertebra) in fetuses at 300 mg/kg bw per day. The frequency of visceral anomalies was significantly increased in F_1 offspring some weeks after birth. The NOAEL for developmental toxicity was 300 mg/kg bw per day, on the basis of an increased frequency of skeletal variations with visceral anomalies in F_1 offspring at 1000 mg/kg bw per day. In a study of developmental toxicity in rabbits, signs of maternal toxicity (abortion and premature delivery) were evident at doses $\geq$ 300 mg/kg bw per day (NOAEL, 100 mg/kg bw per day). No developmental toxicity was observed, the NOAEL being 1000 mg/kg bw per day, the highest dose tested.

The Meeting established an ADI of 0-0.1 mg/kg bw on the basis of the NOAEL of 10 mg/kg bw per day in 1-year studies of toxicity in dogs and a safety factor of 100.

The Meeting concluded that it was not necessary to establish an acute reference dose because of the low acute toxicity of pyriproxyfen.

Toxicological evaluation

Levels that cause no toxic effect

Mouse: 120 ppm, equal to 16 mg/kg bw per day (18-month study of carcinogenicity)

Rat: 600 ppm, equal to 27 mg/kg bw per day (2-year study of

toxicity and carcinogenicity)

5000 ppm, equal to 345 mg/kg bw per day (reproductive toxicity, two-generation study of reproductive toxicity, highest dose tested)

100 mg/kg bw per day (developmental toxicity in a segment 3 study of developmental toxicity)

Rabbit: 100 mg/kg bw per day (maternal and reproductive toxicity in a study of developmental toxicity)

1000 mg/kg bw per day (developmental toxicity in a study of developmental toxicity, highest dose tested)

Dog: 10 mg/kg bw per day (1-year study of toxicity)

Estimate of acceptable daily intake for humans

0-0.1 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information valuable for continued evaluation of the compound

Observations in humans

Toxicological end-points relevant for setting guidance values for dietary and non-dietary exposure to pyriproxyfen

Absorption, distribution, excretion, and metabolism in mammals

Rate and extent of oral absorption	Slow, incomplete absorption (≤ 50%), rat
Dermal absorption	No data (no systemic toxicity up to 1000 mg/kg bw per day by dermal route, rat)
Distribution of total residues	Highest concentrations of radiolabel in fat and, to lesser extent, liver, rat
Potential for accumulation	Possible limited accumulation in fat, rat
Rate and extent of excretion	Rapid, complete, 88-96% within 48 h, primarily in faeces; 4-11% in urine, rat
Metabolism in animals	Extensive. No parent compound detectable in urine; numerous metabolites: main pathway is 4'-hydroxylation; also hydroxylation of the pyridyl ring, ether cleavage, conjugation, mouse, rat, goat, hen
Toxicologically significant compounds (animals, plants and environment)	Pyriproxyfen

Acute toxicity

LD_{50}, oral	> 5000 mg/kg bw, mouse, rat
LD_{50}, dermal	> 2000 mg/kg bw, mouse, rat
LC_{50}, inhalation	> 1.3 mg/L, mouse, rat
Dermal irritation	Not irritating, rabbit
Ocular irritation	Mildly irritating, rabbit
Dermal sensitization	Not a sensitizer, guinea-pig

Short-term toxicity

Target/critical effect	Mouse, rat, dog: liver, increased relative liver weight, mild anaemia, altered lipid metabolism (increased serum cholesterol)
Lowest relevant oral NOAEL	13 weeks, rat, 24 mg/kg bw per day
Lowest relevant dermal NOAEL	21 days, rat, > 1000 mg/kg bw per day
Lowest relevant inhalation NOAEL	28-day, rat, > 1.3 mg/L

