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DATA EVALUATION REPORT

STUDY TYPE: Teratogenicity - Rabbits 83-3 TOX. CHEM. NO.: 663P

MRID NO.: 412906-33

TEST MATERIAL: Fortress Technical (IN 43898); 86% purity; pale yellow liquid

CLASS: Organophosphate

ACTIVE INGREDIENT: Phosphorothioic acid, O,O-diethyl O-(1,2,2,2-tetrachloroethyl) ester

TITLE: Teratogenicity Study of IN 43898 (Fortress Technical) in Rabbits

STUDY NUMBER: 697-88

SPONSOR: E.I. du Pont de Nemours and Co., Inc., Wilmington, DE

TESTING FACILITY: du Pont's Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE

AUTHOR(S): Louis Alvarez

REPORT ISSUED: Report Date: 3/30/89
Report Submitted to Sponsor:
Study initiated: 6/12/88
Study terminated: 7/15/88

CONCLUSION SUMMARY:

Nominal doses of 0, 1, 2, 3, and 3.5 mg/kg/day had to be recalculated based on analysis data to 0, 0.76, 1.38, 2.1 and 2.8 mg/kg/day respectively (see DER for discussion).

Maternal NOEL: 0.76 mg/kg/day
Maternal LEL: 1.38 mg/kg/day (treatment-related mortality associated with cholinesterase inhibition).

Developmental NOEL: 1.38 mg/kg/day
Developmental LEL: 2.1 mg/kg/day (embryo-fetotoxic effect: statistically significant increase in average number of early resorptions per litter relative to controls; supported by an

[1] A Reproductive Outcome Summary table (Table 5), abstracted from the study report has been appended to this DER for presentation at the RED peer review meeting on 11/3/94. KLH 11/3/94

increase in the number of litters with at least one early resorption per total litters observed (relative to controls and lower dose IN 43898-treated groups; see DER for discussion).

No obvious evidence of a teratogenic effect at any dose.

Core Grade: Supplemental. May be upgraded and may be used in fulfilling the requirement for teratogenicity if the sponsor satisfactorily addresses the points listed in the "Discussion" section of this DER with regard to the clinical significance of caudal staining/diarrhea in dose groups II and III and how the impurities in the test material were taken into account for concentration calculations (including batch number and density of test material).

A. MATERIALS

1. Test compound. IN 43898 (Fortress Technical; a pale yellow liquid with purity of 86%). Batch number was not provided. The test material was prepared in a 0.5% aqueous suspension of methyl cellulose.

2. Test animals. Female New Zealand White (Hra:(NZW)SPF) nulliparous rabbits from Hazleton Research Products, Inc. were used in the study. At study initiation, the age of the females was about 26 weeks and group mean body weights ranged from about 3800 g to about 4150 g. Semen for artificial insemination was collected from seven proven fertile male rabbits of the same strain and from the same supplier as the females. The age of the males ranged from 9 months to 3.5 years.

Females were ranked by body weight prior to insemination and then randomly assigned from the ranked lots to dose groups. Females were intravenously injected with 50 U.S.P. units of chorionic gonadotropin 19 days before insemination and with 100 U.S.P. units on the day of but prior to insemination. The males used each day were determined by the volume and quality of semen provided. Insemination day was designated as day 0 of gestation and occurred from June 12 to June 16, 1988.

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B. METHODS

1. Study design. The dosing design was as follows:

Group	Dose of Fortress Tech.[a] (nominal dose in mg/kg body weight/day)	Inseminated Females
I (Control)	0[b]	20
II	1.0	20
III	2.0	20
IV	3.0	20
V	3.5	20

a Fortress technical was administered to inseminated females once daily, by gavage, on days 7-19 of gestation, in a 0.5% aqueous suspension of methyl cellulose at a dosing volume of 2.0 ml/kg body weight. Doses listed are nominal doses only. Actual doses were lower (see "Preparation and Analyses of Test Suspensions", this DER, for details).

b Control animals were gavaged with the vehicle, 0.5% methyl cellulose.

2. Dose level selection. The pilot studies with Fortress technical demonstrated a steep dose response curve and suggested that pregnant rabbits were more susceptible to the toxic effects of the test material than non-pregnant ones. Only a brief, sketchy summary of the pilot work was included in the study report. An overview of the summary data appears below:

At doses of 0, 0.1, 0.4, 0.8, or 2.0 mg/kg body weight/day, no deaths nor toxicity were reported in pregnant rabbits dosed on days 7-19 of gestation (animals/group not reported). The study authors implied that a "maximum tolerated dose" in pregnant rabbits was anticipated with these dose levels since they had been chosen based on the results of previous toxicity studies with Fortress technical (data for these studies were not provided in the study report).

When doses of 3.5, 4.5, and 5.5 mg/kg body weight/day were administered to nonpregnant females (four animals/group) for 13 days, all high dose animals died due to test material administration. No other deaths were reported at lower doses except for one gavage trauma death.

At doses of 0, 3.5, 4.5 or 5.0 mg/kg body weight/day in pregnant rabbits, 3/10, 8/10, and 9/11 animals died respectively of test material-related causes. Dose-related increases in clinical signs indicative of organophosphate poisoning were

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noted. Decreases in group mean body weight gains were noted at all doses, significantly so at the high dose. (The actual percent decreases were not specified).

3. Husbandry. Females and males were individually housed in wire-mesh cages in an environmentally controlled room. Females were given approximately 150 g of Purina Certified Rabbit Chow #5322 daily and water (Wilmington Suburban Water Corp.) was provided on an ad libitum basis. Each female was uniquely identified by ear tattoo and cage card.

4. Preparation and analyses of test suspensions.

The description of the procedures followed for dose preparation and sample analysis was sometimes confusing due to a lack of sufficient detail in parts of the write-up and some vagaries in the wording used. In addition, the data provided did not always reflect what was stated in the procedures.

The protocol appeared to be as follows:
Suspensions of the test material in the vehicle were prepared fresh daily in 300 ml volumes. (It is not clear if separate 300 ml volumes were prepared for each dose level or if one 300 ml stock suspension was first made from which the other dose levels were prepared). The 300 ml mixture of test material and vehicle was homogenized for about 20 seconds with a Polytron mixer at the time of preparation. For test groups I, II, III, IV, and V respectively, nominal concentrations of 0, 0.5, 1.0, 1.5, and 1.75 mg/ml were prepared on the basis of a desired 2.0 ml/kg dosage volume. It was not clear from the description in the study report how the nominal concentration calculations took the 15% impurities in the technical material into account (i.e. did a 1.0 mg/ml nominal concentration contain 1.0 mg/ml active ingredient or 0.85 mg/ml active ingredient?)

It does not appear that the "polytroned" suspensions were immediately analyzed for proper concentration. Apparently, mixing with the Polytron was done only once in the daily preparation procedures. Prior to dosing and sampling, test suspensions were reported to have been manually shaken. Apparently, larger volume containers (500 ml bottles, initially holding 300 ml) as well as smaller (10 ml) volumes of suspension, taken as samples, were manually shaken.

