Reviewed by : Whang Phang, Ph.D. 9/29/87
Section III, Toxicology Branch (TS-769c)
Secondary Reviewer: Marcia van Gemert, Ph.D. 9/30/87
Section III, Toxicology Branch (TS-769c)

DATA EVALUATION REPORT

STUDY TYPE: 3-Generation Reproduction (rats)

TOX. CHEM. NO.: 320 MRID No.: 116484

TEST MATERIAL: 2,4-DP (white powder)

SPONSOR: Amchem Products, Inc.

TESTING FACILITY: Huntingdon Research Center, 216 Congers Rd, New City, NY

CITATION: Calkins, J., Anderson, M., and McElroy, K. (1978). A Three Generation Study of 2, 4-DP Technical Acid in Rats: HRC # 1-361. (Unpublished study received Mar 26, 1979 under 264-231; prepared by Huntingdon Research Center, submitted by Union Carbide Agricultural Products Co., Inc., Research Triangle Park, NC; CDL: 237875-J)

CONCLUSION: This study was previously reviewed by James Holder (Tox. Doc. No. 001995; Attachment). In general this reviewer agrees with the certain conclusions which are derived by Holder except the following points:

1). The previous reviewer considered the increased litter mortality in F2A pups which received 500 ppm of 2,4-DP to be a compound-related effect. This effect was seen only in one mating and one generation, and the standard deviation of the incidence was rather large (Table I of the Attachment). Therefore, this observation could not be considered as biologically significant.

2). This study as reported has many deficiencies which include: (a) purity of the test chemical was never described or tested, (b) no descriptions were given concerning how the treatment diet was prepared, (c) the stability and the actual content of the test compound in the diet were never tested, (d) no histopathology data on reproductive organs of the parental females were reported, (e) no gross necropsy data were presented, (f) no standard deviation or statistical analyses were ever performed, and (g) no clinical observations were reported. Although the previous reviewer classified this study as minimum, under the present standard evaluation procedures a study with the above deficiency could not possibly be classified as a minimum study. Thus, this study is re-classified as supplementary.

Based upon the data presented, the LOEL for parental toxicity is estimated to be 2000 ppm; NOEL 1000 ppm. The LOEL for developmental toxicity is 2000 ppm; NOEL, 1000 ppm.

ATTACHMENT

001995

H. Three Generation Study on 2,4, DP Technical Acid in Rats.
(264-231; 237875, Section I of Submission)

§ 1.0 Conclusions of Three Generation Study in Rats

1.1 Parental rats all survived in all dose groups and in all generations. Intercurrent deseases observed were low and not dose related. Appearance, behavior, and gait were normal in all groups.

1.2 Parental food consumption and weight gains were normal in Groups I, II and III, but were reduced 5-10% in the high dose group (Grp. IV). The same doses were given in the 2-year rat oncology study (section J) and 2000 ppm was toxic in that study indicating that Group IV weight loses were due to general toxicity. When the high dose was reduced to 1000 ppm, weights and food consumption were normal. The toxicity is not certain in so far as 2000 ppm equals a food concentration for 2,4 DP acid of 0.2% which is likely high enough to effect palatability.

1.3 The pregnancy rates (# female rats conceiving/# inseminated) and gestation periods were unaffected amoung the dose groups in any generation.

1.4 The "averages" of litter size were not significantly different among the dose groups, but smaller litters were seen ($n \leq 8$) in F_{1A} and F_{2A} litters at 2000 ppm.

1.5 Fetal mortality was significantly increased at 2000 ppm in F_{1B} and F_{2A} litters. These motality increases were abated when the high dose was reduced to 1000 ppm. Group III (500 ppm) fetal mortalities was slightly increased in F_{2A}, F_{2B}, F_{3A} and F_{3B} litters. There was no increase in fetal mortality at the low dose (125 ppm).

1.6 Mean pups weights at birth and at the end of lactation were affected at the high dose of 2000 ppm, but were unaffected at 1000 ppm. Sex of pups was unaffected.

1.7 Neonatal mortality was increased at the high dose of 2000 ppm. The 500 ppm dose (Grp. III) increased neonatal mortality only in F_{2A} litters and the low dose (125 ppm) only in F_{3B} litters. Although these lower doses had effect in these litters, the high dose (when reduced to 1000 ppm) was the same as controls.

1.8 It is concluded that maternal toxicity has a NOEL = 1000 ppm and LEL = 2000 ppm (reduced weights and increased number of smaller litters, i.e. where the number of pups/litter was 8 or less).

The fetal toxicity LEL = 500 ppm (increased litter mortality), and fetal NOEL = 125 ppm.

