

PYRETHRINS (addendum)

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Explanation

Pyrethrins (pyrethrum extracts) derived from flowers of chrysanthemum of the genus *Chrysanthemum* have been used as insecticides for a long time. Pyrethrum, the active principle containing pyrethrin isomers, was evaluated toxicologically by the JMPR in 1965, 1966, 1967, 1968, 1969, 1970 and 1972. In 1999, the compound was re-evaluated on the basis of new studies that used a blend of refined pyrethrum extracts from plants in four major growing areas, with a total pyrethrin content of 57.6%. The 1999 JMPR established an acceptable daily intake (ADI) of 0–0.04 mg/kgbw on the basis of the no-observed-adverse-effect level (NOAEL) for liver effects in a new 2-year study in rats, and a safety factor of 100. At the same Meeting, an acute reference dose (RfD) of 0.2 mg/kgbw was established on the basis of the NOAEL in a study of acute neurotoxicity in rats, and a safety factor of 100.

The 1999 JMPR concluded that increased incidences of liver and thyroid tumours observed in rats treated with pyrethrins are threshold phenomena of negligible relevance to the low doses to which humans are exposed. In order to confirm this, the Meeting recommended that additional studies be performed to investigate the mechanism by which pyrethrins cause tumorigenesis in the liver and thyroid. The 1999 Meeting also suggested that a test for gene mutation in mammalian cells and more detailed information on case reports of adverse health effects in humans, for which only an abstract was available, should be submitted for evaluation.

The following information was made available to the present Meeting: a new test for gene mutation in mammalian cells, the full report of the mechanistic studies on liver and thyroid tumorigenesis in rats; and the full report of the analysis of case reports of human exposures to consumer products containing pyrethrins and/or pyrethroids.

Evaluation for acceptable daily intake and acute reference dose

1. Genotoxicity

In a study that complied with good laboratory practice (GLP), pyrethrins did not induce gene mutations at the thymidine kinase (*Tk*) locus in mouse lymphoma L5178Y cells (see Table 1). The first assay, conducted in the presence of metabolic activation, was not valid since the frequency of mutation was not increased for the positive control. In the second assay, the frequency of mutation was significantly increased with pyrethrin at a concentration of 85 and 52 µg/ml and was equivocally increased at 61 and 26 µg/ml. In the third assay with duplicates of pyrethrins at a concentration of 72, 61, 52, 26 and 13 µg/ml, no significant increase in the frequency of mutation was observed at any concentration (Steenwinkel, 2001).

2. Studies on mechanism of action: liver and thyroid tumorigenesis in rats

In a 2-year study of toxicity and carcinogenicity in rats, the incidence of hepatocellular adenoma was found to be increased in females given diet containing pyrethrins at a concentration of 3000 mg/kg, while the incidence of thyroid follicular adenoma was increased in males and females given diets containing pyrethrins at a concentration of 1000 or 3000 mg/kg (Goldenthal, 1990). Thus, for mechanistic studies on liver and thyroid tumorigenesis, groups of 15 male and 15 female Sprague-Dawley Crl:CD(SD)IGS BR rats (aged 12–13 weeks) were given diets containing pyrethrins (purity, 57.03%) at a concentration of 0, 100, 3000, or 8000 mg/kg (females) and 0 or 8000 mg/kg (males) for 7 and 14 days. Additional groups were treated for 42 days and sacrificed either directly after completion of treatment or after a 42-day recovery period. Mean intakes of pyrethrins were 0, 6.02–7.68, 163–223 or 262–618 mg/kg bw per day for females, and 0 or 300–503 mg/kg bw per day for males. Further groups of 15 male and 15 female rats were given diets containing phenobarbital (purity, >99%) at a concentration of 1558 mg/kg (equal to 83 mg/kg bw per day) for males or 1498 mg/kg (equal to 85 mg/kg bw per day) for females. However, because of the resulting drowsiness observed in some of the animals, the concentration was reduced to 1200 mg/kg for both sexes on day 8, resulting in mean phenobarbital intakes of 80 mg/kg bw per day for males and 95 mg/kg bw per day for females. All animals were examined for clinical signs, body weights and food consumption assessments. Sampling for analysis of formulated diets was performed during weeks 1, 3 and 6 of the study. Selected animals (eight animals from each group) were given bromodeoxyuridine (BrdU) via a surgically implanted osmotic minipump for 7 days before necropsy. After completion of treatment, animals were killed and blood samples were taken for analysis of clinical chemistry parameters (aspartate amino transferase (AST), alanine transferase (ALT), total protein, total bilirubin), triiodothyronine, thyroxine, reverse triiodothyronine and thyroid stimulating hormone (TSH). A limited necropsy was performed; brain, liver and thyroid glands

