4-6-94

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR - 6 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID# 109701. Permethrin: Review of series 81-8ss and 82-7ss acute and subchronic neurotoxicity screen studies and a literature publication on the neurotoxicity and commentary on a special positive control study with acrylamide.

TOX CHEM No.: 652BB
PC No.: 109701
Barcode No.: D196130 and D197889
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I. CONCLUSION

A. Acute neurotoxicity. The series 81-8ss acute neurotoxicity study submitted by the registrant was determined to be SUPPLEMENTARY and not a candidate for upgrading. The study was determined to have used inappropriate dose levels and dosing volume of corn oil. A study from the literature (McDaniel and Moser, Neurotoxicology and Teratology 15:71-83 1993) was determined to demonstrate **NOEL and LELs of 25 and 75 mg/kg** for acute oral exposure for permethrin induced neurotoxicity based primarily on increased excitability, aggressive behavior, abnormal movement and reduced motor activity. These studies taken together are considered to satisfy the series 81-8 acute neurotoxicity testing requirement. No additional series 81-8ss study data are required at this time.

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B. Subchronic neurotoxicity. The series 82-7ss subchronic neurotoxicity study was determined to be CORE GUIDELINE and to establish **NOEL and LELs of 15.49 and 91.51 mg/kg/day** based primarily on tremors and staggered gait. No indications of neuropathy were evident in males or females dosed with 150 or 189 mg/kg/day (the highest dose tested) respectively for 90 days.

II. Action Requested

The FMC and Zeneca Corporations have submitted both a series 81-88ss (acute neurotoxicity screen) and a series 82-7ss (subchronic neurotoxicity screen) studies with rats as fulfillment of the reregistration requirements for the pyrethroid insecticide permethrin. Refer to letters from Linda Dansbury dated September 15, 1993 and December 2, 1993. In addition, the registrant's have submitted a special positive control study with acrylamide a compound which causes specific neuropathological effects in the rat. A publication found in the open literature addressing the neurotoxicity of permethrin was also reviewed. The following comments apply to these studies and to the issues related to the neurotoxicity of permethrin.

III. Toxicology Branch Comments

1. The DERs for the acute neurotoxicity (series 81-8ss) and subchronic neurotoxicity screen (82-7ss) and the literature publication studies are attached. The DER for the study with acrylamide is attached to the series 81-8ss study. The studies are further identified in Section IV below.

2. Series 81-8ss Acute Neurotoxicity Screen Study.

a. This study was classified as SUPPLEMENTARY and is not a candidate for upgrading. TB-I has determined that the dose levels tested and the volume of corn oil used for this study are inappropriate. The pilot study was reported to indicate clinical signs due to treatment with 50 mg/kg of permethrin when administered as a 10% corn oil solution. The main study was assessed using a 1% corn oil solution and the LEL was determined to be 300 mg/kg or 4 times greater. The 1% corn oil solution required dosing the rats with 30 ml/kg for the control and high dose group and 15 ml/kg for the mid dose group. HED considers that dosing with volumes greater than 10 ml/kg results in confounding the interpretation of the study data because of potential effects on compound absorption.

b. The results of the pilot study indicating effects at 50 mg/kg and the results of the study reported in the literature (refer to item 5 below) which establishes 75 mg/kg as an effect

level both indicate that the dose levels selected for this study would not be useful for risk assessment purposes.

c. The study verified only some of the neurotoxicity responses to permethrin and indicated that tremors and gait impairment are the prominent reactions. The study did not indicate that increased excitability and aggressive behavior results from permethrin treatment. These symptoms have been reported elsewhere (see item 5 below).

d. The registrant should be advised that the large doses of corn oil used for this study should not be used in future studies. In addition, the registrant should be advised that body temperature measurements should be made as a part of the FOB assessments.

3. Series 82-7ss subchronic neurotoxicity screen.

The dietary subchronic neurotoxicity screen study was classified as CORE GUIDELINE and to support NOEL and LELs of 15.49 and 91.51 mg/kg/day based primarily on tremors and staggered gait. There was no evidence of neuropathy at dose levels as high as 150 and 189 mg/kg/day for males and females respectively.

4. Special study with the positive control substance acrylamide.

This study is considered a useful validation for the observation of some aspects of neurotoxicity testing particularly for behavioral, FOB and motor activity assessments and neuropathology. Refer to the special DER appended to the series 81-8ss acute neurotoxicity study for a description of the results. In this study, acrylamide was dosed for 11 days by gavage. Thus, the study is neither an acute or subchronic neurotoxicity study. The study is not considered useful for helping to evaluate the initial responses to treatment of test materials which occur shortly after dosing and are supposed to be assessed at the time of peak effect.

TB-I considers the neurohistopathology assessment in this study to be of limited usefulness in assessing for potential neuropathy caused by pyrethrins or pyrethroids and other chemicals as well. This is because the rats were dosed with the acrylamide for 11 consecutive days and the nerve tissue examined three days later. Whereas in the series 81-8ss studies, the rats will be dosed only once and the tissue examined 14 days later.

5. McDaniel and Moser's publication on permethrin and cypermethrin.

This study established a NOEL and LEL of 25 and 75 mg/kg for acute neurotoxicity of permethrin. This NOEL and LEL set is considerably less than the study submitted by the registrant. TB-I recommends that the NOEL and LEL of 25 and 75 mg/kg be used for acute exposure risk assessments for permethrin.

This publication is considered to be a very useful reference for the description and differentiation of the behavioral and motor neurotoxicity responses to the representative type I pyrethroid (permethrin) and the representative type II pyrethroid (cypermethrin). When other series 81-8ss studies for other type I and type II pyrethroids are reviewed, this paper should be consulted for a description of the expected responses for comparative purposes.

TB-I notes that the study submitted by the registrant (MRID No.: 430463-01) indicated that clinical signs were noted in the group dosed with 50 mg/kg, a dose level lower than the 75 mg/kg in the NOEL and LEL expression based on the McDaniel and Moser study. Since the NOEL is assigned as 25 mg/kg and this level will be used for MOE assessments, TB-I is not concerned with the difference between 50 and 75 mg/kg.

The acute neurotoxicity study submitted by the registrant and the McDaniel and Moser publication differ in their description of the responses to permethrin treatment. The study submitted by the registrant reports that tremors and gait abnormalities are the indicators of toxicity to permethrin at the LEL. The study by McDaniel and Moser does not indicate tremors but recognizes increased excitability and aggressive behavior and abnormal movement as the predominant effects at the LEL. Tremors come at higher near fatal doses. TB-I considers that this may represent the combination of different strains of rats being tested and differences in the observers making the assessments.

6. Pyrethrin and pyrethroid induced neuropathy.

The problem of pyrethrin/pyrethroid induced peripheral neuropathy has been considered since the 1970s. The literature and the results of studies (conventional and special neurotoxicity assessments) do not give consistent results. A recently completed series 81-8ss neurotoxicity screen study with pyrethrins (MRID No.: 429258-01, HED document No.: pending) clearly indicated that males and females developed "myelin/axonal degeneration" in response to treatment. Permethrin is considered closely related to the pyrethrins.

The series 82-7ss subchronic study with permethrin did not

indicate signs of neuropathy at dose levels as high as 150 and 189 mg/kg/day for males and females respectively following 90 days of dosing. Thus, TB-I concludes that since no neuropathy results at these doses the assignment of the NOEL and LEL of 25 and 75 mg/kg for acute exposure and 15.49 and 91.51 mg/kg/day for subchronic exposure will assure protection for any possible neuropathy due to permethrin.

Whether or not permethrin can result in neuropathy following multiple acute exposures such as with the positive control study with acrylamide is considered academic and not necessary for regulatory purposes.

IV. Executive Summaries for Studies Reviewed.

1. Series 81-8ss. FMC Corporation, Study No.: A92-3646, August 27, 1993, MRID No.: 430463-01.

Four groups of 10/sex Sprague-Dawley strain rats were dosed by gavage as either control, 10, 150 or 300 mg/kg of technical grade permethrin in corn oil. The control and high dose groups each received 30 ml/kg of corn oil, the low and mid dose groups received 1 or 15 ml/kg of corn oil. Both the dose levels selected for this study and the volume of corn oil used for dosing were considered inappropriate for this study. Following administration, the rats were assessed for clinical signs daily and FOB and motor assessments were made pretest and at day 0 (at estimated time of peak effect) and days 7 and 14. After day 14, the rats were sacrificed and the nervous system assessed histopathologically.

Reactions to treatment were noted in the males and females in the 300 mg/kg group only. The reactions attributed to treatment included one death, (a female), tremors (all animals), staggered gait and gait impairment (8/sex), splayed hindlimbs (2 males, 6 females), decreased forelimb grip strength (-21% males) as well as other symptoms occurring in 2 or less animals but not in the controls (convulsions, ataxia, exaggerated hindlimb flexion, increased auditory response, uncoordinated landing). No evidence of compound related neurohistopathology was noted in tissues from animals perfused in vivo. The high dose of corn oil confounded the interpretation of the incidence of abdominogenital staining and this condition may also be an effect at 300 mg/kg with a slight effect at 150 mg/kg. **The LEL is 300 mg/kg for acute neurotoxicity based on tremors and gait impairment. The a NOEL of 150 mg/kg.**

Classification: CORE SUPPLEMENTARY (study by itself is not upgradable). When this study is considered with other acute neurotoxicity data, the combined data are considered CORE MINIMUM and to satisfy teh requirement for a series 81-8ss study for permethrin.

2. Series 82-7ss. 90 day neurotoxicity screen. FMC Corporation, Study No.: A92-3647, September 2, 1993. MRID No.: 429337-01.

In this study designed to assess the subchronic neuro-toxicity of permethrin, four groups of 10/sex Sprague-Dawley strain rats (Charles River Portage, Michigan) were dosed with 0, 250, 1500 or 2500 ppm in their diets for 13 weeks. These doses corresponded to 15.49, 91.51 or 150.35 mg/kg/day in males and 18.66, 111.37 or 189.63 mg/kg/day in females. Assessments for

clinical signs were made daily and FOB and motor activity assessments were made at weeks pretest and 4, 8 and 13 of the study. Following sacrifice, the control and high dose group rats were perfused and subjected to histopathological assessments.

Reactions to treatment noted in the 1500 ppm dose group included tremors (in 3 males and 5 females), staggered and/or impaired gait, splayed hindlimbs, increased landing feet splay and abnormal posture and decreased grip strength. Only splayed hindlimb and staggered gait were noted in the FOB battery at 1500 ppm. At 2500 ppm, all of the rats had tremors, staggered gait and splayed hindlimbs. The tremors started at day 1 and persisted throughout the study. Staggered gait and splayed hindlimbs started later. No effects on motor activity or neurohistopathological lesions were noted. Body weight in the high dose group males was 5% decreased and a corresponding slight decrease in food consumption was also noted for this group. **The LEL for neurotoxicity is 1500 ppm (91.51 mg/kg/day in males) based primarily tremors and staggered gait. The NOEL is 250 ppm (15.49 mg/kg/day).**

Classification: CORE GUIDELINE.

3. Series 81-8ss. Acute Neurotoxicity-rats. ManTech Environmental Laboratory as published in Neurotoxicology and Teratology 15:71-83(1993). No MRID No.

A total of sixteen groups of 8/sex Long-Evans rats were dosed with either permethrin (95% a.i., control, 25, 75 or 150 mg/kg) or cypermethrin (97% a.i., control, 20, 60 or 120/100 mg/kg) in corn oil at 1 ml/kg. Separate groups were treated for the FOB and motor activity assessments. Both permethrin and cypermethrin had reported equal ratios of cis and trans isomers. Following dosing, FOB (2 and 4 hours for permethrin and 1.5 and 3 hours for cypermethrin) and motor assessments (4 hours for permethrin and 3 hours for cypermethrin) were made. FOB and motor activity assessments were also made at pretest, and after 24 and 48 hours.

Permethrin:

At 75 mg/kg the rats displayed a general pattern of increased excitability and aggressive behavior. Some of the more pronounced responses included abnormal motor movement (3/8, both males and females) decreased forelimb (males 29%, $p < 0.05$) and hindlimb (males 30%, females 15%, both $p < 0.05$) grip strength and motor activity was decreased (estimated 40% for males) and body temperature was increased about 1 °C. At 150 mg/kg: arousal score, (males), righting reflex (males) and approach response score (females) were all affected and 7/8 of both sexes had abnormal motor movement and motor activity was further decreased and body temperature was increased > 2 °C. Slight decreases in body weight (3-4%) were evident. Recovery from the symptoms was within 24 hours. **The LEL is 75 mg/kg based on several neurotoxicity parameters being affected. THE NOEL is 25 mg/kg.**

Cypermethrin:

The rats displayed gait, muscle effects and choreoathetosis. Motor activity was decreased for all dose groups for males (estimated 45%, 66% and 85% for the 20, 60 and 100 mg/kg dose groups respectively) and gait abnormalities were present in the low dose group. Body temperature was increased about one °C in the low dose male group but decreased for the higher groups. Some ten other parameters were affected at 60 mg/kg and/or above. These included: salivation, urination, arousal, abnormal motor movement, forelimb or hindlimb grip strength, landing foot splay, righting reflex, touch response and tail pinch response. **The LEL and**

NOELs for neurotoxicity are < 20 mg/kg. Decreased motor activity and gait abnormalities resulted at 20 mg/kg.

Classification: SUPPLEMENTARY. Study is in the form of a literature reprint and was not designed to meet a specific guideline protocol.

Note: The data with permethrin when combined with another study are considered CORE MINIMUM and to satisfy the data requirement for a series 81-8ss acute neurotoxciity study with permethrin.

Tox Chem No: 652BB
PC No: 109701

IV. Studies Reviewed

Study Identification	Material	MRID No.:	Executive Summary	Classification
81-8ss. Acute neurotoxicity screen-rats FMC Corporation, Study No.:A92-3646, August 27, 1993.	Technical Permethrin	430463-01	Study conducted at inappropriate dose levels and dosing volume of corn oil. Results should not be used for risk assessment or regulatory purposes [NOEL and LEL = 150 and 300 mg/kg: At 300 mg/kg: death (one female), tremors (all animals), staggered gait and gait impairment (8/sex), splayed hindlimbs, reduced forelimb grip strength, as well as several other symptoms. Study by itself is SUPPLEMENTARY and not upgradable, but when combined with other acute neurotoxicity study data, CORE MINIMUM data for series 81-8ss are achieved. Sprague-Dawley strain rats. Dose levels tested: 0, 10, 150 or 300 mg/kg as 1% in corn oil. 30 ml/kg corn oil used for control and high dose groups.	MIIINIMUM (when combined with other data).

