

**PYRETHRUM EXTRACT (PYRETHRINS)
(addendum)**

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Explanation

Extracts of flowers of the chrysanthemum (genus *Chrysanthemum*) have been used as insecticides for a long time. The insecticidal neurotoxic activity of these extracts is due to a mixture of three naturally occurring, closely related insecticidal esters of chrysanthemic acid (pyrethrins I) and three closely related esters of pyrethric acid (pyrethrins II). Selection of varieties of chrysanthemum rich in pyrethrins and extraction techniques have improved over the years, and the currently available refined pyrethrum extract contains 45–55% total pyrethrins and 23–25% other phytochemical extracts containing triglyceride oils, terpenoids, and carotenoid plant colours. Flavonoids, which have been associated with skin allergies, are not found in the refined extracts. The extracts usually also contain 20–25% light isoparaffins and 3–5% butylated hydroxytoluene, which may be added during and after processing, respectively, for extraction or as antioxidants. The pyrethrin product used in the studies that were evaluated by the present Meeting was a blend of refined pyrethrum extract from the four main growing areas, with a total pyrethrin content of 57.6%. The ratio of pyrethrins I to pyrethrins II in this sample was 1.85. In this document, the product used in the studies is referred to as ‘pyrethrins’ in order to differentiate it from the pyrethrum extract used earlier.

Pyrethrum, the active principle containing pyrethrin isomers, was evaluated toxicologically by the 1965, 1966, 1970, and 1972 Joint Meetings (Annex 1, references 3, 6, 14, and 18). An ADI of 0–0.04 mg/kg bw was allocated by the 1972 Meeting. The compound was reviewed at the present meeting within the periodic review programme of the Codex Committee on Pesticide Residues. This monograph addendum summarizes data on pyrethrins that were not reviewed previously.

Evaluation for Acceptable Daily Intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

Rats

One preliminary and three definitive experiments were conducted with ^{14}C -labelled pyrethrins I in rats to determine their absorption, distribution, and excretion after oral administration of a single or a repeated low dose of 10 mg/kg bw and a single high dose of 100 mg/kg bw in males and 50 mg/kg bw in females. This study was conducted in compliance with guideline 85-1 of FIFRA and in accordance with good laboratory practice (GLP). The concentration of radiolabel in blood peaked between 5 and 8 h. More than 90% of the low dose was absorbed, and < 10% of the parent compound was found in faeces. The mean percentage of the administered radiolabel found in urine was 32–47% in males and 50–57% in females, whereas the mean percentage of the administered radiolabel found in faeces with the various dosing regimens was 55–71% for males and 50–52% for females. More of the administered dose was found in the faeces of male rats given the single high dose (71%) than in those given the single low dose (63%) or repeated doses (55%). No such differences were seen in females. The radiolabel was excreted faster by males and females given repeated low doses than by those given the single dose. The half-lives of pyrethrins I were calculated to be 5 h in males and 7 h in females. The residues were widely distributed in the organs analysed, the highest concentrations being found in fat in all groups. The concentrations were similar in animals of each sex given the single and repeated low dose, but female rats had an approximately two times higher concentration of radiolabel in fat than the males (Selim, 1995).

(b) Biotransformation

Rats

In the phase of the study described above designed to investigate metabolism, the major urinary metabolites were identified, and then the metabolic profile was determined in urine and faeces with quantification of the labelled residues. In the first step, additional groups of five rats were given ^{14}C -labelled pyrethrins I orally as a single dose of 10 mg/kg bw (males and females), 100 mg/kg bw (males), and 50 mg/kg bw (females). The dose administered to the females was lower because it had been shown previously that pyrethrum extract is more toxic to females than males. Chromatographic profiling indicated a quantitatively similar metabolic profile in urine in males and females at all doses and that all of the metabolites present in faeces were also present in the urine. The urine from males at the high dose was therefore used to isolate, purify, and identify the major metabolites by repetitive injections of composited urine onto a semi-preparative high-performance liquid chromatograph and collection of radiolabelled metabolites. After isolation and purification, two major and four minor metabolites were identified by chemical manipulations and mass spectroscopy. The spectrum of metabolites indicated that in rats pyrethrins I are metabolized through two major metabolic pathways: oxidation of the double-bond on the cyclopentene or the cyclopropane side of the molecule to form a diol, and/or oxidation of the methyl groups on the side-chain of the cyclopropane ring to form a carboxylic acid. A second pathway involves hydrolysis of the ester bond to form the corresponding acid and alcohol.

After isolation and identification of the metabolites, the distribution of metabolites was determined in urine and faeces. The major metabolite in urine at all doses was chrysanthemum dicarboxylic acid. In faeces, a significant amount of parent compound was present (< 10% at the low dose), but another metabolite was the most prevalent at all doses. The two metabolites represented over one-third of the total excreted radiolabel. Male and female rats metabolized pyrethrins I in a similar manner, regardless of the dose, and the difference between males and females was quantitative rather than qualitative (Selim, 1995).

The relative rates of microsomal oxidation are similar for the four major pyrethrins, which are readily oxidized by cytochrome P450-dependent oxidases. Multiple sites are involved on each of the pyrethrum constituents. The toxicity of the pyrethrins is attributable to a combination of the effects of the parent esters and the metabolites they generate, and the metabolites might be of relatively low toxicity (Casida & Quistad, 1995).

The principal metabolic pathway of pyrethrins is summarized in Figure 1.