Long-term toxicity and carcinogenicity

Target/critical effect:	Mouse, rat, dog: liver, increased liver weight, decreased body weight, altered lipid metabolism (increased plasma cholesterol) (rat, dog)
Lowest relevant NOAEL	1 year, dog, 10 mg/kg bw per day (diet)
Carcinogenicity	Not carcinogenic, mouse, rat
Genotoxicity	Not genotoxic
Reproductive toxicity	
Reproductive target/critical effect	Reduction in number of implantations and live F_2 fetuses at F_1 developmentally toxic dose, rat
Lowest relevant reproductive NOAEL	345 mg/kg bw per day, rat
Developmental target/critical effect	Retardation of physical development in F_1, rat
Lowest relevant developmental NOAEL	100 mg/kg bw per day, rat
Neurotoxicity/Delayed neurotoxicity	No evidence of developmental neurobehavioural toxicity in rat. No evidence of neurotoxicty or neuropathology in medium- or long-term studies in mouse, rat, dog or during development in rat, rabbit
Other toxicological studies	Possible enzyme inducer, at least in dogs
Medical data	No data

Summary	Value	Study	Safety factor
ADI	0-0.1 mg/kg bw	1-year, dog, toxicity	100
Acute reference dose	Unnecessary		

References

Cardy, R., Moore, M., Murphy, B.S., Thakur, A., Tellone, C., Ito, S., Lang, P., Ginevan, M., Driver, J., Stewart, R. & Wilkinson, C. (1994) Supplemental data and review of oncogenicity study with S-31183 (Sumilarv) in mice (MRID No. 421783-10). Unpublished study from Technology Sciences Group Inc. Reference No. NNT-41-0116. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Chapman, E.A, Lee, P., Virgo, D.M. & Sparrow, S. (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks. Unpublished study from Life Science Research Ltd. Reference No. NNT-11-0081. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Cox, R.H., Zoetis, T., Cardy, R.H., Alsaker, R.D., Kuhlman, S.M., Lewis, S.A., Thakur, A.K. & Phipps, N.G. (1989) Subchronic toxicity Sstudy with S-31183 in rat. Unpublished study from Hazleton Laboratories America, Inc. Reference No. NNT-910045. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Cox, R.H., Zoetis, T., Voelker, R.W., Alsaker, R.D., Kuhlman, S.M., Lewis, S.A., Thakur, A.K. & Phipps, N.G. (1990) Subchronic toxicity study in mice. Unpublished study from Hazleton Laboratories America, Inc. Reference No. NNT-01-0066. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Hara, M., Katoh, H. & Yoshitake, A. (1993a) Reverse mutation test of metabolites of pyriproxyfen, 4'-OH-Pyr, 5"-OH-Pyr, DPH-Pyr, POPA and PYPAC, in bacterial systems. Unpublished study reference No. NNT-30-0104. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Henderson, L.M. & Proudlock, R.J. (1989) Assessment of unscheduled DNA repair synthesis in mammalian cells after exposure to S-31183. Unpublished study from Huntingdon Research Centre Ltd. Reference No. NNT-91-0053. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Hirohashi, A., Kannan, N., Kato, T. & Yamada, H. (1988) Study of S-31183 by oral administration during the period of fetal organogenesis in rabbits. Unpublished study reference No. NNT-80-0033. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Isobe, N., Matsunaga, H., Kimura, K., Yoshitake, A. & Yamada, H. (1988a) Metabolism of S31183 in rats. Unpublished study reference No. NNM-80-0001. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Isobe, N., Matsunaga, H., Kimura, K., Yoshitake, A. & Yamada, H. (1988b) Metabolism of S-31183 in rat (tissue distribution study). Unpublished study reference No. NNM-800002. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kawaguchi, S., Watanabe, T., Suzuki T., Kato, T. & Yamada, H. (1987) Acute inhalation toxicity of S-31183 in rats. Unpublished study reference No. NNT-70-0022. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kawaguchi, S., Yoshioka, K., Ito, S., Suzuki, T., Kato, T. & Yamada, H. (1988) Subacute inhalation toxicity of S-31183 in rats. Unpublished study reference No. NNT-80-0031. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kogiso, S., Hara, M., Yoshitake, A. & Yamada, H. (1988a) Reverse mutation test with S31183 in bacteria systems. Unpublished study reference No. NNT-80-0034. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kogiso, S., Hara, M., Iwawaki, H. & Yamada, H. (1988b) In vitro chromosomal aberration test of pyriproxyfen in Chinese hamster ovary cells (CHO-K1). Unpublished study reference No. NNT-80-0028J. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kogiso, S., Yamamoto, K., Hara, M., Yoshitake, A. & Yamada, H. (1989) In vitro chromosomal aberration test of pyriproxyfen in Chinese hamster ovary cells (CHO-K1). Unpublished study reference No. NNT-90-0054. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kogiso, S., Yamada, T., Hara, M., Yoshitake, A. & Yamada, H. (1990) In vitro gene mutation test of S-31183 in V79 Chinese hamster cells. Unpublished study reference No. NNT-000067. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kogiso, S. Kato, H. & Nakattuka, S. (1992) Reverse mutation test with S-31183 in bacteria systems. Unpublished study reference No. NNT-80-0088J. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Koyama, Y., Kimura, J., Yoshioka, K., Watanabe, T., Seki, T., Hosokawa, S., Yamada, H. & Hagiwara, H. (1989) A six-month chronic dietary toxicity study of pyriproxyfen in rats. *J. Toxicol. Sci.,* 14, 43-64.

