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

pc # 057901

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No.: 057901; Trichlorfon - Two Generation Reproduction Study in Rats

Tox. Chem. No.: 385
Bar Code No.: D177975
Record No. : S417443

FROM: Melba S. Morrow, D.V.M. 9/3/92
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Brigid Lowery, PM 72
Registration Division (H7505C)

THRU: Karl Baetcke, Ph.D. 9/4/92
Branch Chief
Toxicology Branch I
Health Effects Division (H7509C)

Sponsor: Mobay

CONCLUSIONS: Based on the results of this study, when trichlorfon was administered to male and female Sprague Dawley rats at doses of 0, 150, 500 and 1750 ppm (approximately 0, 15, 50 and 175 mg/kg, respectively), the parental NOEL was < 150 ppm and the LOEL was 150 ppm based on decreases in plasma, red cell and brain cholinesterase activity in both generations. In the F_0 generation, females in the high dose group had chronic pneumonia. In the F_1 generation, both sexes of animals in the 1750 ppm group had body weight decrements when compared to controls, and pulmonary (chronic pneumonia characterized by thickened alveolar septa, macrophage accumulation, cholesterol clefts, connective tissue proliferation, type II pneumocyte hyperplasia and neutrophilic infiltration) and renal (mineralization and hydronephrosis) pathology. All of the findings could be associated with the administration of the test material. Chronic pneumonia and kidney mineralization were also noted in mid dose females in the F_1 generation.

The reproductive NOEL was 500 ppm and the LOEL was 1750 ppm based on decreased pup weights on days 7 and 21 and the presence of dilated renal pelvises.

Copies of the DER are attached for your reference.

Reviewed by: Melba S. Morrow, D.V.M. MSM 9/3/92
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES 9/3/92
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Two Generation Reproduction Study - Rats

GUIDELINE #: 83-4

TOX. CHEM. #: 385

MRID #: 422283-01

TEST MATERIAL: Trichlorfon Technical 98%

SYNONYMS: Dylox

STUDY NUMBERS: 89-672 EA

SPONSOR: Mobay
Kansas City, Mo.

TESTING FACILITY: Mobay
Stilwell, Kansas

TITLE OF REPORT: Two Generation Reproduction Study in Rats Using Technical Grade Trichlorfon

AUTHORS: D.A. Eigenberg

REPORT ISSUED: December 20, 1991

CONCLUSIONS: Based on the results of this study, when Trichlorfon was administered to male and female Sprague Dawley rats at doses of 0, 150, 500 and 1750 ppm (0, 15, 50 and 175 mg/kg, respectively), the parental NOEL was < 150 ppm and the LOEL was 150 ppm based on decreases in plasma, red cell and brain cholinesterase activity in both generations. In the F_0 generation, females in the high dose group had chronic pneumonia.

In the F_1 generation, both sexes of animals in the high dose group had decrements in body weight when compared to controls, and pulmonary (chronic pneumonia characterized by thickened alveolar septa, macrophage accumulation, cholesterol clefts, connective tissue proliferation, type II pneumocyte hyperplasia and neutrophilic infiltration) and renal (mineralization and hydronephrosis) pathology. All of these findings were associated with the administration of the test material.

The reproductive NOEL was 500 ppm and the LOEL was 1750 ppm based on decreased pup weights on days 7 and 21 and the increased incidence of dilated renal pelvises.

CLASSIFICATION: Guideline

MATERIALS:

Technical grade trichlorfon, a white crystalline substance with a purity of 98%, was the test material. Male and female Cesarean derived Sprague Dawley rats were the test animals. At the start of the study animals were 7 weeks of age.

METHODS:

Following a two week acclimation period, animals that were to serve as the F_0 (parents) generation were randomly assigned to treatment groups based on weights. Animals were maintained in an environment that allowed a 12 hour light/dark cycle with room temperatures being maintained at 18 to 26°C and a relative humidity range of 40 to 70%. Animals were individually housed during the premating period and food and water were provided ad libitum.

The test material was analyzed prior to and periodically during the study for percent actrive ingredient. The concentration of Trichlorfon in the feed was analyzed using gas chromatography. Feed samples were analyzed periodically during the study to verify stability, and homogeneity was determined from nine samples collected from the top, middle and bottom of the mixing bowl. Homogeneity was determined for the low and high dose levels.

Stability was analyzed at room and freezer temperatures (-23oC) Stability analysis at room temperature was conducted after the samples had been stored unrefrigerated for 1, 3, 7, 10, 14 and 17 days. Frozen samples were analyzed prior to freezing and after being frozen for 7 and 14 days. Stability was based on 80% recovery of the test material.