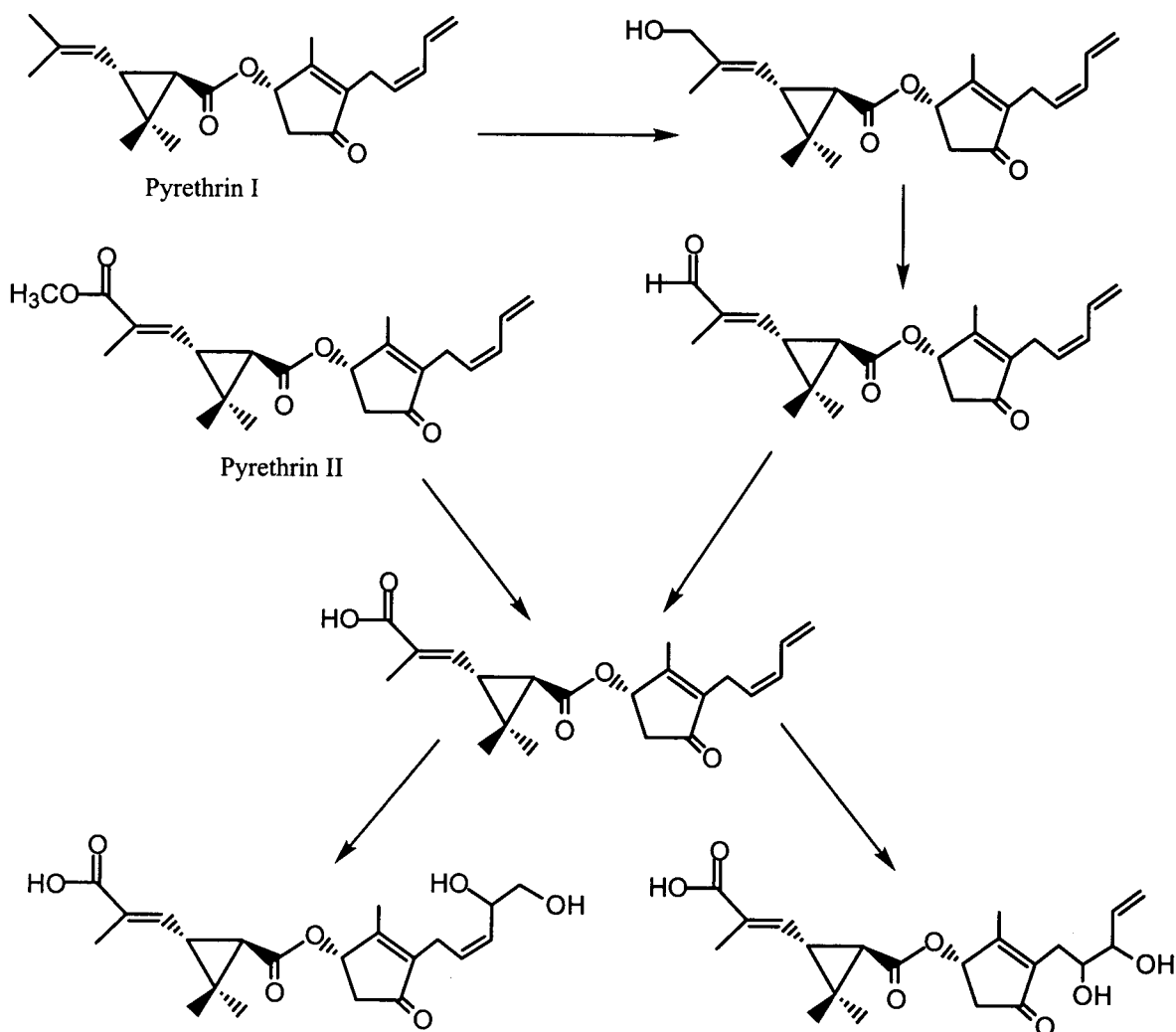
The metabolism of the six natural pyrethrins by mouse and rat microsomes was studied *in vitro*, in a study which provided detailed qualitative and some quantitative information on all six of the active components present in pyrethrins. The metabolism of pyrethrins I was shown to proceed principally through oxidative processes, while that of pyrethrins II was shown to occur through a combination of hydrolytic and oxidative processes. No metabolites were found that would not have been expected on the basis of the chemistry of these chemicals, nor were any of the metabolites found of apparent toxicological concern (Class et al., 1989).

2. Toxicological studies

(a) Acute toxicity

The results of studies on the acute toxicity of pyrethrum extracts are summarized in Table 1. The methods used in these studies complied with the corresponding FIFRA guidelines and with GLP. Pyrethrins have little acute toxicity in rats treated orally, with LD₅₀ values of 2400 mg/kg bw for males and 1000 mg/kg bw for females. The ratio of pyrethrins I and II (1.44 or 2.50) has no effect on the toxicity of pyrethrins in male or female rats. The signs of toxicity included ruffled appearance and tremors within 4–24 h after dosing. Post-mortem examination of animals that died

Figure 1. Pathways for the formation of metabolites in the urine of rats treated orally with pyrethrins I and pyrethrins II



From Caside & Quistad (1995)

Table 1. Acute toxicity of pyrethrins

Species	Strain	Sex	Route	LD ₅₀ or LC ₅₀ (mg/kg bw or mg/L air)	Purity (%) or ratio of pyrethrin I:II	Reference
Rat	Sprague-Dawley	M	Oral	2400	58%	Gabriel (1992)
Rat	Sprague-Dawley	F	Oral	1000	58%	Gabriel (1992)
Rat	Sprague-Dawley	M	Oral	3900	1.4	Gabriel (1992)
Rat	Sprague-Dawley	F	Oral	1300	1.4	Gabriel (1992)
Rat	Sprague-Dawley	M	Oral	3900	2.5	Gabriel (1992)
Rat	Sprague-Dawley	F	Oral	1200	2.5	Gabriel (1992)
Rabbit	New Zealand	M&F	Dermal	> 2000	58%	Gabriel (1991)
Rat	Sprague-Dawley	M&F	Inhalation	3.4	58%	Hoffmann (1991)

M, male; F, female

showed haemorrhagic lungs, tan-to-yellow fluid in the lower gastrointestinal tract and muzzle, and genital staining. The no-effect level for clinical signs was 710 mg/kg bw in males and 320 mg/kg bw in females.

Dermal exposure to 2000 mg/kg bw was tolerated by rabbits, which showed very slight to well-defined erythema, very slight oedema, and stained test sites at 24 h. All animals appeared normal through the 14-day observation period.

Very slight acute toxicity was found in rats exposed to aerosols by inhalation. On the basis of the mean analytical concentration of active ingredient and the resultant mortality, the LC₅₀ was calculated to be 3.4 mg/L for the two sexes. Tremors were seen during exposure to the two higher concentrations. The significant findings *post mortem* included discoloured and oedematous respiratory tissues.

Application of pyrethrins to the skin of albino rabbits produced a minimally irritating skin irritation score of 0.42 (Romanelli, 1991a).

Instillations of the extracts into the conjunctival sac produced irritation in the eyes of albino rabbits, but no irritation was observed by 72 h. No corneal opacity or iritis was seen during the observation period (Bielucke, 1991).

In a study of dermal sensitization in guinea-pigs with a modified Buehler protocol, pyrethrins were not sensitizing (Romanelli, 1991b).

(b) Short-term studies of toxicity

Mice

In order to define a suitable maximum tolerated dose for a longer study, doses of 5000 and 7000 ppm were evaluated in a 2-week study. The method used complied to a certain extent with OECD guideline 407, and the study was conducted in compliance with GLP. One mouse at 7000 ppm died. There was no effect on body weight, but food consumption was slightly decreased at 7000 ppm. Statistically significant increases in the absolute and relative weights of the liver were seen at both doses. On the basis of the results of this study, 7000 ppm was selected as the high dose for the long-term study in mice (Goldenthal, 1987).

Pyrethrins were offered to groups of 15 Charles River mice of each sex in the diet at concentrations of 300, 1000, 3000, or 10 000 ppm for 13 weeks, equal to 47, 160, 460, and 1600 mg/kg bw per day for males and 56, 200, 580, and 1800 mg/kg bw per day for females. The method used complied to a certain extent with OECD guideline 408, and the study was conducted in compliance with GLP. Four males and 10 females at the original high dose of 30 000 ppm died on day 2, and all animals at this dose had died or were killed *in extremis* by day 10. Four males and two females at 10 000 ppm also died on day 2, with clinical signs that included tremors, pale exposed skin, dilated pupils, altered activity, laboured breathing, cold to touch, moribundity, and hunched posture. Tremors and increased activity were seen in several animals at 10 000 ppm during the first 2 weeks of study. No treatment-related clinical signs were observed in the animals at 300, 1000, or 3000 ppm. The group mean body weights and food consumption were similar for

all groups with surviving animals. The absolute weight of the liver and the liver:body weight and the liver:brain weight ratios were all statistically significantly increased in males and females at 3000 and 10 000 ppm, whereas the absolute weights of the liver in females at 300 and 1000 ppm were comparable to those of controls. A treatment-related increase in the incidence and/or severity of congestion in the liver was observed in surviving male and female mice at 10 000 ppm, and an increased incidence but only mild severity was found in < 15% of investigated animals at 3000 ppm. An increased incidence of hepatocellular hypertrophy was present in surviving male and female mice at 3000 and 10 000 ppm; at 1000 ppm, only 2 of 15 mice showed mild congestion of the liver on macroscopic observation. The NOAEL was 1000 ppm, equal to 160 mg/kg bw per day (Goldenthal, 1988a).