Sample taking and analysis procedures appeared to be as follows:
Two samples (10 ml each) of test suspensions for each dose level were taken at the beginning (6/21/88), during (6/28/88), and at the end (7/5/88) of the dosing period to verify concentration and stability of the test substance in the vehicle. On each sampling day, the first sample (described as "Fresh" in the study report) was taken at the time of gavage and was then manually shaken and analyzed. The second sample was taken at the same time as the

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first but was kept at room temperature for five hours, and then manually shaken and analyzed. Sampling was also done in triplicate (on manually shaken suspensions at 0.5 and 1.75 mg/ml) each sampling day after dosing was complete, to determine the uniformity of the suspensions. Samples to check the efficiency of extraction procedures (recovery samples) were taken at the same sampling times from separately prepared 0.5 mg/ml and 1.75 mg/ml concentrations.

Results. Four, instead of one, sampling determinations were reported for each of the three sampling time points for the 0.5 mg/ml and 1.75 mg/ml concentrations of "fresh" samples taken at the time of gavage. For the same two concentrations and sampling time points, four instead of three uniformity sampling determinations were reported. One sample per sampling date was taken for the 1.0 and 1.5 mg/ml concentrations for both the fresh and uniformity samplings.

The average of the recovery samples taken at all three sampling dates for the 0.5 mg/ml and the 1.75 mg/ml concentrations respectively were about 106% and 94% of nominal concentrations. Ranges for the lower concentration were 88 to 128% of nominal and for the higher concentration, 87 to 101% of nominal. The variation noted suggests the possibility of some inconsistency in the sample quantification process and/or sample preparation process.

Actual measured concentrations of "fresh samples" tended to be much lower than nominal concentrations and deviations from nominal varied amongst samplings of the same concentration measured on different days. For example, on 6/21/88, 6/28/88 and 7/5/88 respectively at the 1.5 mg/ml concentration, the percent of nominal concentrations measured were 70%, 80% and 65%.

Variation was also noted amongst samplings of the same concentration taken on the same sampling day.(For "fresh" samples taken at the time of gavage, this involved only the 0.5 mg/ml and 1.75 mg/ml concentrations since they were the only ones from which multiple samples were taken). Example: at 1.75 mg/ml, on 6/28/88, the percent of nominal concentration measured for each of the four samplings was 86%, 89%, 97%, and 102%. On some days, multiple sampling values were closer than on other days and sometimes it seemed that only one value out of four was much different from the other values. However, in the latter case, there was no clear-cut justification to throw this value out in light of the variation seen in much of the analysis data in general. Example: at 0.5 mg/ml on 7/5/88, the percent of nominal concentration measured for each of the four samplings was 66%, 74%, 76% and 76%.

When measurements from "fresh" samples from all three sampling dates were compared, concentrations of many samples were less

than 80% of nominal (findings were confirmed by reanalysis). The range of measured concentrations among all concentration levels and over all sampling dates was from 56 to 102% of nominal. The average and standard deviation of the analysis results at each concentration over all sampling dates were as follows (calculations by Toxicology Branch based on "fresh" sample data collected at the time of gavage and listed in Table 2, Appendix B of the study report):

Group II 0.5 mg/ml 79% ± 8.1% of nominal (n=12)
(1 mg/kg bw/d) (range 66% to 92% of nominal)

Group III 1.0 mg/ml 67% ± 10.6% of nominal (n=3)
(2 mg/kg bw/d) (range 56% to 77% of nominal)

Group IV 1.5 mg/ml 72% ± 7.6% of nominal (n=3)
(3 mg/kg bw/d) (range 65% to 80% of nominal)

Group V 1.75 mg/ml 89% ± 5.5% of nominal (n=12)
(3.5 mg/kg bw/d) (range 82% to 102% of nominal)

Median values of all "fresh" samples determinations (i.e determinations listed in Table 2 of Appendix B of the study report) at each concentration over all sampling dates were as follows (expressed as a percentage of nominal):

0.5 mg/ml	76%	(Note: the mode (most common value) for this dose is also 76%)
1.0 mg/ml	69%	(No mode)
1.5 mg/ml	70%	(No mode)
1.75 mg/ml	89%	(The mode for this dose was also 89%)

A review of the stability sample measurements showed that generally, readings of samples which sat five hours at room temperature before analysis were substantially similar to the analogous fresh sample readings; therefore suggesting that the test material was stable in the vehicle over that time period.

The uniformity determination readings for the 0.5 mg/ml and 1.75 mg/ml concentrations, expressed as a percent of nominal concentration, were similar to the averages of the analogous "fresh" sample determinations. This suggested that the manual shaking of the dosing material at the time of gavage produced a similar degree of suspension as the manual shaking after gavage.

The study authors offered no final explanation as to why administered concentrations of the test material fell so far below the theoretical concentrations although they suggested that it might be sampling error. Based on the data provided, there may have been a combination of factors which contributed to the problem- some possibilities: variability in the extraction

process used to measure the test material and/or failure to completely mix the dosing suspensions at any one of various shaking steps that took place particularly the shaking of the larger volume suspensions.

Although the study measured the stability of the test material in the vehicle, data for the stability of the test material (a liquid) alone was not provided and it was not clear whether the purity of the test material was double checked prior to dose suspension preparation.

5. Statistics. The experimental unit for purposes of statistical evaluation was the litter (i.e., the proportion of affected fetuses per litter or the litter mean). A list of statistical procedures is appended to this report.

6. Statements. Signed statements of quality assurance and compliance with the GLP's were provided.

C. RESULTS

1. Observations. Clinical signs were recorded each morning on gestation days 0-29 and also in the afternoon during the dosing period (gestation days 7-19). Morbidity and mortality were monitored daily.

a. Mortality. The mortality in this study, most of it treatment-related was extensive.

Dose (mg/kg bw/day)	Mortality (deaths/number in group)	Deaths of pregnant animals	Gestation Day [dosing day]
0	1/20 (due to gavage)	1	7 [1]
1	1/20 (due to gavage)	1	8 [2]
2	2/20 (treatment related)	1	13,15 [7,9]
3	10/20 (treatment related)	8	10-20 [4-14]
3.5	13/20 (treatment related)	12	9-16 [3-10]

Deaths increased with increasing dose; the increase was dramatic between 2 and 3 mg/kg/day. For those deaths that were treatment related, deaths, on the average, tended to occur earlier in gestation with increasing dose. The average death day for the high dose group was gestation day 11.8, was gestation day 12.6 for the 3 mg/kg/day group, and was gestation day 14 for the 2 mg/kg/day dose group.

Clinical observations with relationship to mortality are discussed below.

b. Clinical observations. Interestingly, deaths were often preceded by no, sometimes few, or seemingly mild clinical

findings (i.e., tail staining). Sometimes, the findings observed prior to a death (i.e., alopecia, tail or perineal staining, single incidence of diarrhea) were not those primarily or solely associated with toxicity related to cholinesterase inhibition. There was a tendency for clinical findings which might more obviously be connected to cholinesterase inhibition (i.e. tremor, respiratory difficulty, prostration, salivation, abnormal gait, miosis) to be first noted on the day of death or within a few days of it.