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82-7ss. Subchronic neuro-toxicity screen-rats FMC Corporation Toxicology Laboratory. Study No.: A92-3647 September 2, 1993	Technical Permethrin	429337-01	NOEL and LEL = 15.49 and 91.51 mg/kg/day. At 91.51 mg/kg/day: tremors (3 males and 5 females), staggered and/or impaired gait, splayed hindlimbs, increased landing foot splay, abnormal posture and decreased grip strength.; decreased body weight. No evidence of neuropathy. Sprague-Dawley strain rat. Dose levels tested: 0, 250 1500 or 2500 ppm corresponding to 15.49, 91.51 or 150.35 and 18.66, 111.37 or 189.63 mg/kg/day for males and females respectively.	GUIDELINE
Non-guideline. Special positive control (acrylamide) neurotoxicity study-rats FMC Corporation, Study No.: A91-3482, July 20, 1993.	Acrylamide	430463-01 and 429337-01	An informal DER has been prepared for this study and is attached as Appendix I to the series 81-88ss acute neurotoxicity study above. This study provides a useful example of the effects of a positive control substance on FOB and motor activity and also indicates that pathological changes ("myelin bubbles") in the peripheral nerves result from treatment with acrylamide for 11 days by gavage. Sprague-Dawley strain rats. Dose levels tested = control and 40 mg/kg/day by gavage.	No classification (Not related to a specific pesticide)

Non-Guideline. Special acute toxicity study in rats. McDaniel and Moser, Neurotoxicology and Teratology 15:71-83 (1993)	Permethrin	None	NOEL and LEL =25 and 75 mg/kg. At 75 mg/kg: Increased excitability. aggressive behavior, abnormal motor movement, increased forelimb and hindlimb grip strength, deceased motor activity and increased body temperature, at 150 mg/kg: arousal score, impaired righting reflex, approach response and others slight (3-4%) decrease in body weight, Recovery from symptoms was within 24 hours. SUPPLEMENTARY because study is in fomr of a publication and does not follow guidelines. Long Evans strain rat. Dose levels tested Permethrin: 0, 25, 75 or 150 mg/kg in corn oil; cypermethrin: 0, 20, 60 and 120 (FOB) and 100 (motor activity).	MINIMUM (when combined with other data)
Non-Guideline. Special acute toxicity study in rats. McDaniel and Moser, Neurotoxicology and Teratology 15:71-83 (1993) Tox Chem No: 271DD PC No.. 109702	Cypermethrin technical (97% purity)	None	NOEL and LEL < 20 mg/kg. At 20 mg/kg: gait, muscle effects and choreoathetosis, motor activity decreased (45%), body temperature increased. Some 10 FOB parameters affected at 60 mg/kg and above and body temperature decreases. Long Evans strain rat. Dose levels tested Permethrin: 0, 25, 75 or 150 mg/kg in corn oil; cypermethrin: 0, 20, 60 and 120 (FOB) and 100 (motor activity).	SUPPLEMENTARY

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Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Linnea Hansen 3/25/94
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DATA EVALUATION REPORT

STUDY TYPE: 82-7ss. 90-day Neurotoxicity Screen - rats

MRID NO.: 429337-01 TOX. CHEM. NO.: 652BB
PC No.: 109701

TEST MATERIAL: Technical grade permethrin. 95.3% purity, sample No.: PL90-269.

STUDY NUMBER: A92-3647

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Corporation Toxicology Laboratory, Princeton, New Jersey.

TITLE OF REPORT: "Permethrin Technical: Subchronic Neurotoxicity Screen in Rats"

AUTHOR(S): Cristine Freeman

REPORT ISSUED: September 2, 1993

STUDY DATES: August 3, 1992 to November 6, 1992.

Executive Summary:

In this study designed to assess the subchronic neurotoxicity of permethrin, four groups of 10/sex Sprague-Dawley strain rats (Charles River Portage, Michigan) were dosed with 0, 250, 1500 or 2500 ppm in their diets for 13 weeks. These doses corresponded to 15.49, 91.51 or 150.35 mg/kg/day in males and 18.66, 111.37 or 189.63 mg/kg/day in females. Assessments for clinical signs were made daily and FOB and motor activity assessments were made at weeks pretest and 4, 8 and 13 of the study. Following sacrifice, the control and high dose group rats were perfused and subjected to histopathological assessments.

Reactions to treatment noted in the 1500 ppm dose group included tremors (in 3 males and 5 females), staggered and/or impaired gait, splayed hindlimbs, increased landing feet splay and abnormal posture and decreased grip strength. Only splayed hindlimb and staggered gait were noted in the FOB battery at 1500 ppm. At 2500 ppm, all of the rats had tremors, staggered gait and splayed hindlimbs. The tremors started at day 1 and persisted throughout the study. Staggered gait and splayed hindlimbs started later. No effects on motor activity or

neurohistopathological lesions were noted. Body weight in the high dose group males was 5% decreased and a corresponding slight decrease in food consumption was also noted for this group. **The LEL for neurotoxicity is 1500 ppm (91.51 mg/kg/day in males) based primarily tremors and staggered gait. The NOEL is 250 ppm (15.49 mg/kg/day).**

Classification: CORE GUIDELINE. No additional series 82-7ss neurotoxicity testing is required for permethrin at this time. The study did not assess body temperature but this parameter is not considered essential to assess the neurotoxicity of permethrin, although permethrin is known to increase body temperature.

Quality Assurance Statement: Provided
Good Laboratory Practice Statement: Provided

REVIEW

Experimental Constants:

Test Chemical: Technical grade permethrin, identified as reference #PL90-269 and described as a brown solid at room temperature. Based on chromatogram presented in the analytical report, the cis/trans isomer ratio was approximately equal.

Test System: Sprague-Dawley CD rats were obtained from the Charles River Laboratories, Portage, Michigan. They were approximately 5 weeks old at receipt and were approximately 7 weeks old at initiation of dosing. They were housed individually and fed Certified Purina Rodent Chow 5002.

Analytical Chemistry and Dietary Analysis: The analytical report indicated that stock permethrin was stable at room temperature for a period of three months (there was no change in the percentage). Test diets containing the test material were prepared in mid August and mid September and stored covered at room temperature until usage. Test diets were assessed for homogeneity and each layer (top, middle and bottom) were assessed to be 101% of target. The concentration analysis of four diet preparations indicated t^At 95 to 104 percent of the target dose was present. A test diet (2500 ppm) was assessed for stability over a 90 day period and only -3.69% change from the original permethrin concentration was noted.

Basic Experimental Design: Four groups of rats (10/sex) were dosed as control (0 ppm), 250, 1500 or 2500 ppm permethrin in their diet for 90 days. The rats were assessed for general signs of toxicity, FOB and motor activity at pretest and at weeks 4, 8 and 13. After week 13, the animals were sacrificed and perfused, necropsied and the brain and peripheral nervous system were prepared for histopathology (see below for details).

The above dose levels were selected based on a range finding study (Study No.: A92-3645, the study was not submitted and no DER was prepared). In this study Sprague-Dawley rats (5/sex/group) were dosed with 0, 100, 750, 1500, 300, 4000 or 5000 ppm for 28 days. This study established a NOEL of 750 ppm and an

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estimated MTD of 3000 ppm. 5000 ppm resulted in deaths of all rats. The predominant clinical signs included tremors, splayed hindlimbs, staggered gait and chromorhinorrhea as well as others.

Statistics: The study report asserts that the following statistical tests were performed.

Statistical Test	Parameter
Welch trend test	Body weight, body weight gain and motor activity.
Categorical modeling	Handling ease, ocular discharge, exophthalmos, pupilary function, salivation, general appearance, alopecia, arousal/alertness, auditory response, lacrimation, palpebral closure, pupillary state, chromorhinorrhea, fur appearance, piloerection, gait impairment, righting reflex.
ANOVA and Trend test	fecal boli, landing foot splay, hind and fore limb grip strength, urine pools, tail flick latency.
Not analyzed statistically	home cage behavior, open field behavior, home cage gait, open field "gate" description.

Specific Methods and Results

1. Deaths.

The study report asserts that no deaths were attributed to the test material. Four rats were sacrificed for "humane reasons"; these were two males (on days 21 and 30) and one female (day 10) in the 1500 ppm dose group and one male (day 31) in the 2500 ppm dose group. The rats were said to have broken their noses by biting the wire mesh cages.

The occurrence of these injuries in the higher dose groups only raises a question of possible test compound involvement such as some form of agitation or increased aggression. The random pattern of time of death and failure to demonstrate a dose response does not support the possibility of test compound involvement.

2. Body Weight and Gain and Feed and Compound Consumption.

Body weight was assessed weekly. The study report asserts that only the high dose group males had reduced (up to 5%, statistically significant) body weight at weeks 3 to 9 and again at week 12. Body weight gains or female bodyweights were

not obviously affected. Food consumption in the high dose male group was also decreased especially for weeks 1 to 4 and 8 and 9. Compound consumption (based on mean weekly body weight and food consumption values) data were reported as follows in Table 1.

Table 1. Permethrin consumption.

	Permethrin consumed (mg/kg/day)			
	Males		Females	
Group	Range	Mean[1]	Range	Mean
Control	-		-	
250 ppm	12.60 to 20.82	15.49±2.56	16.13 to 22.08	18.66±1.91
1500 ppm	73.95 to 123.67	91.51±15.73	99.46 to 129.92	111.37±10.5
2500 ppm	123.24 to 194.62	150.35+22.3	164.10 to 214.36	189.66+17.4

1. The mean value ± the s.d. listed is the mean based on weekly values as presented in Table 5 of the study report (page 99).

The wide range of compound consumption is related to the decrease in food consumed as the rats matured. The mean value represents the compound intake in time weighted average.

3. Clinical signs. The rats were said to be inspected for reactions to treatment at 8 AM by observers "blind" to the treatment of the test animals. The observations included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory and excitatory systems, autonomic and central nervous systems, somatomotor activity and behavior patterns.

The study report asserts that effects in both males and females were noted at dose levels of 1500 and 2500 ppm. Table 2 illustrates the principle reactions and their onset and duration.

Table 2. Clinical signs and their onset in rats dosed with permethrin.

Symptom	Sex	Control	250 ppm	1500 ppm	2500 ppm
Tremors	M	0	0	3/25[1] (day 68)[2]	10/462 (day 1)
	F			5/16 (day 51)	10/246 (day 1)
Staggered Gait	M	0	0	7/216 (day 38)	10/586 (day 16)
	F	0	0	9/429 (day 35)	10/591 (day 18)
Splayed Hindlimbs	M	0	0	6/135 (day 38)	10/572 (day 15)
	F	0	0	9/411 (day 18)	10/569 (day 10)

Data are from Table 2 pages 29 to 67 and are based on 10 animals per dose except for the decedents described in part 1 above.
1. Numerator = number of animals reportedly affected, denominator = total number of incidents reported.
2. Day first animal reported with symptom.

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Tremors in the mid dose group were first observed in the later part of the study; in one male they were noted on only one day. In the high dose group, the tremors started as early as day 1 in most animals and as late as day 3 in others. They generally were noted in most animals throughout the study.

Staggered gait and splayed hindlimbs did not start immediately as did tremors in the high dose group but once they were noted they generally persisted throughout the study period.

Another symptom that occurred in the high dose group only in some animals that were considered probably related to treatment was unkempt appearance (3 males and 2 females). Pink staining or bloody mucoid discharge in bedding (1 female in each of the mid and high dose groups) was also noted in test animals only but not considered by TB-I to be of a defined toxicological significance.

4. Functional Observational Battery and Motor Activity.

Assessments were reportedly made by technicians that were "blind" to the treatment at pretest, and at weeks 4, 8 and 13. The endpoints assessed are described in Attachment I. The study report asserts that abnormalities were noted in the FOB at dose levels of 1500 and above in both sexes. There were, however, no effects on motor activity reported. Table 3 illustrates the predominant FOB abnormalities noted at weeks 4, 8 and 13 assessments.

Data sets for home cage observations and open field observations were reported separately. Although some of the same types of symptoms were noted there was not always consistency between the home cage and open field observations with more incidents of abnormalities being reported in the open field data sets. Moreover, the clinical observations made daily at cage side indicated more animals affected than did the FOB assessments.

FOB data (Table 3) indicated that several of the listed parameters were affected at 1500 ppm. Some comments on these parameters are as follows:

Landing foot splay. High dose group males were increased at weeks 4 (33%, $p < 0.05$) and 8 (54%, $p < 0.05$). The mid dose group males, the low and mid dose female groups were also elevated (i.e. about 20%) but statistical significance was not attained. Similarly the high dose group females were also elevated (44-48%, $p < 0.01$). The low and mid dose groups were also elevated (14 to 34%) but not statistically significantly different.

Hindlimb and forelimb grip strength. Females in the mid and/or high dose group at weeks 8 and 13 had roughly 25% less forelimb grip strength and at week 13 hindlimb grip strength was similarly decreased. These decreases were statistically significant ($p < 0.05$ or smaller).

Table 3. Predominant FOB parameters determined to be affected by permethrin based on open field observations.

Parameter	Week	Males				Females			
		Control	250	1500	2500	Control	250	1500	2500
Tremors	4	0	0	0	1	0	0	0	2
	8	0	0	1	6	0	0	0	2
	13	0	0	0	4	0	0	0	3
Staggered gait	4	0	0	0	4	0	0	0	5
	8	0	0	3	9	0	0	3	9
	13	0	0	1	7	0	0	2	7
Splayed hindlimbs	4	0	0	0	2	0	0	0	2
	8	0	0	0	4	0	0	2	5
	13	0	0	0	2	0	0	1	3
Abnormal posture	4	0	0	0	2	0	0	0	2
	8	0	0	0	4	0	0	2	5
	13	0	0	0	2	0	0	1	3
Gait Impairment (slight)	4	0	0	0	4	0	0	0	4
	8	0	0	3	9	0	0	3	9
	13	0	0	1	7	0	0	2	7
Landing foot splay (cm)	4	4.63	5.56	5.58ns	6.17*	3.56	4.07	4.77*	5.22*
	8	4.50	5.71	5.34ns	6.04*	4.06	4.18	4.17	6.01*
	13	5.85	5.97	5.74	6.05	4.32	4.37	5.52	5.10
Forelimb Grip Strength (kg)	4	0.955	0.952	0.877	0.941	0.789	0.841	0.786	0.721
	8	1.092	1.037	0.969	0.959	0.859	0.872	0.812	0.657*
	13	0.892	0.941	0.811	0.736ns	0.789	0.783	0.583*	0.596*
Hindlimb Grip Strength (kg)	13	0.678	0.705	0.642	0.634	0.590	0.519	0.445*	0.436*

Data are from Table 6 (pages 105 to 128 of the study report).

Other indications of neurotoxicity noted but not listed in Table 3 because of their arbitrary nature or low frequency are:

Unkempt fur. 3 males in the high dose group at week 13 only and 2 females, one each in the mid and high dose groups.

Fecal boli. In males there were more fecal boli associated with the mid and high dose groups at weeks 8 and 13. TB-I does not consider this observation to be an abnormality in the absence of other indications of treatment related gastro intestinal disturbance. Other, unrelated effects at the same dose level are considered more important in defining the toxicity in this study.

In conclusion, the FOB indicated some reactions to treatment at the 1500 ppm, but TB-I considered that there is poor concordance between the clinical signs and the FOB assessments.

E. Motor Activity.

Assessments were made "immediately" following the FOB testing. Eight animals at a time were placed in individual San Diego Instruments, Inc. Figure 8 mazes. Motor activity sessions were run for 30 minutes and activity (as indicated by the times the rat crossed a beam) was presented as mean activity/5 minute interval and as total session activity. Fine movement, ambulation and rearing were not distinguished.

The study report asserts that there were no effects on motor activity in either sex. Table 7 of the study report indicates that at week 4 the control male group had total counts of 247 ± 12 and the high dose group had 235 ± 10 counts. At week 13 the control male group had a mean total count of 149 ± 27 and the high dose group had a mean total count of 134 ± 24 counts. The female test groups were similar in their relationship to the controls. TB-I concurs with the study report regarding a lack of an effect on motor activity. Inspection of the session mean scores indicated that for both males and females, habituation was essentially the same for all dose group.

F. Histopathology and Perfusion Studies.

After ninety days the rats were sacrificed and necropsied. No obvious compound related lesions were noted.

Five/sex rats were perfused with glutaraldehyde/ paraformaldehyde after the week 13 motor activity assessments. The perfused carcasses were stored refrigerated and shipped to Experimental Pathology Laboratories, Inc, Herndon, Virginia for tissue slide preparation. The following tissues from the control and high dose groups were embedded on paraffin and sections stained using hematoxylin and eosin or Bodian stains.

Brain	three sections including pons, cerebrum and cerebellum.
Spinal cord	cervical (cross and longitudinal)
Sciatic nerve	left and right proximal (cross and longitudinal)

The following tissues were embedded in glycol methacrylate and one-micron sections were stained with toluidine blue:

Gasserian ganglia
Tibial nerve (cross and longitudinal)
Cervical dorsal root ganglia with dorsal nd ventral root
Lumbar dorsal root ganglia with dorsal and ventral root
Sural nerve (cross and longitudinal)

The slides from the control and high dose group only were assessed. A separate pathology report prepared by Lucas H.