Rats

Pyrethrins were offered to groups of 15 Charles River rats of each sex for 13 weeks in the diet at concentrations of 300, 1000, 3000, 10 000, or 20 000 ppm, equal to 17, 57, 170, 590, and 1200 mg/kg bw per day for males and 22, 74, 220, 710, and 1400 mg/kg bw per day for females. The method used in this study complied to a certain extent with OECD guideline 408, and the study was conducted in compliance with GLP. During the first week of the study (days 3–7), one female at 10 000 ppm, one male at 20 000 ppm, and 12 females at 20 000 ppm died; all other animals survived to scheduled sacrifice. The signs seen before death in all animals that died included decreased defaecation, increased respiration rate, tremors, and decreased or increased activity. Convulsions were seen in some animals. The signs in most animals at 10 000 and 20 000 ppm that survived until the end of the study were decreased defaecation, tremors, increased respiration rate, and increased activity. Convulsions occurred mainly in males at 20 000 ppm. Most of the signs were seen only during the first two weeks of the study. Statistically significant decreases in mean body weights were observed during most or all of the study in males and females at 10 000 and 20 000 ppm., and statistically significant decreases in mean food consumption were seen during most or all of the study in females at 10 000 ppm and males and females at 20 000 ppm when compared with the respective control groups. Statistically significantly decreased mean values for haematocrit and haemoglobin were found in males at 20 000 ppm and for erythrocytes, haematocrit, and haemoglobin in females at 10 000 and 20 000 ppm. Females at 3000 ppm also showed a decreased mean haemoglobin value. The treatment-related macroscopic findings consisted of enlargement and congestion of the liver in both males and females, but primarily in males, at 10 000 and 20 000 ppm; however, the macroscopic observation could not be confirmed microscopically. The liver weight was statistically significantly increased in males at 10 000 and 20 000 ppm and in females at 3000, 10 000, and 20 000 ppm. The weight of the kidney was increased in males and females at 3000, 10 000, and 20 000 ppm. The only microscopic finding that was possibly related to treatment was small focal or multifocal areas of tubular degeneration and regeneration in the renal cortex in animals at 3000 and 10 000 ppm, but primarily in males. The NOAEL was 1000 ppm, equal to 57 mg/kg bw per day (Goldenthal, 1988b).

In a study designed to assess the toxic effects of pyrethrins administered by whole-body inhalation as a liquid aerosol, groups of 15 Charles River rats of each sex were exposed for 6 h/day, generally 5 days per week, a minimum of 65 times to target concentrations of the active ingredient corresponding to 0.010, 0.030, 0.10, and 0.35 mg/L. The mean analytical concentrations to which the groups were exposed were 0 (control), 0.038, 0.068, 0.23, and 0.83 mg/L, respectively. Determinations of particle size distribution showed an overall mass median aerodynamic diameter of 2.7 µm. The controls were exposed only to conditioned room air on the same schedule. The study was conducted in compliance with FIFRA Guideline 824 and with GLP.

Two animals at the highest dose died, one animal on day 3. Because this death was accidental and occurred very early in the study, this animal was replaced with another animal from the same shipment. A second animal died on day 15, having shown laboured breathing similar to that of other animals in this group, and its death was considered to be potentially related to exposure. Dose-related increases in the frequency of clinical signs were observed in animals at the three higher doses during observations in the chamber and at detailed weekly examinations. These signs included secretory signs such as nasal discharge and dried material in the facial area in both males

and females. Animals at the highest dose showed laboured breathing, excess lachrymation, tremors, increased activity, and matted coats. There were no ocular effects. The absolute body weights and body-weight gains of both males and females at the two higher doses were decreased, and after 13 weeks of exposure the body-weight gains of males at these doses were about 9% lower than those of controls. In females, the body-weight gain after 13 weeks of exposure was lower than that of controls, by 13% at 0.10 mg/L and 17% at 0.35 mg/L. Food consumption was also slightly decreased during the first 1 or 2 weeks in males and females in these groups. Nonregenerative anaemia was observed in males at the three higher doses and in females at the highest dose, with significant decreases in haemoglobin, haematocrit, and erythrocyte values. An increased leukocyte count was also seen in the females at the highest dose. Significant differences in clinical chemical parameters were seen primarily at the highest dose, including decreased total protein and globulin and an increased albumin:globulin ratio in males and decreased glucose in females. Other changes occurred sporadically and were considered to be unrelated to exposure. Several significant increases in organ weights or ratios were observed. The increases in liver weight were clearly related to treatment. Increases in the organ:body weight ratios of the kidneys and lung might be a reflection of a marginal increase in absolute organ weights and the decreased body weights. Morphological abnormalities in the larynx, nasoturbinate, nasopharynx, and lungs observed by light microscopy were considered to be localized responses indicative of a treatment-related effect. The NOAEL for systemic effects was 0.01 mg/L (Newton, 1992).

Rabbits

Pyrethrins were administered dermally to five male and five female New Zealand white rabbits in the form of a 25% (w/v) mixture in vegetable oil at doses of 0, 100, 300, or 1000 mg/kg bw once daily, 5 days per week for 3 weeks. Animals in the vehicle control group were given vegetable oil on the same regimen and at the same volume as the group receiving the high dose. This study was conducted in compliance with OECD Guideline 410 and GLP. One rabbit at 1000 mg/kg bw was sacrificed *in extremis* on day 10 after showing emaciation, decreased activity, and decreased defaecation. Macroscopic examination of this animal did not reveal the cause of death. A low incidence of desquamation and/or red raised areas on the skin at the application site was observed in all groups, including the vehicle controls. Several animals in the treated groups showed very slight to well-defined erythema of the skin at the application site, but no clear pattern with regard to treatment was seen for any of these findings. Microscopic evaluation revealed no evidence of systemic toxicity. The microscopic lesions at the application site included acanthosis, haemorrhage, hyperkeratosis, and chronic inflammation, although haemorrhage was observed only in the group given the vehicle alone. Thus, all of the dermal reactions appeared to be due to the vegetable oil. The NOAEL for systemic effects was 1000 mg/kg bw, the highest dose tested (Goldenthal, 1992).

This result is supported by that of a previous study performed to assess dermal irritation with pyrethrins at concentrations of 25%, 50%, and 75% w/v in corn oil on rabbits (Myer, 1991).

