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A Comparative Analysis of the Acute Toxicity of Technical-Grade Pyrethroid Insecticides and Their Commercial Formulations[1]

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Seventy-two-hour LD_{50} studies involving the fenvalerate formulation, Pydrin 2.4 E.C., and the permethrin formulation, Ambush, were performed with male Swiss mice to compare the toxicity of the commercial formulations to that of the technical-grade pyrethroids. Comparison of the formulation ip and po LD_{50} values and the lethality of technical-grade pyrethroids revealed an increased toxicity of the technical-grade material when administered as the commercially formulated products. The calculated ip and po LD_{50} values for Pydrin 2.4 E.C. and Ambush were calculated to be 62 and 72 mg/kg, respectively, whereas those for Ambush were 429 and 424 mg/kg. Administration of doses of technical-grade fenvalerate which corresponded to the amount of fenvalerate contained in the calculated LD_{99} value of Pydrin resulted in no deaths. Administration of the LD_{99} value of Ambush, as the technical-grade product, resulted in no deaths following ip administration, whereas the po value resulted in 100% death. The data indicate an effect of the Pydrin formulation vehicle on fenvalerate toxicity, whereas the Ambush vehicle did not enhance permethrin toxicity. Technical-grade material in general was more toxic following po than ip administration suggesting the corn oil vehicle may have reduced ip absorption.

INTRODUCTION

Extracts from the common flowering ornamental *Chrysanthemum cineraiaefolium,* termed pyrethrins, have long been known to have insecticidal activity. These naturally occurring compounds have a very favorable insect-to-mammal lethality ratio; however, they are expensive and rapidly undergo photodegradation. These problems have been circumvented by the development of the pyrethroid class of compounds, synthetic analogs of the pyrethrins (Chambers, 1980). The development of these compounds has resulted in their increased use in various situations, including field pest control and household use, and as veterinary and human pediculicides (Herve, 1985).

Pharmacologically, the pyrethroids interact with the nervous system of both target and nontarget organisms to produce membrane depolarization progressing to a spontaneous firing of nerve impulses. This is thought to occur via involvement of the sodium/potassium channels, possibly through a prolonged opening of the sodium channel gates (Lawrence and Casida, 1982). The acute toxicities produced by this

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class of compounds may be divided into two major types. The T-type toxicity syndrome is observed following administration of various pyrethroids classified as Type I pyrethroids (Lawrence and Casida, 1982). This syndrome consists of aggressive behavior, tremor (T) in the extremities, increased body temperature, and ultimately whole-body tremors, tonic–clonic seizures, and death. The CS-type toxicity is observed following administration of only those pyrethroids which contain an α-cyano moiety (Type II pyrethroids) (Litchfield, 1985). The Type II toxicity syndrome proceeds from a pawing/burrowing behavior to profuse salivation (S), normal or depressed body temperature, whole-body tremors, splayed gait involving the hind limbs, and finally choreoathetosis (C) and death associated with tonic–clonic seizures (Gray, 1985; Verschoyle and Aldridge, 1980; White *et al.*, 1976). Type I pyrethroids include permethrin, cismethrin, and allethrin. Fenvalerate, deltamethrin, and cypermethrin are examples of Type II pyrethroids (Lawrence and Casida, 1982).

The majority of the available literature involves studies which utilized only technical-grade material. Therefore, there is no indication of the possible contribution of the formulation vehicle to the toxicity of the ultimate trade product. Components in the vehicle could be expected to enhance the toxicity of the active ingredient via a toxicokinetic interaction. The reported LD_{50} for oral administration of technical grade permethrin in corn oil in mice is 490 mg/kg (Litchfield, 1985). The LD_{50} of orally administered fenvalerate in corn oil in male mice was reported to range from 190 mg/kg (Parker *et al.*, 1985) to 300 mg/kg (Litchfield, 1985). The LD_{50} values that are reported in the literature indicate a definite vehicle contribution. The oral LD_{50} for permethrin in female mice ranges from 540 mg/kg in corn oil to 4000 mg/kg in water (Litchfield, 1985).