Brennecke, DVM described the pathology assessments and results. Dr. Brennecke concluded that there were no test article lesions present in the high dose group and there was no need for further assessment of the low and mid dose groups. The minimal lesions that were present were stated by Dr. Brennecke to be common spontaneous findings in this strain of rat.

Pyrethrins and pyrethroids have been demonstrated to suspected of causing "myelin/axonal degeneration" in rats in response to treatment. Thus, this lesion merits some special discussion with respect to the incidence of this or related lesions in this study. TB-I notes that among the males in this study, two high dose group rats had "axonal degeneration"[1], one in the cervical spinal cord and one in the left sciatic nerve. The control group also had one incidence of this condition in the cauda equina. Among females, axonal degeneration was present in 2 animals in the left and right sciatic nerve of the controls but only a single incidence was present in the left sciatic nerve of the high dose group. TB-I does not consider there is sufficient basis to conclude that the males or females were affected by treatment.

E. Immunochemistry: No immunochemistry for GFAP were done.

STUDY CONCLUSION: This study is classified as CORE GUIDELINE. It is noted, however, that no body temperature measurements were assessed. Body temperature is known to increase in response to acute permethrin administration. This deficiency is not considered critical. The study establishes NOEL and LEL for neurotoxicity or 250 and 1500 ppm based primarily on tremors and staggered gait. The neurotoxicity symptoms resulting from subchronic exposure were similar to the symptoms resulting from acute exposure (refer to the series 81-8ss acute neurotoxicity study in MRID No.: 430463-01).

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[1]Described as randomly scattered foci of axonal degeneration which presented as disrupted myelin, myelin degeneration, vacuolation ("myelin bubbles") and axonal fragmentation. In most cases the vacuoles or bubbles appeared within cells adjacent to or surrounding the axon. They were described as minimal.

Reviewed by: John Doherty, Ph.D., D.A.B.T. 3/28/94
Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Linnea Hansen, Ph.D. 3/25/94
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DATA EVALUATION REPORT

STUDY TYPE: 81-8. Acute neurotoxicity - rats

MRID NO.: 430463-01[1] TOX. CHEM. NO.: 652BB
PC No.: 109701

TEST MATERIAL: Technical grade permethrin (code PL90-269). (3-phenoxybenzyl (±cis - trans -3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate. 95.3% total cis and trans).

STUDY NUMBER: A92-3646

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Corporation Toxicology Laboratory, Princeton, New Jersey.

TITLE OF REPORT: "Permethrin Technical Acute Neurotoxicity Screen in Rats"

AUTHOR: Christine Freeman

REPORT ISSUED: August 27, 1993

STUDY DATES: August 3, 1992 to August 28, 1992

Executive Summary:

Four groups of 10/sex Sprague-Dawley strain rats were dosed by gavage as either control, 10, 150 or 300 mg/kg of technical grade permethrin in corn oil. The control and high dose groups each received 30 ml/kg of corn oil, the low and mid dose groups received 1 or 15 ml/kg of corn oil. Both the dose levels selected for this study and the volume of corn oil used for dosing were considered inappropriate for this study. Following administration, the rats were assessed for clinical signs daily and FOB and motor assessments were made pretest and at day 0 (at estimated time of peak effect) and days 7 and 14. After day 14, the rats were sacrificed and the nervous system assessed histopathologically.

Reactions to treatment were noted in the males and females in the 300 mg/kg group only. The reactions attributed to treatment included one death, (a female), tremors (all animals), staggered gait and gait impairment (8/sex), splayed hindlimbs (2 males, 6 females), decreased forelimb grip strength (-21% males) as well as other symptoms occurring in 2 or less animals but not in the controls (convulsions, ataxia, exaggerated hindlimb flexion, increased auditory response, uncoordinated landing). No evidence of compound related neurohistopathology was noted in tissues from animals perfused in vivo. The

[1]Includes a separate study for methods validation using acrylamide.

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high dose of corn oil confounded the interpretation of the incidence of abdominogenital staining and this condition may also be an effect at 300 mg/kg with a slight effect at 150 mg/kg. **The study supports a LEL of 300 mg/kg and a NOEL of 150 mg/kg for acute neurotoxicity.**

Classification: CORE SUPPLEMENTARY (not upgradable). Refer to page 8 for list of study deficiencies. This study when taken together with another acute oral neurotoxicity study (McDaniel and Moser, Neurotoxicology and Teratology 15:71-83 (1993), for clinical signs, FOB and motor activity assessments) plus the 90-day series 82-7ss (MRID No.: 429337-01, for neurohistopathology) are considered CORE MINIMUM data to satisfy the requirement for a series 81-8ss acute neeeurotoxicity screen study. No additional series 81-8ss acute neurotoxicity study data are required at this time.

Quality Assurance Statement: Provided
Good Laboratory Practice Statement: Provided
Data Confidentiality Statement: No claim of Confidentiality

REVIEW

Experimental Constants:

Test Chemical:

Chemical:	Technical grade permethrin: (3-phenoxybenzyl (±) +cis - trans -3-(2,2-dichlorovinyl)-2,2 dimethyl-cyclopropane-1-carboxylate).
Purity:	95.3% total cis and trans. The percentage cis and trans was not specifically stated but based on the Analytical Report in Appendix B, about equal amounts of cis and trans isomers were present.
Source:	FMC Corporation
Description:	Dark brown solid at room temperature.
Lot#:	PL90-269

Appended data (Appendix B) indicated that the sample of corn oil solution/suspension of permethrin was homogeneous and stable for a three month period and that the target concentration was achieved.

The test material was administered by gavage dissolved in corn oil. A constant concentration of permethrin in corn oil was used and the

different groups of rats received different volumes of corn oil.

Test System:

Species:	Rat
Strain:	Sprague-Dawley CD
Source:	Charles River Laboratories, Portage, Michigan
Age at dosing:	Approximately 45 to 50 days
Weight (day 0):	Males: 242-245, females: 165-170.
Randomization:	Computerized weight stratification
Housing:	Individual
Diet:	Purina Rodent Chow 5001

Basis for dose level selection. The dose levels were selected based on a preliminary study which indicated clinical reactions (unspecified) at 50 mg/kg and above when dosed as a 10% permethrin mixture. Additional preliminary studies assessed permethrin as a 1% mixture requiring a larger volume of corn oil to be administered. On page 13 of the report, the study author states that permethrin is less toxic when administered in undiluted forms. No data or explanation were presented to illustrate this principle. The results of the preliminary studies in the diluted mixture (1% in corn oil) were not described, the report only states that the dose levels (see below) were chosen and that 12 hours was the approximate time of peak effect.

Basic Experimental Design: Four groups of 10 rats/sex were dosed as either control, 10, 150 or 300 mg/kg of permethrin. The rats were deprived of food for approximately 12 hours prior to dosing. Food was made available about one half hour after dosing. The control and high dose groups received 30 ml/kg and the low and mid dose groups received 1 and 15 ml/kg of corn oil respectively. The large volumes (15 and 30 ml/kg) are in excess of the maximum volume of 10 ml/kg for gavage studies recommended by the Agency. Such large volumes can confound the study results in that the large volume of corn oil may serve as a laxative and promote rapid passage of the test material resulting in a lower bioavailability.

Following administration, the rats were observed for reactions and FOB and motor activity assessments were made at the time of peak activity (12 hour, predetermined in a preliminary study), at day 7 and at day 14. Dosing of the rats was distributed over a five day period in order to allow time for the FOB and motor assessments. After 14 days the rats were sacrificed, necropsied and their peripheral and central nervous systems prepared for histopathology.

Statistics: The study report asserts that the following statistical tests were performed. The statistical analyses were conducted using Neurostat™ System developed by Statistics Unlimited, Inc. Wellesley, Ma.

Statistical Test	Parameter
Welch trend test	Body weight, body weight gain and motor activity
Categorical modeling	Handling ease, ocular discharge, exophthalmos, pupilary function, salivation, general appearance, alopecia, arousal/alertness, auditory response, lacrimation, palpebral closure, pupillary state, chromorhinorrhea, fur appearance, piloerection, gait impairment, righting reflex.
ANOVA and Trend test	fecal boli, landing foot splay, hind and fore limb grip strength, urine pools, tail flick latency.
Not analyzed statistically	home cage behavior, open field behavior, home cage gait, open field "gate" description.

The criteria for statistical significance was $p < 0.05$.

A methods validation study with acrylamide was also submitted and a brief overview of this study is appended to this DER.

Specific Methods and Results

1. Deaths. One high dose group female died on day 0 as a result of treatment. Prior to death, staggered gait, tremors and diarrhea were noted.

2. Clinical signs. The rats were inspected for reactions by technicians "blind" to their treatment once daily.

There were no treatment related signs reported in the groups dosed with 10 or 150 mg/kg.

Symptoms of diarrhea (3 controls and 2 high dose males and females) and abdominogenital staining (9 control, 0 low dose, 4 mid dose and all high dose males; and 4 control, 0 low dose, 2 mid dose and 8 high dose females) were noted. These conditions were attributed to the volume of corn oil used and suggest rapid passage of the test material in the mid and high dose test groups.

The following signs were noted in the high dose 300 mg/kg male and/or female groups only and were considered by TB-I

to be related to treatment:

Tremors- All[2] males and all females.

Staggered gait- 6 males and 8 females affected.

Splayed hindlimbs- 3 males and 5 females.

Chromorhinorrhea- 3 males and 1 female.

Exaggerated hindlimb flexion- two females only.

Hypersensitivity to sound- one male only.

Chromodacryorrhea- one female only.

In males these signs were noted to be present on day 0 only. In females all clinical signs except staggered gait were noted on day 0 only. Staggered gait persisted to day 2 in two females and three others had staggered gait persisting to day 1. The other four had this sign on day 0 only.

The large volume of corn oil administered (30 ml/kg) to the control and high dose group confounds the interpretation of the presence of abdominogenital staining. None of the low dose group animals which received only 1 ml/kg of corn oil were reported as having abdominogenital staining. 9 of 10 males and 4 of 10 females in the control groups were reported as having abdominogenital staining. 4 males and 2 females in the mid dose group (dosed with 15 ml/kg of corn oil) also had this condition whereas 10 males and 8 high dose group females had this condition. Abdominogenital staining was noted on day 0 only except for one female which also had this condition on day 1. Since so many other symptoms occured at the high dose, TB-I does not consider it critical to assign abdominogenital staining as a definite effect of permethrin treatment. Its presence cannot be clearly distinguished from the volume of corn oil used or a combination of the permethrin and the corn oil.

Other clinical signs reported did not indicate evidence of compound related response. These included diarrhea (for both males and females the most animals affected were in the controls), scab on tail or shoulder (one male and one female affected).

2. Body Weight and Gain. Assessments were made at pretest, day 0, 7 and 14. No effects on body weight were noted.

3. Functional Observational Battery. Assessments were made at pretest, days 0 (12 hours after dosing), 7 and 15. The time of

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[2]Data are based on 10 rats/sex/dose level.

peak effect was determined in a preliminary study to be 12 hours post dosing. The assessments were made for 2/sex/group/day by technicians "blind" to the treatment of the animals. Appendix I (attached, photocopied from the study report, describes the endpoints used for assessment.

The rats were assessed to be equivalent for all dose groups at the pretest, day 7 and day 15 evaluations.

At day 0, responses to treatment were evident only in the 300 mg/kg dose groups for both sexes. The following parameters were noted to be affected based on open field observations.

Whole body tremors: All 10 males and females.

Staggered gait: 8 males, 8 females.

Gait impairment: 8 males (5 slight, 3 moderate), 8 females (6 slightly, one each moderate and severe).

Abnormal posture: 2 males, 6 females.

Splayed hindlimbs: 2 males, 6 females.

Convulsions: 1 female only.

Ataxia: 1 male only,

Decreased activity: 1 female.

Exaggerated hindlimb flexion: 1 male, 2 females.

Uncoordinated landing: 1 female.

Exaggerated auditory response: 2 males (not statistically significant).

Forelimb grip strength: males 21% decreased ($p < 0,05$), females 13.5% decreased but not significant.

Concordance with clinical signs observations. These or related responses were also evident in the daily clinical signs assessments thus indicating reasonably good concordance with the FOB observations. Male and female responses to treatment were similar.

C. Motor Activity. Assessments were made "immediately" following the FOB testing. Eight animals at a time were placed in individual San Diego Instruments. Inc, Figure 8 mazes. Motor activity sessions were run for 30 minutes and activity (as indicated by the times the rat crossed a beam) was presented as mean activity/5 minute interval and as total session activity.

The study report asserts that there were no effects on motor activity in either sex. At day 0 when other effects were noted in the high dose group, mean total counts in the control

and high dose male groups were 205 ± 20 and 190 ± 12 or 7% decreased but not statistically significant. The curves for individual interval scores (habituation) plotted vs time were very close together for the control and high dose group. The low dose group was 12% higher than the control and also not statistically significant. Control female mean total counts were 267 ± 17 and the high dose group was only 208 ± 32 or 23% lower but not statistically significant (i.e. p = 0.379). The habituation data indicated that the high dose was consistently lower and maintained the same levels of activity for the entire session. The other groups started higher and habituated to a lower activity level that was similar to the high dose group activity level. At day 7 when the rats were otherwise clear of symptoms, the female high dose group total counts were 24% higher than the control. TB-I considers these data suggestive of decreased motor activity in the high dose females but not of sufficent magnitude to be a definite effect.

D. Histopathology and Perfusion Studies.

After 14 days the rats were sacrificed and necropsied. No compound related lesions were noted at necropsy.

Five "adequately" perfused rats/sex were prepared by perfusing with glutaraldehyde/paraformaldehyde. The following organs were prepared embedded in paraffin and examined following staining with hematoxylin and eosin or Bodian-stains.

Brain	three sections including pons, cerebrum and cerebellum.
Spinal cord	cervical (cross and longitudinal)
Sciatic nerve	left and right proximal (cross and longitudinal)

The following were embedded in glycol methacrylate and processed to one-micron sections and stained with toluidine blue:

Gasserian ganglia
Tibial nerve (cross and longitudinal)
Cervical dorsal root ganglia with dorsal nd ventral root
Lumbar dorsal root ganglia with dorsal and ventral root
Sural nerve (cross and longitudinal)

The slides from the control and high dose group only were assessed. A separate pathology report prepared by Lucas H. Brennecke, DVM described the pathology assessments and results. Dr. Brennecke concluded that there were no lesions related to treatment present in the high dose group and there was no need for further assessment of the low and mid dose groups. The minimal lesions that were present were stated by Dr. Brennecke to be common spontaneous findings in this strain of rat. The following animals were affected with "axonal degeneration":

Controls: 2 males and 3 females affected

Males had a total of four incidents all minimal: Rat number AC6883 had axonal degeneration in the left sciatic nerve and tibial nerve. Rat number AC6884) had axonal degeneration in the cervical spinal cord and right sciatic nerve.

Females had a total of six incidents all minimal: Rat number AC6920 had axonal degeneration in the caudal equina and right sciatic nerve. Rat number AC6923 was affected in the left sciatic nerve and tibial nerve. Rat AC6924 was affected in the left and right sciatic nerve.

High Dose: 3 males and 4 females were affected.

Males had a total of six incidents all minimal: Rat AC6871 had only the left sciatic nerve affected. Rat AC6872 had the caudal equina, left sciatic nerve and sural nerve affected. Rat AC6874 had the caudal equina and right sciatic nerve affected.

Females had a total of 7 incidents with some being graded as mild. Rat AC6911 had cauda equina only affected. Rat AC6913 had only the left sciatic nerve affected. Rat AC6914 had only the sural nerve affected. These three rats had only grades of minimal. Rat AC6915 had the left sciatic nerve (minimal), right sciatic nerve (mild), tibial nerve (minimal) and sural nerve (mild) all affected.