More specific comments on clinical observations appear below:

Alopecia in various areas of the body was frequently noted in all dose groups including the control group throughout the study. Although treatment may have been directly or indirectly associated with some of the alopecia noted, no treatment-related pattern was obvious.

Control group
Occasional incidences of brown and yellow staining of the tail or perineum were noted in control females at various times during the study. One animal had several occurrences of diarrhea.

Group II
In the 1.0 mg/kg/day dose group, brown and yellow tail staining were noted in some animals. Total incidences of staining were slightly higher in Group II than in Group I. Generally, staining commenced during the dosing period but sometimes persisted or recurred after dosing was complete. Single instances of diarrhea were noted in two animals, in one animal at the end of the gestation period and in the other during dosing. It would be difficult to definitively attribute the increased incidence of staining noted in this group to treatment based on descriptions in the study report.

Group III
At 2.0 mg/kg/day, more animals had occurrences of staining (brown and/or yellow staining of the perineal area or tail) compared to Groups I and II, however a number of the staining occurrences were observed either before dosing, started during dosing but persisted long after dosing ended toward the end of the study, or commenced near the end of the study. Therefore, when these factors were taken into account, it would be difficult to definitively ascribe the increase to treatment based on descriptions in the study report. It could not be determined if the exophthalmus and pale left eye noted in one animal was related to treatment. Alopecia was the only observation noted prior to the death of one of the animals dying at this dose and no observations were reported at any time prior to the death of the other. Three animals in this group were listed as having occasional diarrhea, two during dosing and one post-dosing.

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Groups IV and V
The large number of deaths in the top two dose groups hampered comparison of incidences of some clinical signs with other groups (such as various types of staining in the caudal region). The study authors attributed tail staining noted in the two top dose groups to treatment and stated that it was probably due to diarrhea (even though, in the study report, the authors tabulated diarrhea separately from tail staining; the incidences of diarrhea reported at lower doses were too low to have much impact on total incidences of tail staining data there).

Other findings observed at 3.0 mg/kg/day were clearly associated with treatment-related cholinesterase inhibition (i.e. tremors, salivation, pupil constriction, irregular respiration, prostration, abnormal gait or mobility, wet perioral area). Four animals had one reported incidence each of diarrhea. Two of the animals with diarrhea during the dosing period also had other signs of cholinesterase inhibition.

No cageside observations were reported for five of the ten animals dying in Group IV. The observations occasionally noted in two other animals which died were of a type that did not appear to indicate that the life of the animal was in danger (i.e.one instance each of brown tail staining and slight diarrhea in one animal and numerous observations of alopecia in the other). Animals in Group IV with definite symptoms of cholinesterase inhibition all died unscheduled deaths.

In Group V, definitive signs of cholinesterase inhibition were not restricted to animals that died during the study. Such signs included: salivation, tremors, miosis, wet perineum and wet perioral area, prostration, brown staining of the perioral and perinasal area, red discharge, pupil constriction, irregular respiration, brown and/or yellow staining of tail, underbody, perineum, hind quarters, rear. Four animals had occasional instances of what was described as diarrhea mostly during the dosing period. Two animals were found dead with no preceding clinical signs. Five animals were found dead with minimal symptomology (occasional brown or yellow tail or hind quarter staining, alopecia, red discharge, one instance of diarrhea).

One animal in Group V, which survived this study, aborted what were described as 6 "apparently late resorptions and 1 viable fetus" over the last four days of the gestation period and another survivor delivered early on day 29 of gestation (there were 3 nidations listed for this animal and 2 additional nidations for the first animal but the fates of the nidations were not reported.

2. Body Weights. Individual body weight determinations were reportedly made on gestation days 0, 7-20, 24 and 29 but, in the

study report, data were tabulated on days 0,7,10,13,16,20, and 29 of gestation. Data from females that were not pregnant, aborted, delivered early, had total resorptions, or died were excluded from the study author's tabulations.

No statistically significant changes were noted over predosing days 0-7 of gestation or during post-dosing days 20-29 of gestation. Statistically significant decreases in body weight gains were noted during the dosing period (gestation days 7-20):

Body weight changes were 63% and -187% of control values in the 3 mg/kg and the 3.5 mg/kg groups respectively. The change in the top dose group (-217.8) was statistically significantly different from the control value (116.3) at the $p \leq 0.5$ level and the value for the 3.0 mg/kg dose group (73.5) was described as being part of a significant downward trend. Values in other two dose groups for this time interval showed no pattern of decline. After the dosing period there appeared to be a compensatory increase in body weight gain in the top two dose groups.

3. Food Consumption. Individual food consumption was apparently measured daily but was reported in the study over the following gestation day intervals: 0-7, 7-10, 10-13, 13-16, 16-20, 7-20 and 20-29. Data from females that were not pregnant, aborted, delivered early, had total resorptions, or died were excluded from the study author's calculations.

With regard to gestation day intervals of 0-7, 7-20 and 20-29, food consumption was only statistically significantly lower than the control group value during the dosing interval (gestation days 7-20) in high dose group. During the dosing interval, food consumption in Group V was 64% of the control value. Food consumption in other groups was similar to the appropriate control value for the three time intervals.

4. Sacrifice and Postmortem Examinations

a. Exam/Reporting -Adult females. The study report described procedures to be as follows:

Animals were euthanized on day 29 of gestation and examined for gross anatomical abnormalities. The gravid uterus and liver were removed and individually weighed. The uterus was opened and examined for live and dead fetuses and resorptions and their relative positions. Uteri of seemingly non-pregnant animals were stained with ammonium sulfide and examined to detect very early resorptions. Corpora lutea were counted for each ovary. Nidations (described as live and dead fetuses, and resorptions) were tabulated. Animals delivering early or aborting were maintained until their scheduled sacrifice.

Females dying during the study were also examined for gross

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anatomical abnormalities, pregnancy status (by presence or absence of implantations), and, if possible, implantations were counted and their contents described.

Evaluation of the material presented in the study report revealed the following:

Total nidations were recorded for each litter. However, individual maternal or litter data for corpora lutea, early resorptions, late resorptions, dead fetuses, live male and female fetuses, and mean fetal weights were not recorded nor included in the study author's calculations for animals that died gavage or unscheduled deaths, or for those which delivered early, aborted fetuses or totally resorbed litters. Dams which delivered early, totally resorbed litters or aborted fetuses were identified, but it is not clear if any fetuses or fetal material from the early deliveries or abortuses were examined for abnormalities.

b. Exam/Reporting-Fetuses. The study report indicated the following:

Live fetuses from females surviving to day 29 of gestation were weighed and examined for external abnormalities. Live fetuses were euthanized by injection and examined for visceral alterations by the technique of Staples (Staples, R.E., Detection of Visceral Alterations in Mammalian Fetuses, Teratology 9(3):A37-A38 (1974)) and sexed. The brain was examined by making a transverse section between the parietal and frontal bones of the unfixed fetal head. The eyelids of each fetus were removed and the eyes were examined separately. All fetuses were fixed, processed, and stained with alizarin red S prior to examination for skeletal alterations.