The neonatal toxicity NOEL = 1000 ppm and LEL = 2000 ppm (increased pup mortalities during lactation period).

2

§ 2.0 Recommendations

2.1 A complete chemical analyses should be submitted to the Agency of the technical grade 2,4 DP used to dose the rats in this three generation study.

2.2 A palatability study of the test material should be done and the results reported to the Agency.

§3.0 Introduction

Weedone® contains the active ingredient butoxyethanol ester of 2,4 DP acid. The acid moeity was fed in this study to Sprague Dawley rats at doses in rat chow: I (0 ppm), II (125 ppm) III (500 ppm), and IV (2000 ppm). The study was performed by the Huntingdon Research Center, 216 Congers Rd., NY. NY. 10956 (the center is now closed) for the Amchem Division of Union Carbide in order that the potential to affect the reproduction performance in rats may be assessed. Effects on the parental, fetal, and neonatal survivability and weight gains were also observed in order to assess the oral toxicity of 2,4 DP acid.

The three generation study was started on April 12, 1977 and completed on October 2, 1978. The composition of the technical grade 2,4 DP fed to the rats was not submitted by Union Carbide.

§4.0 Methods:

4.1 One hundred twenty (40 male, 80 female) Crl:COBS CD (SD) BR weaning rats were obtained from Charles River Breeding laboratories, Inc., 251 Ballardvale Street, Wilmington, Massachusetts 01887. Animals were housed five (5) per sex per cage in suspended wire meshed stainless steel cages. Light cycle was 0700 through 1900 hours. Urine and feces dropped onto DACB papter (Upjohn). DACB papter changed at least three (3) times per week. GLP was followed in these experiments.

During the mating phase one male and two females were housed per suspended wire meshed stainless steel England breeding box. After twenty days, and when sperm was observed microscopically in the vagina of the female, the males were housed five/cage. Females were individually housed in plastic breeding boxes containing approximately three inches of Sani-Chips (Saint Regis Paper Co.) for bedding. Presumably, these Sani-Chips were acceptable to the dams for nesting.

After a seven (7) day acclimation period to laboratory conditions the animals were assigned to the following groups and received the indicated diet: Group I, 0 ppm; Group II, 125 ppm; Group III, 500 ppm; Group IV, 2000 ppm into F_{1B} premating phase and 1000 ppm thereafter. In each dose group there were 20 males and 20 females. Basic diet given was Micro Mix Rat Diet.

§5.2 F_0 Generation:

Animals in the F_0 generation were maintained on their respective diets for sixty (60 days). One male and one female were then placed into mating cages for twenty (20) days. Upon the identification of sperm in the vagina of the female, the female was placed into a breeding box and allowed to give birth to the F1A generation. The F1A generation was raised until weanling and then sacrificed. Approximately one-third

3

(1/3) of the F1A generation was subjected to necropsy examination at twenty-one (21) days of age.

After a seven day rest period, the F0 animals were remated, using different male/female pairings for no longer then twenty days. This mating producted the F1B generation. When the F1B generation was twenty-one days old, twenty (20) male and twenty female rats were selected from the litters of each group to become the parents of the next generation. Remaining weanling animals and the animals in the F0 generation were sacrificed. Approximately one-third (1/3) of the weanling animals from the F1B generation were subjected to a gross necropsy examination.

F1B Generation

Due to the reduced numbers of young and low body weights of parents the high dose level was reduced to 1000 ppm of the test material on October 19, 1977. Animals in the F1B generation were maintained on their respective diets for sixty days. The animals in the F1B generation were mated as described for the F0 generation and produced the F2A and F2B generations.

When the F2B generation was twenty-one days old, twenty male and twenty female rats were selected from litters of each group to become parents of the next generation. Remaining weanling animals and the animals in the F1B generation were sacrificed. Approximately one-third of the weanling animals from the F1B generation were subjected to a gross necropsy examination.

F2B Generation

Animals in the F2B generation were maintained on their respective diets for sixty days. The animals in the F2B generation were mated as described for the F0 generation and produced the F3A and F3B generations.

When the F3B generation was twenty-one days old, animals in the F3B generation and F2B generation were sacrificed. Animals in the F3B generation were subjected to a gross necropsy examination.