Dogs

Pyrethrins were incorporated into the basal diet of groups of two pure-bred beagle dogs of each sex at concentrations of 600, 1000, 3000, or 6000 ppm for 8 weeks, equal to 18, 30, 86, and 170 mg/kg bw per day for males and 19, 29, 94, and 200 mg/kg bw per day for females. The method used complied to a certain extent with OECD guideline 409, and the study was conducted in compliance with GLP. One male and both females at 6000 ppm died or were killed *in extremis* during the study. The treatment-related clinical signs observed in animals at this dose included inappetence, thin appearance, ataxia, trembling, oily coat, impaired limb function, shallow breathing, moribundity, and death. With the exception of moribundity and death, similar signs were observed at 3000 ppm. Males and females at 6000 ppm lost weight during the study, but the body-weight gains of animals in all other treated groups were comparable to those of controls. The average food consumption was decreased for males at 3000 ppm and for males and females at 6000 ppm when compared with controls. Decreased haematocrit, haemoglobin, and erythrocyte values were seen at the end of the study in males at 3000 and 6000 ppm, but there were no other treatment-related haematological finding. One male and one female at 6000 ppm killed *in extremis*

had increased leukocyte counts and decreased haematocrit, haemoglobin, and erythrocyte values. Slightly decreased glucose, calcium, phosphorus, and cholesterol values were found at the end of the study in males at 6000 ppm, and males and females at 3000 ppm had slightly decreased cholesterol concentrations. The aspartate and alanine aminotransferase activities of males at 6000 ppm were slightly increased at the end of dosing, and the surviving male at this dose had a very high creatinine phosphokinase value. There were no other treatment-related biochemical findings at the end of the study. Males and females at 6000 ppm killed *in extremis* showed some variation in electrolyte levels, and the urea nitrogen concentration was increased in both dogs. The female showed large increases in aspartate and alanine aminotransferase and creatine phosphokinase activities. The absolute weights of the liver in both males and females at 1000 and 3000 ppm were increased in a treatment-related fashion, and the absolute weight of the testis at these doses appeared to decrease in a similar manner. There were no macroscopic or microscopic lesions attributable to the administration of pyrethrins. The NOAEL was 600 ppm, equal to 18 mg/kg bw per day (Goldenthal, 1988c).

Pyrethrins were administered to groups of four beagle dogs of each sex for 52 weeks in the diet at concentrations of 0, 100, 500, or 2500 ppm, equal to 2.6, 14, and 66 mg/kg bw per day for males and 2.8, 14, and 75 mg/kg bw per day for females. Four dogs of each sex were evaluated at each dietary concentration. This study was conducted in compliance with FIFRA Guideline 83-1 and with GLP. All animals survived to the end of the study, and no remarkable clinical signs of toxicity were found at any dose. Overall, the mean body weights of the treated animals were similar to those of controls. The mean food consumption of males at 2500 ppm and of females at 500 and 2500 ppm was lower than that of controls during the first week of the study, but consumption was similar for the remainder of the study, except that males at 500 ppm consumed greater amounts of food than the controls. Increased total leukocyte and segmented neutrophil counts were found in females and decreased erythrocyte, haemoglobin, and haematocrit values in males at 2500 ppm. Alanine aminotransferase activity was statistically significantly increased in females at 2500 ppm at both the 6- and 12-month evaluations. The slight increase in the activity of this enzyme in males was not statistically significant. No treatment-related changes in urinary parameters were seen at either interval. The relative and absolute weights of the liver were significantly increased in males at 2500 ppm when compared with controls, but no statistically significant differences in organ weights were seen in females at this dose. No macroscopic or microscopic treatment-related changes were observed in tissues. The NOAEL was 500 ppm, equal to 14 mg/kg bw per day (Goldenthal, 1990a).

(c) *Long-term studies of toxicity and carcinogenicity*

Mice

Groups of 60 male and 60 female Charles River CD-1 mice received diets containing pyrethrins at concentrations of 100, 2500, or 5000 ppm for 18 months, equal to doses of 14, 350, and 690 mg/kg bw per day for males and 17, 410, and 830 mg/kg bw per day for females. This study was conducted in compliance with FIFRA Guideline 83-2(b) and with GLP. One male and one female at 5000 ppm were found dead during the first week of the study, but no other treatment-related deaths occurred, and survival was similar in the control and treated groups throughout the study. All animals at 5000 ppm exhibited increased activity when stimulated during the first week of the study but not later. There were no other clinical signs seen that differentiated the treated from the control groups. Statistically significant differences in mean body weight and food consumption were seen between control and treated groups sporadically throughout the study, but none of the differences was considered to be related to treatment. No treatment-related effect was observed in other parameters examined at 12 and 18 months of study. At necropsy, discoloured, dark livers were more common in males at 5000 ppm and in females at 2500 and 5000 ppm, and treatment-related increases in the absolute and relative weights of the liver were seen in males and females at 2500 and 5000 ppm. Microscopically, vacuolar fatty change was found in the livers of males at these doses, and this change was considered to be related to treatment. The dark discoloration seen macroscopically could not be explained by the microscopic findings. The incidence of nodules and masses in the lungs appeared to be slightly increased in animals at 5000 ppm. When

the lungs were examined microscopically according the original test protocol, the incidences of alveolar bronchiolar adenomas were increased in females at 5000 ppm, and the increase was statistically significant, exceeding the range in historical controls. Treated males showed an apparent, not clearly dose-related increase in the incidence of alveolar bronchiolar carcinomas which exceeded the upper limit of 95% of the historical controls (Table 2). The sponsor asked the testing laboratory to conduct serial sectioning of the remaining lung tissue of female mice in the control and highest-dose groups, which did not have diagnoses of lung tumours in the original examination. As this was considered not to be an acceptable toxicopathological practice, the results of the first evaluation were taken into consideration for the risk assessment, and the increased incidence of lung tumours was considered to be a treatment-related effect. The NOAEL was 100 ppm, equal to 14 mg/kg bw per day (Goldenthal, 1990b).

Rats

Groups of 60 Charles River CD rats of each sex received diets containing pyrethrins at concentrations of 100, 1000, or 3000 ppm for 104 weeks, equal to 4, 43, and 130 mg/kg bw per day for males and 5, 56, and 170 mg/kg bw per day for females. This study was conducted in compliance with FIFRA Guideline 83-5 and with GLP. Survival was similar in the treated and control groups, and there were no clinical findings attributable to treatment. Statistically significant decreases in body weight which were considered to be related to treatment were observed during the first 78 weeks of the study in both male and female rats at 3000 ppm, with a difference from controls of 7% in males and 10% in females. A slight, treatment-related decrease in food consumption was seen at the same time. No treatment-related ophthalmological findings or organ weight changes were detected during the study, and no haematological or urological changes were found. The activities of serum transaminases were substantially increased at most intervals of analysis in males at 3000 ppm, most of the values reaching statistical significance. Increased incidences of benign tumours of the liver, thyroid, and skin were also observed (Table 3), and a statistically significantly higher incidence of hepatocellular adenomas was described in females at the high dose. Follicular adenomas and carcinomas were initially seen in the thyroid glands of rats at the high dose, which appeared to be related to treatment, but during a re-evaluation some of the carcinomas were reclassified as adenomas and some adenomas were reclassified as hyperplasia. After the re-evaluation, the incidence of hyperplasia was found to be enhanced in males and females, and the incidence of follicular adenomas was statistically significantly increased only in females at 3000 ppm. Nevertheless, the tumour incidences in animals of each sex were higher than the upper range seen in historical controls. The results of a further, full histopathological peer review confirmed these increased tumour incidences. Macroscopic examination of the skin showed a slight increase in the incidence of cystic lesions in the skin and subcutis, and the microscopic assessment showed an apparently higher incidence of keratoacanthomas in males at the high dose, which was statistically significant in comparison

Table 2. Comparison of first and second evaluations of microscopic neoplastic lesions in the lung of mice treated with pyrethrins for 18 months

