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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

ADDENDUM TO DERS For Fortress

From: Karen L. Hamernik, Ph.D. Karen Hamernik 12/12/94
Section Head, Section 3, Tox Branch I

Titles: 1. Acute Neurotoxicity Of DPX-43898 [Fortress Tech] in Rats (MRID 425592-10)
2. Subchronic Neurotoxicity: 90-Day Study of DPX-43898 Feeding Study in Rats (MRID 425592-17)

Dr. William Sette (HED/SACB) reviewed the Fortress neurotoxicity database and made a number of recommendations which were accepted by Toxicology Branch I. Thus:

1. For the rat acute neurotoxicity study (MRID 425592-10):

a) the study is considered to be Supplementary and not upgradeable (as was the conclusion in the DER). A major deficiency in the study is the absence of histopathological examinations (following perfusion) as specified in the guidelines. In addition, there appeared to be either a problem with dose solution preparation accuracy or a problem with the analytical method;

b) NOELs/LOELS for neurotoxicity can be established for the study as performed (e.g. for males NOEL = 1.14 mg/kg and LOEL = 1.18 mg/kg based on decreases in horizontal and vertical motor activity and for females NOEL = 0.32 mg/kg and LOEL = 0.76 mg/kg based on decreased vertical motor activity). However, with no histopath evaluation, confidence in these conclusions is decreased.

c) because of recent concerns about neurotoxicity associated with exposure to the chemical chlorpyrifos, it is prudent for the Agency to have a reasonable degree of confidence in the neurotoxicity databases associated with other organophosphate chemicals, including Fortress and to ensure that the databases for these chemicals are as complete as possible under existing guidelines and data requirements. Therefore, a new acute neurotoxicity study with Fortress technical by the gavage route in the rat is being required which includes a histopathological exam (following perfusion), evaluation of

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plasma, rbc and brain cholinesterase activities following a single dose of the test material, and body temperature evaluations. It is recommended that the protocol be submitted to the Agency for comment prior to starting the study.

In addition, an NTE (neurotoxic esterase) study in the hen (including the assay of NTE activity) as per the 1991 Neurotoxicity Guidelines is also being required. It is recommended that the protocol be submitted to the Agency for comment prior to starting the study.

2. For the rat subchronic neurotoxicity study (MRID 425592-17):

a) the study is considered to be Supplementary and not upgradeable (as was the conclusion in the DER). Confidence in the results of this study was reduced because of inconsistences with two other Fortress 90-day feeding studies in the rat (MRID 412906-27 and MRID 425592-15). Obvious clinical signs of toxicity and mortality were noted at doses of 10 ppm and above in these two studies while no frank signs of toxicity were apparent in the neurotoxicity even at 12.8 ppm. Since cholinesterase determinations were not made in the study values, were not available to serve as points of reference with other studies.

b) variations in analytical results (homogeneity and concentration) were noted, but the severity of the variability was probably not enough, in and of itself, to compromise the study;

c) the requirement for a new subchronic neurotoxicity study in the rat (probably by gavage) is Reserved pending the receipt and evaluation of the new rat acute neurotoxicity study and NTE study in the hen;

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Reviewed by: John Doherty, Ph.D., D.A.B.T. John Doherty 5/13/94
Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Linnea Hansen, Ph.D. Linnea J. Hansen 5/13/94
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DATA EVALUATION REPORT

STUDY TYPE: 81-8. Acute neurotoxicity - rats

MRID NO.: 425592-10 TOX. CHEM. NO.: 663P
PC No.: 129006

TEST MATERIAL: DPX-43898 (Fortress)

STUDY NUMBER: 193-92 (Laboratory Report No.)

SPONSOR: E.I. Dupont de Nemours

TESTING FACILITY: Haskell Laboratory, Newark, Delaware

TITLE OF REPORT: "Acute Neurotoxicity Study of DPX-43898 in Rats"

AUTHOR: Elizabeth A. Lochry, Ph.D.

REPORT ISSUED: August 6, 1992 (as revised)
In-life phase September 19, 1991 to November 1/91.