One hundred twenty males and 120 females were assigned to one of four treatment groups and served as F_0 parental animals. Dose groups for this study were selected based on the results of a two year reproduction study, a chronic toxicity/oncogenicity study and a pilot study designed to examine cholinesterase activity. The pilot study was not reviewed and the results were not provided in the report.

The F_1 parents were randomly selected from the offspring to allow for 30 pairs per dose group. These F_1 adults received the same dose of trichlorfon as their parents, and brother/sister matings were avoided.

Dose groups for both generations were as follows:

Dose Group	Conc. (ppm)	# Animals (F_0 and F_1)
Control	0	30M, 30F
Low	150	30M, 30F
Mid	500	30M, 30F
High	1750	30M, 30F

Trichlorfon was dissolved in corn oil and was mixed in the diet. Control animals received diets with only corn oil. Diet batches were prepared weekly and, once a week, the test animals received a fresh ration. Previously frozen preparations were administered for the remaining six days.

All parental animals (F_0 and F_1) received the test material for 10 weeks prior to mating. For the F_1 animals, the first dose was administered following weaning.

Breeding

For both the F_0 and F_1 animals, vaginal smears were collected from 10 females per dose group to determine the stage of the estrous cycle. Breeding was accomplished by cohabitating one female and one male from the same dose group. Females were examined daily for the presence of a vaginal plug and smears were examined for the presence of sperm. Once sperm were observed, females were removed from the males and were placed in individual cages. Females remained in these cages throughout the gestation and lactation periods. The maximum period of male/female cohabitation was 21 days. After this period, if mating did not occur, females were co-housed for one week with a different proven male from the same dose group.

Observations:

Cage side observations were conducted twice daily for moribundity, mortality and clinical signs of toxicity. Detailed physical examinations were conducted weekly and body weights and food consumption were measured weekly for the ten week period prior to mating. Body weights were measured for dams on days 0, 6, 13 and 20 of gestation and on days 4, 7, 14 and 21 of lactation. Food consumption was measured weekly during gestation, twice weekly during the first week of lactation and once weekly during weeks 2 and 3 of lactation.

For males, body weights and food consumption were measured weekly. No food consumption measurements were made during the cohabitation period.

Plasma and red cell cholinesterase levels were measured for 10

animals per sex per group for both the F_0 and F_1 adults during the eighth week of treatment and just prior to sacrifice. Brain cholinesterase levels were measured at terminal sacrifice for 10 animals per sex per group in both generations.

Plasma, red cell and brain cholinesterase levels were also measured for one male and one female pup from 10 litters/dose group at culling and weaning. Blood was often pooled to obtain sufficient amounts for analysis. When this was done, brain tissue was also pooled for the same animals.

For each litter, the number of live and stillborn pups were recorded on days 0 and on days 21. Pup weights were recorded on days 4, 7, 14 and 21. Culling was conducted on day 4 to reduce litter size to 8 pups. No adjustments were made if the litters were 8 pups or less at the time of birth. All culled animals, all animals found dead or all animals sacrificed <u>in extremis</u> were subjected to a gross necropsy.

Pups that were dead on day 0 post- partum, were examined to determine if they were alive or stillborn. This was done by determining lung floatation or by observing the presence of fluid in the stomach. Males were sacrificed after the litters were delivered. If dams went beyond day 24 of gestation, males were sacrificed at that time. Females were sacrificed after the pups were weaned or after day 24 of gestation.

The uterus from each dam was examined and the number of implantation sites were counted. For all F_0 and F_1 adults, terminal body weights and gonad weights were recorded. For F_1 adults, liver, kidney and lung weights were recorded. Mating, fertility, gestation, birth, live birth and viability (on and after day 4) indices and gross lesions were recorded for both generations.

Gross examinations were conducted and tissues were collected from the reproductive organs of adults in both generations. In the F_1 adults, tissue samples were also collected from the stomach, duodenum, liver, kidney, lungs and gross lesions.

STATISTICAL ANALYSIS:

ANOVAs were performed on data for body weights and food consumption, and were followed by Dunnett's test to identify significant differences. The Kruskall- Wallis Test was used to analyze the number of estrous cycles, litter size and gestation length in the dams, and viability, birth and live birth indices and gross lesions for the offspring. This test was also used to evaluate data on the number of implantation sites, time to mating and gross lesions. Significant differences between groups subjected to the Kruskall-Wallis Test were identified using the Mann-Whitney U Test.