TB-I notes that one more male and one more female were affected in the high dose groups than in the controls and that only in the high dose group was the severity of the axonal degeneration graded as mild. Although this is noted, TB-I does not consider this sufficient evidence to conclude that the axonal degeneration was related to treatment.

E. Immunochemistry: No GFAP assessments were made.

STUDY CONCLUSION: This study is classified as SUPPLEMENTARY (not upgradable). The following deficiencies were noted. The rationale for dose selection and dosing dilution are considered by TB-I to be inappropriate. On page 10 of the study report it clearly states that a dose level of 50 mg/kg used in the range finding study and administered in a 10% corn oil dosing solution results in "clinical signs". Based on experience with permethrin, TB-I considers these to be neurotoxicity responses. The dosing solution was changed to 1% and no reactions were noted except at the higher dose of 300 mg/kg. On page 13 of the study report, it is stated that "experience with oral dosing of permethrin in diluted and undiluted forms demonstrates that permethrin is significantly less toxic when administered in the latter form". This statement contradicts the reported results of the pre-liminary experiments on page 10 of the report which indicate that permethrin is more toxic in the undiluted form. The published data of McDaniel and Moser, Neurotoxicology and Teratology 15:71-83 (1993) also indicate that less dilute permethrin (7.5% in corn oil) resulted in symptoms at 75 mg/kg. The large volume of corn oil used for the control, mid and high

dose (15-30 ml/kg) is also considered a confounding factor. No body temperature recordings or skeletal muscle pathology assessments were made. Additional data will be required to satisfy the series 81-8ss study requirement.

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Secondary reviewer: Linnea Hansen, Ph.D.
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3/28/94

Linnea P. Hansen 3/28/94

WC

DATA EVALUATION REPORT FOR A LITERATURE PUBLICATION

Note: A copy of the paper is attached.

STUDY TYPE: Special Non-Guidelines Acute neurotoxicity - rats

MRID NO.: None TOX. CHEM. NO.: 652BB and 271DD
PC No.: 109701 and 109702

TEST MATERIAL: Technical grade permethrin (95% purity) and technical grade cypermethrin (97% purity) obtained from the FMC Corporation. Both were reported to have equal amounts of cis and trans isomers.

STUDY NUMBER: None

SPONSOR: US EPA

TESTING FACILITY: ManTech Environmental Technology, Inc. Research Triangle Park, North Carolina.

TITLE OF REPORT: "Utility of a Neurobehavioral Screening Battery for Differentiating the Effects of Two Pyrethroids, Permethrin and Cypermethrin"

AUTHOR(S): Katherine L. McDaniel and Virginia C. Moser

REPORT ISSUED: 1993. As published in Neurotoxicology and Teratology 15:71-83 (1993).

STUDY DATES: Not provided.

Executive Summary:

A total of sixteen groups of 8/sex Long-Evans rats were dosed with either permethrin (95% a.i., control, 25, 75 or 150 mg/kg) or cypermethrin (97% a.i., control, 20, 60 or 120/100 mg/kg) in corn oil at 1 ml/kg. Separate groups were treated for the FOB and motor activity assessments. Both permethrin and cypermethrin had reported equal ratios of cis and trans isomers. Following dosing, FOB (2 and 4 hours for permethrin and 1.5 and 3 hours for cypermethrin) and motor assessments (4 hours for permethrin and 3 hours for cypermethrin) were made. FOB and motor activity assessments were also made at pretest, and after 24 and 48 hours.

Permethrin:

At 75 mg/kg the rats displayed a general pattern of increased excitability and aggressive behavior. Some of the more pronounced responses included abnormal motor movement (3/8, both males and females) decreased forelimb (males 29%, $p < 0.05$) and hindlimb (males 30%, females 15%, both $p < 0.05$) grip strength and motor activity was decreased (estimated 40% for males) and body temperature was increased about 1 °C. At 150 mg/kg: arousal score, (males), righting reflex (males) and approach response score (females) were all affected and 7/8 of both sexes had abnormal motor movement and motor activity was further decreased and body temperature was increased > 2 °C. Slight decreases in body weight (3-4%) were evident. Recovery from the symptoms was within 24 hours. **The LEL is 75 mg/kg based on several neurotoxicity parameters being affected. The NOEL is 25 mg/kg.**

Cypermethrin:

The rats displayed gait, muscle effects and choreoathetosis. Motor activity was decreased for all dose groups for males (estimated 45%, 66% and 85% for the 20, 60 and 100 mg/kg dose groups respectively) and gait abnormalities were present in the low dose group. Body temperature was increased about one °C in the low dose male group but decreased for the higher groups. Some ten other parameters were affected at 60 mg/kg and/or above. These included: salivation, urination, arousal, abnormal motor movement, forelimb or hindlimb grip strength, landing foot splay, righting reflex, touch response and tail pinch response. **The LEL and NOELs for neurotoxicity are < 20 mg/kg. At 20 mg/kg decreased motor activity and gait abnormalities resulted.**

Classification: SUPPLEMENTARY. Study is in the form of a literature reprint and was not designed to meet a specific guideline protocol. Refer to page 8 for discussion of study deficiencies. The data with permethrin from this study taken together with another study (MRID No.: 430463-01) are considered CORE MINIMUM and to satisfy the requirement for a series 81-8ss acute neurotoxicity screen study, The NOEL and LEL from this study with permethrin should be used for acute neurotoxicity risk assessment.

Quality Assurance Statement: Not provided.
Good Laboratory Practice Statement: Not provided.

REVIEW

[Most of the data is presented in a semi-quantitative manner and no individual animal data are presented to verify the observations. A copy of the article is attached to this DER for reference for additional details.]

Basic Experimental Design:

Eight groups of 8/sex Long-Evans rats (Charles River Laboratories, Raleigh, NC, 70-90 days of age at testing) were utilized for each of the experiments with permethrin and cypermethrin. Both chemicals were administered the test material by gavage in a dosing volume of 1 ml/kg of corn oil. Permethrin treated rats were dosed with 25, 75 or 150 mg/kg and cypermethrin treated rats were dosed with 20, 60 or 120 (FOB experiment) or 100 (motor activity) mg/kg. Two corn oil control groups were run. Separate sets were treated for the FOB and motor assessments. Following treatment, the rats were assessed at 2 and 4 hours for permethrin and 1.5 and 3 hours for cypermethrin for FOB assessment. Motor activity was assessed 3 and 4 after dosing with cypermethrin and permethrin respectively. FOB and motor assessments were also made at pretest and at 24 and 48 hours after dosing for all groups.

The statistical methods used are described on page 73 of the paper.

Specific Methods and Results

1. Deaths.

Permethrin: Five rats died on the day of dosing as a result of treatment. During the FOB assessments, one male in the high dose group died and was replaced. During the motor activity assessment, one male and three female rats died. They were not replaced.

Cypermethrin: During the FOB assessments, one male and six of 12 (8 original and four replacements) female rats dosed with 120 mg/kg of cypermethrin died. The cypermethrin dose was thus reduced to 100 mg/kg for the motor assessments and at this level 2 males and 1 female died. The symptoms preceding death were not described for each animal.

Becasue deaths result in the high dose group for each chemical, the symptoms at the high dose levels are thus considered reactions at fatal or near fatal doses. The high dose group for series 81-8ss studies are not supposed to result in deaths.

2. Body Weight and Gain.

Data were not presented but comments on bodyweight effects were made for the high dose groups.

Permethrin: Transient weight losses of 4% and 3% for males and females were noted four hours after dosing in the high dose group.

Cypermethrin: Transient weight losses of 7.5 and 9% for males and females were noted 24 hours postdosing in the high dose group which persisted to 48 hours.

In general non-fatal doses did not result in body weight effects.

3. General reactions to treatment (clinical signs in the home cage or open field).

Table 1 below lists differences and similarities in the responses to permethrin and cypermethrin without regard to dose level since for many of these parameters the actual response at each dose level was not presented in the paper. The purpose of this table is to present a description of the differences in response to treatment by each pyrethroid. In general, no symptoms were noted in the low dose group with permethrin. Symptoms of decreased motor activity, gait effects and an increase in body temperature were noted in the low dose group dosed with cypermethrin.

In general, permethrin produced aggressive behavior, had a lesser effect on neuromuscular parameters and autonomic function and males and females were about equally affected. Cypermethrin more significantly affected gait, muscle function tone and equilibrium and produced burrowing behavior and autonomic signs (salivation and urination) and females were more sensitive than males for some parameters.

3. Functional Observational Battery.

Home cage and open field assessments were made. Numerous parameters were investigated that were described in Appendix 1 of the article (attached). This Appendix provides a very useful overview of the assessments to be made in a FOB test.

Table 4 (from the study report) presents the responses to treatment in a more quantitative and with respect to time manner as assessed in the FOB tests. Permethrin: The low dose group was not affected. Statistically significant differences in "abnormal motor movements" (males and females), decreased forelimb (males only) and hindlimb (males and females) grip strength and touch response (males) were noted at

Table 1. Parallel table of predominant responses in Long-Evans rats to treatment with permethrin and cypermethrin.

Parameter	Permethrin	Cypermethrin
General clinical signs	Basic response: **Aggressive behavior.** -positioned with backs against corner -rearing and assuming a "boxing" stance" or "aggressive sparring" -unprovoked agitated behavior (dart around cage, retreat to corner, bounce on haunches, spontaneous vocalization -pronounced shaking of head and forelimbs -difficulty in removal from cage. -whole body tremors (abnormal clonic movements	Basic response: **gait, muscle effects and choreoathetosis.** -lying flat on home cage with tremors (spasmodic, clonic movements and tonic extensions and flexion of body musculature or choreoathetosis -shovelling or burrowing into the bedding (females more sensitive) -muzzles appeared swollen -excessive lateral head movements -retropulsion -salivation and urination (autonomic function).
Autonomic	Little if any effects.	Profuse salivation and increased urination.
Sensory	Increased click, touch and approach response (males).	Increased click response but decreased touch and tail pinch response (males and females).
Neuromuscular	Decreased forelimb and hindlimb grip strength. Ataxic "tip-toe" gait highest dose only). "Slight" alterations in righting reflex. Landing foot splay not affected.	Decreased forelimb and hindlimb grip strength. More severe effects on gait (all dose levels), "tip-toe" gait progressed to combination of limb dragging and flattened posture. Equilibrium and muscle tone with righting reflex markedly impaired (couldn't do landing foot splay assay).
Motor activity	Decreased	Decreased
Body temperature	Increased	Apparently biphasic, lower doses can increase but higher doses decrease.
Duration	Most if not all symptoms regressed after 24 hours.	Some symptoms persist beyond 48 hours.
Gender differences	No definite differences but males may be more sensitive to some parameters as noted by this reviewer	Females more susceptible to lethal effects. Otherwise differences are indefinite and in some cases males seem more affected.

the 75 mg/kg dose level. At higher (near fatal) doses, these same parameters were also affected and indications of effects on the righting reflex (males only) and handling reactivity (females

only) became evident. The low dose group was reportedly unaffected. The symptoms were reported only at the 2 and 4 hour observations.

Cypermethrin. None of the 10 parameters listed in Table 4 of the study report were statistically significantly affected at 20 mg/kg for either sex. For males in the mid dose group 9 of these ten parameters were affected (urination was not). For the high dose group, all ten were affected. The direction of the change is indicated in Table 1 above. For females in the mid dose group, 5 of these parameters were statistically affected while in the high dose group all but urination were affected.

Gait abnormalities were presented separately (in Figure 3 of the study report).

Permethrin: Only the high dose group was affected and recovery was by 24 hours.

Cypermethrin: The low dose group had a significantly higher median score for gait abnormalities at 1.5 hours post dosing and the next two higher dose groups had progressively higher median scores. Thus, it is concluded that males are affected at the low (20 mg/kg) dose group. The high dose males and females continued to show gait abnormalities to 24 and probably also to 48 hours.

4. Motor Activity:

Motor activity was assessed in a figure 8 maze composed of a series of interconnected alleys with two blind alleys projecting from the central arena. Six phototransmitter/diode pairs were equally spaced in the apparatus. Apparently only a single session of 1 hour was recorded. TB-I notes that the session should be divided into epochs to assess for habituation and early and late effects patterns of motor activity. The data for males were presented graphically (Figure 4 of the report) for all dose levels but only the data for the high dose group females were included in the graphs.

Permethrin: Motor activity was depressed at the 75 (estimated 40% decrease) and 150 (estimated 60% decrease) mg/kg dose levels for males but was essentially equivalent to the controls at 24 and 48 hours. The percentage decrease in female activity could not be estimated since the control, low and mid dose female data were not on the graph presented. The female high dose group was stated as being 16% of the control group at four hours (84% reduced). Recovery was evident at 24 and 48 hours. TB-I notes that even though the motor activity was decreased, the general pattern of response to treatment with permethrin was increased excitability and aggression. This does not seem to be consistent with a decrease in motor activity. In this regard, it would have

been interesting to see the results of the epoch data within the session.

Cypermethrin: Motor activity was depressed for all treatment groups at 3 hours postdosing. For example males and females were decreased an estimated 46% and 43% respectively for the low dose group. Males and the females in the high dose group appeared to be 80% decreased. At 24 hours, motor activity for the mid (estimated 25%) and high (estimated 45%) dose male and high dose female (estimated 55%) groups was also decreased. At 48 hours, motor activity appeared comparable to control levels.

5. Body temperature. Data were presented in the form a graph (Figure 5 of the publication). Data for the males were presented for all dose groups but only data for the high dose female group were presented.

Permethrin: Mean body temperature was increased at 2 and 4 hours postdosing to nearly 40 degrees C for the male high dose permethrin treated animals with the mid dose group being increased to 39 degrees (control and low dose groups were 38 degrees). The high dose females also had a mean temperatures of 40 degrees C but the mean control group temperature was 39 degrees. Temperatures were apparently within normal range after 24 hours.

Cypermethrin: The effects of cypermethrin were apparently biphasic. The male low dose group was increased (statistically significant) at 1.5 hours from 38.5 degrees to slightly over 39 degrees C. Male and female high dose and male mid dose body temperature was decreased to below 37 degrees C at 1.5 hours for females and 3 hours for males. Females were still decreased after 24 and 48 hours.

D. Histopathology and Perfusion Studies.

No histopathology or perfusion data were generated.

DISCUSSION/CONCLUSION: These data are classified as SUPPLEMENTARY. The study is in the form of a literature reprint and is in summary form only without individual animal data. The study did not include histopathology of the nervous system which is required for the series 81-8ss acute neurotoxicity screen study.

This paper (copy attached) is considered a very useful source of additional information to the guideline studies submitted for permethrin and cypermethrin. The paper defines the behavioral and neurotoxicology responses to the type I (permethrin) and type II (cypermethrin) pyrethroids and will be a useful reference for aiding the review of series 81-8ss studies with other pyrethroids.

The DER as above contains some of the reviewer's interpretation of the data. In some cases, there may be more information in the actual paper.

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Utility of a Neurobehavioral Screening Battery for Differentiating the Effects of Two Pyrethroids, Permethrin and Cypermethrin

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McDANIEL, K. L. AND V. C. MOSER. *Utility of a neurobehavioral screening battery for differentiating the effects of two pyrethroids, permethrin and cypermethrin.* NEUROTOXICOL TERATOL 15(2) 71–83, 1993.—The ability of a neurobehavioral screening battery to differentiate the effects of two pyrethroids, permethrin and cypermethrin, was assessed in this experiment. Although the structures of these pesticides differ only in the α-cyano group, the behavioral syndromes associated with the Type I and II pyrethroids are quite different. The tests included a functional observational battery which is a series of subjective and quantitative measures of neurological function and behavior, and an automated measure of motor activity. Our results verified previous reports in the literature describing these different syndromes, i.e., aggressive sparring behavior, fine to whole-body tremor, hyperthermia, and decreased motor activity for the Type I pyrethroid permethrin, and pawing, burrowing, salivation, whole body tremor to choreoathetosis, hypothermia, and lowered motor activity for the Type II pyrethroid cypermethrin. In addition, we report that permethrin produced decreased grip strengths, increased resistance to capture, increased reactivity to a click stimulus, and induced head and forelimb shaking and agitated behaviors, whereas cypermethrin produced pronounced neuromuscular weakness and equilibrium changes, retropulsion, lateral head movements, alterations in responses to various stimuli, and increased urination. Although there were similarities in some effects (e.g., decreased motor activity), the pesticides differed sufficiently in their overall behavioral profiles, and severity and time course of effects, to discriminate these two compounds. Thus, this type of screening approach is sensitive enough to differentiate these pyrethroids for hazard identification purposes.