Sex	Dose (ppm)	No. of lungs examined	Alveolar bronchiolar neoplasm	
			Adenoma	Carcinoma
Male	0	60	14	0 ^a
	0	60	16	0
	100	60	15	1
	2500	60	13	3 ^{b,c}
	5000	60	17	3 ^{b,c}
Female	0	60	8	1
	0	60	4	3
	100	60	11	0
	2500	60	5	2
	5000	60	19 ^{c,d}	2

^a $p < 0.05$; Cochran trend test

^b Significant differences in pair-wise comparison of the high-dose group with controls at $p < 0.05$

^c > 95% of the upper range of historical controls

Table 3. Incidences of microscopic neoplastic lesions in rats fed diets containing pyrethrins for 104 weeks

Sex	Dose (ppm)	Liver			Thyroid				Skin		
		Total examined	Hepatocellular tumours		Total examined	Hyperplasia	Follicular tumours		Total examined	Cystic lesions	Keratoacanthomas ^b
			Adenoma	Carcinoma			Adenoma ^a	Carcinoma ^a			
Male	0	60	6	1	60	2	2 ^c	0	60	5	4
	0	60	1	0	60	0	1	1	60	2	5
	100	60	0	0	60	2	4	1	24	1	4
	1000	60	3	0	59	5	5 ^d	2	14	5	4
	3000	60	3	1	60	7	5 ^d	2	60	9	11 ^{d,e}
Female	0	60	0	1	60	0	0	1	60	0	0
	0	60	1	0	60	2	1	1	59	3	0
	100	60	0	0	60	1	2	0	8	2	1
	1000	60	1	0	60	1	3 ^d	0	8	2	1
	3000	60	5 ^{d,f}	0	60	5	5 ^d	1	60	0	0

^a Results of the histopathological peer review

^b Results of the re-analysis only in males

^c $p < 0.05$; Cochran trend test

^d Greater than the upper limit of historical control data

^e Significant difference in the pair-wise comparison of the high-dose group with the controls at $p < 0.05$

^f $p < 0.05$; Fisher exact test

with both control groups. A peer review of pathological lesions in all male rats resulted in removal of several keratoacanthomas from the table of incidence in treated groups but confirmed that the incidence of this lesion clearly exceeded the upper limit of the range in historical controls (1.4–10%). The increased incidences of liver and thyroid tumours and of keratoacanthomas of the skin were considered to be treatment-related effects but to be threshold phenomena of negligible relevance to the low doses to which humans are exposed. The NOAEL was 100 ppm, equal to 4 mg/kg bw per day (Goldenthal, 1990c).

(d) Genotoxicity

The results of tests for the genotoxicity of pyrethrins *in vitro* are summarized in Table 4.

(e) Reproductive toxicity

(i) Multigeneration reproductive toxicity

Rats

In a two-generation study, groups of 28 male and 28 female Charles River rats received diets containing pyrethrins at concentrations of 100, 1000, or 3000 ppm, equivalent to 10, 100, and 300 mg/kg bw per day. A control group received the basal laboratory diet on an identical regimen. The F₀ parental generation were treated for a minimum of 77 days before the first of two matings. The same numbers of weanlings from the F_{1b} litters were selected randomly to become parents of the F₁ generation and were treated for a minimum of 95 days before being mated twice to produce the F_{2a} and F_{2b} litters. This study was conducted in compliance with FIFRA Guideline 83-4 and with GLP. No treatment-related effects were noted with respect to clinical signs, body weights, or food consumption in the parental rats of the F₀ generation, but the body weights and food

Table 4. Results of assays for the genotoxicity of pyrethrins *in vitro*

Test system	Test object	Concentration	Purity (%)	Results	Reference
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	8.8–8772 µg/plate in acetone	58	Negative ± S9	San & Springfield (1989)
Chromosomal aberration ^a	Chinese hamster ovary cells	0.02–0.32 Fl/ml + S9 0.005–0.08 Fl/ml – S9 in DMSO	58	Negative ± S9	Putman & Morris (1989)
Unscheduled DNA synthesis	Rat primary hepatocytes	0.0–1.0 Fl/ml in acetone	58	Negative	Curren (1989)

^a Study conducted in compliance with GLP and to a certain extent with OECD guidelines

consumption of the parental rats of the F₁ generation were significantly reduced at 3000 ppm and sporadically reduced at 1000 ppm. These reductions were considered to be treatment-related. Treatment at 3000 ppm resulted in significantly reduced body weights at birth for F_{1a} males and F_{2a} pups of each sex and during lactation for the male and female offspring of both matings of both generations. The mean body weights of pups at 1000 ppm were also lower than those of controls for male F_{2a} pups at birth and for F_{1b} and F_{2a} pups during lactation. Reproductive performance and other litter parameters were not affected by the treated diet at any dose. The NOAEL for parental and reproductive toxicity was 100 ppm, equivalent to 10 mg/kg bw per day (Schardein, 1989).

(ii) *Developmental toxicity*

Rats

Groups of five mated female Charles River rats were used in a range-finding study to determine the doses of pyrethrin to be used in a later study. Doses of 37.5, 75, 150, 300, or 600 mg/kg bw per day were administered orally by gavage on days 6–15 of gestation at a volume of 3 ml/kg. The control group received only the vehicle, 0.5% methylcellulose, on a comparable regimen. Uterine examinations were performed on all surviving females on day 20 of gestation. The method used in this study complied to a certain extent with OECD Guideline 414, and the study was conducted in compliance with GLP. Treatment-related maternal toxicity, observed as deaths, convulsions, and/or tremors, occurred at 150, 300, and 600 mg/kg bw per day. No treatment-related maternal deaths occurred at 75 mg/kg bw per day, although tremors were observed in this group. No treatment-related clinical signs were observed at 37.5 mg/kg bw per day. On the basis of these results, doses of 5, 25, and 75 mg/kg bw per day were selected for use in the following study (Schardein, 1987a).

Groups of 25 mated female Charles River rats were given pyrethrins suspended in 0.5% methylcellulose orally by gavage at doses of 5, 25, or 75 mg/kg bw per day on days 6–15 of gestation at a volume of 3 ml/kg. The control group received the vehicle only on a comparable regimen. On day 20 of gestation, the fetuses were removed surgically for evaluation. The method used in this study complied to a certain extent with OECD Guideline 414, and the study was conducted in compliance with GLP. No animals died or were killed *in extremis* during the study, and no treatment-related clinical signs were observed. The body-weight gains of the treated groups were comparable to those of the controls during treatment. No evidence of fetotoxicity was found, and morphological examination revealed no teratogenic effects at any dose tested. The NOAEL for maternal toxicity was 75 mg/kg bw per day, and that for developmental toxicity was 75 mg/kg bw per day, the highest dose tested (Schardein, 1987b).

Rabbits

Groups of five inseminated female New Zealand white SPF rabbits were used in a range-finding study to determine the doses of pyrethrins for use in a later study. Doses of 37.5, 75, 150, 300, or 600 mg/kg bw per day were administered orally by gavage on days 7–19 of gestation at a volume of 3 ml/kg. The control group received only the vehicle, 0.5% methylcellulose, on a comparable regimen. Uterine examinations were performed on all surviving females on day 29 of gestation. The method used complied to a certain extent with OECD Guideline 414, and the study was conducted in compliance with GLP. Treatment-related maternal toxicity, seen as deaths, tremors, convulsions, and weight loss, and fetal toxicity, seen as high postimplantation loss, were found at 600 mg/kg bw per day. Maternal toxicity, in terms of weight loss during treatment and tremors was seen at 300 mg/kg bw per day. No clear treatment-related effects were observed at 37.5, 75, or 150 mg/kg bw per day. On the basis of these results, doses of 25, 100, and 250 mg/kg bw per day were selected for use in the following study (Schardein, 1987c).

