We would like to comment on

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US EPA:

Pyrethrins: Report of the Cancer Assessment Review Committee (Third Evaluation)

PC Code 069001

The Cancer Assessment Review Committee (CARC) reviewed the data on a chronic bioassay with Pyrethrins in rats and two subsequent mechanistic studies (Goldenthal 1990, Finch et al. 2002, Lake 2002).

In the bioassay an increased incidence in thyroid follicular tumours in male and female rats at higher dose levels and an increased incidence of hepatic adenoma in female rats were noted in the highest dose group only. In a range of short term tests it was established that pyrethrins are non-genotoxic.

In line with the current view in the scientific community (IARC 1999, Cohen et al. 2003, Meek et al. 2003) and considering the data obtained in a mechanistic study the CARC regarded the thyroid tumour incidence as "*following a mechanism that has been established for a number of pesticides*" (Hurley et al. 1998). … *Pyrethrins, like most antithyroid pesticides, operate at an extrathyroidal site by increasing hepatic metabolism and excretion of thyroid hormone*."

Thus, it is concluded in the Report that these thyroidal effects are specific to rats and are of no concern for human exposure.

1. Weight of Evidence Descriptor

Based on the slight increase of benign liver adenoma in the high dose females observed in the bioassay the committee formulated the descriptor

"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"

We believe this descriptor is unfortunate and highly misleading.

Clearly, the minimal tumourigenic potential is confined to chronic high dose levels, several orders of magnitudes higher than expected human exposure levels, and thus poses no carcinogenic risk to humans.

The aim of cancer risk assessment is to aid risk managers to differentiate agents that pose a carcinogenic risk to humans from those agents that do not pose a carcinogenic risk

In the most recent version of the EPA Guidelines for carcinogen risk assessment there are only five descriptors recommended in order to facilitate risk management for humans:

- Carcinogenic to Humans
- Likely Carcinogenic to Humans
- Suggestive Evidence of Carcinogenic Potential
- Inadequate Information to Assess Carcinogenic Potential
- Not likely to Be Carcinogenic to Humans

In the case of Pyrethrins the descriptor "Not likely to Be Carcinogenic to Humans" would be more in line with the evidence and also consistent with the JMPR assessment concluding that "the mechanism by which Pyrethrins induce tumours in the liver and thyroid is similar to other non-genotoxic agents.(…) Such agents exhibit a clear threshold for tumour formation and produce tumours by non-genotoxic mechanisms that are most unlikely to occur in humans" (JMPR 2003)

2. Mechanism of liver tumour formation

- There is a consensus that pyrethrins are not genotoxic.
- Only benign tumours (adenoma) were observed – no carcinoma – in the 2-year oral toxicity bioassay in rats (table 1).
- There was no clear dose response, e.g. as a singularly phenomenon only in females maintained for two years on the (maximum tolerated) high dose (3000 ppm) a minimal increase in adenoma was noted.
- Incidence of hepatic adenoma in the highest dose group (5 of 60 females = 8%) was only just outside the range of historical control data (0-6% ~ 4 of 60 animals) in this rat strain (table 1)
- In male rats the incidences of adenoma were 5% (3 of 60 males) in both, the highest dose and the mid dose (3000 ppm and 1000 ppm) (table 1).
- Subchronic studies indicated that Pyrethrins induced signs of hepatotoxicity in male and female rats, this was confined to high dose levels ($\geq$ 3000ppm) (table 3)
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The mechanistic study was conducted with different concentrations of pyrethrins investigating several time points (7 d, 14 d, 42 d and 42 d treatment + 42 days recovery) including a negative control group and a phenobarbital-treated positive control group.

The parallelism of effects observed in the positive control (~1500 ppm phenobarbital) and the high dose Pyrethrins-treated groups is evident (Figure 1) and the conclusion may be drawn that both compounds induce tumours in the liver by a similar non-genotoxic mechanism at high dose levels. Notably, phenobarbital appears to be far more potent as compared to Pyrethrins, and indeed the effect of pyrethrins on the incidence of hepatic adenoma was only minimal.

Phenobarbital has been shown to induce liver enzymes and also hepatic cell proliferation via an activation of the constitutive androstane receptor (CAR), a nuclear receptor that forms heterodimers with the retinoid acid receptor to induce a number of proteins (Wei et al. 2000,Swales and Negeshin, 2004, Elrick et al. 2005) but not hepatic cytotoxicity. Similar mechanisms have been reported for synthetical analogs of Pyrethrins (Bauer et al. 2004).