Reporting details were as follows:

Incidences of abnormalities were reported as malformations, fetal developmental variations, and variations due to retarded development (definitions of these categories were not provided). These main categories were further divided into external, visceral, head and skeletal subcategories. Statistical analyses were performed on these subcategories of abnormalities.

Relative positions of fetuses in the uterus were not reported. Information on nidations from dams which died unscheduled deaths were not reported and the fate of nidations from animals which delivered early or aborted fetuses were incompletely described.

5. Maternal Findings

a. Reproductive Outcome. Twenty animals were inseminated in each dose group. Pregnancies per number inseminated were as follows for groups I, II, III, IV and V respectively 60%, 90%, 80%, 85%

and 95%. The number of pregnant animals which survived to the end of the study were for the same groups: 11, 17, 15, 9, and 7 and the corresponding number of litters were 11, 17, 15, 8, and 5. Differences in the two sets of values were accounted for by a total resorption in group IV and one early delivery and an abortion in Group V.

b. Organ Weights and Gross Findings. There were no statistically significant differences in absolute or relative liver weights. Gravid uterine weight determinations were made but the findings were not reported. External gross postmortem findings, if any, were consistent with reported clinical signs. No outstanding internal gross findings were reported.

c. Litter data. The large number of deaths in the top two dose groups resulted in smaller relative sample sizes for some of the fetal and litter parameters measured.

There was no statistically significant difference in the average number of corpora lutea per litter or in the average number of nidations per litter. Although not statistically significant, there was a slight decrease in the average number of live fetuses per litter in the high dose group (5.8 in the high dose group versus 7.5 in the control group and a range of 6.4 to 6.8 in the other groups).

By the Mann-Whitney U test ($p \leq 0.05$), there was a statistically significant increase in the average number of early resorptions per litter in Groups IV and V. The values and standard errors for Groups I (11 litters),II (17 litters),III (15 litters),IV (8 litters), and V (5 litters) respectively were 0.0 ± 0.0, 0.6 ± 0.3, 0.1 ± 0.07, 0.4 ± 0.18 and 1.0 ± 0.77. The animal with total resorptions in the 3.0 mg/kg group (animal #22898) was not included by the study authors in the calculations for early resorptions. However,there was only one nidation in this animal and this in turn became the early resorption. Inclusion of this additional resorption in the calculations for Group IV brings the value to 0.625 ± 0.11.
The study authors said that no trend was detected statistically whether or not the additional resorption in the 3.0 mg/kg group was included.

Apparently, pregnant animals which died unscheduled deaths were not examined for evidence of fetal resorptions even though there does not seem to be a reason why they couldn't have been. Therefore, the possibility of there having been additional resorptions in these animals cannot be excluded.

The study author's position on the biological significance of the early resorption data was that only the early resorptions at the high dose group represented a treatment-related sign of developmental toxicity. They argued that the statistical

significance of the average resorptions per litter calculated for dose group IV, 0.4, was due to the absence of early resorptions in the control and therefore did not imply biological significance. In further support of this, they stated that 0.4 fell within the range of early resorption values reported in six other studies. The values were 0, 0.1, 0.3, 0.3, 0.3, and 0.6 resorptions per litter but the usefulness of these numbers could not be ascertained because no information about their source was provided. Since the value in the top dose group (1.0) was outside of this "historical range", the study authors stated that it was probably indicative of a compound-related effect.

There were no statistically significant differences in the sex ratios of the live fetuses or in the average number of late resorptions as tabulated for statistical analysis by the study authors. However, 6 late resorptions were reported for the animal which aborted. There were no dead fetuses or stunted fetuses reported in individual litters. However, crown to rump length comparisons between control and Fortress-treated fetuses were not made.

There were no statistically significant differences in average fetal weights (male, female, total) although average fetal weight in the high dose group was slightly lower than the control and other group values (i.e. For Groups I,II,III,IV,V respectively 44, 45, 48, 46, and 41 g).

6. Fetal Findings

a. Malformations. The control group had no malformations. In the 1.0 mg/kg/day dose group there was one incidence each (in different litters) of distended aorta, fused rib (3-4 left), and hemivertebra (thoracic 13 with rib fusions: #12-13). At 2.0 mg/kg/day, There was one incidence of a fused vertebra (fused arches and centra, thoracic 8-9 left. There were no reported malformations in the 3.0 mg/kg/day group. In the high dose group there was one incidence of fused sternebra (fused (#2-5) with asymmetry of sternocostal articulation. There was no obvious relationship between malformations and treatment in this study. The study authors found no statistically significant relationships between malformations and treatment.

b. Fetal developmental variations. Evaluating the possible significance of fetal variations was hampered by the low number of litters at the top two doses relative to the other groups (due to maternal deaths).

In the summary table, the study authors did not provide separate tabulations of variations by type (for example: within a particular dose group, all incidences of extra ribs were counted together regardless of which ribs were involved). However, any individual abnormalities for a particular fetus were reported in

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an appendix. Fetuses with malformations were omitted from calculations of fetal variation frequencies.
No external or head variations were detected. Common visceral developmental variations in all groups including controls were Great Heart Vessels (Lt. Carotid off Innominate) and Supernumerary Vessels. The study authors should have provided a description of these abnormalities. Common skeletal findings were: rudimentary and extra ribs and extra thoracic vertebra with ribs. Although tabulations of these abnormalities (both visceral and skeletal) included all sites, generally, only a few sites predominated in each case. Incidences of these findings are listed in Table I below. Any increases in the top dose groups were probably related to the relatively small sample sizes in these groups. In light of this, none of the above abnormalities nor occasional occurrences of other developmental variations were clearly related to treatment. The study authors reported no statistically significant increases for either skeletal or visceral variations.

c. Variations due to Retarded Development

No external, visceral or head variations were reported. The only two skeletal observations were partially ossified and unossified sternebrae (Table I below). In each case, all sites were combined but there were not many total observations. The high dose increase appeared to be related to the relatively small sample size in this group and therefore, there was no obvious relationship to treatment. No statistically significant increases were reported in the skeletal variations subcategory by the study authors.

Table I

Frequency of Developmental and Retarded Development Variations[1]
(litters affected[2]/litters examined)

Developmental Variations	Groups I	II	III	IV	V
Visceral					
Great Heart Vessels (left carotid off Innominate)	7/11 (63)[3]	9/17 (53)	9/15 (60)	4/8 (50)	4/5 (80)
Supernumerary Vessels	4/11 (36)	5/17 (29)	2/15 (13)	3/8 (37)	1/5 (20)
Skeletal					
Rudimentary Lumbar Rib	9/11 (81)	10/17 (59)	12/15 (80)	6/8 (75)	5/5 (100)
Extra Lumbar Rib	8/11 (72)	10/17 (59)	11/15 (73)	5/8 (63)	2/5 (40)
Extra Thoracic Vertebra with ribs	2/11 (18)	4/17 (23)	2/15 (13)	3/8 (37)	1/5 (20)
Variations Due to Retarded Development					
Skeletal					
Partially Ossified Sternebrae	2/11 (18)	3/17 (18)	1/15 (7)	0/8 (0)	1/5 (20)
Unossified Sternebrae	2/11 (18)	1/17 (6)	1/15 (7)	2/8 (25)	2/5 (40)

1 Data in this table were extracted from the study report
2 Litters with at least one incidence of the abnormality
3 Data expressed as a percentage

D. DISCUSSION

There are a number of issues of concern with regard to this study and/or the test material which will be discussed below. The reader should consult the text above for details on these areas.