Groups of 16 inseminated female New Zealand white SPF rabbits were randomly assigned to receive pyrethrins at doses of 25, 100, or 250 mg/kg bw per day orally by gavage on days 7–19 of gestation at a volume of 3 ml/kg. The control group received only the vehicle, 0.5% methylcellulose, on a comparable regimen. On day 29 of gestation, the fetuses were removed

surgically for evaluation. The study was conducted in compliance with OECD Guideline 414 and with GLP. All animals survived to the end of treatment. One doe at the high dose aborted near term, on day 28 of gestation, after showing decreased or absent defaecation on several days previously. No gross lesions were present at necropsy. Excessive salivation, arched head, and/or laboured breathing were observed in a few females at the high dose on days 18–19 of gestation. One female at the intermediate dose had excessive salivation and arched head on gestation day 19. No apparent treatment-related clinical signs were seen at the low dose. There were no treatment-related gross pathological changes in any of the animals at the end of the study. Body-weight loss was seen in does at the high dose throughout treatment, and slightly reduced body-weight gain relative to the control value was observed in animals at the intermediate dose. The body-weight gains of treated groups were comparable to that of the control group throughout gestation (days 0–29). When the body weights on day 29 were adjusted by subtracting the uterine weights to reflect only maternal weight change, mean weight losses were evident in all groups, including the control, over the entire gestation period. There were no biologically meaningful or statistically significant differences in the mean numbers of viable fetuses, postimplantation losses, total implantations, or corpora lutea and in fetal body weight or fetal sex distribution in the treated groups in comparison with the control values. One doe at the high dose resorbed its litter, but it is not clear if this finding was related to treatment. There was no treatment-related or statistically significant difference in the incidence of fetal malformations or variations. The NOAEL for maternal toxicity was 25 mg/kg bw per day and that for developmental toxicity was 250 mg/kg bw per day, the highest dose tested (Schardein, 1987d).

(f) *Special studies*

(i) *Effects on the central nervous system*

Rats

Male Sprague-Dawley rats were treated once by oral gavage with a 10% or 25% solution of pyrethrins in corn oil at doses of 0, 40, 100, 200, 400, 800, 1400, or 2000 mg/kg bw, and females received a 2.5, 5, or 10% solution of pyrethrins in corn oil at doses of 0, 25, 50, 100, 150, 200, 400, or 800 mg/kg bw. This study was conducted in compliance with FIFRA Guideline 81-8 and with GLP. The clinical signs were characterized by mild-to-severe tremors. On the basis of the occurrence, severity, and onset of these reactions, a solution of 10% pyrethrins in corn oil and doses of 40, 125, and 400 mg/kg bw were selected for the study of acute neurotoxicity in males and a solution of 5% pyrethrins in corn oil and doses of 20, 63, and 200 mg/kg bw in females (Hermansky & Hurley, 1993a).

In the main study, groups of 15 male Sprague-Dawley rats received by gavage a 10% w/v solution of pyrethrins in corn oil at doses of 0, 40, 125, or 400 mg/kg bw, and the same numbers of females received a 5% w/v solution of pyrethrins in corn oil at doses of 0, 20, 63, or 200 mg/kg bw. This study was conducted in compliance with FIFRA Guideline 81-8 and with GLP. Five males and two females at the high dose died on the day of treatment, and a variety of acute neurological signs were observed in the other animals at this dose, including tremors, urogenital area wetness, salivation, perinasal encrustation, exaggerated startle response, decreased grip strength, hind leg splay, and increased body temperature. Tremors were also observed in three females at the intermediate dose. Measurements of motor activity on the day of treatment indicated increased fine movement and decreased rearing and ambulation in animals of each sex at the high dose and decreased fine movement, rearing, and ambulation in males at the intermediate dose. In addition, slight, statistically nonsignificant decreases in body weight were seen in males at the high dose on days 7 and 14. There was no evidence of any gross, treatment-related lesion. The microscopic changes were limited mainly to sections of the sciatic nerve and its branches. The histomorphological changes within the peripheral nerve sections indicated the presence of scattered degenerating nerve fibres or myelin sheaths. These changes were seen in only a few animals, were graded as minimal, and were not dose-related. The NOAEL was 20 mg/kg bw (Hermansky & Hurley, 1993b).

(ii) *Effects on hepatic microsomal enzymes*

Rats

Oral administration of pyrethrins to male rats at 85, 200, or 500 mg/kg bw per day for 3 weeks resulted in liver enlargement and decreased hepatic DNA concentrations. Significantly decreased hexobarbital-induced hypnosis without concomitant changes in barbital-induced hypnosis suggested an alteration in hepatic drug metabolism. The activities of hepatic microsomal enzymes responsible for detoxification of *O*-ethyl-*O*-(4-nitrophenyl)phenyl-phosphonothioate, *para*-nitroanisole demethylation, and hexobarbital oxidation were increased at 200 mg/kg bw per day to 150, 173, and 241% of the control values, respectively. Increased liver weight, the detoxification of pyrethrins, and demethylation of *para*-nitroanisole were found to be dose-related. Small increases in enzyme activities were observed when the lowest dose was given for 15 days. At 500 mg/kg bw per day, the liver weight and enzyme activities were increased up to 17 days of treatment but returned to the control level within 7 days after cessation of treatment. NADPH cytochrome c reductase activity and the cytochrome P450 concentration were also increased. It was suggested that pyrethrins caused induction of microsomal enzymes. The LOAEL was 85 mg/kg bw per day (Springfield et al., 1973).

3. Observations in humans

The main adverse effects seen after exposure to pyrethrum extracts in older studies were those manifesting as either skin or respiratory reactions. Much of the research performed to date has focused on the dermatological effects of pyrethrins, although mention has been made of the respiratory reactions that have often accompanied those of the skin. Studies by Ramirez (1930) and Feinberg (1934), in which the association between sensitivity to ragweed and to pyrethrins was delineated, contributed much to the understanding of allergic responses to pyrethrins. Several investigations have since been undertaken to isolate and characterize the allergen responsible for the dermal reactions. Mitchell et al. (1972) isolated a sesquiterpene lactone, pyrethrosin, from pyrethrins which induced positive dermal responses in humans given a patch test. None of the other fractions induced reactions, with the exception of pyrethrins II, which elicited a weak response. Zucker (1965) demonstrated that a dermal reaction to unrefined pyrethrum extract does not result in allergy to refined pyrethrins. The previous evaluation by the Joint Meeting in 1970 cited an unpublished report in which 200 people were given patch tests with a 1% water dispersion of pyrethrins, and no evidence of primary irritation or of sensitization was found. Rickett et al. (1972) concluded that the refined extracts that have been marketed since 1957 do not induce skin allergies when tested on sensitive subjects and that the dermal effects reported in the early literature are not relevant to an assessment of refined pyrethrins.