Epidemiological studies showed no human cancer risk for Phenobarbital (IARC 2001), a widely used barbiturate and antiepileptic drug that is taken at considerable (pharmacologic) dose levels, therefore it is highly unlikely that exposure to Pyrethrins at levels below the ADI (0.04 mg/kg bw/d) will have an influence of human cancer risk.

The lack of correlation observed for liver enzyme induction and carcinogenicity was cited by the CARC from a study by Elcombe et al (2002). These authors investigated nine non-genotoxic rodent carcinogens three of these are targeting the rat liver (diethylhexyl phthalate, chlorendic acid, monuron) but none with a mechanism similar to phenobarbital and the pyrethrins.

The CARC noted the lack of hepatotoxicity in female rats in the bioassay was not consistent with the prposed mode of action. However, increased liver weights were observed a high dose levels in both male and female rats in the 2-year chronic bioassay (table 2) and hepatotoxicity was also observed both in male and female rats in a subchronic study (90 days oral administration, table 3). Increased liver weights were noted at the high dose level for male rats and for female rats both at 10000 ppm and 3000 ppm Pyrethrins (Goldenthal 1988).

This is in concordance with the view hepatotoxicity was the mechanism in a 2 year feeding study with rats were in a marginal increase benign tumours was noted in the liver of females rats.

It is a general consensus in the scientific community that benign liver tumours induced in the rat that follow a non-genotoxic mechanism are highly specific to rodents and of no relevance for humans.

While the mode of action concept (Cohen 2003, Meek 2003, Cohen 2004) helps to differentiate and evaluate the human relevance of animal tumour data this concept apparently disregards the carcinogenic potency of an agent. Thus, in the present case Pyrethrins with only a marginally tumourigenic activity in rodents act apparently by a mechanism similar to Phenobarbital, but the potency is about 5-10 fold lower with regard to biochemical effects. Phenobarbital has been shown to be non carcinogenic to humans even when administered at pharmacologic levels.

It may be concluded that exposure to low levels of Pyrethrins (below an ADI of 0.04 mg/kg bw/d) are *Not likely to Be Carcinogenic to Humans*.

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Table 1: Affected numbers of animals to selected parameters in a 2-year rat feeding study with Pyrethrins (Goldenthal 1990)

Values in ( ) incidence expressed in %; (n= 60)

Parameter	Sex	Control 1	Control 2	Pyrethrins ppm		
				100	1000	3000
Adenoma	m	6 (10 %)	1 (2 %)	0	3 (5 %)	3 (5 %)
	f	0	1 (2 %)	0	1 (2 %)	**5 (8 %)[1)2)]**
Carcinoma	m	1 (2 %)	0	0	0	1 (2 %)
	f	0	0	0	0	0

[1]Significantly different from control 1
[2]Significantly different from control 2

Table 2 Terminal body weights and relative liver weights in male and female rats in a 2-year rat feeding study with Pyrethrins (Goldenthal 1990)

Parameter	Sex	Control 1	Control 2	Pyrethrum extract, ppm		
				100	1000	3000
Terminal body weight	m	646	616	592	607	589
	f	419	**506[1]**	478	439	408
Relative liver weight	m	3.83	3.64	4.25	4.08	**4.25[2]**
	f	4.18	3.99	3.86	4.09	**4.67[2]**

[1]Significantly different from control 1

[2]Significantly different from control 2

Table 3 Terminal body weights and liver weights in male and female rats in a 90 day rat oral toxicity study with Pyrethrins (Goldenthal 1988)

Parameter	Sex	Control	Pyrethrum extract, ppm				
			300	1000	3000	10000	30000[1)]
Body weight	m	588	594 (+1.0)[2)]	569 (-3.2)	548 (-6.8)	523 (-11.1)	460 (-21.8)
	f	276	280 (+1.4)	277 (+0.4)	267 (-3.3)	233 (-15.6)	234 (-15.2)
Liver weight	m	25.6	27.21	25.53	28.8	**36.42[3)]**	**39.71 [3)]**
	f	11.12	11.6	11.91	**14.0 [3)]**	**17.97 [3)]**	**23.11 [3)]**