EXECUTIVE SUMMARY:

Six groups of male and 4 groups of female rats (Crl:CD^{R}BR strain, 12/sex/group) were dosed with DPX-43898 (Fortress, 86% purity) by gavage at dose levels (expressed based on the analytical concentrations) of control (two groups), 1.14, 1.18, 1.95 or 2.78 mg/kg for males and as control, 0.32, 0.76 or 0.746 mg/kg for females in 0.5% methyl cellulose administered at 10 ml/kg. The rats were assessed daily for general signs of toxicity and for FOB and motor activity at pretest, day 0 ("immediately" after dosing), day 7 and day 14.

Several effects all attributed to secondary neurotoxicity resulting from inhibition of ChE/AChE were observed. At the lowest doses body weight gain was decreased (transiently) for both males (-14%) and females (-13%). Males displayed decreased motor activity (-22% for horizontal and -24% for vertical) and their righting reflex was impaired (one rat) at 1.14 mg/kg and one animal had wet perineum (assumed to be related to increased excretory activity. At the mid dose levels, tremors and gait impairment and associated effects on locomotion and coordination were noted in males (4 to 5 animals) and females (3 to 4 animals). Other symptoms noted included salivation, pupil response, decreased arousal and tail pinch response. These effects were transient and noted on the day of dosing only although motor activity for the higher dose groups may have persisted to at least week 1. Deaths resulted within several hours of dosing at dose levels of 1.95 mg/kg (2 rats) and 2.78 mg/kg (10 rats) among

the males and at 0.746 mg/kg (5 rats) among the females. No necropsy or histopathology reports were presented. **The LEL for acute neurotoxicity is < 0.32 mg/kg based on decreased body weight gain in females. No NOEL for acute neurotoxicity was established for either sex.**

Classification: SUPPLEMENTARY. No NOEL was established and no histopathology was conducted. Since the rats were not prepared for histopathology by perfusing at sacrifice, this study cannot be upgraded. Additional series 81-8ss study data may be required pending a need for such data for acute neurotoxicity risk assessment.

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

REVIEW

Experimental Constants:

Test Chemical:

Chemical:	Fortress. DPX-43898, phosphorothioic acid, O,O-diethyl)-(1,2,2,2-tetrachloroethyl)ester. Also known as chlorethoxyfos.
Purity:	86% (list of contaminants not provided).
Lot No.:	4-3-0-0
Description:	Pale yellow liquid
Vehicle:	0.5% methyl cellulose

Analytical Chemistry.

The content of DPX-43898 in the dosing solutions was assessed gas chromatographically. Dosing formulations of DPX-43898 in 0.5% methyl cellulose were assessed for preparations of nominal concentrations of 0.05, 0.075, 0.10, 0.125, 0.16, 0.20 and 0.275 mg/ml for stability and homogeneity and concentration.

Stability. Samples were assessed following five hours of storage at room temperature. The study report asserts that the compound was stable over this period. TB-I notes that based on the data presented, the high dose group had some indications of compound degradation since the 0 time samples were about 103% and the 5 hour samples were about 76% of the nominal concentration. The other samples did not show indications of decomposition.

Concentration verification. The 0.05 mg/ml sample was consistently low or about 56% of the nominal. The 0.10 and 0.125 mg/ml samples were also low or 75 to 80% of the nominal. The other samples tended to be more acceptably near the nominal although wide ranges in individual samplings were noted (71 to 102%).

Homogeneity. The study reports that acceptable coefficients of variation (<20%) were attained for the samples except for the 0.200 mg/ml sample on the first trial. A repeat preparation resulted in a more homogeneous sample.

In conclusion, in some cases low readings resulted and this may have been due to difficulty in extracting the test material from the methyl cellulose. The median analytical values for each of the dose levels are

listed in Table 1 below. Overall, the analytical data are within acceptable limits only for some of the dose solutions. TB-I has determined that in the absence of more definite evidence of faults in the analytical procedures (i.e extracting from the methyl cellulose) the median analytical values should be used for expressng the dose levels.

