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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID# 069001. Pyrethrins. Review of a series 81-8ss acute neurotoxicity screen study.

TOX CHEM No.: 715
PC No.: 069001
Barcode No.: D195297, D19550
Submission No.: S448789, S 442433

FROM: John Doherty 3/28/94
Section IV, Toxicology Branch I
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TO: Bruce Sidwell
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THROUGH: Marion Copley, DVM, Section Head
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I. CONCLUSION

The series 81-8ss acute neurotoxicity study with pyrethrins (MRID No.: 429258-01) was reviewed and determined to be CORE GUIDELINE. The study demonstrated NOEL and LELs for neurotoxicity of 20 and 63 mg/kg based on the presence of tremors in females. Pyrethrins were demonstrated to show neuropathy with LELs of 200 mg/kg in females and 400 mg/kg in males. No additional series 81-8ss neurotoxicity data are required at this time.

II. Action Requested

The Chemical Specialties Manufacturers Association on behalf of their client the Pyrethrin Joint Venture (refer to letter from Ralph Engel dated September 17, 1993) has submitted a series 81-8ss acute neurotoxicity study as a part of the requirements for the reregistration of pyrethrins. The study was reviewed and the following comments apply.

C10583

III. Toxicology Branch Comments

1. The study is further identified in Section IV below. A copy of the DER is attached.

2. The study was classified as CORE GUIDELINES.

3. The study demonstrated that in the higher dose levels (200 mg/kg in females and 400 mg/kg in males) peripheral neuropathy in the form of myelin/axonal degeneration resulted. This condition was described as minimal meaning that only a few fibers were affected and no more than 5 females and 3 males were affected. in the high dose groups. Possible but indefinite indications that lower does may be threshold doses for this condition were noted because one female in the 20 mg/kg dose group and one male in the 125 mg/kg dose group also had this condition but the controls did not. The problem of the potential for pyrethrins to cause neuropathy in rats has been considered since the 1970s. There does not seem to be consistent results among existing studies. This study can be interpreted as demonstrating that pyrethrins can cause a detectable neuropathy. The low degree of response (minimal) at the highest and only dose level affected (400 mg/kg), however, does not indicate that special considerations be given for this endpoint in toxicity. For example, the study NOEL (20 mg/kg) and LEL (63 mg/kg) will probably be used for risk assessments.

The potential for pyrethrins to induce neuropathy will be further evaluated in the series 82-7ss subchronic neurotoxicity study when this study is submitted.

Acute and subchronic neurotoxicity studies with other pyrethroids should be critically assessed for the methodology and techniques used for histopathology of the peripheral nerves to evaluate for myelin/axonal degeneration or similar lesions resulting form exposure to these chemicals.

IV. Study Reviewed.

Study Identification	Material	MRID No :	Results	Classification
81-8ss. Acute neurotoxicity screen - rats Bushy Run Research Center Study No.: 92N1026 (main study) and 91N0122 (range finding study) Sept. 14, 1993	Pyrethrin extract 57.467% Lot LS-92-3?	429258-01 (main study) 429304-01 (range finding study)	NOEL and LEL = 20 and 65 mg/kg. At 63 mg/kg: fine tremors in females (2/15). At 125 mg/kg (males): decreased motor activity. At 200 (females) and 400 (males) mg/kg: deaths; coarse tremors, exaggerated startle response, increased body temperature, and decreased grip strength, anogenital staining and salivation; increased motor activity (total and fine movement) but decreased ambulation and rearing. Neuropathy (minimal focal or multi focal myelin/axonal degeneration in sciatic, peroneal or tibial nerves. Charles River CD strain rats. Dose levels tested: 0, 20, 63 or 200 mg/kg in females and 0, 40, 111125 or 400 mg/kg in males in corn oil.	GUIDELINE

(A)

[81-8. pyrethrin extract/1993]

Reviewed by: John Doherty, Ph.D., D.A.B.T. 3/16/94
Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Linnea Hansen, Ph.D. 3/16/94
Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 81-8. Acute neurotoxicity - rats

MRID NO.: 429258-01 (main study) TOX. CHEM. NO.: 715
429304-01 (range finding study) PC No.: 069001

TEST MATERIAL: Pyrethrin Extract (57.467%)

STUDY NUMBER(S): 92N1036 (main study), 91N0122 (range finding study).

SPONSOR: Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association

TESTING FACILITY: Bushy Run Research Center, Export, Penn.