§5.3 Observations

5.31 Parental Animals:

a. Daily Observations: All animals were observed each morning and afternoon for mortality and were observed at approximately the same time each day for appearance, behavior, gait, and signs of toxic effect.

b. Food Consumption: Food consumption was measured weekly during the sixty day premating phase of each generation.

c. Body Weight: Body weights were recorded for each parental rat in all generations on a weekly basis during the sixty day premating phase. Maternal animals were weighed on days 0, 7, 14 and 20 of pregnancy. Maternal animals producing the F1B, F2B and F3B generations were weighed on days 0, 7, 14 and 21 post partum. New born animals were weighed on days 0, 4, 12 and 21.

d. Pregnancy Rate: The pregnancy rate was calculated for each generation as the ratio of the numbers litters born to the number of females paired.

e. Mating Performance: During the mating periods, vaginal smears were prepared daily and examined microscopically for the presence of sperm. The day that sperm were seen was termed the day of mating and day zero of pregnancy. Rats were observed to determine if there were any effects upon the estrous cycle.

f. Gestation Period: The length of gestation was recorded for all generations and data for test and control animals compared.

g. Litter Data: As soon as possible after birth, pups were counted, litters weighed, mortality determined where possible, and pups examined for external abnormalities. The pups were again weighed on days 4, 12, and 21 post partum. Pups were sexed at day 21.

§ 6.0 Results

6.1 Parental Animal Data

Survival - All rats survived (except one killed by a falling cage) in all close groups in all three generations. Thus, 2,4 DP did not effect survival of either male or female rats. Only a few rats were affected were affected by intermittant disease and was random in the dose groups and considered normal for this strain of rat. Appearance, behavior, gait were all normal.

Food consumption - Male and female rats were affected (less consumed) at the high dose of 2000 ppm in the F0 generation. Males in the Group III F1B and F2B generations ate less than controls. All other food consumption was the same among controls and treated rats. In those groups affected, food consumption was lowered 5-10%.

Body Weights - Commensurate with less food sonsumption, body weights in the high dose group were decreased 5-10% compared to controls. The low and mid dose group weights were the same as controls.

Pregnancy Rate - The rates of pregnancies is each group and generation were normal (90 - 100%) and no differences occur due to 2,4 DP treatments.

Gestation period - The gestation periods were unaffected by 2,4 DP acid and were 21 or 22 days.

6.2 Litter Data

Litter size - The litter sizes are given in Table I. There appears to be no significant differences in "averages" amoung the groups or amoung the generations, therefore 2,4 DP does not seem to affect litter number of pups. However, if the number of litters which have 8 or less pups are scored (Table I), it is seen that 2000 ppm in the F1A and F2A litters produce these smaller litters.

001995

Fetal Mortality - At day zero (at parturition) the percent of dead fetuses show definite 2,4 DP affects at 2000 ppm in F0 --> F1B and F1B ---> F2A (Table I). This increase is dead detuses is not seen in successive generations where the high dose was reduced from 2000 to 1000 ppm. It should be noted that a slight fetal effect* at 500 ppm (Group III) is seen after the F1B ---> F2A generation which is missing altogether in the F0 whelpings.

Neonatal Mortality - Groups III (500 ppm) and IV (2000 ppm) show significant neonatal mortality responses to 2,4 DP in the F1B---> F2A generation whereas only the 2000 ppm shows neonatal responses in the F0 generations.

After the high dose was reduced from 2000 to 1000 ppm the mortalities are same in the high dose as controls. The mortality from 500 ppm treatments tend to show slight increases* (compared to controls). The low dose (125 ppm) produces increased neonatal mortality only in the F3B generation.

Mean Pup Weight - The mean pup weights are recorded in Table II pups at birth and at the end of the lactation period (at 21 days).

At birth (day zero) - a slight reduction (Group IV) in mean pup weight is seen in the F0 --->F1B generation. Mean pup weights are the same among the treatment groups in all other generations.

At the End of Lactation - A definite reduction was seen in Group IV weights in F1A and F1B pups. After the high dose reduction no decrease in pup weights were seen compared to controls in any other generation.

Sex - There was no change in the proportion of males and females in each litter with treatment of 2,4 DP in any of the generations.

§ 7.0 Classification of Study: Core Minimum (because of the deficiencies indicated in § 2.0).

*Not statistically significant from controls when compared by a standard 2x2 contingency test.

TABLE I
Three Generation Study
Litter Mortality Incidence
(Average Cumulative Percent)