Table 1. Results of a study of genotoxicity with pyrethrins in vitro

End-point	Test object	Concentration	Purity (%)	Results	Reference
Gene mutation	L5178Y mouse lymphoma cells; <i>Tk</i> locus	–S9, 0–50 µg/ml	57.03	Negative ± S9 ^{a,b}	Steenwinkel (2001) ^c
		+S9, 0–85 µg/ml (in DMSO)			

S9, exogenous metabolic activation from 9000 × g supernatant fraction of rat liver induced with Aroclor; DMSO, dimethyl sulfoxide

^aTest in duplicate (–S9) or in triplicate (+S9); positive controls included

^bCytotoxicity seen at concentrations of >25 µg/ml; relative total growth was 6% at 50 µg/ml (–S9) and 3% at 85 µg/ml (+S9)

^cStudy complied with GLP (OECD GLP principles 1997) and was performed in accordance with OECD guideline 476 (adopted 21 July 1997)

being weighed and preserved from all animals. Liver and thyroid glands were processed and examined histologically. Examination of slides also allowed a qualitative and quantitative assessment of cell proliferation in the liver and thyroid to be made on the basis of the staining index with BrdU (Finch et al., 2002).

Frozen samples of the liver were sent to another laboratory for analysis of microsomal enzymes; this was performed using a separate protocol. Liver microsomes were prepared and assayed for protein, total cytochrome P450 content and activities of phase I markers of hepatic xenobiotic metabolism (7-ethoxyresorufin *O*-deethylase, 7-pentoxoresorufin *O*-deethylase and testosterone 7 α -, 16 β - and 6 β -hydroxylases) and the phase II enzyme thyroxine UDP glucuronosyltransferase. To allow for any changes in microsomal protein content and liver weight, total cytochrome P450 content and all the enzyme activities measured were expressed as specific content or activity (i.e. per unit of microsomal protein), per gram of liver (i.e. specific activity or content \times microsomal protein content) and by using the absolute and relative liver weight of each animal, i.e. total liver and per liver weight/kgbw, respectively (Lake, 2002).

With the exception of three female rats that were sacrificed prematurely after 7 days of treatment at 8000 mg/kg because of adverse clinical signs, there were no treatment-related clinical effects. In the animals treated with phenobarbital, subdued behaviour and rolling gait were apparent. Body weight and body-weight gain were statistically significantly decreased in males and females at 8000 mg/kg throughout the treatment period; the effect had resolved at the end of the recovery period. A slight but statistically significant decrease in body weight was noted in females at 3000 mg/kg during the first 14 days of treatment, but no significant changes were seen at 100 mg/kg. Animals given phenobarbital showed decreased body weight and/or body-weight gain during the first 7 days of treatment. There was a statistically significant decrease in food consumption in males and females at 8000 mg/kg and in females at 3000 mg/kg over the first 7 days of treatment. In the animals treated with phenobarbital, food consumption was decreased in males for the first 4 days and in females for the first 7 days of treatment, while food consumption was increased at the end of the 14-day period of treatment. Both studies complied with GLP (United States Environmental Protection Agency GLP regulations, 40 CFR Part 160; MHLW, MAFF and METI Japan).