[1)] Mortalities at highest dose level

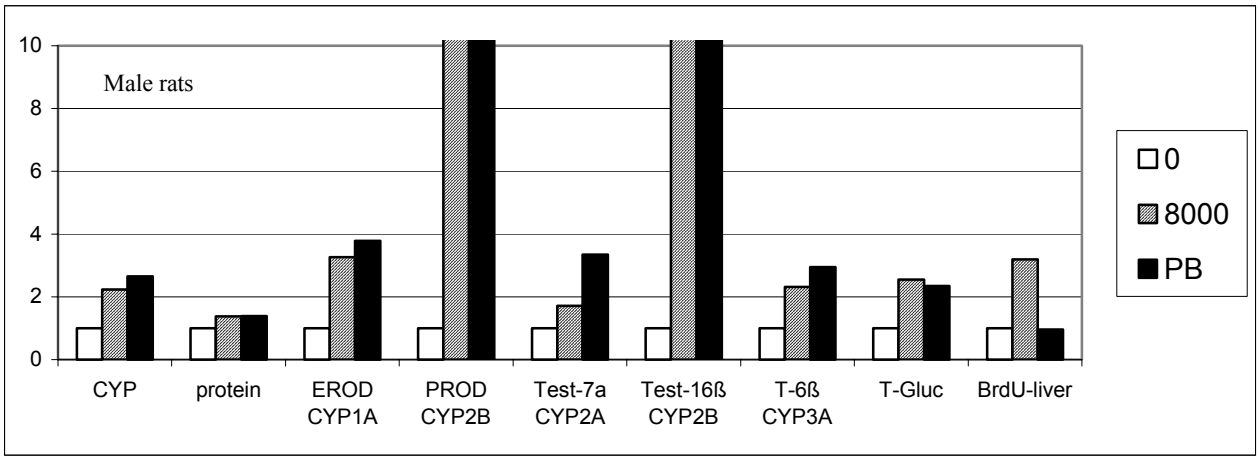
[2)] Difference from control (%)

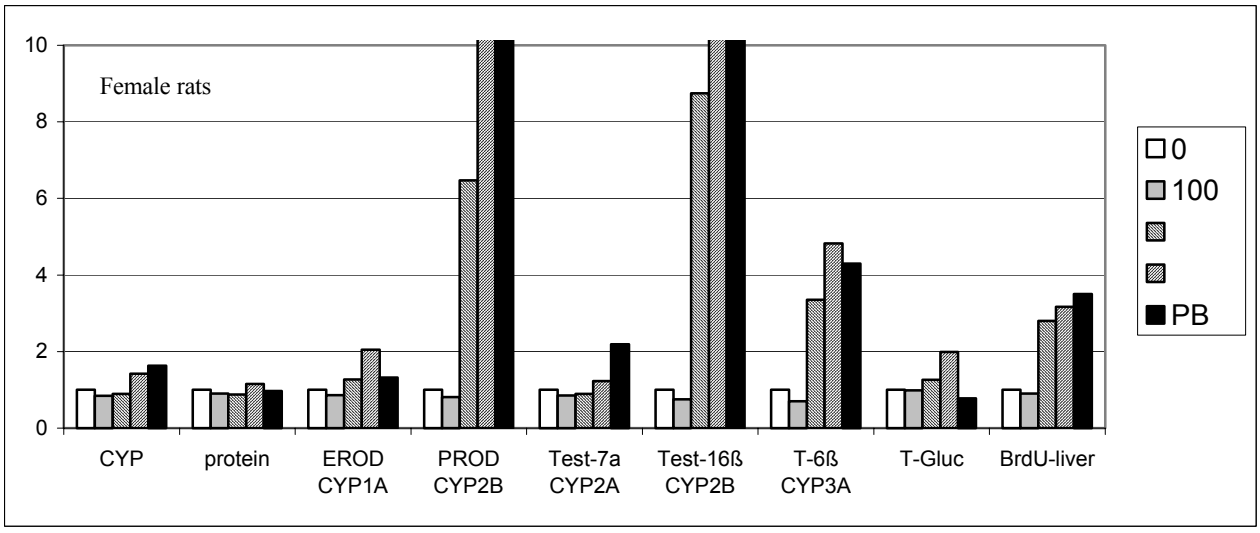
[3)] Significantly different from control

Figure 1

Graphical presentation of the data obtained in the mechanistic study (14day of treatment).

Data are normalised for values of control animals. A clear parallelism is obvious for high dose Pyrethrins (hatched bars) and Phenobarbital (black bars) while low dose Pyrethrins (grey bars) parallel the untreated controls (white bars).





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