Bartlett's test was used to evaluate terminal body weights, organ weights and cholinesterase data. The Kruskall-Wallis test and the Mann- Whitney U tests were used if these data were found to be non-homogeneous.

The Chi-Square test, followed by Fisher's Exact with Bonferonni's Correction was used to analyze mating, fertility and gestation indices and the number of dams with cannabalized pups. Chi-Square was also used to evaluate the frequency of gross and microscopic lesions that occered with uncertain significance.

Significance was at $p < 0.05$ for all test except Bartlett's ($p< 0.001$).

QUALITY ASSURANCE:

A signed Statement of Quality Assurance dated December 18,1991 was included in the summary.

RESULTS:

Homogeneity, Stability and Concentration

The active ingredient concentration ranged 97.6 to 98.5%.

Based on the results of the tests conducted to evaluate the homogeneity, stability and concentration, it was determined that Trichlorfon was homogeneously distributed in the ration. It was also determined that the compound was stable after being stored at room temperature for 17 days and under refrigeration for 21 days.

It is noted that at 100 ppm, only 69% of the chemical was recovered at day 14. At the next sampling interval, the recovery was greater than 80%. Lack of stability beyond one week would not adversely affect the outcome of the study, as new rations were formulated on a weekly basis. Although the sponsor states that stability was observed up to day 17 under room conditions, the data indicate that the compound may have only been stable up to day 10.

For the three dose groups, the concentration ranged from 90 to 108%. (See Table I).

F_0 Generation:

No deaths were reported in this generation prior to sacrifice. Clinical signs that were observed with the highest frequency included alopecia and lacrimation. These signs were present in control and treated groups and were not believed to be associated

with the administration of the test material. No treatment related effects were observed on body weight or food consumption. During the pre-treatment period in males and females, decreases in mean body weights were reported in the mid dose group at days 49 (6%) and 56 (6.8%) and in the high dose group at day 56 (6%). Reductions in mean food consumption were reported in low dose and high dose females on day 56. All of the reductions were transient in nature and none were considered statistically or biologically significant.

In males, increases in mean body weights were recorded for mid dose and high dose groups at day 0 and for high dose groups at day 7. None of the observed changes in body weight were associated with the administration of the test material.

In F_0 dams, there were no effects on body weight during the gestation or lactation periods. Decreases in food consumption were recorded for the high dose groups on days 7, 14 and 21. These decreases ranged from 13 to 30% lower than the values reported for the control groups.

No treatment related effects on time to mating, mating index, fertility index and gestational length were reported. Additionally, no effects on F_1 litter size were reported. (See Table II for reproductive parameters).

In the F_1 pups, a higher number of pups in the high dose group were cannabalized by their dams. In the high dose group, 9/25 dams cannabalized pups compared to 4/27 in the control group.

The F_1 mean pup weights were significantly lower for the high dose group when compared to controls on days 7, 14 and 21. This represented percentage reductions of 10, 18, and 26, respectively, when compared to controls at the same intervals. A slightly lower viability index was reported for high dose animals (97%) when compared to 100% for controls. This finding was neither statistically nor biologically significant.

In high dose adult animals, plasma cholinesterase activity was inhibited in both males and females at premating. In the high dose females, plasma cholinesterase levels were also inhibited at termination (40 to 75% inhibition). In mid dose females, decreases (30 to 47%) in plasma cholinesterase levels were reported at premating and termination. In low dose females, inhibition of plasma cholinesterase activity (24% lower than controls) was only present at termination.

In the low dose animals the observed inhibition of plasma cholinesterase at termination, only, may have been the result of an additive effect of the compound on this parameter.

Erythrocyte cholinesterase levels were decreased in high dose males (17 to 25% less than control values) and females (20 to 43%) and in mid dose females (27% less than controls) at termination. Brain cholinesterase activity was decreased in high dose males (12% < controls), and in high, mid and low dose females (59%, 38% and 12%, respectively).

No treatment related lesions were found in the reproductive organs. Discolored lungs were present in high dose females in the F_0 generation, with 13/30 animals in this group being affected. Microscopically, these animals had chronic pneumonia, characterized by multifocal regions of thickened alveolar septa with increased infiltration of alveolar macrophages and increased amounts of fibrous connective tissue. Perivascular neutrophilic infiltration and hyperplasia of Type II pneumocytes were also present.