	Average No. of Pups/Litter	(Parturition) At Birth	At 4 Days	At 12 Days	At 21 Days	No. of Litters with 8 or less Pups/No. of Litter in Group
		Litter Mortality Incidence % (Cumulative)				
F_0–F_{1A}						
I	13.8 ± 2.7	1.21	3.10	3.42	3.42 ± 4.45	1/19
II	13.0 ± 1.8	0.39	2.05	2.05	2.05 ± 4.19	0/18
III	13.6 ± 2.3	0.00	2.78	2.78	3.47 ± 6.98	1/19
IV	11.2 ± 3.4	0.83	4.32	5.00	5.00 ± 6.49	5/18
F_0–F_{1B}						
I	13.2 ± 4.1	0.00	1.33	6.39	8.38 ± 12.07	3/18
II	11.9 ± 3.6	0.79	0.79	2.32	3.5 ± 6.05	3/19
III	14.7 ± 1.8	0.39	2.50	5.06	5.66 ± 5.32	0/18
IV	12.3 ± 3.5	18.15	18.90	33.85	37.95 ± 37.31	3/20
F_{1B}–F_{2A}						
I	11.94 ± 3.8	0.00	1.06	5.12	6.88 ± 12.04	3/17
II	14.1 ± 2.0	0.00	2.21	4.32	7.95 ± 11.20	0/19
III	11.9 ± 3.9	3.53	17.11	26.05	26.88 ± 35.44	4/17
IV	11.3 ± 3.9	11.15	13.10	19.80	26.00 ± 34.81	6/20
[Following F_{2A} Birthing — High Dose Group Lowered 2000 ppm to 1000 ppm]						
F_{1B}–F_{2B}						
I	13.6 3.1	1.50	2.06	5.12	5.12 ± 7.59	1/16
II	15.0 ± 3.0	0.32	3.63	9.63	11.44 ± 13.93	1/19
III	14.1 ± 2.5	3.52	9.32	11.42	12.77 ± 22.79	2/19
IV	13.1 ± 2.3	1.37	4.52	8.32	9.32 ± 12.50	1/19
F_{2B}–F_{3A}						
I	13.8 ± 2.0	1.50	1.95	3.35	4.26 ± 7.21	0/20
II	13.2 ± 1.7	0.39	1.61	1.72	2.66 ± 5.61	0/18
III	12.2 ± 2.5	4.65	5.00	5.30	5.30 ± 5.11	1/20
IV	12.2 ± 3.5	.70	0.70	0.70	1.65 ± 3.54	2/20
F_{2B}–F_{3B}						
I	14.6 ± 2.5	0.65	1.80	2.45	3.55 ± 7.53	1/20
II	13.6 ± 2.6	1.80	5.80	10.10	10.40 ± 19.04	1/20
III	14.3 ± 2.2	3.95	4.30	9.20	9.50 ± 15.04	0/20
IV	14.8 ± 1.1	0.70	1.30	1.90	1.90 ± 3.65	0/20

TABLE II.

Mean Pup Body Weight (Grams) ± Standard Deviation (%)

Generation / Treatment	Group I (0 ppm)	Group II (125 ppm)	Group III (500 ppm)	Group IV (2000/1000 ppm)
$F_0 \rightarrow F_{1A}$	(g) (%)	(g) (%)	(g) (%)	(g) (%)
At Birth (zero days)	6.18 ± 6.8	6.18 ± 15.7	6.22 ± 12.9	6.27 ± 14.2
End of Lact. (21 days)	43.1 ± 17.2	45.3 ± 17.8	43.0 ± 16.6	34.5 ± 27.2
$F_0 \rightarrow F_{1B}$				
At Birth (Zero days)	6.51 ± 11.1	6.34 ± 10.4	6.26 ± 8.9	6.00 ± 14.5
End of lact. (21 days)	46.4 ± 20.4	43.8 ± 17.7	40.1 ± 14.3	33.5 ± 31.1
$F_{1B} \rightarrow F_{2A}$				
At Birth (Zero days)	6.72 ± 13.7	6.34 ± 7.25	6.49 ± 12.4	6.70 ± 15.3
End of Lact. (21 days)	36.9 ± 24.4	33.19 ± 12.8	36.6 ± 20.3	34.4 ± 30.5
$F_{1B} \rightarrow F_{2B}$			Switch to 1000 ppm	
At Birth (Zero days)	6.56 ± 9.1	6.31 ± 7.3	6.46 ± 14.4	6.34 ± 10.4
End of Lact. (21 days)	39.8 ± 21.3	38.3 ± 16.9	39.6 ± 21.3	37.1 ± 20.8
$F_{2B} \rightarrow F_{3A}$				
At Birth (Zero days)	6.13 ± 17.9	5.99 ± 9.7	6.28 ± 8.9	6.18 ± 12.0
End of Lact. (21 days)	38.8 ± 16	39.0 ± 13.7	42.5 ± 27.0	39.7 ± 28.2
$F_{2B} \rightarrow F_{3B}$	6.73 ± 15.9	6.65 ± 9.3	6.75 ± 13.2	6.38 ± 7.1
End of Lact (21 days)	39.7 ± 17.5	38.3 ± 18.0	39.6 ± 20.0	33.9 ± 11.8

Note: Each value is given in grams weight ± the % variation in each group.