Pyrethroid Permethrin Cypermethrin

THE use of rapid screening methods to assess the neurotoxic potential of chemicals has been recommended by expert panels (e.g., 16,31,46) and reviewed by the Office of Technology Assessment (41). This approach required by the U.S. Environmental Protection Agency for testing under the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act (39,42), and is under consideration by the Organization for Economic Cooperation and Development (OECD) for inclusion in standard toxicity testing (OECD ad hoc Meeting on Neurotoxicity Testing; March 27–29, 1990). Furthermore, the validation and utility of neurotoxicity screening batteries has been the topic of workshops and symposia (e.g., 1,5,11,38,40). Ongoing research in our laboratory has been directed toward applying these methods to a broad variety of chemicals and testing conditions to assess how well such tests perform to our expectations. We are also developing a database of chemical effects against which the actions of unknown chemicals can be compared (21–28). Our neurobehavioral screening battery consists of a functional observational battery (FOB), which is a series of tests to assess sensory, neuromuscular, and autonomic function, and an automated measure of motor activity. These tests are primarily intended to provide dose-response and time-course data, as well as profiles of neurological effects which can then be used to make decisions concerning a compound's neurotoxic potential and lead to subsequent studies for risk assessment (24).

In the present study we examined the ability of our neurobehavioral screening battery to characterize and differentiate the previously-described effects of two pyrethroids. Pyrethroids comprise a large class of widely-used pesticides, and the toxicity of pyrethroids in insects and vertebrates involves their effects on motor and sensory fibers in the central and peripheral nervous systems (for reviews see 4,13,45,47). The pyrethroids in general produce numerous neurobehavioral signs and alter the gating mechanism of the sodium ion channel in neural tissues (4,15,30,43,44). However, differences in the specific actions of these chemicals have been reported and

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these are summarized in Table 1. Pyrethroids have been classified as Type I or II depending on the particular behavioral syndrome they produce ('T' for tremors, or 'CS' for choreoathetosis and salivation, respectively; 15,43) and on their action on sodium ion channels (29,44). The major structural feature which differentiates the subgroups is that Type II pyrethroids usually contain an α-cyano moiety while the Type I pyrethroids do not (4,13).

In this experiment, we obtained dose-response and time-course data for the effects of permethrin (Type I pyrethroid) and cypermethrin (Type II) using our neurobehavioral screening battery, and profiles of effect on functional domains were established. The data obtained using this approach were shown to differentiate the actions of these two pyrethroids.

METHOD

Animals

Adult Long-Evans hooded rats of both sexes (Charles River Laboratories, Raleigh, NC) were used in all experiments and were 70–90 days of age when testing began. Rats used in the motor activity study were singly-housed in wire mesh cages, whereas for the FOB experiments rats were singly-housed in solid polycarbonate cages with hardwood chip bedding (Beta-Chip, Granville Milling Co., Creedmoor, NC). All rats were allowed free access to Purina rodent chow (Rat Chow No. 5002, Ralston Purina Co., St. Louis, MO) and deionized tap water in the home cages. The animal facility was maintained at 22 ± 1°C, 55 ± 5% humidity, and had a 12 L : 12 D cycle with lights on at 0630 h. FOB testing took place in the animal housing room. For the motor activity tests rats were transported to another isolated room in the animal facility. Testing of each chemical was conducted within a time frame of 2 to 3 weeks, with the dose groups counterbalanced across days. When lethality occurred in the FOB studies, extra rats from within the same shipment were added to have the same number of rats per dose group. There were no available rats with which to replace those that died in the motor activity studies.

Chemicals

Technical grade permethrin (95% purity) and cypermethrin (97% purity) were kindly donated by Drs. D. Gammon and L. Smeltz (FMC Corp., Princeton, NJ). Both samples contained approximately equal quantities of *cis* and *trans* isomers. Chemicals were dissolved in corn oil (Mazola^{R}) and administered by oral gavage in a volume of 1 ml/kg.

Functional Observational Battery

Procedure. The FOB consists of home-cage, handling, open-field, and manipulative behavioral measures, as well as physiological measures. The FOB protocol used in this study was based on a previous version (27). Since that initial report, however, experience with these tests has led to many modifications. For example, some FOB measures have been changed from descriptive to rank-order (such as the sensorimotor responses), and other measures (such as limb rotation, catalepsy, and extensor thrust) that were found to be insensitive to treatment or otherwise unreliable have been eliminated. Because fairly extensive changes have been made, the current protocol is described in Appendix 1.

The FOB tests were completed in approximately 6–8 min per rat. All data were recorded on standardized datasheets and later entered into a computer for further analysis. The observer was blind with respect to the dose level.

Experimental design. Eight rats of each sex were treated by gavage (po) with either corn oil (vehicle), 25, 75, or 150 mg/kg permethrin. For cypermethrin, test groups received either corn oil (vehicle), 20, 60, or 120 mg/kg. The selection of doses was based on motor activity dose-response data from Crofton and Reiter (8).

Preliminary studies using arousal and gait score assessments were conducted to determine times of peak effect for each compound on the day of dosing. It was observed that somewhat different effects were evident at different times after dosing; thus assessments were made at two time points on the day of dosing. These times were 2 and 4 h for permethrin, and 1.5 and 3 h for cypermethrin. In addition, rats were

TABLE 1

DIFFERENCES AND SIMILARITIES IN SELECTED BIOLOGICAL EFFECTS OF PYRETHROIDS*

	Pyrethroid	
	Type I	Type II
Behavioral syndrome	T syndrome: aggressive sparring, increased sensitivity to external stimuli, fine to whole body tremor, hyperthermia	CS syndrome: pawing, burrowing, salivation, coarse whole body tremor progressing to choreoathetosis
Motor activity	decreased	decreased
Acoustic startle response	increased amplitude, no effect on latency	decreased amplitude, increased latency
Operant performance	decreased responding	decreased responding, altered patterning
Action on Na^+ channel	repetitive firing; keeps channel in open state for msec	frequency-dependent depolarizing block; keeps channel in open state for minutes
Action on GABA system	none	inhibits GABA-induced chloride influx

*References: 2,7,8,10,12,13,15,17,32,33,43,44,45.

tested immediately prior to dosing (time 0) and at 24 and 48 h after dosing.

Data analysis. A severity scoring scheme was used to determine chemical effects on specific domains of neurological function (i.e., autonomic, activity, excitability, neuromuscular, and sensorimotor). This method of analysis normalized individual data for all measures to a 1-to-4 scale, where a score of 1 reflected what was often observed in control rats and a score of 4 reflected a rare occurrence in control rats (24,28). The conversion of individual data to a severity score was based on the data for control rats. Table 2 illustrates that for measures which produce continuous data, score assignments were based on the range defined by the mean and SD of the control group, whereas for the rank and quantal data the score assignments were based on the frequency of occurrence of each score in the control rats. Measures such as lacrimation, gait score, etc., were already defined in terms of severity of effect and no conversions were necessary.

For each rat at each time point, these severity scores were summed across the measures that comprised each functional domain. The composite scores were then averaged across treatment groups and analyzed across time using a repeated-measures analysis of variance (ANOVA). Because motor activity data were not collected in the same rats in these studies and motor activity is an important indicator of the activity domain, chemical effects on the activity domain were not analyzed.

Analysis of individual FOB measures within a functional domain was carried out as previously described (6,27). In addition, physiological measures such as body weight and body temperature were analyzed for each study. For all measures, two-way ANOVAs were carried out using a between-subject factor of dose and within-subject repeated measures across time. If the ANOVA produced a significant overall effect of dose or dose × time interaction, subsequent univariate analyses were conducted at each time point which included group comparisons to control values using Dunnett's *t* test. In all analyses, resulting p values ≤ 0.05 were considered significant. Continuous data were analyzed by a linear model (GLM; 37) using each rat's time-0 value as a covariate. Descriptive and rank data were analyzed using a categorical procedure (CATMOD; 37). Due to the small dose-group size, analyses were conducted separately for males and females (6,27).

Motor Activity

Apparatus. Motor activity was measured in a maze composed of a series of interconnected alleys shaped like a figure-8 with two blind alleys projecting from the central arena (see ref. 36). Six phototransmitter/diode pairs were equally spaced around the figure-8 portion of the maze, and one pair was located in each of the blind alleys. Motor activity was recorded by a microprocessor as the number of photocell interruptions over a 1-h session.

Experimental Design. The motor activity experiments were designed to conform to the FOB studies, but a few modifications were necessary. Eight rats of each sex were dosed with either corn oil (vehicle), 25, 75, or 150 mg/kg permethrin. For cypermethrin the highest dose was lowered due to lethality noted in the FOB study and the resulting dose groups included corn oil (vehicle), 20, 60, or 100 mg/kg. Motor activity testing took place 4, 24, and 48 h after dosing for permethrin, and at 3, 24, and 48 h after dosing for cypermethrin. (Earlier time points of 1.5 and 2 h were excluded from the motor activity studies to avoid having two test sessions occurring so closely together.)

Data analysis. Total photocell counts over the session were subjected to a two-way ANOVA using a grouping factor of dose and repeated measures across time, followed by Dunnett's *t* test.

RESULTS

Lethality occurred during the testing of both compounds. During FOB testing, one male rat from the high dose groups of permethrin and cypermethrin died on the day of dosing (those rats were subsequently replaced using available extras).

TABLE 2

CRITERIA FOR CONVERSION OF FOB DATA TO SEVERITY SCORES

Severity Score	Measure		
	Continuous	Scalar	Other
1	within X ± 1 × SD*	Score which ≥ 50% receives	considered normal
2	within X ± 1.5 × SD	Score which >1 but <50% subjects receives	considered slightly deviant from normal
3	within X ± 2 × SD	Score which 1 rat receives, or is 1 rank from mode	considered somewhat deviant from normal
4	outside X ± 2 × SD	Score >1 rank from mode	considered severely deviant from normal
	forelimb and hindlimb grip strength, landing foot splay, rears, activity counts, urination, defecation	handling reactivity, ease of removal, arousal, touch response, click response, tail pinch response, approach response	home-cage posture, lacrimation, pupil response, salivation, palpebral closure, clonic and tonic movements, gait score, righting reflex

*Within the range defined by mean ± SD. See refs. 24, 28.

Six out of 12 female rats that were dosed with 120 mg/kg cypermethrin died (of these, four were replaced, therefore $n = 6$ females at that dose for the FOB). During the motor activity tests, one male and three female rats administered 150 mg/kg permethrin died. A slightly lower dose of cypermethrin was used for the motor activity test (100 mg/kg instead of 120 mg/kg), and two male and no female rats died at that dose.

Overall, cypermethrin was the more potent compound since the low dose (20 mg/kg) had considerable activity on some measures, whereas the low dose of permethrin (25 mg/kg) had no detectable effects (no-observable effect level, or NOEL). The magnitude of effects seen with the high dose of permethrin (150 mg/kg) on some measures was similar to that for the middle cypermethrin dose (60 mg/kg).

Functional Changes

The pyrethroids significantly affected the excitability, neuromuscular, and sensorimotor functional domains in both male and female rats. The autonomic domain was also affected by cypermethrin in both sexes but was only marginally significant ($p < 0.042$) in male rats treated with permethrin. A summary of the severity scoring analysis and general changes in individual measures is presented in Table 3. Data for the FOB measures which were significantly altered by pyrethroid exposure are illustrated in Figures 1–5 or listed in Table 4. Most effects were obtained on the day of dosing, although a few residual effects (most notably neuromuscular) were still significant 1 to 2 days after dosing.

Close examination of the profiles of effect for these two compounds revealed both qualitative and quantitative differences. For example, permethrin produced aggressive behaviors, only affected a few of the neuromuscular measures, and had no clear autonomic activity. On the other hand, cypermethrin affected gait, muscle function, tone, and equilibrium, and produced pronounced autonomic signs and burrowing behaviors. Female rats were generally more sensitive to the effects of cypermethrin, but there were no gender-related differences in the effects of permethrin.

Both compounds produced somewhat bizarre behaviors which have been described in the literature and the nature of these behaviors were very different. While in the home cage, rats dosed with permethrin were positioned with their backs against a corner, rearing up and assuming a boxing stance. Although these rats were individually housed, the postures met the description of "aggressive sparring" which has been reported for Type I compounds (43). They also displayed unprovoked agitated behaviors, i.e., dart around the cage and then retreat and cower in the corner or bounce on their haunches, and sometimes vocalize spontaneously. On the open field, permethrin-treated rats showed other unusual behaviors involving pronounced shaking of the head and forelimbs, slapping their own forelimbs, and the same unprovoked agitated reactions which were observed in the home cage.

In marked contrast, rats administered cypermethrin were often observed lying flat in the home cage with tremors. Shoveling or burrowing into the bedding was also observed, and this was more prevalent in females. In many cases, the rat's muzzle also appeared swollen. On the open field, these rats displayed excessive lateral head movements, a high incidence of retropulsion, and continued to shovel persistently with their noses (analogous to the burrowing behavior observed in the home cage).

The effects of cypermethrin on autonomic function in both sexes included profuse salivation. This initial sign of intoxication was significant only at 1.5 h after dosing (Table 4). Urina-

TABLE 3

PROFILE OF EFFECTS OF PERMETHRIN AND CYPERMETHRIN

Domain*	Cypermethrin	Permethrin
Autonomic	M:† 1.5,3 h F:† 1.5,3 h	M: 4 h
	salivation	–
	increased urination	
Excitability	M: 1.5,3,24 h F: 1.5,3 h	M: 2,4 h F: 2,4 h
	increased removal resistance	increased removal resistance
	decreased arousal	increased arousal
	choreoathetosis	whole body tremors
		increased handling reactivity
Neuromuscular	M: 1.5,3,24 h F: 1.5,3,24,48 h	M: 2,4,24 h F: 4 h
	splayed limbs, flattened posture	ataxia
	decreased grip strengths	decreased grip strengths
	altered righting	altered righting
	increased landing foot splay	
Sensorimotor	M: 3h F: 3h	M: 4,48 h F: 4 h
	increased click response	increased click response
	decreased touch response	increased touch response
	decreased tail pinch response	increased approach response

*Could not include motor activity in functional domain, therefore analysis of severity scores not applicable.

†M: males; F: females.

For each functional domain, times during which that domain was significantly affected are indicated. Also listed are the general effects within each domain.