TITLE OF REPORT: "Acute Oral Neurotoxicity Study with Pyrethrum Extract in Rats"

AUTHOR(S): S.J. Hermansky and J.M. Hurley

REPORT ISSUED: September 14, 1993 (main study) and September 16, 1993 (range finding study)

STUDY DATES: April 20 to May 8, 1993.

Executive Summary:

In this study designed to assess potential acute neurotoxicity, 4 groups 15/sex of Charles River CD® strain rats (Portage, Michigan facility) with 0, 40, 125 or 400 mg/kg (males) or 0, 20, 63 or 200 mg/kg (females) total pyrethrins once by gavage (derived from pyrethrum extract containing 57.467% pyrethrins) in corn oil. Specific neurotoxicity assessments included FOB and motor activity assessments at pretest, 3 hours post dosing and on days 7 and 14. On day 15, the rats were perfused and selected regions of their nervous system assessed histologically.

Females were more sensitive than males. At 63 mg/kg, 2 of 15 females developed fine tremors; at 125 mg/kg/day males had decreased motor activity (35% total activity, 39% fine movements 35% ambulation and 25% rearing based on cumulative session counts). At 400 mg/kg an increase in total (34%) and fine movement (146%, presumably related to the tremors) was evident but ambulation (-30%) and rearing (-54%) were still decreased. At 400 mg/kg the first intrasession motor activity in males

indicated decreased activity but distinctly higher activity was noted after the rats acclimated to the chamber. In females, the 200 mg/kg dose group had increased motor activity (46% total and 161% fine movement) whereas ambulation and rearing (both -60%) were decreased. At the 400 (males) and 200 (females) mg/kg dose levels deaths (5 males and two females), coarse tremors (10 of 13 males and 7 of 10 females), exaggerated startle response (9 males, 6 females), increased body temperature (about 1 degree C), decreased grip strength in males (21%) and anogenital wetness and salivation were noted in some rats. Body weight was at most 5% decreased and not statistically different. Neurohistological effects attributable to the test material in the high dose groups such as **minimal focal or multifocal myelin/axonal degeneration** in sciatic, tibial or peroneal nerves (no more than 5 females and 3 males of the 10/sex examined were affected). A single female in the low and male in the mid dose group were affected with myelin axonal degeneration but were not considered definite respo to treatment. **The LEL is 63 mg/kg based on the presence of tremors in females. The NOEL is 20 mg/kg.**

The study is classified as CORE GUIDELINE and satisf the requirement for series 81-8ss acute neurotoxicity testing. N additional series 81-88ss data are required at this time. It is noted that the study did not include GFAP assessments to verify the low grade histopathological changes noted.

Quality Assurance Statement: Provided
Good Laboratory Practice Statement: Provided

REVIEW

Experimental Constants:

Test Chemical: Pyrathrum extract from Lot No.: LS-92-37, Task Force Blend FEK-99 obtained from the Fairfield American Corporation. It was described as a viscous liquid and stored refrigerated at 5 °C. The percent active ingredient was determined by the sponsor to be 57.467%. The test material was dissolved in corn oil (Mazola®) prior to gavage and the dosages are in total pyrethrins. The same solutions were reportedly used over the four day period.

Analytical Chemistry: Data on homogeneity indicated that the 5% solution was 91.8 to 92.8% of nominal and for the 10% sample 93.2 to 96.2% of nominal. The concentrations of the test solutions were assessed to be within 9% of nominal concentration.

The pyrethrin dosing solutions were also said to be stable over the four day period when stored at room temperature and protected from UV light (by means of black electrical tape covering the container).

Test System: Charles River CD® strain rats were obtained from the Charles River Company, Portage, Michigan. They were reportedly 28 days old on arrival and they were approximately 7 weeks old at dosing. Males were 219 to 284 gms and females were 136 to 182 gms at the first day of treatment. They were housed individually during the experiment.

Basic Experimental Design: The dose levels were selected based on a preliminary range finding study (MRID No.: 429304-01, no DER has been prepared for this study) which demonstrated that females were more sensitive than males and that the peak of symptoms was at 3-5 hours after test material administration. In the definitive study, testing was conducted in 8 replicate groups tested over a four consecutive day period, 2 replicates/group/day. The specific dosing conditions and dose levels used for the definitive study are as follows:

Group	No./ Sex	Males Dose Level	Males ml/kg[1]	Females Dose Level	Females ml/kg
Control	15	0	4.00	0	4.00
Low	15	40 mg/kg	0.40	20 mg/kg	0.40
Mid	15	125 mg/kg	1.25	63 mg/kg	1.25
High	15	400 mg/kg	4.00	200 mg/kg	4.00

1. Males were dosed with 10% and females were dosed with 5% total pyrethrin in oil. ml/kg-dose volume administered.
Note: Doses are in total pyrethrins, not extract.