There were slight but statistically significant reductions in AST activity in males at 8000 mg/kg and in females at 3000 and 8000 mg/kg after 42 days of treatment; the decrease was also observed in females at the highest dose after a 42-day recovery period. Activity of ALT was statistically significantly decreased in females at 8000 mg/kg after 7 days and at 3000 and 8000 mg/kg after 42 days of treatment. Total bilirubin concentration was slightly but statistically significantly increased at 8000 mg/kg in males after 14 days and in females after 42 days of treatment. The females treated with phenobarbital showed a statistically significant increase in AST activity after 7 and 14 days and in ALT activity after 14 days, while total bilirubin concentration was slightly increased after 7 days of treatment.

In males receiving diets containing pyrethrins at a concentration of 8000 mg/kg, there were statistically significant reductions in concentrations of thyroxine on days 7, 14 and 42; concentrations of triiodothyronine were reduced on days 7 and 14. In females, concentrations of triiodothyronine were reduced statistically significantly in all treated groups on day 42, while concentrations of reverse triiodothyronine were increased at 3000 and 8000 mg/kg at all time-points, including during the recovery period. Concentrations of thyroid

Table 2. Serum concentrations of thyroxine/triiodothyronine (ng/ml) in rats given diets containing pyrethrins or phenobarbital

Sex	Dietary concentration (mg/kg of feed)	Treatment period			
		7 days	14 days	42 days	42 days +42 days recovery
Males	0	3.69 / 83.12	2.91 / 72.98	3.96 / 75.11	3.21 / 79.92
	8000	2.55***/61.62***	2.24** / 65.23*	3.11** / 68.18	3.63 / 78.07
	Phenobarbital	2.51*** / 71.34**	2.22** / 59.98***	—	—
Females	0	2.36 / 89.24	2.59 / 89.44	2.34 / 98.81	2.48 / 90.05
	100	2.78 / 88.03	2.13* / 83.82	1.89 / 87.44*	2.21 / 89.53
	3000	2.67 / 83.19	2.23 / 86.27	2.82 / 84.09**	2.00 / 85.46
	8000	2.19 / 76.31	2.79 / 84.71	3.00* / 88.90*	2.16 / 85.46
	Phenobarbital	1.64** / 80.48	1.41*** / 84.63	—	—

From Finch et al. (2002)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ **Table 3. Serum concentrations of thyroid stimulation hormone (ng/ml) in rats given diets containing pyrethrins or phenobarbital**

Sex	Dietary concentration (mg/kg of feed)	Treatment period			
		7 days	14 days	42 days	42 days +42 days recovery
Males	0	4.26	4.46	2.68	4.50
	8000	6.28	7.69**	6.95**	2.92
	Phenobarbital	6.11	10.25***	—	—
Females	0	1.87	1.97	2.07	1.86
	100	2.57	2.25	2.14	1.77
	3000	3.79***	4.47***	3.57	1.73
	8000	4.77***	7.88***	7.82***	1.75
	Phenobarbital	4.13***	3.99**	—	—

From Finch et al. (2002)

** $p < 0.01$, *** $p < 0.001$

stimulating hormone were statistically significantly increased in males on days 14 and 42, while in females concentrations of thyroid stimulating hormone were statistically significantly increased at 3000 and 8000 mg/kg on days 7 and 14 and at 8000 mg/kg on day 42. No effects were observed in females at 100 mg/kg. For analysis of thyroxine, triiodothyronine and thyroid stimulating hormone, the results for animals treated with phenobarbital were similar for both sexes.