The widespread use of these pesticides in the agricultural setting indicates that a spill and consequent exposure to these products in their place of manufacture or in the field would expose workers to the pesticide in its formulation vehicle, rather than to the active ingredient and the inert test vehicle, corn oil, which most toxicity studies have utilized. The purpose of this research was to conduct a comparative evaluation of the acute toxicities of fenvalerate and one of its commercial formulations, Pydrin 2.4 E.C., and permethrin and its commercial preparation, Ambush, in mice following po and ip administration.

MATERIALS AND METHODS

Male Swiss mice (25–30 gm) obtained from Charles River Co. (Wilmington, MA) served as experimental subjects following a 14-day acclimation period. Subjects were individually housed in stainless-steel hanging cages equipped with wire screen floors. A 12-hr light/dark cycle was in effect throughout the study. All testing procedures were performed during the light portion of the cycle. Environmental temperature was maintained at 21 ± 2°C. Food in block form (Lab Chow, Ralston Purina Co.) and water were available *ad libitum* during the 14-day acclimation period. Subjects were food deprived for 16 hr and water deprived for 1 hr prior to dosing. Food and water were again provided *ad libitum* 8 hr after exposure.

Technical-grade fenvalerate and its formulation vehicle were donated by Shell Chemical Co. (Houston, TX). The technical-grade material was determined to be 95.6% fenvalerate by the Mississippi State Chemical Laboratory, Mississippi State University. Pydrin 2.4 E.C. and Ambush (minimum 35% (±) *cis*/maximum 65%

TABLE 1

ACUTE ORAL AND INTRAPERITONEAL 72-hr TOXICITY OF THE FENVALERATE FORMULATION PYDRIN AND THE PERMETHRIN FORMULATION AMBUSH IN MALE MICE

Compound	Route	LD_{50} (mg/kg)	95% C.L. (mg/kg)	LD_{99} (mg/kg)	95% C.L. (mg/kg)
Pydrin 2.4 E.C.	IP	62	57–67	89	79–118
	PO	72	61–82	128	105–209
Ambush	IP	429	381–594	838	602–2321
	PO	424	322–584	1327	850–4162

Note. Numbers correspond to the amounts of active ingredient (pyrethoid) and not to the amount of the entire formulation.

(±) *trans*) were obtained from local commercial sources. Technical-grade permethrin (95.1%) was obtained from FMC Corp. (Princeton, NJ).

Subjects were randomly assigned to treatment groups of 10 to 12 mice. All test substances were administered in a volume of 0.4 ml and were administered by the ip or po routes. The 72-hr LD_{50} of Pydrin was determined by administering doses ranging from 50–100 mg/kg ip and from 25–125 mg/kg po. The 72-hr LD_{50} of Ambush was determined by administering doses ranging from 100–1600 mg/kg ip and 100–2000 mg/kg po. Doses of the formulated products were prepared by diluting the stock material with water. Doses of technical-grade pyrethroids corresponding to the amount of active ingredient in the calculated LD_{99} and LD_{99} upper confidence limit of Pydrin and Ambush were administered by both routes. The technical-grade products were diluted to the appropriate concentrations with corn oil for a total dose volume of 0.4 ml. The volumes of Pydrin formulation vehicle contained in the calculated LD_{99} and LD_{99} upper confidence limit for both routes were also evaluated. The vehicle formulation for Ambush was unavailable. Since personal communication had indicated that xylene was the major constituent of similar vehicles, the decision was made to use xylene as the vehicle control for Ambush. Assuming that the commercial vehicle was no greater than 85% xylene, the amount of xylene that would be present in the largest doses of Ambush administered po and ip served as the Ambush vehicle control. The doses of formulation vehicle, xylene, and commercial product were diluted with distilled water to a total volume of 0.4 ml. Control groups consisted of formulation vehicle control (for Pydrin), xylene vehicle control (for Ambush), distilled water, corn oil, and no treatment. The number of deaths were recorded hourly from 1 through 8, and at 24, 48 and 72 hr following dosing. All of the mice that died during the experiment were necropsied for evidence of gavage error or intestinal puncture. LD_{50}, LD_{99}, and 95% confidence limits were calculated by probit analysis (Finney, 1971).