Test System:

Species/Strain:	Rat/Crl:CD^{R}BR.
Source:	Charles River Breeding Labs, Kingston, New York.
Age:	Approximately 80 days at dosing. Some may be only 63 days old at dosing.
Weight:	Males: 368 ± 15 to 371 ± 16 gms; females: 260 ± 7 to 264 ± 12 gms.
Randomization:	Computer driven weight ordered. Initial body weights were not statistically significantly different among the test groups.

Basic Experimental Design: Four groups of rats were dosed with DPX-43898 and observed for 14 days. FOB and motor activity was assessed at pretest, day zero (reportedly "immediately" after dosing), day 7 and day 14. The fate of the animals after the 14 day period was not specified. There was no indication that necropsy or histopathol-ogy was attempted.

The differential sensitivity of the male and female rats to DPX-43898 required that different dose levels be administered. Table 1 indicates the dose levels and number of rats used for males and females.

Table 1. Dose levels and experimental design.

Males			Females		
Nominal Conc.	Median Conc.[1]	Number of rats	Nominal Conc.	Median Conc.	Number of rats
Control-1	0	12-a	Control	0	12
Control-2	0	12-a	0.5 mg/kg	0.32	12
1.25 mg/kg	1.14	12	0.75 "	0.76	12
1.6 "	1.18	12-a	1.0 "	0.746	12
2.0 "	1.95	12			
2.75 "	2.78	12-a			

a. Due to excessive mortality in the original high dose males (2.75 mg/kg) a lower dose group (1.6 mg/kg) was added along with 12 more controls.
1. The median analytical concentration is listed and the dosing levels are discussed in terms of the analytical median level.

Basis for dose level selection. The dose levels for this study were selected based on a preliminary study that is described in the report of the main study (page 18, no separate study number for this study was provided). In the range finding study, 2 or 3/sex fasted rats were dosed per group. At dose levels of 1.0 and 1.25 mg/kg (in 0.5% methyl cellulose, administered as 10 ml/kg by gavage), DPX- 43898 was fatal to 4 of 6 female rats but doses as high as 0.8 mg/kg were reported as not producing death or clinical signs. In males, dose levels of 3 mg/kg or higher were fatal to all rats. Dose levels of 2.0 and 2.5 mg/kg in males produced clinical signs (not defined). This study indicates that DPX-43898 is very toxic and has a steep dose response curve. No information on the time to onset and duration of the symptoms was indicated. Thus, a basis for the selection of the time of maximum response

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for the FOB and motor assessments was not provided.

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

Statistical Test	Parameter
Cochrane Armitage trend test, then by Fisher's Exact test (with a Bonferroni correction). Significant at 0.05 level.	Clinical signs, descriptive FOB parameters.
Bartlett's test for homogeneity of variances, if not significant, data analyzed by Analysis of Variance (ANOVA), with Dunnett's test used to identify which groups were significantly different from the control group. If Bartlett's test significant, Kruskall-Wallis test with the Wilcoxson test was used to identify which groups were different from the control. Significance at 0.005 level for Bartlett's test and 0.05 level for others.	Body weights, body weight gains, feed consumption, as well as continuous data from the FOB (fore- and hindlimb grip strength, landing foot splay) analyzed as parametric data.
Univariate Analysis of Variance with dose as a between subjects factor and block as a repeated measure. Dunnett's test was used to determine if there was a significant effect of dose or if there was an interaction of dose and block. Significant at 0.05 level.	Motor activity

Specific Methods[1] and Results

1. Deaths and clinical signs.

Deaths: Among the males, 2 of 12 in the 1.95 mg/kg and 10 of 12 in the 2.78 mg/kg dose groups died and among the females, 5 of 12 in the "0.746" mg/kg dose group died as a result of treatment. These rats were said to have died several hours after dosing and the "majority" were found dead after the motor activity assessments. The report states that "no clinical signs were observed in any of the rats prior to death". The rats that died, however were described at necropsy as having "wet areas" present on the nose and/or chin, chest, perineum or forelimbs. Foamy light liquid and/or light tan fluid were present in the stomach of each rat.