TABLE 4

EFFECTS OF CYPERMETHRIN AND PERMETHRIN ON MEASURES OF THE FOB WHICH WERE SIGNIFICANTLY ALTERED BY EITHER CHEMICAL

	Cypermethrin							
	Males				Females			
Dose (mg/kg)	0	20	60	120	0	20	60	120
Salivation† 1.5 h	1	1	1.5*	2.5*	1	1	2*	3*
Urination‡ 1.5 h	.13	.13	.63	.86*	.13	0	.38	.38
Arousal† 3 h	3	3	2.5*	2*	4	4	2.5*	2*
Abnormal motor movements§ 1.5 h	0/8	1/8	6/8*	7/8*	0/8	0/8	4/8*	7/8*
3 h	0/8	1/8	7/8*	7/8*	0/8	0/8	4/8*	8/8*
24 h	0/8	0/8	2/8*	2/8*	0/8	0/8	0/8	1/6
Forelimb grip strength (kg)‡ 3 h ± SEM	1.012 ± .069	.973 ± .074	.654 ± .155*	.533 ± .152*	.993 ± .044	1.022 ± .053	.835 ± .114	.275 ± .051*
Hindlimb grip strength (kg)‡ 3 h ± SEM	.966 ± .054	.851 ± .034	.521 ± .066*	.511 ± .107*	.866 ± .033	.825 ± .054	.582 ± .063*	.379 ± .050*
Landing foot splay (mm)‡ 24 h ± SEM	66.1 ± 4.1	65.2 ± 4.0	81.6 ± 8.4*	80.8 ± 6.1*	59.3 ± 4.4	58.5 ± 3.9	62.4 ± 4.0	78.5 ± 4.5*
Righting reflex† 3 h	1	1	3.5*	4*	1	1	3*	4*
Touch response† 3 h	3	3	1.5*	1.5*	3	2*	2.5	1*
Tail pinch response† 3 h	4	4	1.5*	2*	3	2	4	1*

continued

TABLE 4 *Continued*

	Permethrin							
	Males				Females			
Dose (mg/kg)	0	25	75	150	0	25	75	150
Arousal†								
4 h	3	2*	2.5	3.5*	4	4	4	3
Handling reactivity†								
2 h	2	2	2.5	2.5	2	2	2	2.5*
Abnormal motor movements§								
2 h	0/8	0/8	0/8	1/8	0/8	0/8	0/8	4/8*
4 h	0/8	0/8	3/8*	7/8*	0/8	0/8	3/8*	7/8*
Forelimb grip strength (kg)‡								
4 h	1.038 ± .048	1.021 ± .078	.739 ± .090*	.778 ± .039*	.774 ± .032	.771 ± .039	.789 ± .051	.599 ± .07*
Hindlimb grip strength (kg)‡								
4 h	1.178 ± .057	1.121 ± .085	.820 ± .080*	.751 ± .060*	.934 ± .063	.847 ± .058	.794 ± .060*	.737 ± .05*
Righting reflex†								
4 h	1	1	1	2*	1	1	1	1
Approach response†								
4 h	1.5	1.5	2	2	1.5	2	2	2*
Touch response†								
2 h	3	3	3*	3.5*	3	2	2.5	2

Data are provided for only the time at which the peak effect occurred. Dose groups statistically different from vehicle are indicated (*).
†Median; ‡Mean ± SEM; §Number of rats showing signs/number of rats in dose group.

tion was also increased in these rats on the day of dosing (males at 1.5 h; females at 3 h). Permethrin was without significant effect on any individual autonomic measure, even though the composite autonomic score showed a significant effect of treatment in male rats at 4 h. This was probably because the transformed severity scores for defecation were somewhat higher than controls; however, statistical analysis of the raw data was not significant.

Permethrin greatly increased excitability, whereas the effects of cypermethrin, though significant, were not as prominent. Ease of removal from the home cage (i.e., resistance to capture) was significantly affected by permethrin (both sexes) and cypermethrin (males only). However, the magnitude of this effect was much greater with permethrin. Scores for the ease of removal measure are shown in Fig. 1. Median scores are given since these scores are nonparametric. In this and all figures, dose-response and time-course data are shown for male rats; data for female rats in the high dose group are included for comparison. Most male rats administered 60 or 120 mg/kg of cypermethrin were rated as moderately difficult to remove (rank = 3, rat follows investigator's hand and does not sit quietly), whereas rats (male and female) dosed with 75 and 150 mg/kg permethrin appeared agitated, darted around

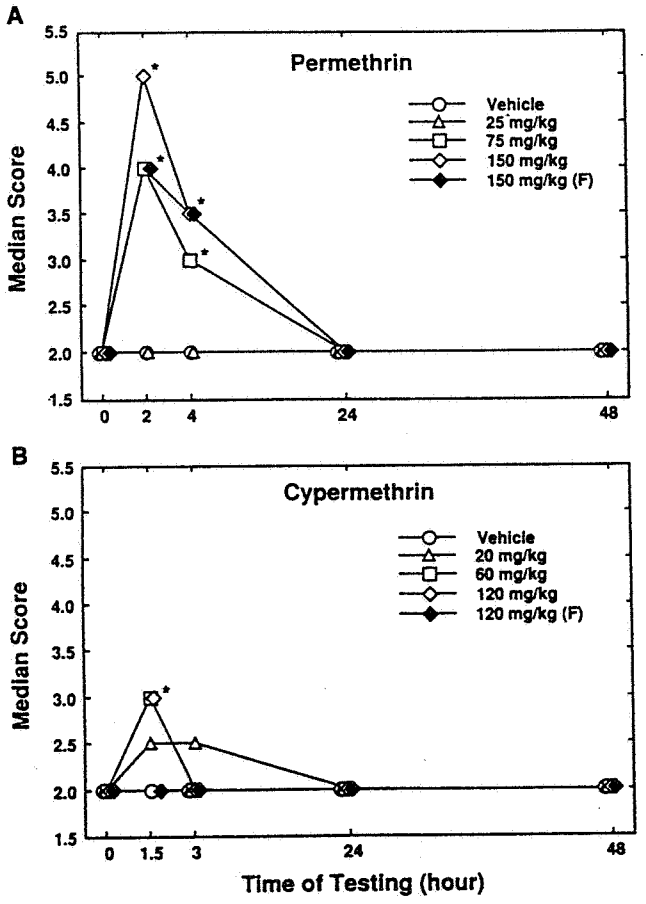

FIG. 1. Median score for ease of removal from the home cage for male rats treated with permethrin (A) and cypermethrin (B). Data for female rats treated with the high dose only are included. See Appendix for scoring criteria. n = 8/dose for all dose groups, except n = 6 for females given cypermethrin 120 mg/kg. Dose groups statistically different from the appropriate control (i.e., male or female) are indicated (*).

the cage, were very difficult to grab, and in a few cases, showed aggression toward the observer's hand (ranks of 5 or 6). Once out of the cage, however, cypermethrin-treated rats were no different to handle than vehicle-treated rats, whereas females in the high permethrin dose group showed an increased reactivity and resistance in being handled (handling reactivity, Table 4). Furthermore, permethrin-treated male rats in the high dose group appeared more alert, and darted about in the open field, in contrast to cypermethrin-treated rats which were less alert (arousal, Table 4).

Permethrin produced moderate to severe whole-body tremors, the incidence and severity of which were dose-related at 2 and 4 h after dosing (abnormal clonic movements, Table 4). Cypermethrin caused a combination of spasmodic, clonic movements and tonic extensions and flexions of the body musculature (choreoathetosis). This occurred at the two higher doses on the day of dosing and 24 h later (more so in males than females; Table 4).

Sensorimotor reactivity was also altered by both pyrethroids and these effects were transient, occurring at 3 h for cypermethrin and 4 h for permethrin (Table 4). For some stimuli, differences between the two compounds were evident in the direction of change for specific measures. Both pyrethroids significantly increased the response to the click stimulus, shown in Fig. 2. With permethrin, reactivity was uniformly increased, as evidenced by increased reactions to the touch stimulus (males only) and the approach of a pencil (females only). In contrast, responses to the touch and tail pinch stimuli were significantly decreased by cypermethrin (both sexes).

Both compounds produced gait abnormalities and weakness (i.e., decreased forelimb and hindlimb grip strength), but the effects of cypermethrin were more severe and persistent (Table 4). All of the neuromuscular changes were more prominent at the later time points on the day of dosing (3 h for permethrin, 4 h for cypermethrin). Gait scores are presented in Fig. 3. Cypermethrin significantly altered gait in all dose groups on the day of dosing, and the effects of the high dose were still significant at 48 h. The effects of cypermethrin on gait were described as ataxia and tip-toe walking that progressed to a combination of limb dragging and flattened posture. On the other hand, only the highest dose of permethrin produced an ataxic tip-toe gait. In male rats treated with either chemical, there was no differential sensitivity between forelimb and hindlimb grip strength. Furthermore, the middle and high doses of each compound produced approximately equal decreases (Table 4). In females, significant decreases in hindlimb grip strength were obtained at the middle and high doses, but only the high doses affected forelimb grip strength.

Cypermethrin but not permethrin also greatly affected equilibrium and muscle tone. Righting reflex was markedly impaired at 3 h in rats of both sexes (Table 4). Data for landing foot splay are incomplete for the day of testing, because most rats were too impaired to land properly and the test was not conducted on those rats. However, increased foot splay was significant in female rats in the high dose group at 24 and 48 h, and in male rats in the middle and high dose groups at 24 h. Permethrin produced slight alterations in righting reflex, but only in the high dose group (males), and landing foot splay was not affected at any time.

Motor Activity

Motor activity was markedly depressed on the day of dosing, and the effects of both pyrethroids on total counts are

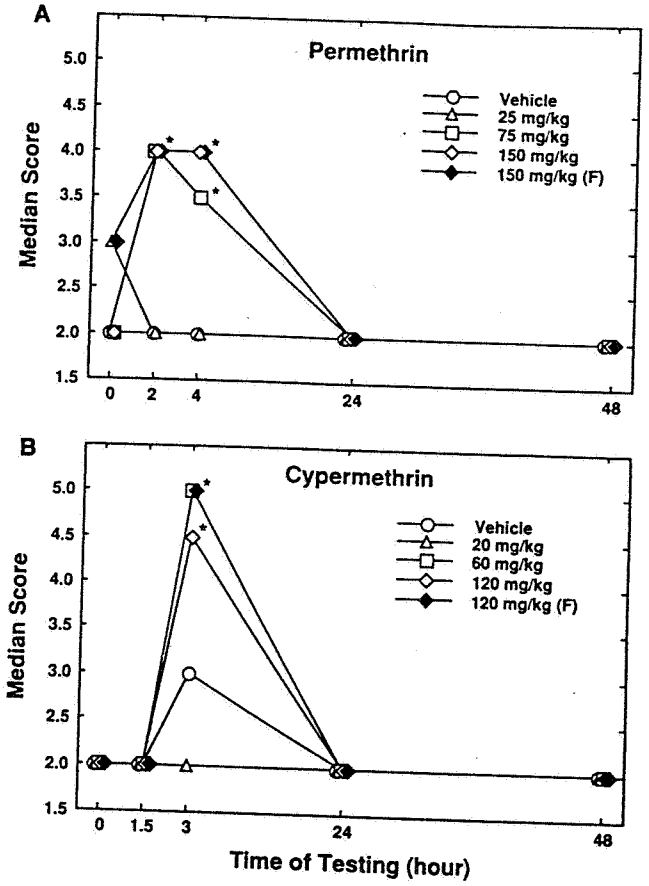

FIG. 2. Median score for the rating of the click response for male rats treated with permethrin (A) and cypermethrin (B). Data for female rats treated with the high dose only are included. See Appendix for scoring criteria. n = 8/dose for all dose groups, except n = 6 for females given cypermethrin 120 mg/kg. Dose groups statistically different from the appropriate control (i.e., male or female) are indicated (*).

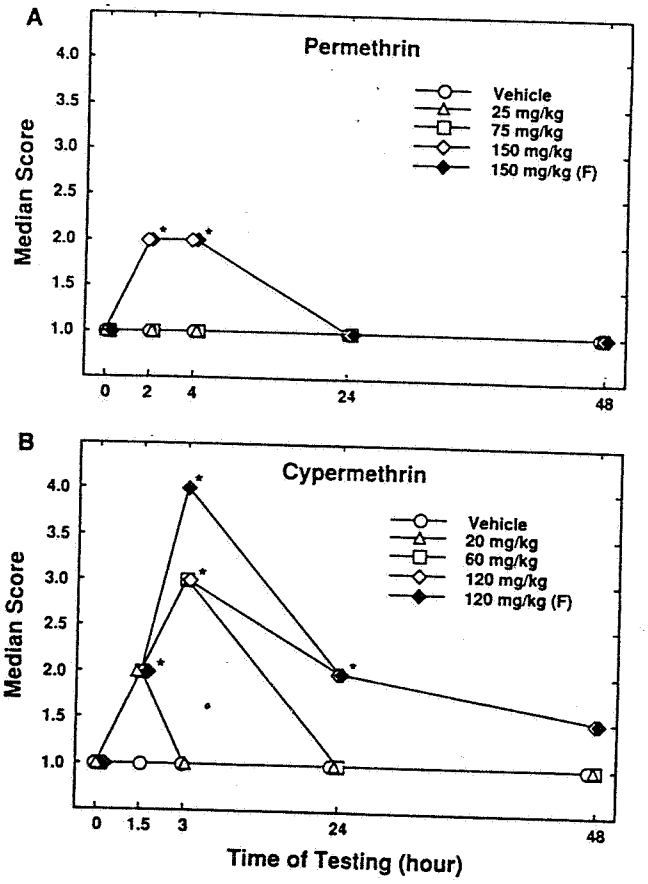

FIG. 3. Median score for the rating of gait abnormality for male rats treated with permethrin (A) and cypermethrin (B). Data for female rats treated with the high dose only are included. See Appendix for scoring criteria. n = 8/dose for all dose groups, except n = 6 for females given cypermethrin 120 mg/kg. Dose groups statistically different from the appropriate control (i.e., male or female) are indicated (*).

presented in Fig. 4. At 4 h after permethrin administration, the middle and high doses significantly decreased activity counts. Total counts in the high dose group were 39% and 16% of vehicle control values for males and females, respectively. Recovery was evident at 24 and 48 h, and activity was even significantly increased in the female high dose group at 48 h. At 3 h after dosing with cypermethrin, all doses significantly decreased activity in both sexes, and the high dose was still effective at 24 h. Furthermore, the low dose (20 mg/kg) was close to the ED_{50} value for this measure, in that it decreased activity by 46% and 43% in males and females, respectively. No effects of cypermethrin were obtained at 48 h.

Physiological Measures

Body weight loss was produced by cypermethrin in both sexes, and the effect of the high dose was still evident at 48 h. Permethrin produced only a transient weight loss in male rats on the day of dosing. The magnitude of the weight loss was greater with cypermethrin, with rats in the high dose group losing a maximum (at 24 h) of 7% and 9% of the predosing weight in male and female rats, respectively. Male rats dosed with the high dose of permethrin lost a maximum of 4% body weight (at 4 h), and the 3% weight loss recorded in females was not statistically significant.

Body temperature was markedly increased by the two higher doses of permethrin on the day of dosing, as shown in Fig. 5. Cypermethrin produced hypothermia in both sexes. In addition, the low dose of cypermethrin (20 mg/kg) produced a small but significant increase (<1°C higher than vehicle) in temperature at 1.5 h (male rats only).

DISCUSSION

Differences and similarities between permethrin and cypermethrin were evident by their effects on a neurobehavioral screening battery. Behavior representing all of the functional domains assessed were affected by both compounds, indicating their broad neurological activity. These effects were generally seen in both sexes with peak effects occurring within hours of dosing. Differences between the two chemicals emerged when their effects on individual measures of each domain were compared in terms of (a) qualitative signs of toxicity, (b) magnitude of effect, and (c) time course and effective dose range.