Case reports of adverse respiratory effects, such as anaphylactoid reactions and asthma, attributed to pyrethrins indicate that these responses often occur in individuals with a history of asthma. Although investigations of the dermal effects of pyrethrum extracts suggests that pyrethrins are not the causative agent, no thorough investigation of the agent(s) responsible for the adverse respiratory responses has been conducted.

Comments

Absorption, distribution, and excretion in rats were investigated only for pyrethrins I. After oral administration, more than 90% of a low dose of pyrethrins I was absorbed, and the concentration of radiolabel in blood peaked between 5 and 8 h. The radiolabelled residues were widely distributed in the organs analysed, with the highest concentrations in fat in females. The elimination half-time of pyrethrins I in males and females was approximately 6 h. The mean percentage of administered radiolabel found in the urine ranged from 32 to 47% in males and from 50 to 57% in females, the remainder being excreted in faeces.

The substance is extensively metabolized, the residues of the parent compound in faeces and urine representing only 10%. Six metabolites were identified and two major metabolic pathways were suggested, the first involving oxidation of the double-bond and/or the methyl groups and the second involving hydrolysis of the ester bond. Pyrethrins I are metabolized mainly through

oxidative processes, while pyrethrins II are metabolized through a combination of hydrolytic and oxidative processes.

Pyrethrins show little acute toxicity, with an oral LD₅₀ in rats of > 1200 mg/kg bw and NOAELs for clinical signs of 710 mg/kg bw for males and 320 mg/kg bw for females, a dermal LD₅₀ in rabbits of > 2000 mg/kg bw, and an inhalation LC₅₀ in rats of 3.4 mg/L. The compounds are minimally irritating to the skin and eye and show no potential for skin sensitization. Pyrethrum extracts have not been classified by WHO for acute toxicity.

In short-term tests for toxicity in mice, rats, and dogs, the lowest relevant NOAELs after oral administration were 1000, 1000, and 600 ppm, equal to 160, 57, and 18 mg/kg bw per day, respectively, for the three species. Statistically significant decreases in mean body weight or body-weight gain were observed at the high doses throughout most or all of the studies.

The liver is the main target organ in mice, rats, and dogs, and an increased liver weight was frequently accompanied by changes in serum transaminase activity. In mice, increased liver weights were associated with a higher incidence of hepatocellular hypertrophy. In the livers of rats and dogs, generally unremarkable histopathological changes were observed. At doses of 85 mg/kg bw per day and above, a pyrethrum extract containing 20% pyrethrins induced microsomal enzymes in rats. Furthermore, anaemia was observed in rats and dogs at doses of 3000 ppm and above. The kidney was another target, but only in rats. In a 13-week study, rats at doses greater than 1000 ppm had increased kidney weights associated with tubular degeneration and regeneration in the renal cortex.

In a 13-week study in rats exposed by inhalation, the NOAEL for systemic toxicity was 0.011 mg/L. The increases in liver weight were clearly related to exposure and were accompanied by changes in serum transaminase activity. Nonregenerative anaemia was also observed. The weights of the kidney and lung were increased in relation to body weight. The morphological abnormalities observed in the larynx, nasoturbinates, nasopharynx and lungs by light microscopy were considered to be localized responses indicative of a treatment-related effect.

Dermal administration of pyrethrins at doses up to 1000 mg/kg bw per day for 21 days caused no systemic toxicity in rabbits.

In a two-year study of toxicity and carcinogenicity in rats and an 18-month study of carcinogenicity in mice, the NOAEL was 100 ppm in both species, equal to 14 and 4 mg/kg bw per day in mice and rats, respectively. The liver was the main target. A treatment-related effect on the incidence of lung tumours was seen in mice and increased incidences of benign tumours of the skin, liver, and thyroid were observed in rats. The increased incidences of hepatocellular adenomas were associated with persistent induction of cytochrome P450 enzymes and hepatocellular hypertrophy, suggesting that pyrethrins are rodent-specific hepatoproliferative carcinogens. Enzyme induction leading to increased clearance of thyroid hormones would also be consistent with the higher incidence of follicular hyperplasia and follicular adenomas. However, additional studies on the mechanism of formation of the liver and thyroid tumours are required. The Meeting concluded that the increased tumour incidences caused by pyrethrins are threshold phenomena of negligible relevance to the low doses to which humans are exposed (see Appendix 1 to this monograph addendum).

Pyrethrins did not induce reverse mutagenicity in *Salmonella typhimurium* with metabolic activation, did not induce chromosomal aberration in Chinese hamster ovary cells, and did not induce unscheduled DNA synthesis in rat primary hepatocytes. The Meeting concluded that pyrethrins have no genotoxic or mutagenic potential, but a test for gene mutation in mammalian cells is lacking.

Pyrethrins did not show developmental toxicity in rats or rabbits at the highest maternally toxic doses tested, which were 75 and 250 mg/kg bw per day, respectively. The only effects on the offspring, observed in a two-generation study of reproductive toxicity in rats, were reduced body weights at the parentally toxic doses of 1000 and 3000 ppm, with a NOAEL of 100 ppm, equivalent to 10 mg/kg bw per day.

In a study of neurotoxicity in rats given single oral doses, acute neurological disorders (tremors, wetness of the urogenital area, salivation, perinasal encrustation, exaggerated startle response, decreased grip strength, and hind-leg splay) and behavioural effects (increased motor activity and decreased rearing and ambulation) were noted, with a NOAEL of 20 mg/kg bw.

The available data on humans did not show a causal relationship between exposure to modern pyrethrin-containing products and significant adverse health effects.

An ADI of 0–0.04 mg/kg bw was established for the tested blend of refined pyrethrum extract, which was based on the NOAEL of 100 ppm, equal to 4 mg/kg bw per day, observed in the long-term study of toxicity and carcinogenicity in rats and a safety factor of 100. This figure is identical to the ADI derived by the 1972 Meeting, which was based on a NOAEL of 200 ppm, equivalent to 10 mg/kg bw per day, in a long-term study in rats and a safety factor of 250.

The acute and long-term toxicity of the pyrethrins differ significantly. The acute toxicity of orally administered pyrethrins is expressed as neurotoxic effects. The longer-term toxicity is based principally on effects on the liver. Therefore, an acute reference dose of 0.2 mg/kg bw was allocated for the tested blend of refined pyrethrum, which was based on the NOAEL of 20 mg/kg bw for acute neurotoxicity in rats and a safety factor of 100.