Clinical signs. The following noteworthy clinical signs

[1] Results of positive control (DDT, acrylamide, carbaryl and amphetamine) from this laboratory are appended.

were noted.

Tremors: In males, tremors were noted at the dose of 1.18 mg/kg and above. 4 of 12 in each of the 1.18 and 1.95 mg/kg dose groups were affected and these were noted on day 0. In the high dose group, 2 surviving rats were affected and tremors were reported as late as day 2. In females, 2 and 4 rats dosed with "0.76 and "0.746" mg/kg were affected on day 0.

Salivation: In males, salivation was noted at dose levels of 2.0 mg/kg (4 affected) and 2.75 mg/kg (2 affected) on day 0 only. In females, only rats dosed with 1.0 mg/kg (2 rats) were affected on day 0 only.

Male rats with stained and wet areas on their body were noted in 0, 5, 1, 6, 4 and 2 animals in the control (first control group), control (second control group), 1.14, 1.18, 1.95 and 2.78 mg/kg dose groups respectively. The second control group was high because for undefined reasons 5 rats had stained appearance on days 11 or 12. The single male rat affected in the 1.14 mg/kg dose group had wet perineum. Among females, only three rats in the high dose group had wet or stained areas.

Other signs that were present in some of the dosed animals (2 highest dose levels for males and the high dose level for females) in 2 or less animals and were attributable to treatment included: weak appearance, lethargy, irregular respiration, lung noise, gasping, licking, discharge from eye or nose, These were reported usually on day 0 or as late as day 2 for some.

2. Body Weight and Gain and Food Consumption. The effects of treatment on body weight gain are illustrated in Table 2.

Table 2. Body weight gain.

Group[1]	Males		Females	
	Day 0-2 gain(gms)	Final wt.(gms)	Day 0-2 gain(gms)	Final wt.(gms)
Control1	37.0±4.9[2]	477.7[3]	22.3±4.7	299.2
Control2	42.6±7.6	448.4	--	
Low	31.8±7.3 (-14%)	473.7	19.4±4.2 (-13%)	305.5
Mid-1	17.9±11.5*(-58%)	438.1	16.8±6.7 (-25%)	294.0
Mid-2	5.7±26.4*(-85%)	470.9	--	
High	-49.4±1.2* (neg)	437.6	7.0±18.8* (69%)	297.6

Data are from Tables 3 and 4 (males) and 5 and 6 (females) on pages 39 to 44 of the study report.
1. Refer to table 1 for the numerical value of the dose level. Data are based on 12/sex/group except for the mid (10) and high (2) dose males and high (7) dose female groups which have decedents.
2. Mean weight gain for days 0-2 are presented with the s.d. and the percent difference in ().
3. Terminal body weight is presented to illustrate the recovery. Mean value only is presented, the s.d. were 5-7% of the mean.
* $p < 0.05$.

As indicated in Table 2, both male and female low dose groups have decreased weight gain for the 0-2 day interval but

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statistical significance was not attained. Gains were progressively decreased as the dose level was increased reaching statistical significance at 1.18 mg/kg for males and 0.7460 mg/kg for females. As indicated by the final body weight, the treated rats recovered from the initial loss in weight gain. This was evident in body weight gain data which showed larger weight gains in intervals following the 0-2 day interval.

Food consumption data generally paralleled the body weight data for the higher dose groups. Although body weight decreases were noted in the low dose groups for each sex, these decreases were not accompanied by decreased food consumption. There was a decrease in feed consumption followed by a tendency for an increase with the recovery that was most pronounced in the high dose group males. The largest decrease was noted on days 0-2 and the 1.18 mg/kg dose group for males was reduced 27% ($p < 0.05$). The 1.95 mg/kg dose group was reduced 42%, $p < 0.05$) and the high dose group was reduced 98%. The low dose male group consumed only 5% less feed (not significant). Only the high dose female group consumed significantly less feed (41%, $p < 0.05$) although the mid dose group was lower (14%).