No histopathological effects were seen in animals receiving pyrethrins at a dietary concentration of 100 mg/kg. Follicular cell hypertrophy of thyroid glands was found at 3000 or 8000 mg/kg in 40–100% of animals at days 14 and 42 or at each time-point, respectively, correlating with increases of approximately 20–50% in organ weight. BrdU-labelling indices in the thyroid in animals killed after 14 days were three- to seven-fold greater than those in controls. Liver cell hypertrophy was found in 60–100% of animals at 3000 or 8000 mg/kg at each time-point, correlating with increased liver weights of approximately 30–60% greater than values for controls. BrdU-labelling indices in the liver in the animals killed after 7 or 14 days were three- to five-fold greater than the values for the controls. Results were similar for animals treated with phenobarbital.

Table 4. Weight of the thyroid gland (mg)^a/incidence^b of animals with histological thyroid follicular cell hypertrophy in rats given diets containing pyrethrins or phenobarbital

Sex	Dietary concentration (mg/kg of feed)	Treatment period			
		7 days	14 days	42 days	42 days +42 days recovery
Males	0	21.8 / 0	23.4 / 0	28.2 / 0	30.5 / 0
	8000	25.6 / 7	29.4** / 15	37.4*** / 13	33.9 / 0
	Phenobarbital	26.8* / 9	29.4*** / 15	—	—
Females	0	19.4 / 0	19.8 / 0	22.5 / 0	22.9 / 0
	100	19.0 / 0	19.1 / 0	21.7 / 0	22.7 / 0
	3000	21.4 / 3	24.0** / 11	25.1* / 10	25.0 / 0
	8000	19.9 / 6	29.0*** / 14	29.8*** / 14	27.8** / 0
	Phenobarbital	20.1 / 0	22.6* / 5	—	—

From Finch et al. (2002)

^aUsing body weight as covariate^bNo. of animals out of 15* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ **Table 5. Liver weight (g)^a/incidence^b of animals with histological liver cell hypertrophy in rats given diets containing pyrethrins or phenobarbital**

Sex	Dietary concentration (mg/kg)	Treatment period			
		7 days	14 days	42 days	42 days +42 days recovery
Males	0	18.09 / 0	17.66 / 0	21.66 / 0	21.77 / 0
	8000	23.13*** / 14	26.43*** / 9	30.73*** / 11	22.89 / 0
	Phenobarbital	23.21*** / 15	24.60*** / 15	—	—
Females	0	10.94 / 0	11.79 / 0	13.18 / 0	13.09 / 0
	100	10.77 / 0	11.96 / 0	12.35* / 0	13.38 / 0
	3000	13.65*** / 11	15.00*** / 11	16.04*** / 14	13.82 / 0
	8000	14.70*** / 13	18.51*** / 12	18.95*** / 12	14.09 / 0
	Phenobarbital	13.12*** / 14	14.94*** / 15	—	—

From Finch et al. (2002)

^aUsing body weight as covariate^bNo. of animals out of 15* $p < 0.05$, *** $p < 0.001$

Treatment with pyrethrins at a dietary concentration of 3000 or 8000 mg/kg significantly increased hepatic microsomal cytochrome P450 content (114–161% of values for the controls) and significantly induced the activities of 7-ethoxyresorufin *O*-deethylase (133–255% of values for the controls), 7-pentoxoresorufin *O*-deethylase (537–3957% of values for the controls), testosterone 16 β -hydroxylase (567–2633% of values for the controls), testosterone 6 β -hydroxylase (155–440% of values for the controls) and of thyroxine UDP glucuronosyltransferase (146–246% of values for the controls) on days 7, 14 or 42. Treatment with phenobarbital for 7 and 14 days significantly increased hepatic microsomal cytochrome P450 content (153–206% of values for the controls) and significantly induced the activities of 7-ethoxyresorufin *O*-deethylase (140–383% of values for the controls), testosterone 7 α -hydroxylase (222–276% of values for the controls), 7-pentoxoresorufin *O*-deethylase (3160–6057% of values for the controls), testosterone 16 β -hydroxylase (2367–3767% of values for the controls), testosterone 6 β -hydroxylase (211–480% of controls) and thyroxine UDP glucuronosyltransferase (147–232% of values for the controls). Overall, the effects of pyrethrins on the investigated hepatic drug-metabolizing enzymes were qualitatively similar to those of phenobarbital. On the basis of intake in mmol/kg per