1. The first issue concerns the characteristics of test material toxicity. Mortality occurred at a very low nominal dose, 2 mg/kg/day (1 pregnant and 1 non-pregnant animal). A steep dose response curve was noted in this study as evidenced by the sudden

increase in mortality particularly between nominal doses of 2 and 3 mg/kg/day. Comparison of this study and the information provided about the dose ranging studies indicates variability in the approximate dose at which mortality commenced (the possible contribution of error in dosing suspension concentration to the differences observed is not known; concentration error was not reported for the dose ranging studies; see below for further discussion of dose concentration).

One of the more troubling aspects about the toxicological character of this test material is that deaths were often preceded by no, few or seemingly mild cageside findings (i.e. tail staining) thus presenting a clinical picture that would not obviously indicate that a life-threatening condition existed. Unfortunately, no cholinesterase-inhibition data were available (and are not required under FIFRA guidelines) for this study so that there is no clue about what was happening at the molecular level in the absence of clear symptomology indicative of cholinesterase poisoning.

Also troubling is the frequently observed lag time between the onset of dosing and death (with no apparent warning). Consider that at the lowest dose at which treatment-related deaths occurred (nominal 2 mg/kg/day), the lag time between the onset of dosing and death was 7-9 days and that there were no clinical signs indicative of toxicity nor impending death (the only cageside observation reported was alopecia in one animal and this had commenced prior to dosing).

Even when characteristic symptoms of cholinesterase inhibition were present, they frequently did not commence until the day of death or one or two days prior to it. Therefore, a common scenario might be that an animal showed no outward sign of toxicity for days after dosing onset, then suddenly developed clinical indicators of poisoning and died that day or shortly thereafter. But as indicated above, death was frequently preceded by no or innocuous-appearing clinical observations.

2. One aspect of the clinical findings that was difficult to interpret in reviewing this study was the various types of caudal staining versus diarrhea. The study authors tabulated diarrhea separately from tail staining, however, in the text of the study report, for top test groups IV and V, they attributed tail staining noted to treatment and stated that it was probably due to diarrhea. The authors did not make this connection between the various types of caudal staining noted in the lower dose groups with diarrhea or with treatment. Based on incidences of these observations in the lower groups (caudal staining alone or plus incidences of diarrhea), the general times of occurrence, comparison with the control group, and the information provided in the study report, it would be difficult to definitively attribute the increased incidence of staining noted in these

lower dose groups to treatment.

However, the study authors need to clarify this issue, especially since caudal staining and/or diarrhea could be a symptom of cholinesterase inhibition.

3. Measured concentrations of dosing suspensions were often much lower than nominal concentrations and significant variability was noted in deviations from nominal among doses measured on a given day and in the same dose sampled on different days. Apparently the study authors could not determine why the problem occurred.

The study authors' description of dose suspension preparation and analysis was sometimes vague and confusing making reconstruction of the procedures followed up until the actual insertion of the sample in the gas chromatograph tedious. In addition, the study authors need to explain the discrepancy between the number of "fresh" and uniformity sampling determinations the study report said were taken at 0.5 mg/ml and 1.75 mg/ml and the number actually reported.

However, a reasonable amount of analysis data was presented over the relatively short dosing period. For all dose levels during the dosing interval, samples were collected three times at approximately weekly intervals. Measured concentrations of "fresh samples" did not fall below 56% of nominal and generally fell over all doses and time points from 74% to 92% of nominal.

Because it is not clear what caused the problem (i.e sampling error, variability in extraction process or inadequate mixing, etc.), if this data is to be used at all as the basis for determining NOELs/LELs, it should be used conservatively. The median values for each dose level calculated earlier in this DER would, overall, provide the most conservative estimations of test material intake based on the analysis data in the study report. Statistical analysis of the data by HED supports the use of the median values. Therefore, nominal doses would be altered as follows (see "Preparation and Analyses of Test Suspension", this DER, for previous calculations):

Group	Nominal Dose (mg/kg bw/day)	Decrease in Nominal Concentration (based on median decrease)	Recalc. Dose (mg/kg bw/day)
II	1	76%	0.76
III	2	69%	1.38
IV	3	70%	2.1
V	3.5	80%	2.8

The study sponsor has the option of performing a new study.

To further clarify the issue of dose administered, the study authors should specify how the concentration calculations took the 15% impurities in the technical material into account (i.e. did a 1.0 mg/ml nominal concentration contain 1.0 mg/ml active ingredient or 0.85 mg/ml active ingredient?)

4. Evidence to support embryo/fetotoxicity due to either indirect (via maternal toxicity) and/or direct effects of the test material were noted in the high dose group. The high dose group was the only one with an aborted litter (which contained 6 apparent late resorptions, 1 viable fetus and 2 unknown nidations) and an early delivery (fate of nidations unknown). In addition, a statistically significant increase in early resorptions (average number of early resorptions per litter) relative to controls was found in group V and was judged by the study authors to be an indication of developmental toxicity.

A statistically significant increase in the average number of early resorptions per litter relative to controls was also observed in dose group IV. The study authors argued that the increase was not biologically relevant because the value fell in the range of historical control data they provided and because the control value was 0.0 (see previous discussion under "Maternal Findings, Litter data" in this DER).

The study authors' argument is weakened by several factors:

a. The significance of the historical control data cannot be adequately judged because the data were not presented within an acceptable context;

b. The inclusion of the additional early resorption from animal #22898 brings the average number of early resorptions per litter just outside of the study authors' "historical control data" (i.e. from 0.4 ± 0.18 to 0.625 ± 0.11 S.E.);

c. When early resorptions are looked at on the basis of the number of litters with at least one early resorption per total litters observed, an increase is noted in the two top dose groups compared to controls and the other IN 43898-dosed groups. There are increases whether or not the additional early resorption is included from animal #22898.

Control	0/11 litters	(0%)
Group II	4/17 litters	(23%)
Group III	1/15 litters	(7%)
Group IV	4/9 litters	(44%)with; 3/8 litters (37.5%)without
Group V	2/5 litters	(40%)

Although the percentage in Group V is about the same as group IV, it should be remembered that the fate of some nidations were unaccounted for in the top two dose groups. There were many mortalities in Groups IV and V but pregnant animals which died unscheduled deaths were apparently not routinely examined for evidence of fetal resorptions. Therefore, the possibility of additional resorptions cannot be excluded in the top two dose groups.

In light of these points, Toxicology Branch is of the opinion that a conservative approach to the data should be taken. Therefore, the NOEL for developmental toxicity will be set on the premise that there is a embryo/fetotoxic effect occurring in Group IV (3.0 mg/kg/day nominal dose) as well as in Group V. (No obvious evidence of a teratologic effect was noted at any dose level). Treatment-related mortality was still noted at the next lower dose, in group III (2.0 mg/kg/day nominal dose).

e. Examination or reporting omissions noted in this DER for maternal or litter data generally involved animals that died unscheduled deaths or which delivered early or aborted. This really involves only the two top dose groups (Groups IV and V) because only one pregnant animal died a treatment-related death in Group III so that only three nidations were unaccounted for in that group (leaving a total of 15 litters from which data could be obtained).