3. Neurobehavioral Testing: A. Functional Observational Battery. Tests were run prior to dosing (pretest), "immediately after dosing" and on days 7 and 14. The time "immediately after dosing" is vague and not clarified by the preliminary data. There is no indication that the rats were assessed at the time of peak response. The FOB assessment was three phased: i. inside the home cage; ii. upon removal from the home cage; and iii. in an open field arena (45 x 31 x 12 cm. The following parameters were mentioned as being assessed for each phase:

Observations in Home Cage:
Posture, palpebral closure, writhing, circling and biting.

Removal from Home Cage:
Fur appearance, ease of removal, and handling, vocalizations, piloerection, bite marks, palpebral closure, lacrimation and salivation.

Open field arena:
Activity level, grooming, coordination, locomotion, gait, righting reflex, convulsions/tremors, and assessments of sensory response to approach/touch, finger snap, and tail pinch.

The FOB also included pupillary constriction (light beam), fore- and hindlimb grip strength (using a Chatillon Dial Push-Pull gauge) and landing foot splay.

4. Neurobehavioral Testing: B. Motor Activity. An 8 station automated motor activity monitoring device (Omnitech Digiscan) was utilized and both horizontal and vertical (rearing) counts were recorded. Four consecutive blocks of 10 minutes each were recorded.

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Results: Many FOB parameters were affected and the parameters noted in the low and mid dose groups are listed in Table 3 below. Since the high doses in males (2.78 mg/kg) and females (0.746 mg/kg) were lethal, FOB changes at these doses that were not seen at lower dose levels are not included in Table 3 below. These parameters will be discussed following Table 3. Table 3 is designed to assist in determining the NOEL and LEL for FOB and motor activity parameters.

The study author asserts that the NOEL for males is less than the low dose group (1.14 mg/kg) based on the presence of impaired or lost righting reflex (one animal affected), decreased horizontal (-22%) and vertical (-24%) movement and stained or wet perineum (one animal affected, noted in the cage side clinical signs observation and not shown in Table 3). As indicated in Table 3, the decrease in motor activity for males shows a clear dose response. Although the total count data at 1.14 mg/kg do not reach statistical significance, the 22% decrease in horizontal motor activity is considered by TB-I to be sufficient to support an effect level. In addition, the habituation data indicated that some sessions were statistically significantly lower (i.e. 36%, $p < 0.05$, for the second session at day 0). In general the habituation data (individual session counts) indicated that the treated rats started at lower activity levels and had lower activity levels at the end of the session. Table 17 (data for males) is attached to illustrate this observation. Evidence for persistence of decreased motor activity in the two highest dose groups to days 7 and 14 for males was also indicated. These dose levels, however, are lethal or near lethal.

Among females, a NOEL and LEL of 0.32 and 0.76 mg/kg can be assigned since there were no animals affected at the 0.32 mg/kg dose level for either FOB parameters or motor activity. Motor activity, particularly in the high dose group, was considered to be decreased as late as day 7.

In addition to the symptoms reported in Table 3 above, the high dose male group was associated with decreases in forelimb (67%) and hindlimb (38%) grip strength. The forelimb grip strength for the 1.18 mg/kg dose group was also reportedly decreased (11%) on day 0 of testing and was statistically decreased on days 7 (12%) and 14 (14%) when compared to its concurrent control. When compared to baseline but not the absolute value and when compared with the main study control the 1.18 mg/kg dose group was not reduced. The 1.95 mg/kg group was not reduced. Thus, grip strength is considered by TB-I to be affected only at the high (fatal) dose level in males. Female grip strength was not affected.

The high dose male dose groups also had decreased landing foot splay (days 0, 7 and 14, -21%, -28% and -44%). The female high dose group on the contrary had increased landing foot splay at day 0 (28%) and at day 7 (25%) when expressed as percent of

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Table 1. FOB (open field) and motor activity parameters determined to be affected by Fortress acute administration assessed on the day of dosing.