Toxicological evaluation

Levels that cause no toxic effects

Mouse:	100 ppm, equal to 14 mg/kg bw per day (18-month study of carcinogenicity)
Rat:	20 mg/kg (study of acute neurotoxicity) 100 ppm, equal to 4 mg/kg bw per day (2-year study of carcinogenicity) 100 ppm, equivalent to 10 mg/kg bw per day (parental and reproductive toxicity in a two-generation study of reproductive toxicity) 75 mg/kg bw per day (maternal toxicity in two studies of teratogenicity, no developmental toxicity in a study of teratogenicity at the highest dose tested)
Rabbit:	25 mg/kg bw per day (maternal toxicity in a study of teratogenicity, no developmental toxicity in a study of teratogenicity at the highest dose tested)
Dog:	500 ppm, equal to 14 mg/kg bw per day (toxicity in a 1-year study)

Estimate of acceptable daily intake for humans

0–0.04 mg/kg bw

Estimate of acute reference dose

0.2 mg/kg bw

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

1. Gene mutation test in mammalian cells (required for submission to WHO by 2001)
2. Mechanistic study on liver and thyroid tumorigenesis (see Appendix 1; required for submission to WHO by 2001)
3. Further observations in humans

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

Absorption, distribution, excretion, and metabolism in mammals

Rate and extent of oral absorption	Immediately (peak between 5 and 8 h) and nearly complete (> 90%) in rats
Distribution	Widely distributed in rats, highest concentrations in fat
Potential for accumulation	None
Rate and extent of excretion	Nearly complete excretion in urine (32–47% and 50–57% in male and female rats) and in faeces

Toxicological end-points (contd)

Metabolism in animals	Extensively metabolized in rats, six metabolites identified; two major metabolic pathways.
Toxicologically significant compounds (animals, plants and environment)	Parent compound and metabolites
<i>Acute toxicity</i>	
Rat, LD ₅₀ , oral	> 1200 mg/kg bw
Rabbit, LD ₅₀ , dermal	> 2000 mg/kg bw
Rat, LC ₅₀ , inhalation	> 3.4 mg/L (4 h)
Dermal irritation	Non, rabbits
Ocular irritation	None, rabbits
Dermal sensitization	Not a sensitizer (Buehler test in guinea-pigs)
<i>Short-term toxicity</i>	
Target/critical effect	Liver (mice, rat, dog), erythrocytes (rat, dog), kidney (rat)
Lowest relevant oral NOAEL	90 days, dog: 600 ppm (18 mg/kg bw per day)
Lowest relevant dermal NOAEL	3 weeks, rabbit: > 1000 mg/kg bw per day
Lowest relevant inhalation NOAEL	3 months, rat: 0.01 mg/L
<i>Long-term toxicity and carcinogenicity</i>	
Target/critical effect	Liver
Lowest relevant NOAEL/NOEL	2 years, rat: 100 ppm (4 mg/kg bw per day)
Carcinogenicity	Increased tumour incidences in liver, thyroid, skin (rats) and lungs (mice)
<i>Genotoxicity</i>	In an incomplete range of studies, no genotoxic or mutagenic potential identified
<i>Reproductive toxicity</i>	
Reproductive target/critical effect	Reproductive effects (reduced pup body weights) at parentally toxic doses
Lowest relevant reproductive NOAEL	Rat: 100 ppm (10 mg/kg bw per day)
Developmental target/critical effect	No developmental effects at maternally toxic doses
Lowest relevant developmental NOAEL	Rat: 75 mg/kg bw per day
<i>Neurotoxicity/Delayed neurotoxicity</i>	
Acute neurotoxic NOAEL	Acute clinical disorders and behavioural effects Rat: 20 mg/kg bw
<i>Other toxicological studies</i>	Induction of hepatic microsomal activity
<i>Medical data</i>	Available human data do not show causal relationships between exposure to modern pyrethrin-containing products and significant adverse health effects.

<i>Summary</i>	<i>Value</i>	<i>Study</i>	<i>Safety factor</i>
ADI	0–0.04 mg/kg bw	Long-term toxicity, rats	100
Acute reference dose	0.2 mg/kg bw	Acute neurotoxicity, rats	100

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Appendix 1: Application of the Conceptual Framework for Cancer Risk Assessment

(Revised on the basis of discussions at the IPCS Workshop on Developing a Conceptual Framework for Cancer Risk Assessment, 16–18 February 1999, Lyon, France)

This framework, developed by an IPCS working group, provides a generic approach to the principles commonly used in evaluating a postulated mode of action for tumour induction by a chemical. Thus, the framework was used by the 1999 JMPR to provide a structured approach to the assessment of the overall weight-of-evidence for the postulated mechanism of the increased incidences of benign tumours of the liver, thyroid, and skin in rats and a treatment-related effect on the incidence of lung tumours in mice observed after long-term administration of pyrethrins.

The framework guidelines suggested 10 section headings. The introduction (see monograph section 2(c), 'Long-term studies of toxicity and carcinogenicity') describes the cancer end-points that have been observed. Three of these (rat liver and thyroid neoplasms, rat keratoacanthomas, and mouse lung tumours) are addressed separately in the analysis. An appropriate mode of action is postulated and the key events identified; the observed dose–response relationships and temporal relationships are discussed. The strength, consistency, and specificity of the association of tumour response with key events and the biological plausibility are analysed. Alternative modes of action are identified and found not to be supported. The three postulated modes of action are discussed below, with some uncertainties about both the biology of tumour development and the database on the compound and any inconsistencies in the method that were identified.

- The increased incidences of hepatocellular adenomas were associated with persistent induction of cytochrome P450 enzymes and hepatocellular hypertrophy, suggesting that pyrethrins are rodent-specific hepatoproliferative carcinogens. Enzyme induction leading to increased clearance of thyroid hormones would also be consistent with the increased incidence of follicular hyperplasia and follicular adenomas seen. The level of confidence in this postulated mode of action is moderate, because some uncertainties and inconsistencies were introduced by the method. Therefore, additional studies to identify more key events in the liver and thyroid (e.g. measurements of enzyme induction with purified pyrethrins, estimation of thyroid hormone changes, and estimation of thyroid and pituitary weights) are required.
- The higher incidence of keratoacanthomas in male rats at the high dose, which exceeds the upper limit of the range in historical controls, was considered to be related to treatment. The slight increase in the incidence of cystic lesions in the skin and subcutis in males observed macroscopically provided some support that the increased incidence of neoplastic lesions is a result of irritating chronic injury of the skin. There were some uncertainties introduced by the method, and differing opinions on the biology of these tumours were found in the literature. The level of confidence in this postulated mode of action is very low, and no further studies were identified to support it.
- The increased incidence of lung tumours in mice might be a result of proliferative processes following chronic injury of the respiratory epithelium or activation of microsomal mixed-function enzymes, especially in Clara cells, which contain high concentrations of P450 enzymes. Such chronic injury might be followed by cell proliferation with a dose–response relationship. The level of confidence in this postulated mode of action is, however, very low.

There was also little confidence in other possible modes of action. Uncertainties in the database on pyrethrins were identified. Improvement of the method used in the study of carcinogenicity (e.g. detailed histological sectioning of each lung lobe cut at the level of bronchi and additional microscopic examination of step-sections of the remaining lung tissue beginning at the level of the bronchi in all animals of each sex, followed by a re-evaluation of all histological slides in the absence of knowledge of their origin; reporting of the number and size of neoplastic and preneoplastic lesions in each animal) could serve to increase confidence that these tumours have no relevance at the low concentrations to which humans and animals are exposed.

The Meeting concluded that the increased tumour incidences associated with exposure to pyrethrins are threshold phenomena of negligible relevance to the low concentrations to which humans are exposed and that pyrethrins have no genotoxic or mutagenic potential. Therefore, no classification of cancer risk is necessary. However, additional studies are required.

The discussion of the postulated mode of action of tumour induction by pyrethrins was helpful in the overall process of hazard characterization and risk assessment and contributed to consideration of the relevance of the findings in animals to the human situation. Application of the framework also promoted confidence in the conclusions reached, as it represents use of a defined procedure which mandates consistent documentation of the facts and reasoning that includes consideration of inconsistencies and uncertainties. The Meeting concluded that the framework could be developed for use both by regulators and by researchers in identifying research needs on the basis of clear delineation of data gaps and inconsistencies.