As in previous reports (e.g., 13,15,43), detailed descriptions of pyrethroid-induced clonic motor movements and bi-

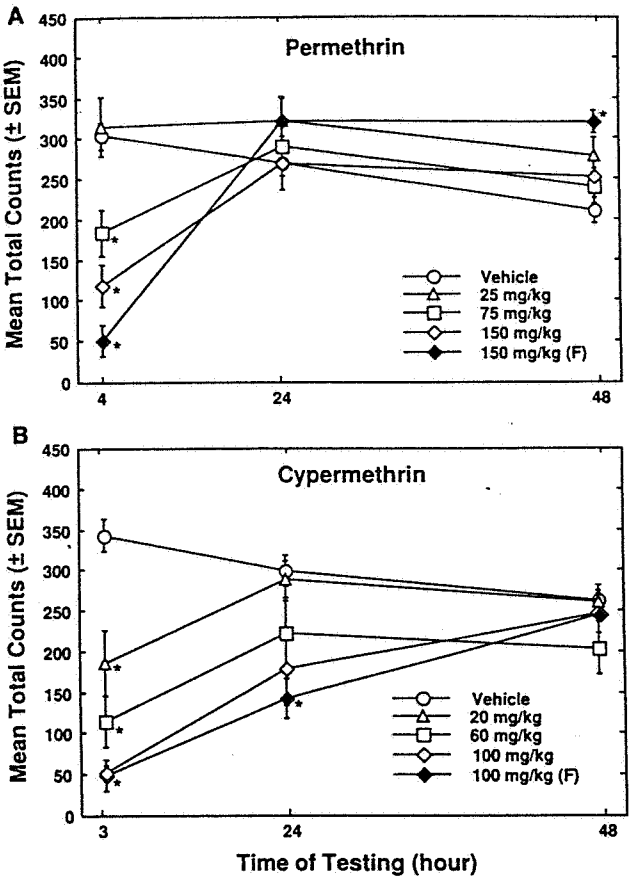

FIG. 4. Mean (± SEM) total activity counts during a 1-hour session for male rats treated with permethrin (A) and cypermethrin (B). Data for female rats treated with the high dose only are included. n = 8/dose for all dose groups, except: n = 6 for males given cypermethrin 100 mg/kg, n = 7 for males and n = 5 for females given permethrin 150 mg/kg. Dose groups statistically different from the appropriate control (i.e., male or female) are indicated (*).

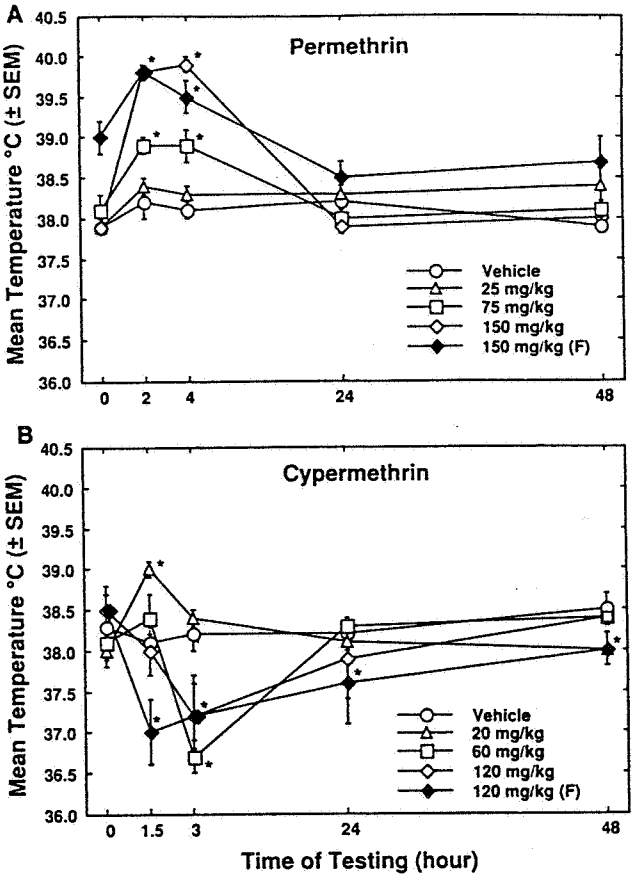

FIG. 5. Mean (± SEM) body temperature data for male rats treated with permethrin (A) and cypermethrin (B). Data for female rats treated with the high dose only are included. n = 8/dose for all dose groups, except n = 6 for females given cypermethrin 120 mg/kg. Dose groups statistically different from the appropriate control (i.e., male or female) are indicated (*).

zarre behaviors served to differentiate the syndromes produced by these chemicals. These included whole body tremor and aggressive sparring behavior after permethrin (a Type I pyrethroid) administration, and pawing, burrowing, and tremor progressing to choreoathetosis after cypermethrin (a Type II pyrethroid). Permethrin-treated rats displayed postures resembling sparring behavior even though they were individually housed. Head shaking and slapping of the forelimbs were also observed effects of permethrin, and these behaviors are similar to the shaking of head and forelimbs reported with intraventricularly-administered Type I pyrethroids (14). After cypermethrin administration, rats displayed pawing and burrowing behavior even while on the open field, indicating that these may be stereotypic rather than "purposeful" behaviors. The swollen muzzles, which were quite prominent after cypermethrin exposure, may have been a direct effect of the chemical or due to irritation possibly produced by the excessive burrowing actions. It is interesting to note that, in our experience, these pyrethroids are the only compounds which induce spontaneous vocalizations.

Only anecdotal accounts have been reported in the literature for some of the behavioral changes produced by pyrethroids. In this study, rating scales for assessing these behaviors provided semiquantitative data which exhibited dose-response and allowed statistical analyses to be conducted. These behaviors included those indicative of: increased sensitivity to external stimuli (seen for both types of pyrethroids); ataxia (permethrin); splayed hind limbs and abnormal locomotion (cypermethrin). For example, ataxia and abnormal locomotion were ranked using the gait and mobility scores; splayed hind limbs were quantified using the landing foot spread test. Reactivity to various stimuli (specific and nonspecific) was quantified by scoring the reactivity to being removed from the cage, and the responses to the tail-pinch, click, touch, and approach stimuli. Analysis of these measures also revealed differences between the effects of these compounds. For instance, permethrin markedly increased resistance to removal, whereas cypermethrin produced only a small effect on this measure (Fig. 1). These data probably indicate that permethrin generally increases overall excitability, since other sensorimotor responses were also increased. In contrast, cypermethrin generally decreased sensorimotor reactivity, with the exception of the click response which was increased.

Both chemicals produced neuromuscular changes as evidenced by decreased grip strengths, gait changes, and altered righting ability. However, the effects of cypermethrin were

more severe and persistent (e.g., see Fig. 3). Cypermethrin, but not permethrin, decreased muscle tone as evidenced by splayed legs, increased landing foot spread, and flattened posture. These differences were not simply a matter of potency and/or the doses utilized. In comparing the effects of the high dose of permethrin (150 mg/kg) with those for the middle dose of cypermethrin (60 mg/kg), which were approximately equi-effective on hindlimb grip strength (38% and 43% decrease for permethrin and cypermethrin, respectively), cypermethrin produced a greater decrease in forelimb grip strength (Table 4), greater degree of gait abnormality (Fig. 3), and in addition significantly altered righting, impaired mobility, and increased foot splay (Table 4). Although deltamethrin has been shown to increase landing foot splay (14), we could not find literature reports of the pronounced neuromuscular changes which we present here for cypermethrin.

The behavioral effects of both pyrethroids were significant at both the middle and high doses. Neuromuscular dysfunction produced by cypermethrin was evident at even the low dose. Most previous, purely-descriptive studies of the behavioral effects of these pesticides have employed only near-lethal doses (3,34,43). This provides evidence that behavioral evaluations conducted using standardized rating and testing criteria are sufficiently sensitive to evaluate behavioral effects of chemicals at lower doses, such as those used in first-tier screening studies. The behavioral data reported here generally show cypermethrin to be more potent than permethrin, and indeed addition of the α-cyano group has been shown to increase the potency of pyrethroids (13).

Reported LD50 values in rodents for cypermethrin are around 250–300 mg/kg (19,35). However, in the FOB study the high dose (120 mg/kg) produced 11% lethality in male rats and 50% in females. Although there is no previous evidence of this suggested gender difference in sensitivity, most studies have not compared males and females and reported LD50 values often do not specify sex. Lethality data for permethrin are difficult to derive from the literature, and LD50 values in rodents range from 410 mg/kg (35) to >5 g/kg (18). These differences may be due to isomeric potency differences. For permethrin, the *trans* isomer is not neurally active (4,13), and batches of permethrin may vary in the ratios of *cis* and *trans* isomers. Thus, comparison of effective dose ranges of permethrin between studies can be misleading.

Though not statistically tested, there were differences in time course of effects between permethrin and cypermethrin. For cypermethrin, two phases of toxicity were evident. Salivation and increased removal reactivity (males) were most evident at 1.5 h. By 3 h these signs were subsiding, and the pronounced motor and sensory effects were apparent (e.g., compare the time course in Fig. 1 with those in Figs. 2 and 3). It is possible that different mechanisms are acting to produce these differences in time course. For permethrin, most effects were maximal at 4 h, or else they were approximately equal at 2 and 4 h. There were also differences in the duration of action of the two pyrethroids. Rats dosed with permethrin recovered by 24 h, but in those given cypermethrin several motor effects (e.g., gait changes, lowered grip strengths, decreased motor activity) were still evident 1–2 days later (especially in females).

The profiles of the neurobehavioral effects produced by these two structurally similar compounds were quite different. The signs of toxicity reported in this study correspond well to those of earlier reports. In addition, we report here pronounced neuromuscular changes produced by cypermethrin, and increased excitability and reactivity produced by permethrin. Thus, the neurobehavioral test battery clearly detected neurological activity, defined dose-response and time-course characteristics, and provided additional information for differentiating the distinct syndromes produced by these two pyrethroids.

ACKNOWLEDGEMENTS

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REFERENCES

1. American College of Toxicology. Symposium: Screening for neurotoxicity II (October 1990). J. Am. Coll. Toxicol. 10:661–736; 1991.
2. Bloom, A. S.; Staatz, C. G.; Dieringer, T. Pyrethroid effects on operant responding and feeding. Neurobehav. Toxicol. Teratol. 5:321–324; 1983.
3. Brodie, M. E.; Aldridge, W. N. Elevated cerebellar cyclic GMP levels during the deltamethrin-induced motor syndrome. Neurobehav. Toxicol. Teratol. 4:109–113; 1982.
4. Casida, J. E.; Gammon, D. W.; Glickman, A. H.; Lawrence, L. J. Mechanisms of selective action of pyrethroid insecticides. Ann. Rev. Pharmacol. Toxicol. 23:413–438; 1983.
5. Cranmer, J. M. Seventh international neurotoxicology conference: Neurotoxicity and risk assessment (September 1989). Neurotoxicology 11:177–390; 1990.
6. Creason, J. P. Data evaluation and statistical analysis of functional observational battery data using a linear models approach. J. Am. Coll. Toxicol. 8:157–169; 1989.
7. Crofton, K. M.; Reiter, L. W. Effects of two pyrethroid insecticides on motor activity and the acoustic startle response in the rat. Toxicol. Appl. Pharmacol. 75:318–328; 1984.
8. Crofton, K. M.; Reiter, L. W. The effects of Type I and II pyrethroids on motor activity and the acoustic startle response in the rat. Fund. Appl. Toxicol. 10:624–634; 1988.
9. Edwards, P. M.; Parker, V. H. A simple sensitive and objective method for early assessment of acrylamide neuropathy in rats. Toxicol. Appl. Pharmacol. 40:589-591;1977.
10. Eldefrawi, M. E.; Sherby, S. M.; Abalis, I. M.; Eldefrawi, A. T. Interactions of pyrethroid and cyclodiene insecticides with nicotinic acetylcholine and GABA receptors. Neurotoxicol. 6:47–62; 1985.
11. Gad, S. C. Symposium: Screening for neurotoxicity (April 1988). J. Am. Coll. Toxicol. 8:5–239; 1989.
12. Gammon, D. W.; Lawrence, L. J.; Casida, J. E. Pyrethroid toxicology: Protective effects of diazepam and phenobarbital in the mouse and cockroach. Toxicol. Appl. Pharmacol. 66:290–296; 1982.
13. Gray, A. J. Pyrethroid structure-toxicity relationships in mammals. Neurotoxicol. 6:127–138; 1985.
14. Gray, A. J.; Rickard, J. Toxicity of pyrethroids to rats after direct injection into the central nervous system. Neurotoxicol. 3: 25–35; 1982.
15. Lawrence, L. J.; Casida, J. E. Pyrethroid toxicology: Mouse intracerebral structure-toxicity relationships. Pestic. Biochem. Physiol. 18:9–14; 1982.
16. Leukroth, R. W. Predicting neurotoxicity and behavioral dysfunction from preclinical toxicologic data. Bethesda, MD: Life

Sciences Research Office, Federation of American Societies for Experimental Biology; 1986.
17. Lund, A. E.; Narahashi, T. Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroids and DDT analogs. Pestic. Biochem. Physiol. 20: 203–216; 1983.
18. Material Safety Data Sheet. Permethrin. Philadelphia: FMC Corporation, Agricultural Chemical Group; 1980.
19. Material Safety Data Sheet. Cypermethrin. Philadelphia: FMC Corporation, Agricultural Chemical Group; 1983.
20. Meyer, O. A.; Tilson, H. A.; Bird, W. C.; Riley, M. T. A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav. Toxicol. 1:233-236; 1979.
21. Moser, V. C. Screening approaches to neurotoxicity: A functional observational battery. J. Am. Coll. Toxicol. 8:85–93; 1989.
22. Moser, V. C. Approaches for assessing the validity of a functional observational battery. Neurotoxicol. Teratol. 12:483–488; 1990.
23. Moser, V. C. Application and assessment of neurobehavioral screening methods in rats. In: B. L. Johnson, ed., Advances in neurobehavioral toxicology: Applications in environmental and occupational health. Chelsea, MI: Lewis Publishers; 1990: 419–432.
24. Moser, V. C. Applications of a neurobehavioral screening battery. J. Am. Coll. Toxicol. 10:661–669; 1991.
25. Moser, V. C.; MacPhail, R. C. Comparative sensitivity of neurobehavioral tests for chemical screening. Neurotoxicol. 11:335–344; 1990.
26. Moser, V. C.; McDaniel, K. L.; Phillips, P. M. Rat strain and stock comparisons using a functional observational battery: Baseline values and effects of amitraz. Toxicol. Appl. Pharmacol. 108:267–283; 1991.
27. Moser, V. C.; McCormick, J. P.; Creason, J. P.; MacPhail, R. C. Comparison of chlordimeform and carbaryl using a functional observational battery. Fund. Appl. Toxicol. 11:189–206; 1988.
28. Moser, V. C.; Sumrell, B. M.; MacPhail, R. C. Profiles of chemical effects using a neurobehavioral screening battery. The Toxicologist 12:349; 1992.
29. Narahashi, T. Site and types of action of pyrethroids on nerve membrane. In: J. E. Casida; M. Elliott, eds. Pyrethroid insecticides: Chemistry and action. Paris:Table Rounde Roussel Uclaf; 1980.
30. Narahashi, T. Nerve membrane ionic channels as the primary target of pyrethroids. Neurotoxicol. 6:3–22; 1985.
31. National Academy of Sciences. Toxicity testing: Strategies to determine needs and priorities. Washington, DC: National Academy of Sciences Press: 169–174; 1984.
32. Peele, D. B.; Crofton, K. M. Pyrethroid effects on schedule-controlled behavior: Time and dosage relationships. Neurotoxicol. Teratol. 9:387–394; 1987.
33. Ramadan, A. A.; Bakry, N. M.; Marei, A. S. M.; Eldefrawi, A. T.; Eldefrawi, M. E. Action of pyrethroids on $GABA_A$ receptor function. Pestic. Biochem. Physiol. 32:97–105; 1988.
34. Ray, D. E.; Cremer, J. E. The action of decamethrin (a synthetic pyrethroid) on the rat. Pestic. Biochem. Physiol. 10:333–340; 1979.
35. Registry of Toxic Effects of Chemical Substances. Department of Health and Human Services (NIOSH): Publication #87-114. Washington, DC: US Government Printing Press; 1987.
36. Reiter, L. W.; Anderson, G. E.; Laskey, J. W.; Cahill, D. F. Developmental and behavioral changes in the rat during chronic exposure to lead. Environ. Health Perspec. 12:119–123; 1975.
37. SAS Institute, Inc. SAS User's Guide: Statistics, version 5. Cary, NC: SAS Institute; 1985.
38. Scalzo, F. M.; Holson, R. R.; Paule, M. G. Symposium: Methods in behavioral toxicology and teratology (October 1989). Neurotoxicol. Teratol. 12:411–566; 1990.
39. Sette, W. F. Adoption of new guidelines and data requirements for more extensive neurotoxicity testing under FIFRA. Toxicol. Ind. Health 5:181–194; 1989.
40. Society of Toxicology. Poster-discussion session: Approaches to neurotoxicity screening (February 1992). The Toxicologist 12: 348–350; 1992.
41. U.S. Congress, Office of Technology Assessment. Neurotoxicity: Identifying and Controlling Poisons of the Nervous System. OTA-BA-436. Washington, DC: US Government Printing Office; pp. 105–144; 1990.
42. U.S. Environmental Protection Agency. Neurotoxicity Test Guidelines Addendum 10, Pesticide Assessment Guidelines Subdivision F. Publication number PB 91-154617. Springfield, VA: National Technical Information Services; 1991.
43. Verschoyle, R. D.; Aldridge, W. N. Structure-activity relationships of some pyrethroids in rats. Arch. Toxicol. 45:325–329; 1980.
44. Vijverberg, H. P. M.; van den Bercken, J. Action of pyrethroid insecticides on the vertebrate nervous system. Neuropath. Appl. Neurobiol. 8:421–440; 1982.
45. Vijverberg, H. P. M.; van den Bercken, J. Neurotoxicological effects and the mode of action of pyrethroid insecticides. Crit. Rev. in Toxicol. 21:105–126; 1990.
46. World Health Organization. Principles and methods for the assessment of neurotoxicity associated with exposure to chemicals. Finland: Environmental Health Criteria 60; pp. 32–58; 1986.
47. Wouters, W.; van den Bercken, J. Action of pyrethroids (rev.). Gen. Pharmacol. 9:387–398; 1978.