While the possibility that some greater degree of developmental toxicity than that already discussed for the top two dose groups might have been missed by the omissions, such effects would have occurred at doses that were lethal to the mother.

E. SUMMARY OF FINDINGS

Nominal Doses: 0,1,2,3,3.5 mg/kg/day IN 43898 in 0.5% methyl cellulose vehicle by gavage in 20 inseminated female New Zealand White (Hra:(NZW)SPF) rabbits, during gestation days 7-19.

Recalculated Doses: 0, 0.76, 1.38, 2.1, 2.8 mg/kg/day (based on analysis data)

Treatment-related mortality was noted from 2 mg/kg (nominal) up (gestation day 9-20). Deaths increased with increasing dose and were associated with cholinesterase poisoning. Deaths were often preceded by no, sometimes few, or seemingly mild clinical findings. At 2 mg/kg (nominal), lag time between dosing and death was 7-9 days with no obvious clinical signs of toxicity.

Clinical signs clearly associated with treatment were noted only in the top two dose groups and included: tremor, salivation, pupil constriction, irregular respiration, prostration, abnormal gait or mobility, wet perioral and perinasal area, wet perineum, diarrhea, and staining (yellow and brown) in the caudal region.

Cholinesterase determinations were not made (nor are they currently required with this type of study).

Body weight gains were limited to 63% and -187% of the control value at 3.0 and 3.5 mg/kg/day (nominal) respectively. These decreases were statistically significant as was the decrease in food consumption in the high dose group. Post-mortem findings in adults were consistent with the clinical findings and no statistically significant differences were noted in liver weights.

Total numbers of litters in the two top dose groups, relative to controls and the other dose groups, were significantly decreased due to mortality of dams; one abortion and one early delivery further depleted the litters in the high dose group (both dams survived the study).

Statistically significant increases in the average number of early resorptions per litter relative to controls was observed in the top two dose groups (see DER for discussion). The increase was also seen when early resorptions were looked at on the basis of the number of litters with at least one early resorption per total litters observed (i.e about 40%-44% in the top two groups versus 0%, 23% and 7% in the control 1 and 2 mg/kg/day (nominal) groups respectively). The possibility of additional resorptions could not be excluded because animals which died unscheduled deaths were apparently not routinely examined for evidence of fetal resorptions.

The increase in early resorptions at 3 and 3.5 mg/kg/day (nominal) were judged by Toxicology Branch to support an embryo/fetotoxic effect.

There was no obvious evidence of a teratologic effect at any dose level or effects on other maternal or fetal/litter parameters However, the fate of nidations from animals which died unscheduled deaths or which delivered early or aborted were not or were incompletely followed up. Since this generally involved only the top two dose groups (only one pregnant animal died a treatment-related death at a lower dose) any findings which might have been missed at the top two doses would have occurred at doses lethal to the mother.

Doc MRID# 41394433

Page_____ is not included in this copy.

Pages 21 through 22 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.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__✓__ FIFRA registration data.

_____ The document is a duplicate of page(s) _________.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Karen L. Hamernik, Ph.D. KLH 3/28/91
Section II, Tox. Branch III (H7509C)
Secondary reviewer: Henry Spencer, Ph.D.
Section II, Tox. Branch III (H7509C) 1/30/91

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation-Request for Data Waiver (Guideline 81-3) TOX. CHEM. NO.: 663P

MRID NO.: None

TEST MATERIAL: Fortress 5G granular insecticide

CLASS: Organophosphate

ACTIVE INGREDIENT: phosphorothioic acid, O,O-diethyl O-(1,2,2,2-tetrachloroethyl)ester at 5%, by weight)

STUDY NUMBER: None

SPONSOR: Agricultural Products Dept., E.I. du Pont de Nemours and Co., Inc., Wilmington, Delaware

TESTING FACILITY: Not applicable.

AUTHOR(S): Not applicable.

REPORT ISSUED: Report Date: Not applicable
Report Submitted to Sponsor: N/A
Study initiated:N/A
Study terminated:N/A

CONCLUSION:

The sponsor's argument for a waiver of the requirement for an acute inhalation study with the formulation Fortress 5G has been determined to be unacceptable. An acute inhalation study with Fortress 5G will be required. Specific points which the sponsor must address in designing and performing the study are discussed below.

Evalution of Request for Waiver of Data Requirement

The sponsor (E.J. Du Pont de Nemours and Co., Inc.) was previously notified by the Agency that an acute inhalation study with Fortress 5G would be required to support an EUP with a temporary tolerance for this formulation.

Du Pont submitted a request for a waiver of the study. The rational is appended to this report (see Attachment 1).

The request was reviewed by Health Effects Division (HED) scientist Stanley Gross, Ph.D., DABT, CIH. He concluded that the company's rationale does not adequately support a waiver of the acute inhalation toxicity data requirement and that an acceptable acute inhalation study must be performed. His comments about the appropriateness of a data waiver and about acute inhalation toxicity study design and performance are appended to this report (see Attachment 2). The HED SEP referred to in his comments is NTIS Document No. PB89-100366/As, Inhalation Toxicology Testing, EPA-540/09-88-101, August 1988. Dr. Gross's comments also mention a memo he authored, dated April 18, 1988. This memo is appended to this report (as Attachment 3). The sponsor may consult the Agency with questions related to study design, if necessary.

SAG. 1/16/91

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SUBJECT: DuPont Fortress 5G inhalation testing. Caswell 663P
EPA# 352-...

TO: Karen Hamernick, PhD, DABT
HED/TOX (7509C)

FROM: Stanley Gross, PhD, DABT, CIH
HED/TOX (7509C)

SBG 4/11/90

cc: Marion Copley, DVM, DABT; HED/TOX

DuPont is asking to "relax" (waive?) the requirement for doing an acute inhalation test on Fortress 5G because:

1) Insignificant amount of dust in product.

2) Difficulty in maintaining during grinding of the formulation because the a.i. is volatile.

3) Difficulty in maintaining stable aerosols during testing.

Response for the Company.

1) We ask for inhalation testing on granulars regardless of the small amount of fines in the product. (See SEP and Gross memo of April 18, 1989.) This is important in this case because the a.i. is quite toxic as indicated by Sanford Bigelow's memo of 3/23/89.

2) DuPont indicated that the a.i. is volatile and is driven off during the grinding process. The company can carry out the grinding in a cool environment and should provide data on the loss of a.i. from the granular particles.

We are toxicologically concerned with the a.i. leaching out of the granules as well as the mount that might vaporize off the granules during inhalation.

3) If it is difficult to maintain the small particles during generation of the aerosol during testing, then this should be shown in the results of testing. If the clay tends to agglomerate during testing, the fines would be expected to agglomerate during the use of the granular.