RESULTS

Classical pyrethroid toxicities, including tremors, ataxia, salivation, and convulsions, were observed following administration of the commercial preparations. The severity of these signs and latency of recovery appeared to be dose-dependent. The calculated LD_{50} and LD_{99} values for Pydrin 2.4 E.C. and Ambush are illustrated in Table 1. The LD_{50} values for both routes of administration for each of the pyrethroids

TABLE 2

CUMULATIVE DEATHS AFTER FENVALERATE TREATMENT

Treatment	Route	Dose of fenvalerate (mg/kg)	N	Hour posttreatment										
				1	2	3	4	5	6	7	8	24	48	72
Pydrin 2.4 E.C.	PO	25	11	0	0	0	0	0	0	0	0	0	0	0
		50	12	0	0	1	1	1	1	1	1	1	1	1
		75	12	0	1	6	6	6	6	6	6	6	6	6
		100	11	0	2	10	10	10	10	10	10	10	10	10
		125	12	0	8	12	12	12	12	12	12	12	12	12
	IP	50	12	0	0	0	0	0	0	0	0	0	0	0
		60	11	0	0	0	6	6	6	6	6	6	6	6
		70	11	0	1	6	8	8	8	8	8	8	8	8
		80	12	0	5	8	10	11	11	11	11	11	11	11
		100	12	0	10	12	12	12	12	12	12	12	12	12
Fenvalerate	PO	128	11	0	0	0	0	0	0	0	0	0	0	0
		209	12	0	0	2	2	3	6	6	6	9	9	9
	IP	89	11	0	0	0	0	0	0	0	0	0	0	0
		118	12	0	0	0	0	0	0	0	0	0	0	0
Pydrin vehicle[a]	PO	0.014 ml	12	0	0	0	0	0	0	0	0	0	0	0
	IP	0.008 ml	12	0	0	0	0	0	0	0	0	0	1	1
Corn oil	PO	0.4 ml	12	0	0	0	0	0	0	0	0	0	0	0
	IP	0.4 ml	12	0	0	0	0	0	0	0	0	0	0	0
Distilled water	PO	0.4 ml	12	0	0	0	0	0	0	0	0	0	1	1
	IP	0.4 ml	11	0	0	0	0	0	0	0	0	0	0	0
No treatment			12	0	0	0	0	0	0	0	0	0	0	0

[a] Volumes correspond to the amount of vehicle contained in the Pydrin LD_{99} upper-confidence-limit dosage. These volumes were diluted to 0.4 ml with distilled water.

were similar, indicating that the route of administration was not a significant variable in determining the acute toxicity of the formulations. The LD_{50} values for Ambush were much greater than those for Pydrin 2.4 E.C. for both routes of administration demonstrating the greater potency of Pydrin 2.4 E.C.

Table 2 also illustrates the similar potency of Pydrin 2.4 E.C. administered by both the ip and the po route. Pydrin produced 100% lethality in the highest doses given by the oral and parenteral routes. These results follow a typical dose: response curve of increased dose resulting in concomitant increases in mortality. There appeared to be no differential effect of route of administration on the time course of the toxicity. Animals that died as a result of treatment did so within 3 hr postadministration. Deaths (75%) occurred in only one of the technical-grade fenvalerate treated groups, that group whose dose corresponded to the po LD_{99} upper confidence level of Pydrin. The amount of the pyrethroid which would be contained in the calculated LD_{99} of the formulation when administered as the technical-grade material, resulted in no deaths when given by either po or ip route. Therefore, the technical-grade material was much less toxic than the formulation. A single death was recorded in the ip formulation vehicle control group and no deaths occurred following oral administration of the formulation vehicle.

Table 3 illustrates the lethality data resulting from acute administration of permethrin and its formulation, Ambush. Unlike the case with Pydrin and fenvalerate,