Appendix 1

Protocol for conducting a functional observational battery (modified from Moser et al., ref. 25).

HOME-CAGE MEASUREMENTS
CARRIED OUT WHILE RAT IS IN HOME CAGE

Posture (Descriptive)

1. Sitting or standing
2. Rearing
3. Asleep, lying on side or curled up
4. Flattened, limbs may be spread out
5. Lying on side, limbs in air
6. Crouched over
7. Head bobbing

Note: Only 1, 2, or 3 are "typical"

Involuntary Motor Movements (Descriptive)

Clonic

1. Repetitive movements of mouth and jaws
2. Quivers of limbs, ears, head, or skin (sometimes seen in untreated rats)
3. Mild tremors
4. Severe or whole body tremors
5. Myoclonic jerks
6. Clonic convulsions
7. Wet dog shakes

Tonic

1. Contraction of extensors such that limbs are rigid and extended
2. Opisthotonus: head and body rigidly arched backward
3. Emprosthotonus: head and body rigidly extended forward
4. Explosive jumps into the air with all feet leaving the surface
5. Severe clonic and/or tonic convulsions resulting in dyspnea, postictal depression, or death

Vocalizations (Quantal):

Spontaneous, not in reaction to being handled. Also includes spontaneous vocalizations in the open field.

Palpebral Closure (Ranked)

1. Eyelids wide open
2. Eyelids slightly drooping
3. Eyelids drooping approximately half-way
4. Eyelids completely shut

REMOVE RAT FROM CAGE

Ease of Removing Rat From Cage (Ranked)

1. Very easy (rat sits quietly, allows investigator to pick it up)
2. Easy (vocalizations, without much resistance to being picked up)
3. Moderately difficult (rat rears, often following investigator's hand.
4. Rat flinches (with or without vocalizations)
5. Difficult (runs around cage, or is hard to grab, with or without vocalizations)
6. Very difficult (tail and throat rattles, with or without vocalizations)

Reactivity to Being Handled (Ranked)

1. Low (no resistance, rat is easy to handle)
2. Moderately low (slight resistance to being handled, with or without vocalizations)
3. Moderately high (rat may freeze, or be tense, or rigid in hand, with or without vocalizations)
4. High (squirming, or twisting, or attempting to bite, with or without vocalizations)

MEASUREMENTS MADE WHILE HANDLING RAT

Remove from cage and hold in hand. Note (under "Other" on data sheet) such things as increased or decreased body tone, bite marks, soiled fur appearance, missing toe nails, piloerection, emaciation (shallow stomach, prominent spinal vertebrae), or death. (Observations such as piloerection may also be made while rat is on open field)

Lacrimation (Ranked)

1. None
2. Slight
3. Severe

Palpebral Closure

Same as in home-cage measurements.

Salivation (Ranked)

1. None
2. Slight
3. Severe

Piloerection (Quantal)

"+" indicates presence of piloerection (i.e., coat does not lie down after stroking).

OPEN-FIELD MEASUREMENTS

Rat is placed in the center of a flat surface with a perimeter barrier covered with clean absorbent paper for exactly 3 min. During this time, the number of rears is counted and other observations are made. (Suggested size of cart: approximately 60 × 90 cm with a 6.5 cm rim):

Rearing (Count)

Defined as each time the front legs of the rat come completely off the surface, although the rat does not necessarily have to raise itself up (i.e., this is a measure of the ability of the rat to place its weight on its haunches). Includes when the rat uses the side or lip of a cart top as support.

Involuntary Motor Movements

Same as in home-cage measurements.

Gait (Descriptive)

Note, if rat did not move during the 3-min observation period, it may be gently prodded (after the 3 min is over) in order to observe the gait.

1. Ataxia, excessive sway, rocks, or lurches
2. Hindlimbs show exaggerated or overcompensated movements, drag, or are splayed
3. Feet markedly point outward from body
4. Forelimbs drag, are extended, or unable to support weight
5. Walks on tiptoes
6. Hunched or crouched body position
7. Body drags or is flattened against surface

Gait Score (Ranked)

Ranking of gait abnormalities.

1. No abnormal gait
2. Slightly abnormal
3. Moderately abnormal
4. Severely abnormal

Mobility Score (Ranked)

Ability of rat to locomote despite gait abnormalities (different from gait score)

1. No impairment
2. Slightly impaired
3. Somewhat impaired
4. Totally impaired

Arousal (Ranked)

Level of unprovoked activity and alertness in the open field

1. Very low (stupor, coma)
2. Low (somewhat sluggish, some head or body movement)
3. Somewhat low (slightly sluggish, some exploratory movements with periods of immobility)
4. Alert, exploratory movements
5. Somewhat high (slight excitement, tense, excited, sudden darting or freezing)
6. Very high (hyperalert, excited, sudden bouts of running or body movements)

Stereotypy

Record any behaviors that are excessive or repetitive such as circling, stereotypic grooming, pacing, repetitive sniffing, or head weaving.

Bizarre Behavior

Record any unusual behaviors such as self-mutilation, Straub tail, retropulsion, writhing, flopping.

Excretion

At the end of 3 min, measure defecation and urination.

Defecation (Count)

Number of fecal boluses on paper. "D" will be recorded if diarrhea is present

Urination (Count)

Number of pools of urine on the paper. "X" will be recorded if polyuria, or overlapping pools, is present

STIMULUS REACTIVITY

Performed while rat is sitting on cart surface.

Approach Response (Ranked)

Approach rat head-on with the end of a blunt object, such as a pencil, hold approximately 3 cm from face for 4 s

1. No reaction
2. Rat slowly approaches and sniffs or turns away
3. Rat flinches, actual muscle contractions
4. More energetic response than 2) or 3)
5. Exaggerated reaction—jumps, bites, or attacks

Touch Response (Ranked)

Coming in from the side, touch rump gently with blunt object, such as a pencil

1. No reaction
2. Rat may slowly turn or walk away, or vocalizations with little or no movement
3. Rat flinches, actual muscle contractions
4. More energetic response than 2. or 3)
5. Exaggerated reaction: jumps, bites, or attacks

Click Response (Ranked)

Position clicker approximately 5 cm above the back of the rat and make sudden sound.

1. No reaction
2. Slight reaction, some evidence that noise was heard
3. Rat flinches, actual muscle contractions
4. More energetic response than 2) or 3)
5. Exaggerated reaction—jumps, bites, or attacks

Tail Pinch Response (Ranked)

Metal tweezers are used to squeeze the tail approximately 2-3 cm from the tip

1. No reaction
2. Rat may turn or walk forward, or vocalizations with little or no movement
3. Rat flinches, actual muscle contractions
4. More energetic response than 2 or 3
5. Exaggerated reaction: jumps, bites, or attacks

Pupil Response (Quantal)

The beam of a penlight flashlight is brought in from the side of the rat's head. Constriction of the pupil is noted with a "+", and "−" indicates lack of response. (This may be difficult to observe in some strains of rats and may be dependent on ambient lighting conditions)

Righting Reflex (Ranked)

Rat is held supine, then dropped from approximately 30 cm. Score ease of landing. Note, if the rat is paralyzed or severely affected, this test and the landing foot splay will not be carried out so as not to injure the rat.

1. Rat lands on feet
2. Slightly uncoordinated
3. Lands on side
4. Lands on back

Forelimb and Hindlimb Grip Strength (Continuous)

Strain gauges are used with wire mesh screens for the rats to grab. Screen for the forelimb grip measurement is oriented horizontally from the strain gauge, while the hindlimb grip screen is placed at a 45 angle from the gauge to allow full contact with the hind feet when the rat is pulled off the support platform. Two readings are taken and averaged (modified from Meyer et al., ref. 9).

Body Weight (Continuous)

Body Temperature (Continuous)

Rectal temperature is taken and thermister allowed to stabilize before reading

Landing Foot Splay (Continuous)

Fourth digit pads of hind feet are dotted with Tempra paint. Rat is dropped twice from prone position 40 cm from paper, and ink spots where he lands are noted. Measure distance between middle of ink blots, and average (modified from Edwards and Parker, ref. 20).

Other

Includes torn toenails, broken teeth, soiled fur, fur discoloration, convulsions at any time other than in the home cage or open field, crustiness around face or eyes, red pigmented excretions from eyes, or any findings which may impact the data.

PERMETHRIN

Page_____ is not included in this copy.

Pages 51 through 55 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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Appendix

Review of a special neurotoxicity study with the positive control substance acrylamide.

Study Title: "Validation Study for a Neurotoxicity Screen in Rats With Acrylamide"

Author: Christine Freeman

Laboratory: FMC Corporation Toxicology Laboratory, Princeton, New Jersey

Study No.: A91-3482

Study Date: July 20, 1993

MRID No.: 429337-01 and 430463-01 (study was submitted twice and has two MRID Nos.

This study used most of the same procedures and apparatus as the acute neurotoxicity screen study with permethrin (MRID NO.: 430463-01) which this Appendix accompanies. Details of this review are confined only to the special use of acrylamide and the neurotoxicity results associated with this chemical.

Basic Experimental Design

In this study, two groups of 10/sex Sprague-Dawley CD rats (Charles River Laboratories, Kingston, New York, about 6 weeks of age at start of dosing) were dosed as either controls or with 40 mg/kg/day with acrylamide (a white powder obtained from the Sigma Chemical Company, lot #129F-0046, prepared as a 1% weight/volume solution/suspension in water) for 11 days. Dosing was by gavage at a rate of 4 ml/kg. The controls received water only. The rats were assessed daily for 14 days for clinical signs. FOB and motor assessments were made at pretest, day 0, 7 and 14. After day 14 the rats were sacrificed, perfused and subjected to neurohistopathological assessments.

The experimental procedure of dosing the animals for 11 days means that this study is neither an acute or a subchronic neurotoxicity study. The study is, however, considered useful in validating some methods used for neurotoxicity assessment.

Results

There were no deaths. Body weight at day 14 was statistically significantly ($p < 0.001$) decreased for both males (13%) and females (15%). Female body weight was also reduced at day 7 (6.7%, $p < 0.05$) and males were 3.3% lower but not significantly.

5B

Males gained 46% less weight than the controls and females gained 70% less than the controls.

The following clinical signs were noted in the dosed animals but not in the controls:

Clinical Sign	Males	Females
Ataxia	10/51	10/60
Splayed hindlimbs	10/51	10/54
Staggered gait	10/66	10/83
Toes curled in feet	9/24	10/39
Tremors	8/31	9/45
Decreased locomotion	7/11	5/15
Ventral surface on floor	6/10	4/8
Piloerection	10/34	6/18
Abdominogenital staining	3/11	2/5
Unkempt	2/4	-/-
Rales	3/10	5/10
Chromorhinorrhea	2/2	1/1
Chromodacyorrhea	1/1	1/1
Hematuria	-/-	1/1

Numerator = Number of animals affected/Denominator = number of times sign was noted over the duration of the study.

In general the first sign to appear was staggered gait which was noticed in females as early as day 6 and in males on day 7. The observation of tremors and splayed hindlimbs usually came one or two days later.

No acrylamide related effects were noted in the FOB assessments on day zero. Based on the stated time of dosing (between 8 and 9 AM) and the time stated for initiating the FOB assessments (10 AM), effects might not be expected to be noted within the 1-2 hours after initial dosing.

At day 7 and/or 14, FOB assessments indicated the following signs which were not also noted in the controls of either sex:

Signs	Males		Females	
	Day 7	Day 14	Day 7	Day 14
Ataxia-of	5	10	8	10
Splayed hindlimbs-of	0	10	0	9
Impaired gait-of	5 (s)	10 (m)	8 (s)	10 (m)
Landing foot splay-of	+46%*	+96%*	+22%*	+75%*
Decreased activity-of	0	10	0	8
Whole body tremors	0	0	0	6
Poor righting reflex-of	0	10	0	10
Aggressive/tense rigid-hc	0	2	0	5
Unkempt/soiled-hc	0	2	0	2
Piloerection-hc	0	10	0	6
Urine pools-hc	(+?)	++	-	-
Forelimb grip strength-of	-9%ns	-35%*	-13%ns	-39%*
Hindlimb grip strength-of	-8%ns	-64%*	+1%ns	-51%*

Data from Table 4 pages 396 to 415.
Based on ten animals per sex. - = not observed. + slight amount, ++ more.
ns = not significant. * statistically significant $p < 0.05$ or less.
of = based on open field observation, hc = based on home cage observation.
% = percent different form the controls.

Concordance between clinical signs and FOB observations is considered reasonable. The FOB at day 7 indicated some signs (such as landig foot splay, poor righting reflex, aggressive and tense disposition, urine pools and grip strength that were not noted in clinical observations.

Motor activity was reduced relative to the controls as indicated in the following table.

Interval	Males	Females
Day 0	-39%***	+2%
Day 7	-66%**	-63%***
Day 14	-79%***	-64%***

Data are from Table 5 pages 460 to 467.

Five males and five females were perfused and prepared for histopathology as described in the preceding DER. The sections were assessed for pathology at the Experimental Pathology Laboratories, Inc. by Drs. Beverly Y. Cockrell and Dr. Jerry Hardisty of EPL's North Carolina Laboratory. Based on the consensus of the two pathologists, the only neuropathological lesion attributed to treatment was "myelin bubbles" in either the sciatic, tibial or sural nerves. A photograph of these bubbles was presented in the study report. In summary the following animals were affected.

Control: One female had myelin bubbles in the sural nerve that was graded minimal.

Treated males: A total of 4 males with a total of 7 nerve structures had myelin bubbles (2 in each (left and right) of the

sciatic and sural nerves and 3 in the tibial nerve) were affected. Severity ranged from minimal to slight.

Treated females: All five females assessed were affected with myelin bubbles for a total of 12 nerve structures (4 in each of the sciatic, tibial and sural nerves). Severity ranged from minimal to moderate.

SUMMARY: This study does not assess the effects of a pesticide and is therefore not classified. The study provides a very useful demonstration of the neurotoxicity effects of acrylamide when dosed daily for 11 days by successive gavage administrations. The clinical signs were noted to be in reasonable concordance with the FOB battery assessments and the FOB assessment indicated signs of toxicity for some parameters at earlier times than did the clinical observations. Neurohistopathological findings were confined to the peripheral nerves and consisted of "myelin bubbles".

PERMETHRIN

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