Parameter	Day	Males[2] Control 1	Control 2	1.14	1.18	1.95	2.78	Females Control	0.32	0.76	0.746
tremors		0	0	0	6	4	2	0	0	2	5
alter. Coordinat.		0	0	0	5*	4	2*	0	0	4	8*
pupil response		0	0	0	4	2	4	0	0	0	2
gait-abnor/ataxic		0	0	0	5	5	2	0	0	3	5*
impair locomotion		0	0	0	5	4	2	0	0	3	4*
salivation		0	0	0	4	4	2	0	0	1	3
tail pinch (reduced resp.)		1	0	0	6	5	2	0	0	3	6
arousal		0	2	1	4	5	2	0	0	3	6
righting reflex		0	0	1	3	3	0	0	0	0	1
motor activity (horizontal)	0[1]	8198	4949	6402	2640*	3321*	2474*	10512	10755	8366	3231*
	7	11180	10352	10727	9936	8945	6407*	14394	13749	13869	12258
	14	10473	11760	10304	10662	8024*	7901	12452	13186	13224	11513
motor activity (vertical)	0	544	275	415	111*	140*	6*	829	818	503*	122*
	7	108	67	91	52	62	35	1087	1040	1083	1140
	14	791	805	982	711	576	725	962	1120	957	1091

FOB data are from Tables 15 and 16 of the study report (pages 54 to 59) and are based on open field observations. Motor activity data are from Tables 17 and 18 (pages 60 to 67).

1. Day 0, 7 and 14 assessments.

* $p < 0.05$.

2. Data are based on 12 rats/group/sex. Most rats that died, died after the first motor assessments. Thus, day 7 and 14 assessments have less than 12 rats for the higher dose groups (see Results Part 1 above).

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baseline and compared to the control. The study author did not consider this to be related to treatment. TB-I notes that the male group was severely affected by the test material and only 2 survivors were assessed and believes the effect in males is probably related to treatment.

Other FOB parameters present in 2 or less animals in the high dose groups that were likely related to treatment included: labored respiration, decreased touch response, rigid muscle tone (one animal affected).

CONCURRENCE: TB-I considers the concurrence between the clinical signs observations and the FOB and motor activity assessments to be reasonable with regard to the predominant symptoms noted. There is also good concurrence between the observations in each sex with the females being more sensitive than the males.

D. Histopathology and Perfusion Studies. No necropsy or histopathology report was presented. The fate of the animals after day 14 was not defined.

E. Immunochemistry: No data on immunochemistry for detection of GFAP were presented.

STUDY CONCLUSION/DISCUSSION: The study is classified as SUPPLEMENTARY. No NOEL was established for either sex. Since the rats were not perfused and assessed histologically, it is not a candidate for upgrading to CORE MINIMUM or higher. Other deficiencies in this study include that the time after dosing for starting the FOB assessments was described only as "immediately". There was no basis for this time selection based on preliminary experimental data. Also body temperature and ChE/AChE inhibition were not assessed. The study protocol was designed before the requirement for inclusion of ChE/AChE inhibition for organophosphates in series 81-8ss studies was made. Consistently low readings for some of the analyses for the concentration (or otherwise) of the test material in the dosing solutions led TB-I to use the analytical median values for describing the dose levels rather than the nominal dose levels. The registrant may wish to reevaluate the analytical procedures used to account for the low values obtained.

The study established that Fortress is highly toxic and has a steep dose response curve and that females are more sensitive than males to lethality and neurotoxicity. TB-I concurs with the study author's conclusions that no NOEL is established for

either males or females with the LEL being 0.5 mg/kg based on decreases in body weight. A decrease in body weight is considered a neurotoxic response to acute intoxication by organophosphates that is secondary to the inhibition of ChE/AChE or possible other reactions to the organophosphate with the nervous system. For males the LEL is 1.25 mg/kg (the lowest dose level tested) based on decreased body weight gain, decreased motor activity and affected righting reflex as well as one rat having wet perineum.