TABLE 3

CUMULATIVE DEATHS AFTER PERMETHRIN TREATMENT

Treatment	Route	Dose of permethrin (mg/kg)	N	Hour posttreatment										
				1	2	3	4	5	6	7	8	24	48	72
Ambush	PO	100	12	0	0	0	0	0	0	1	1	1	1	1
		200	8	0	0	0	0	0	0	0	0	0	0	0
		300	11	0	0	1	2	2	2	2	2	2	2	2
		650	9	0	0	1	1	3	4	5	6	6	6	7
		1100	9	0	2	2	3	4	9	9	9	9	9	9
		1500	10	1	1	1	1	3	4	5	9	9	9	9
		2000	8	0	0	1	2	2	3	3	4	8	8	8
	IP	100	9	0	0	0	0	0	0	1	1	1	1	1
		200	9	0	0	0	0	0	0	0	0	0	0	0
		225	11	0	0	0	0	0	0	0	0	0	0	0
		250	11	0	0	0	0	0	0	0	0	0	0	0
		275	12	0	0	1	1	1	1	1	1	1	1	1
		300	12	0	0	0	0	0	0	0	0	0	1	1
		400	10	0	0	0	0	1	1	1	1	1	1	1
		800	10	1	1	2	3	6	7	7	7	10	10	10
		1600	10	0	4	10	10	10	10	10	10	10	10	10
Permethrin	PO	1327	11	0	0	1	3	4	8	8	8	9	10	11
		4162	11	0	0	4	9	10	10	10	10	10	10	10
	IP	838	11	0	0	0	0	0	0	0	0	0	0	0
		2321	11	0	0	1	1	2	2	2	2	2	2	2
Xylene[a]	PO	0.140 ml	7	1	1	1	1	1	1	1	1	3	4	4
	IP	0.112 ml	10	0	2	7	9	9	9	9	9	10	10	10
Corn oil	PO	0.4 ml	11	0	0	0	0	0	0	0	0	1	1	1
	IP	0.4 ml	11	0	0	0	0	0	0	0	0	0	0	0
Distilled water	PO	0.4 ml	10	0	0	0	0	0	0	0	0	0	0	0
	IP	0.4 ml	10	0	0	0	0	0	0	0	0	0	0	0
No treatment			10	0	0	0	0	0	0	0	0	0	0	0

[a] Values correspond to the volume of xylene (assuming the commercial vehicle was 85% xylene) in the largest PO and IP dosages of Ambush, administered, i.e., 2000 and 1600 mg/kg, respectively.

many of the deaths resulting from permethrin and Ambush administration were delayed in that they occurred 5–24 hr post administration. The oral administration of technical-grade permethrin equivalent to the po LD_{99} and LD_{99} upper confidence limit for Ambush resulted in the expected number of deaths. There did not appear to be any differences in the toxicity of permethrin and Ambush following oral administration. However, ip permethrin was much less toxic than ip Ambush. No deaths occurred in the LD_{99} permethrin group and only two in the LD_{99} upper confidence limit group. Therefore, similar results were seen with ip comparisons between the two dosage forms of both pyrethroids. The administration of xylene po and ip resulted in 57% and 100% lethality, respectively. A single delayed (8–24 hr) death was reported in the po corn-oil control group.

Table 4 summarizes the acute toxicity comparisons between the technical-grade and formulation form of the two pyrethroids. The calculated LD_{99} values of the formulation given as technical-grade material in corn oil are compared to similar amounts of the formulated product. When given by the oral route, fenvalerate is

TABLE 4

CUMULATIVE DEATHS 72-hr AFTER PYRETHROID TREATMENT

Treatment	Route	Dose of pyrethroid (mg/kg)	*N*	Deaths
Pydrin 2.4 E.C.	PO	125	12	12
Pydrin 2.4 E.C. LD_{99} given as fenvalerate	PO	128	11	0
Pydrin 2.4 E.C.	IP	80	12	11
Pydrin 2.4 E.C. LD_{99} given as fenvalerate	IP	89	11	0
Ambush	PO	1550	10	9
Ambush LD_{99} given as permethrin	PO	1327	11	11
Ambush	IP	800	10	10
Ambush LD_{99} given as permethrin	IP	838	11	0

much less toxic than Pydrin. However, Ambush and permethrin possess a similar degree of toxicity when given orally. When tested intraperitoneally both Ambush and Pydrin 2.4 E.C. are much more toxic than similar amounts of active ingredient administered as technical-grade material in corn oil.