day, however, phenobarbital was a significantly more potent inducer of CYP2B (i.e. 7-pentoxoresorufin *O*-deethylase and testosterone 16 β -hydroxylase) and CYP3A (i.e. testosterone 6 β -hydroxylase) forms, being 9.1 and 3.4 times more potent than pyrethrins in male and female rats, respectively. In both sexes, the hepatic effects of pyrethrins were reversible on cessation of treatment.

The Meeting concluded that pyrethrins act via an induction of hepatic microsomal cytochrome P450 enzymes and thyroxine UDP glucuronosyltransferase activity that leads to increased clearance of thyroid hormones, as demonstrated by evidence of a correlation between doses of pyrethrins that do and do not increase liver enzyme activities and perturb the concentrations of thyroid hormone. The studies reviewed also demonstrated that the mechanism by which pyrethrins induce formation of tumours in the liver and thyroid is similar to that of other non-genotoxic agents (i.e. phenobarbital) that induce hepatic drug-metabolizing enzymes in the rat. Such agents exhibit a clear threshold for tumour formation and produce tumours by non-genotoxic mechanisms that are most unlikely to occur in humans (Finch et al., 2002; Lake, 2002).

3. Observations in humans: human exposures to consumer products

The Meeting considered a study designed to analyse incidents of exposure to products containing pyrethrins and pyrethroids reported to the American Association of Poison Control Centers (AAPCC) from 1994 to 1999. The original intent of this review was to focus on natural pyrethrins; however, the method by which the AAPCC categorizes data by does not permit pyrethrins and synthetic pyrethroids to be readily distinguished. AAPCC estimated that a population of 260.9 million people was served by the 64 participating centres in 1999, this representing approximately 95% of the population of the USA. The collected data included information about age and sex of the person exposed, reason for exposure (accidental, intentional, etc.), site of exposure, duration of exposure (acute, chronic, etc.), route of exposure (inhalation, ingestion, etc.), patient management and treatment (treated on site, treated and released, admitted for medical or psychiatric care, etc.), symptoms reported, and medical outcome. Medical outcome was a rating of the severity of the effects, made by a specialist in poison information. The ratings included: no effect (no apparent symptoms exist), minor effect, moderate effect, major effect, and death. Given the self-reporting nature of the poison control system, precise quantification of exposure is not possible.

Sales of household insecticides containing pyrethrins and/or pyrethroids from 1995 to 1999 were estimated to be >250 000 000 product units, suggesting that >1 000 000 000 uses of pyrethrin/pyrethroid-containing products occurred during this period, if one assumes that a unit was used on four occasions. The annual number of exposure reports for these products increased from about 11 000 in 1994 to about 16 000 in 1997, when the number of exposures levelled off; the total number of people exposed by a variety of routes to consumer products containing pyrethrins and/or pyrethroids during the review period was 81 838. Adults (aged ≥ 20 years) accounted for the highest proportion of exposures (45%), while children aged <5 years accounted for 37%. One-third of the exposures were through ingestion, while inhalation, dermal and ocular exposures occurred in 27.8%, 26.2% and 10.7% of the cases, respectively. Unintended exposures were most frequently reported (93.1%) and exposures occurred mostly in or around the home (93%); nearly all cases (95%) involved an acute exposure. Of the 49 331 cases for which medical outcome was known, 30.5 % of cases were asymptomatic and 22.4% of cases were considered to be unrelated to the expo-