Matsunaga, H., Yoshino, H., Isobe, N., Kaneko, H., Nakatuka, I. & Yamada, H. (1995) Metabolism of pyriproxyfen in rats. 1. Absorption, disposition, excretion, and biotransformation studies with [phenoxyphenyl-^{14}C] pyriproxyfen. *J. Agric. Food Chem.,* 43, 235-240.

Misaki, Y. & Nakatuka, I. (1993) Acute oral toxicity study of pyriproxyphen metabolites, 4'-OH-Pyr, 5"-OH-Pyr, DPH-Pyr, POPA and PYPAC in mice. Unpublished study reference No. NNT-30-0107. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Mitchell, D.J., Virgo, D.M., Broadmeadow, A., Chase, K.R., Lee, P. & Sparrow, S. (1993) S31183: Toxicity study by oral (capsule)

administration to beagle dogs for 52 weeks (additional investigation). Unpublished study from Life Science Research Ltd. Reference No. NNT-31-0102. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Moore, M.R., Zoetis, T., Doyle, D., Cardy, R.H., Pearson, R.C., Walker, M.D., Lewis, S.A., Thomas, D.L., Thakur, A.K., Burlew, P.L., Hatcher, C.F. & Vegarra, M. (1993) 21-day dermal toxicity study in rats with S-31183. Unpublished study from Hazleton Washington, Inc. Reference No. NNT-31-0094. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Nakano, M., Yamamoto, T., Kato, T. & Miyamoto, J. (1986) Acute oral toxicity study of S31183 in dogs. Unpublished study reference No. NNT-60-0012. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Nakano, M., Kohda, A., Kato, T. & Yamada, H. (1988) Three-month oral toxicity study of S31183 in dogs. Unpublished study reference No. NNT-80-0037. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Osheroff, M.R., Ziegler, K.A., Cardy, R.H., Alsaker, R.D., Kuhlman, S.M., Lewis, S.A., Thakur, A.K., Burlew, P.L. & Nasca, A.J. (1991a) Oncogenicity study in mice with S31183. Unpublished study from Hazleton Laboratories America, Inc. Reference No. NNT-11-0084. Submitted to WHO by Sumitomo Chemical Co.