DISCUSSION

Comparison of the lethalities of the technical-grade fenvalerate, the Pydrin 2.4 E.C. vehicle, and Pydrin 2.4 E.C. indicates a substantial vehicle effect in the toxicity of the commercial product. The exact composition of the Pydrin formulation vehicle is confidential and may vary. Therefore, the individual vehicle constituents cannot be tested for possible interaction with the pyrethroid. Although the Pydrin 2.4 E.C. label indicates that it contains 70% "inert" compounds, it should be noted that many compounds which are labeled inert by a manufacturer may prove otherwise in the laboratory setting (Gosselin *et al.*, 1984). Since the enhancement of toxicity was seen with both routes of administration, the interaction could be of a toxicokinetic nature. One could presume that the formulation vehicle may contain ingredients to enhance cell membrane penetration of the pyrethroid in the target organism. The same result would likely occur in nontarget species as well.

The po LD_{50} of technical-grade fenvalerate in male mice has been reported to be 190 mg/kg (Parker *et al.*, 1985). Administration (po) of the amount of technical-grade fenvalerate contained in the calculated LD_{99} upper-confidence-limit dosage of orally administered Pydrin resulted in 75% mortality. This amount of technical-grade fenvalerate (209 mg/kg) is within the 95% fiducial limit of the Parker study. Conversely, the amounts of fenvalerate contained in the calculated LD_{50} of Pydrin 2.4 E.C. (72 mg/kg) could be contrasted with the Parker values. The result of these comparisons demonstrates substantial differences in the lethality of the two preparations of this pyrethroid. Strain and weight differences, unreported acclimation, and housing conditions (possibly group versus individual) could have partially contributed to this differential toxicity. However, it is not probable that these factors alone could account for all the reported differences in lethality.

Comparison of the lethality of technical-grade permethrin to that of Ambush following oral administration implies that the Ambush vehicle does not contribute to the toxicity of the pyrethroid, since there was no profound difference in lethality or toxicity of the formulation and similar amounts of the technical-grade material. The oral LD_{50} of permethrin in corn oil has been reported to be 540 mg/kg (Litchfield, 1985) which is well within the 95% confidence interval for the calculated LD_{50} for orally administered Ambush, which was 322–584 mg/kg. An oral dose of 1327 mg/kg of permethrin in corn oil killed all subjects in the current study.

Following ip administration, the formulation Ambush was significantly more toxic than administration of similar doses of technical-grade material, i.e., the same relationship seen between fenvalerate and Pydrin 2.4 E.C. This differential in ip toxicity of the two dosage forms could possibly be due to kinetic differences (absorption, metabolism, etc.). It is possible that the corn oil vehicle used in the study limited the absorption of the pesticide from the peritoneal cavity. The highly lipophilic nature of the pyrethroids could inhibit their absorption by the ip route by sequestration in the corn oil vehicle. Since there was no appreciable difference in toxicity following oral administration of technical-grade permethrin or Ambush, it would appear that the Ambush formulation vehicle does not affect the toxicity of Ambush. However, the formulation vehicle did appear to have a definite affect on fenvalerate toxicity, since fenvalerate is more toxic when administered both orally and intraperitoneally as Pydrin 2.4 E.C. rather than as technical-grade fenvalerate.

The actual contribution of the formulation vehicle to the toxicity of Ambush could not be accurately determined since the formulation vehicle was not provided. If the vehicle contains a high (85%) percentage of xylene, then that amount of xylene alone was toxic and could have contributed significantly to the death seen in the groups treated with the highest doses of Ambush po and ip. One can assume that since no significant difference in lethality existed between groups receiving 1100 and 1500 mg/kg of Ambush and those receiving 1327 mg/kg of permethrin that the formulation vehicle had little effect on permethrin lethality. These data also suggest that the Ambush formulation vehicle is less than 85% xylene.

CONCLUSIONS

On the basis of the data obtained in this research, the formulation vehicle of Pydrin is proposed to be a dominant factor resulting in wide variations in lethality. Since this variation was not observed in Ambush, the implication is that the Pydrin formulation contains some unknown compound which increases fenvalerate toxicity while the Ambush formulation does not.

Additional studies involving the pyrethroid formulations are necessary in order to effectively evaluate the intrinsic potential of the "inert" ingredients of formulation vehicles, as well as the effects that different vehicle controls (corn oil) may have on absorption. These studies could elucidate the toxicodynamic and toxicokinetic effects of the pyrethroid insecticides in relation to their vehicles.

The popularity of commercial pyrethroid insecticides and their widespread replacement of older, more toxic compounds in various settings mandates a complete understanding of both the active and inert components of their formulations.

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