Frtrss.nte 4/11/90; SBG

ATTACHMENT 2

Fortress/

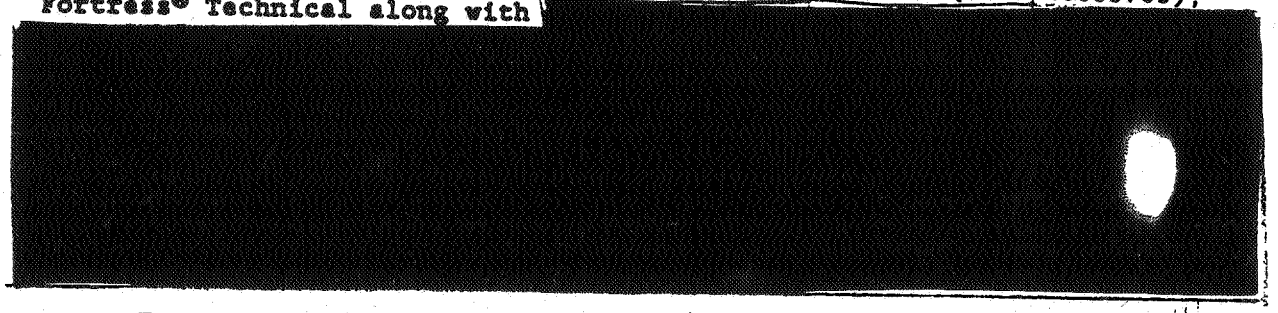
DU PONT FORTRESS® INSECTICIDE 5% GRANULAR FORMULATION

ACUTE TOXICITY

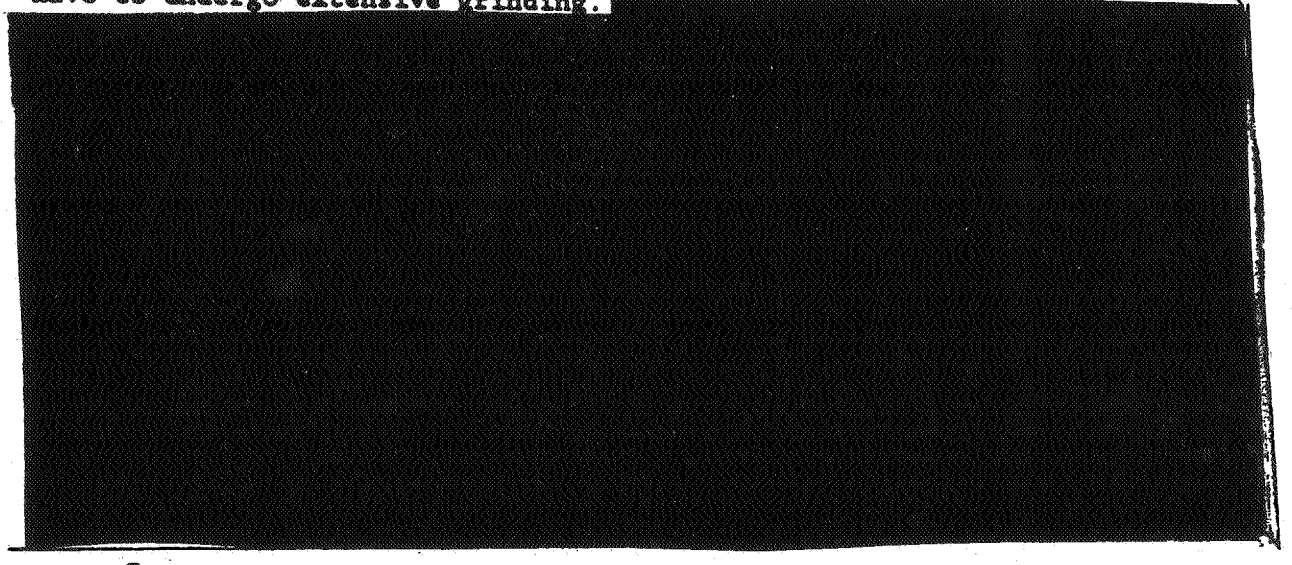
The toxicity of Du Pont Fortress® 5G is consistent with the active ingredient content of the formulation and the toxicity in corresponding tests of the technical.

In the Agency's letter from Mr. Harrison dated April 10, 1989 granting Experimental Use Permit No. 352-EUP-150, Du Pont was notified that an acute inhalation study (81-3) would be required on the TEP prior to granting a temporary tolerance. We request that the Agency reconsider this requirement in that the study presents considerable experimental challenge, while yielding results of questionable value.

The experimental difficulties are these: Fortress® 5G is a granular product. As described in the product chemistry section (MRID 40883703), Fortress® Technical along with



To successfully perform an inhalation experiment, Fortress® 5G would have to undergo extensive grinding.



In summary, we request that the requirement for an inhalation toxicity study of Du Pont Fortress® Insecticide be relaxed because:

1) There is little likelihood of significant dust in the product,

2) it is extremely difficult to maintain formulation concentration while [redacted] to respirable size, and

3) maintenance and measurement of exposure is extremely difficult in that the animals would be treated by a combination of vapor Fortress® and [redacted]

Below is a list of the studies supporting this summary. A listing of the acute toxicity data for the 5G formulation follows, as well as the title page and summary from each report.

Acute Testing - % Granule

Report No.	Study Title
HLR-794-88 (81-1)	Acute Oral Toxicity Study with DPX-43898-26 in Male and Female Rats
HLR-730-88 (81-2)	Acute Dermal Toxicity Study of DPX-43898-26 in Rabbits
HLR-711-88 (81-4)	Primary Eye Irritation Study with DPX-43898-26 in Rabbits
HLR-732-88 (81-5)	Primary Dermal Irritation Study with DPX-43898-26 in Rabbits
HLR-142-89 (81-6)	Closed-Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with DPX-43898-26 in Guinea Pigs

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ATTACHMENT 3

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

APR 18 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Comments on Standard Evaluation Procedure. Inhalation Toxicology Testing (SEP/Inhalation).

TO: Recipients of SEP/Inhalation

FROM: Stanley B. Gross, Ph.D., DABT, CIH
Senior Toxicologist/Industrial Hygienist
TB/HED/OPP, USEPA (H7509C)

You have or are now receiving a copy of our final

"Hazard Evaluation Division Standard Evaluation Procedure. Inhalation Toxicology Testing." EPA-540/09-101, August 1988.

I wish to add here some historical clarifications concerning particle testing sizes and the limit testing which have apparently caused some confusion with testing requirements. Some of this information was discussed in the chapter (Gross, 1987) cited on page 20 of the SEP.

A. Product Particle Sizes and Testing Requirements.

The 1978 proposed FIFRA Guidelines specified limits for particle sizes and percent particles in a product; that is, testing was to be required if 20 percent of the product contained particles of 10 micrometers or less. These proposed limits were dropped and do not apply to the current Guidelines (EPA-540/9-82-025, October 1982; revised, 1984). The 20 percent limit was dropped because small amounts (considerably less than 20 percent) of a highly toxic material can be hazardous if the material can be inhaled. Products applied as large-particle liquid sprays contain small amounts of fine droplets that can be inhaled or may develop inhalable particles by impact on surfaces. Dusts which contain primarily large particles also contain small amounts of fine particles derived, in part, from the manufacturing process, or by the rubbing action between particles during transport. Thus, all dusts, sprays including granulars regardless of the anticipated aerosol sizes are to be tested.