It is noted that different volumes of corn oil were used for the different dose levels. TB-I prefers that the dosing volumes be kept constant in acute toxicity tests. The practice of varying the volume alone, however, in the absence of other compromising factors is not considered sufficient to invalidate the study. The volume of corn oil used is not in excess of the 5 ml/kg recommended by HED.

Statistics:

Statistical Test	Parameter Analyzed
When Levine's test indicated similar variances and ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levine's test indicated heterogeneous variances, all groups were compared by ANOVA for unequal variances and where necessary by a separate variance t-test for pairwise comparisons.	Quantitative continuous variables
Fisher's exact test Gamma, Kendall's Tau-B, Stuart's Tau-C and Somer's D measures of association.	Incidence data Incidence data for select FOB endpoints with ordered severity scores for group differences.
Repeated measures analysis of variance with dose as the grouping factor and test period and test session time as within subject factors. These data were also adjusted using the epsilon adjustment procedure (Greenhouse-Geisser correction).	Motor activity. Parametric tests were also use to assess the cumulative test session activity.

Specific Methods and Results

1. Deaths and Clinical Signs. Cages were reportedly inspected twice daily for deaths and the rats were examined once daily for clinical signs.

Five males and two females all in the high dose group were found dead on the day of dosing. Obvious reactions or clinical signs were noted in the high dose group only on the day of dosing and included tremors, urogenital wetness (one male and 5 females), and salivation (3 females). Some of these are further described under the FOB assessments.

2. Body Weight and Gain. Assessments made at prior to dosing and after 7 and 14 days. Decreases in body weight did not reach statistical significance although the body weight for the high dose group males was 5% less than the control at the 7 and 14 day weighing.

3. Functional Observational Battery. Assessed at pretest, day 0 (at the time of estimated maximum response to the test material, 3 hours for males and females) and on days 7 and 14. The rats were subjected to qualitative and quantitative assessments of condition as observed. The technicians assessed the animals unaware of their treatment and the same observer evaluated the animals from one sex at each testing period. The FOB parameters investigated included:

Posture-c	Convulsions-c,a	Handling reactivity
Tremors-c,a	Vocalization-a	Palpebral closure-c,a
Unusual behavior-a	Gait-a	Body position-a
Breathing pattern-a	Arousal-a	Defecation-a
Urination-a	Rears-a	Approach response-a
Startle response-a	Tail pinch response-a	Pupil response-h
Muscle tone-h	Piloerection-a	Lacrimation-h
Salivation-h	Exophthalmus-h	Emaciation-h
Dehydration-h	Fur appearance-h	Crust(mouth, nose, eyes)-h
Visual placing-h	Grip strength-s	Rectal temperature-s
Body weight	Air righting reflex-s	Hindlimb splay-s

c-evaluated primarily in the home cage
a-evaluated in over a two minute span in a special arena (not described)
h-evaluated while handling the rat
s-special equipment needed for assessment.

4. Motor activity. Motor activity was assessed using an automated recording apparatus (San Diego Instruments Inc), and the test were for 90 minutes duration with 10 minute intrasession intervals. Data for ambulatory and fine motor activity and rearing and total activity were generated. The rats were reportedly assessed for motor activity just after the FOB assessment or at four hours after treatment and again on days 7 and 14.

Table 1 below illustrates the frequency of responses for the FOB and motor activity parameters noted to be affected by treatment.

Table 1. FOB, body temperature and motor activity parameters determined to be affected by pyrethrins following acute oral administration and assessed on the day of dosing.