sure. Where there were symptoms, they were considered to be minor, moderate or major effects for 38.9%, 7.8% or 0.2% of the cases, respectively. There were no deaths reported in the cases included in this analysis. Thus, >90% of the cases with known outcome were either unrelated to exposure, asymptomatic, or featured symptoms of minor severity. Ingestion was the most frequent route of exposure, but was most often associated with minor medical outcome (owing to very low exposure to the product), while inhalation and ocular routes of exposure were more likely to be associated with a more severe medical outcome. Children aged <5 years were most likely to report ocular symptoms, and children aged 5–9 years were most likely to report ocular and dermal symptoms. Adults reported a wider variety of symptoms, including gastrointestinal, dermal, ocular and respiratory effects. Major effects were reported for 114 cases. Detailed records were available for 56 of these cases and they were reviewed in detail. Only 28 cases could be confirmed as “definitely” or “possibly” major outcome, with respiratory and neurological symptoms being reported most frequently (18 and 15 cases, respectively). Of these 28 cases, only seven cases were known to involve pyrethrins, sometimes in combination with other agents. One of these cases reported the outcome of an unsuccessful suicide attempt. Of the remaining six cases, which were major cases associated with exposure to pyrethrins, in only two cases did the patient have a medical history of asthma. One of these reports described a large accidental exposure to a burst can. These data suggest that people with asthma or allergies were not disproportionately represented in the AAPCC reports (PEGUS, 2001).

Comments

In the test for gene mutation evaluated by the present Meeting, pyrethrins did not induce mutations at the *Tk* locus in L5178Y mouse lymphoma cells. The Meeting reaffirmed the conclusion of the 1999 JMPR, which decided that pyrethrins are not genotoxic.

In mechanistic studies of liver and thyroid tumorigenesis, treatment of rats with pyrethrins at a dietary concentration of 3000 or 8000 mg/kg for 7, 14 and 42 days resulted in significant induction of a number of hepatic microsomal cytochrome P450 enzyme activities, thyroxine UDP glucuronosyltransferase activity, decreased triiodothyronine and thyroxine and increased thyroid-stimulating hormone activity. Additionally, increased liver and thyroid weights in association with increased BrdU-labelling indices in the liver and thyroid, and liver cell and thyroid follicular cell hypertrophy were observed. The studies were somewhat limited in that the choice of concentrations used did not thoroughly assess the dose concordance of the mechanistic events with the induction of tumours (e.g. a dietary concentration of 1000 mg/kg, which produced thyroid follicular adenoma in the long-term study of carcinogenicity in rats, was not tested). Nonetheless, the Meeting concluded that pyrethrins induce the formation of liver and thyroid tumours by mechanisms that appear to be similar to those used by other non-genotoxic, mitogenic substances, e.g. phenobarbital, which produce tumours in rodents, and these tumours are not predictive of hazard in humans at relevant exposures. The Meeting thus concluded that the increased tumour incidences caused by pyrethrins are threshold phenomena of negligible toxicological relevance to humans.

Although the data on human exposure (case reports of 81 838 patients exposed by a variety of routes to consumer products containing pyrethrins and/or pyrethroids) did not permit ready distinction between exposure to natural pyrethrins and synthetic pyrethroids, important inferences can be made about the safety of pyrethrins. Of 49 331 cases with known

medical outcomes, >90% of patients had symptoms that were unrelated to exposure, were asymptomatic, or reported symptoms of minor severity. Major effects of exposure were reported in 114 cases, but only in 28 cases (including 7 cases of people exposed to pyrethrins) could these be confirmed as major outcomes after thorough review of the case reports. Among these 28 cases, respiratory and neurological symptoms were reported most frequently (18 and 15 cases, respectively). There was no evidence that having a history of asthma was disproportionately associated with major adverse outcomes after exposure to pyrethrins.

Toxicological evaluation

The Meeting concluded that the ADI of 0–0.04 mg/kg bw established by the 1972 JMPR and reaffirmed by the 1999 JMPR, and the acute RfD of 0.2 mg/kg bw established by the 1999 JMPR are supported by the new data.

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