B. Particle Sizes in Animal Tests.

The Guidelines make reference to particle sizes which might be inhaled by humans, namely 15 micrometers (um). A number of

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laboratories have submitted data based on particle sizes of 10 or 15 um assuming these sizes, which are inhalable for man, are the particle sizes to be tested in animals. This is not the case.

For humans: "Inspirable particles" capable of entering the nose of man have been identified as 185 um aerodynamic diameters. "Inhalable" particles which can pass through the trachea have been identified as 15 um for man. "Respirable" particles, small enough to reach the deep lung or alveoli of man, are considered to be 1, 5 or 10 um or less, depending on different literature sources. Comparable diameters for animals had not been established at the time the Guidelines were finalized, nor have they been at this time. However, 1 um diameter had been recommended for rats by a number of inhalation toxicologists. This number (1 um mass median aerodynamic diameter) is still being recommended for rodent inhalation studies. If the mass median aerodynamic diameter reported in a study is larger than 1 um, we can accept the study if at least 25% of the particles are 1 um or less. If the laboratory is having difficulty in achieving the required diameters, the study needs to indicate what they did and why they were unable to provide the small particles. See the SEP/Inhalation Toxicity Testing for more discussion on this matter.

I need to note that many of the particle size data submitted by registrants are submitted as "optical" diameters or "sieve" diameters, rather than aerodynamic diameters. Data on particle size is to be measured and reported as "aerodynamic" diameters. As noted in the SEP, it is the aerodynamic diameter that determines where a particle is likely to be deposited in the respiratory tract.

C. Limit testing.

The limit test (section g, page 51 of the Guidelines, PB86-108958, Nov.'84) says: "If a test at an exposure of 5 mg/L (actual concentration of respirable substances) for 4 hours or, where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, produces no compound-related mortality, then a full study using three dose levels might not be necessary." The limit test usually applies to the acute 4 hour inhalation test. This limit is set at the Toxicity Category IV in which the material would be considered to have minimal adverse effects during an acute exposure.

In order to favor a reduced use of animals during toxicity testing, the Agency has suggested the use of limit test (when such a test seems appropriate). If deaths are seen during the limit test, a full LC50 test as described in the Guidelines is still required. However, a number of registrants have used the limit test as the only test, as a "yes/no test" and usually at levels below the 5 mg/L concentrations. This does not fulfill the testing requirements for this guideline.

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Further, the limit test can be carried out at the maximum attainable concentration. A number of registrants have reported test results from a limit test at concentrations below 5 mg/L which did not cause any deaths. The concentration was reported as a maximum attainable concentration without any documentation to support this conclusion. This has not been accepted. In order to declare the concentrations as the maximum attainable, the registrant needs to indicate what efforts were made to reach the 5 mg/L concentrations, what problems were encountered and, if possible, try to explain why higher concentrations were not achievable.

D. Data Reporting Guidelines.

These Guidelines are or will be available from the National Technical Information Service (NTIS):

" PESTICIDE ASSESSMENT GUIDELINES. Acute and Subchronic Toxicity Testing." by Stanley B. Gross, Ph.D. DABT, CIH. Addendum 6 to Subdivision F, Hazard Evaluation: Human and Domestic Animals (EPA 540/9-89-007), NTIS #PB89-124077.

Please contact me if you have any further questions on these issues (or any other inhalation toxicity testing matters) at 557-4382.

cc: M. Copley
J. Hauswirth

April 18, 1989
sep.mmo

Reviewed by: Melba S. Morrow, D.V.M. MSMorrow 5-4-90
Section II, Tox. Branch I (H7509C)
Secondary reviewer: Karen Hamernik, Ph.D. for 3/26/91
Section II, Tox. Branch I (H7509C) KHH 3/26/91

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal-Rabbits TOX. CHEM. NO. 663-P

GUIDELINE NO: 81-2

MRID NO.: 412906-23

TEST MATERIAL: DPX-43898-26 (brown, solid granule)

SYNONYMS: Fortress 5G

STUDY NUMBER(S): HLR 730-88

SPONSOR: E.I.duPONT de Nemours and Company, Inc.
Wilmington, Delaware

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine
Newark, Delaware

TITLE OF REPORT: Acute Dermal Toxicity of DPX-43898-26 in Rabbits

AUTHOR: WILLIAM J. BROCK

STUDY DATES: October 5,1988 to October 19, 1988

REPORT ISSUED: NOVEMBER 3, 1988

CONCLUSION: From the data presented, the LD_{50} for DPX-43898-26 was >2000 mg/kg of body weight when topically administered to rabbits.

Toxicity Category: III

Classification: Supplementary (refer to Discussion section for additional information).

MATERIALS: Five male and five female adult, New Zealand White rabbits, weighing between 2396 and 2656 grams were the test animals. DPX-43898-26, containing 5.3% active ingredient by analysis,and moistened with dimethyl phthalate to form a paste served as the test material.

METHODS: At 24 hours prior to administering the test material, the hair on each rabbit was clipped to expose the back. The exposed area ran from the scapular region to the lumbar region.

Each rabbit was fitted with a collar to prevent ingestion of the test material or disruption of the wrappings. The test material was spread evenly over an exposed skin area which measured approximately 190 square centimeters. Sterile gauze pads were placed over the treated sites and animals were wrapped with layers of plastic film, gauze bandages and adhesive bandages.

Rabbits were individually housed and placed in rooms that allowed for a 12 hour light /12 hour dark cycle.

Twenty-four hours after treatment, the wrappings were removed and excess test material was washed from the animals with warm water. Observations for clinical signs of toxicity were made one hour after dosing and daily thereafter for 14 days. No observations were made on weekends. Body weights were taken prior to treatment and on days 1,7 and 14 post-treatment.

RESULTS: All animals survived the treatment and no deaths were reported during the observation period. Slight body weight losses were reported for one male and one female on the first day following treatment. No other clinical signs of toxicity were observed.

At a dosage rate of 2000 mg/kg, total average doses of 5 g and 5.1 g were administered to males and females, respectively.

QUALITY ASSURANCE: A statement of compliance with GLPs, dated November 21, 1988, is included in this submission.

DISCUSSION: The dermal LD_{50} for DPX-43898-26 is > 2000 mg/kg in rabbits. The study was conducted using only a single dose; however, this dose was in accordance with the limit suggested in the Subdivision F Guideline.

The study does not address the results of gross examination of the rabbits following sacrifice. The sponsor should have addressed their reasons for not conducting necropsies on the test animals.

The sponsor should also provide some information to justify the use of the vehicle. The sponsor should also address whether the material is readily absorbed when applied topically.

The study is classified as supplementary. Additional information is required regarding the vehicle used in order to upgrade the study. The sponsor should also explain why the rabbits were not sacrificed and subjected to gross examination.

There is a concern that the vehicle, dimethyl phthalate, a plasticizer, may block or inhibit potential absorbtion of the test material.