	Males				Females			
Parameter N	Control 15	40 15	125 15	400 14	Control 15	20 15	63 15	200 12-15[2]
FOB assessments								
Fine tremors	0	0	0	3	0	0	2	7
Coarse tremors cage/arena[1]	0	0	0	10/13	0	0	0	7/10
Gait (splayed)	0	0	1	2	0	0	0	6
Startle | exagg.	0	1	0	9	0	0	0	6
response | none	0	0	0	0	0	0	0	1
Grip strength fore kg ±sd	0.69 0.099	0.70	0.66	0.59 0.182	0.67 0.112	0.72	0.62	0.48** 0.151
Grip strength hind (kg) ±sd	0.58 0.079	0.57	0.56	0.46** 0.128	0.47 0.092	0.52	0.46	0.44 0.088
Body temperature °C ±sd	38.12 0.323	37.88	38.19	39.10** 0.571	38.22 0.386	38.04	38.09	39.65** 0.434
Crusts-eye/nose	0	0	0	5	0	0	0	5
Motor assessments[3] N	15	15	15	12	15	15	15	13
Total counts ±sd	656 194	548 173	428** 164	878 485	737 306	808 328	665 295	1075 763
Fine movement ±sd	297 111	249 95	182** 76	658* 513	330 132	369 149	325 139	862* 760
Ambulation ±sd	224 74	183 63	146** 60	157* 63	243 113	248 117	195 97	147* 82
Rearing ±sd	135 40	116 36	101* 43	62** 52	164 82	191 97	147 84	66* 48

Data are incidence unless another unit of measure is indicated.
1. Numerator is the incidence noted at home cage observation/denominator is the incidence noted in the arena observation.
2. Due to animals dying the number available for examination differed. Tremors and gait are based on 14 females, startle response and crusts are based on 13 animals and motor and grip strength assessments are based on 12 animals.
3. Data presented are cumulative test session counts. These data are discussed in terms of the intrasession counts below.
* $p < 0.05$ and ** $p < 0.01$ study report statistics. Note the standard deviations (sd) are presented for selected samples but not all to conserve the clarity of the table. When no sds are presented, the sds were similar to those presented.

Table 1 above indicates that the females were affected

with fine tremors (an FOB parameter and referring to whole body tremors) and the males had decreased motor activity (total, fine movement, ambulation and rearing) in the mid dose (63 mg/kg for females and 125 mg/kg for males) levels. Several other parameters as indicated in the table were affected in the high dose group for males and females.

Based on total cumulative test session counts, total motor activity and fine movement were increased in the high dose males and females but were decreased in the mid dose males. The decrease in ambulation and rears in the high dose females is consistent with the decrease in the mid and high dose group males for these two parameters.

Apparently some aspects of the motor activity response are biphasic based primarily on the male data when the individual sessions counts are considered. Figure 3 (page 48 of the report, attached) indicate that for males all four motor activity parameters (total, fine movement, ambulation and rearing) are lower for the mid and high dose groups at the initiation of testing. As the males adjust to the test apparatus, cumulative counts and fine movement are increased relative to the control for the high dose group. A somewhat similar response was noted for females for total counts (Figure 5, page 54 of report attached). Fine movement was consistently higher at all times assessed. The study author presumes that the increase in fine movement is related to the tremors in the high dose group animals. The decrease in ambulation and rearing noted for both sexes means that the treated animals were less mobile. Overall the conclusion is that pyrethrin treatment affects motor activity at the mid and high dose males and high dose females. The mid dose pretreatment scores were lower than controls but not statistically significant as were the effects in this group when assessed at 4 hour posttreatment. In addition rearing was not lower at pretreatment but was at four hours posttreatment. Thus, TB-I concludes that motor activity in the mid dose male group is affected.

In addition to tremors and motor activity effects, the FOB indicated that in the high dose groups the startle response, hind limb (males, 21%)) and fore limb (females, 28%) grip strength was decreased and body temperature was increased (males by 0.98 degree C and females by 1.43 degree C).

The day 7 and day 14 FOB and motor assessments did not indicate test material related effects.

5. Histopathology and Perfusion Studies. A separate pathology report was presented in Appendix 2 of the study report and prepared by Robert H. Garman, DVM.

The rats were anesthetized with sodium pentobarbital 15

days after treatment and all male and female surviving rats were perfused with 10% neutral buffered formalin by intracardiac perfusion. After perfusion, the brain and spinal cord and the peripheral nerves in the hind limb were removed and further fixed in neutral buffered formalin. The brains were weighed. The rats were given a gross necropsy examination. Initially neural tissue from 10 rats/sex from the control and high dose groups were examined histopathologically. Indications of possible pathological effects in the high dose animals later led to the examination of selected tissue from the low and mid dose groups. The following subdivisions of the brain, spinal cord and peripheral nerves were prepared for assessment:

forebrain-cs	spinal cord
center of cerebrum-cs	cervical-cs,ls
center of midbrain-cs	thoracic-cs
cerebellum and pons-cs	lumbar-cs
medulla oblongata-cs	
gasserian ganglia-cs,ls	dorsal and ventral spinal
dorsal root ganglia-cs,ls	nerve roots-cs,ls
proximal sciatic nerve	peroneal (fibular) and sural
(above knee)-cs.ls	nerves (below knee)-ls
tibial nerve (below knee)-ls	

cs = cross section, ls = longitudinal section

The sections of the brain, spinal cord, gasserian ganglia, nerve roots, and dorsal root ganglia were embedded in paraffin, cut into 6 micron sections and stained with hematoxylin and eosin, luxol fast blue and Bielschowsky's techniques. The peripheral nerves were embedded in glycol methacrylate, sectioned at 2 microns and stained with hematoxylin and eosin, toluidine blue and the Bielschowsky's techniques.

Brain weight. Brain weights from all surviving animals (including those which were obtained from perfused animals) were equivalent to the controls for both sexes. Note: brain length and width data were not presented and are not considered necessary although they are recommended by the guidelines.

Neurohistopathology. Some 37 individual substructures were assessed for histopathological changes. These included the various substructures of the brain and spinal cord and peripheral nerves. The occurrence of the lesions as indicated by examination of 10 rats/sex/group is as follows.

Control Groups. [0/10 males and 1/10 females with lesions].

A single female had minimal myelin sheath swelling in the sciatic nerve.

Low Dose Groups. [2/10 males and 1/10 female with lesions]

Two males had minimal myelin/axon degeneration in the sciatic nerve with one incident each of "focal" and "multifocal".

One female had minimal focal myelin/axon degeneration in the peroneal nerve.

Mid Dose Groups. [1/10 male affected and 0/10 females with lesions]

One male had minimal myelin/axonal degeneration in the sciatic nerve.

No females were reported to have lesions.

High Dose Groups. [3/10 males and 5/10 females with lesions].

Three males were reported with lesions in the sciatic nerve. Two had minimal focal myelin degeneration and one had minimal focal myelin/axonal degeneration.

Five females were reported to have lesions in either the sciatic, tibial or peroneal nerves.

One female (rat #2899) had mild multifocal myelin/axonal degeneration in its sciatic, tibial and peroneal nerves as well as pons (vacuolation of the cochlear nucleus moderate focal) and caudate nucleus (mild multifocal of myelinated fibers, not active).

One female rat (rat #2908 had minimal multifocal myelin/axonal degeneration of the sciatic nerve and myelin degeneration of the tibial nerve.

Three other female rats had single lesions. Rats #2913, 2926 and 2928 had minimal multifocal sciatic nerve myelin degeneration.

Note: These five females did not share common patterns for effects on motor activity. Animal #2899 with the most lesions was lower than most other animals in each motor category but was not the lowest and did not have tremors. Animal #2913 was lower in some categories but had some of the highest scores for the test group for other categories. Of these five females, only rat #2908 had tremors.

TB-I notes that one female in the low dose group and one male in the mid dose group also had the degeneration lesion whereas only swelling was noted in the controls. Due to a lack of a dose response for females (none were reported affected in the mid dose group) and only a single animal was affected in the mid dose group males, TB-I considers their association with treatment less definite but acknowledges that these dose levels may be threshold. In conclusion, TB-I considers that only the high dose group has definite compound related neuropathological lesions. These are considered minimal and females are more frequently affected than males.

E. Immunochemistry:

No immunochemistry for assessment of glial fibrillary acidic protein (GFAP) was performed.

DISCUSSION. This study is classified as CORE GUIDELINE and satisfies the requirement for a series 81-8ss acute neurotoxicity screen. No additional series 81-8ss study data are required at this time. The study demonstrated that females were more sensitive than males to induction of tremors and other FOB or motor functions. At the high dose levels (200 mg/kg in females and 400 mg/kg in males), there was evidence of pyrethrin induction of minimum focal or multifocal myelin/axonal degeneration in either the sciatic, peroneal or tibial nerves. None of the controls but one low dose female and one mid dose male had this condition. The potential for pyrethrins and the pyrethroids to potentially cause neuropathy has been considered before. There is a lack of consistency in the various studies in assessing this phenomenon. The current study indicates that pyrethrins have such a potential to induce neuropathy. In this regard, the neurohistopathlogical methods and results of other neurotoxicity screen studies (both acute and subchronic) with pyrethroids should be critically assessed for similar evidence of myelin/axonal degeneration.

Pyrethrin

Page_____ is not included in this copy.

Pages 13 through 14 are not included.

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