Osheroff, M.R., Ziegler, K.A., Machotka, S., Alsaker, R.D., Kuhlman, S.M., Lewis, S.A., Thakur, A.K., Burlew, P.L., Devis, P.J. & Graham, R. (1991b) Combined chronic toxicity and oncogenicity study in rats with S-31183. Unpublished study from Hazleton Washington, Inc. Reference No. NNT-11-0085. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Panthani, A.M., Walsh, K.J. & Turck, P. (1996a) Metabolism of [phenoxyphenyl-^{14}C]S71639 (pyriproxyfen) in lactating goats. Unpublished study from Ricerca, Inc. Reference No. NNM-0043. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Panthani, A.M., Walsh, K.J. & Turck, P. (1996b) Metabolism of [pyridyl-^{14}C]S-7 1639 (pyriproxyfen) in lactating goats. Unpublished study from Ricerca, Inc. Reference No. NNM-0046. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Panthani, A.M., DiFrancesco, P. & Savides, M.C. (1996c) Metabolism of [phenoxyphenyl^{14}C]S-71639 (pyriproxyfen) in laying hens. Unpublished study from Ricerca, Inc. Reference No. NNM-0045. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Panthani, A.M., DiFrancesco, P. & Savides, M.C. (1996d) Metabolism of [pyridyl-^{14}C]S71639 (pyriproxyfen) in laying hens. Unpublished study from Ricerca, Inc. Reference No. NNM-0044. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Proudlock, R.J., Haynes, P. & Goodenough, A.J. (1991) Mouse micronucleus test on S31183. Unpublished study from Huntingdon Research Centre Ltd. Reference No. NNT11-0082. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Robinson, K., Washer, G. & Noveroske, J. (1991) A dietary 2-generation (1 litter) reproduction study of S-31183 in the rat. Unpublished study from Bio-Research Laboratories Ltd. Reference No. NNT-11-0087. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Saegusa, T., Kitajima, S. & Narama, I. (1988a) Study on administration of a test substance prior to and in the early stages of pregnancy in rats. Unpublished study from Hamamatsu, Seigiken Research Co., Ltd. Reference No. NNT-80-0036. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Saegusa, T., Kitajima, S. & Narama, I. (1988b) Study on administration of S-31183 during the perinatal and lactation periods in rats. Unpublished study from Hamamatsu, Seigiken Research Co., Ltd. Reference No. NNT-80-0030. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Saegusa, T., Kitajima, S. & Narama, I. (1988c) Study by administration of S-31183 during the period of fetal organogenesis in rats. Unpublished study from Hamamatsu, Seigiken Research Co., Ltd. Reference No. NNT-80-0029. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, T., Sako, H., Okuno, Y., Kato, T. & Yamada, H. (1987a) Acute oral toxicity of S31183 in mice. Unpublished study reference No. NNT-70-0014. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, T., Misaki, Y., Okuno, Y., Kato, T. & Miyamoto, J. (1987b) Acute oral toxicity of S31183 in rats, Unpublished study reference No. NNT-70-0005. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, T., Sako, H., Okuno, Y., Kato, T. & Yamada, H. (1987c) Acute dermal toxicity of S31183 in mice. Unpublished study reference No. NNT-70-0015. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, T., Misaki, Y., Okuno, Y., Kato., T. & Miyamoto, J. (1987d) Acute dermal toxicity of S-31183 in rats. Unpublished study reference No. NNT-70-0006. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, T., Kawaguchi, S., Watanabe, T., Kato, T. & Yamada, H. (1987e) Acute inhalation toxicity of S-31183 in mice Unpublished study reference No. NNT-70-0023. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, T., Nakanishi, T., Kato, T. & Miyamoto, J. (1987f) Skin sensitization test with S31183 in guinea pigs. Unpublished study reference No. NNT-70-0003. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, T., Nakanishi, T., Kato, T. & Miyamoto, J. (1987g) Primary eye and skin irritation tests with S-31183 in rabbits. Unpublished study reference No. NNT-70-000. Submitted to WHO by Sumitomo Chemical Co., Ltd.

WHO (1999) *Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1998-1999* (WHO/PCS/98.21/Rev. 1), Geneva, International Programme on Chemical Safety.

Yoshino, H. (1993a) Metabolism of pyriproxyfen in rat (high-dose, ^{14}C-concentrations in tissues). Unpublished study reference No. NNM-30-0028. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Yoshino, H. (1993b) Metabolism study of (pyridyl-2,6-^{14}C)pyriproxyfen in rat (pyridyl^{14}C-labeled test compound, single oral administration at low- and high-doses). Unpublished study reference No. NNM-30-0025. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Yoshino, H., Kaneko, H., Nakatsuka, I. & Yamada, H. (1995) Metabolism of pyriproxyfen. 2. Comparison of in vivo metabolism between rats and mice. *J. Agric. Food Chem.*, 43, 2681-2686.

Yoshino, H., Kaneko, H., Nakatsuka, I. & Yamada, H. (1996) Metabolism of pyriproxyfen. 3. In vitro metabolism in rats and mice. *J. Agric. Food Chem.*, 44, 1578-1581.

See Also:
Toxicological Abbreviations
Pyriproxyfen (ICSC)