In the high dose group there was an increase in the incidence of dilated renal pelvises in the F_1 pups. At this dose level, a total of 15 pups had this condition that was either unilateral or bilateral in its presentation. This compares to 7, 5 and 9 pups in the control, low and mid dose groups, respectively.

F_1 Generation

In high dose F_1 females, mean body weights were 5 to 10% lower than those reported for control animals throughout the premating period. No concurrent decreases in food consumption were reported except on day 70. Lower body weights were also reported for males in the high dose group and were correlated to decreased food consumption in this group.

During gestation, lower body weights were reported on days 0, 6 and 13; however, body weight gains were unaffected. Food consumption was 12 to 28% lower than controls on days 7, 14 and 21 in the F_1 dams in the high dose group. The observed decreases in body weights in the high dose group, were believed to be related to lower pup weights during the lactation period.

The only reproductive parameter that was affected in this generation was the birth index. This index was significantly reduced for low and high dose groups and translated into a statistically significant decrease in the litter size for both of these groups. No treatment related effects were observed on gender, gestation, mating and fertility indices, or on the number of implantation sites and gestation length. Mean pup weights were lower on days 14 (26%) and 21 (19%) when compared to controls. (See Table III for reproduction and litter data).

There was an increase in the incidence of cannabalism in high dose dams (7/26) when compared to controls (3/21). Additionally,

"purple coloration" was reported in 11 pups, ten of which were from the same litter. The significance of this finding is unknown.

Plasma cholinesterase was decreased during premating in high dose males and females and in mid dose females. Erythrocyte cholinesterase was also decreased in high dose females throughout the study and in high dose males during pre-mating. In mid dose females, erythrocyte cholinesterase was depressed during pre-mating and at terminal sacrifice. Brain cholinesterase was affected (decreased) in high dose males (12% lower than controls), and in high, mid and low dose females (63%, 41% and 14 % lower than controls, respectively).

Cholinesterase levels were also affected in pups born to dams in the high dose group.

Grossly, there was a reported decrease in mean terminal body weights in adult high dose animals of both sexes. Discolored lungs were observed in 24/30 high dose females and in 8/30 high dose males. Dilated renal pelvises were also present in 16/30 high dose females and in 10/30 high dose males. Microscopically, the lesions in the lungs were characterized by thickened alveolar septa, alveolar macrophage accumulation, cholesterol clefts, fibrous connective tissue proliferation, type II pneumocyte hyperplasia and neutrophilic infiltration. The kidney lesions were characterized by renal mineralization and hydronephrosis. Microscopic lesions could be correlated to gross findings and a diagnosis of chronic pneumonia and hydronephrosis were made based on histopathology.

In the F_2 pups from the high dose group, there was an increase in the incidence of dilated renal pelvises. In this group 52 pups were diagnosed with this condition compared to 5, 16 and 10 pups in the control, low and mid dose groups, respectively.

DISCUSSION:

It was noted that during lactation in the F_0 females, the decrease in food consumption could be related to a decrease in the level of lactation. This decrease in food consumption was reflected in the offspring as decreased pup weights. It appears as though the dams decreased their caloric intake and compensated for the increased caloric requirements of lactation by decreasing their milk production.

The low body weights observed in adult F_1 animals appeared to be a carry over from low birth weights and from low body weight gains prior to mating.

Although cannabalization was not considered statistically significant in F_0 or F_1 dams, the possible relationship of this

finding with the administration of the test compound is unclear. In the F_0 generation, 9/25 high dose dams had cannabalized pups compared to 4/27 dams in the control group. Similar findings occured in the next generation, where cannabalization was reported in 7/26 high dose dams compared to 3/21 controls. In the other groups cannabalization was similar to controls. (F_0: 1/27@ LD, 4/28 @ MD; F_1 : 3/26 @ LD, 4/20 @ HD).

There is also the possibility of an association with the administration of Trichlorfon and the presence of lung lesions. Similar lesions were observed both grossly and microscopically in high dose females in the F_0 generation and in high dose males and females in the F_1 generation. Additionally, pneumonia was observed in the combined chronic toxicity/ oncogenicity study conducted in Fischer 344 rats. Although there were slight differences in the histology of the lungs, the observed differences were probably related to strain variations. In both studies females were affected more than males with regard to frequency and severity of lesions.

The lesions that were observed in the kidneys appeared to be related to trichlorfon and involved a possible maturation delay. This conclusion is based on the fact that no lesions were present in the F_0 animals, but were prominent in offspring of F_0 and F_1 adults in the high dose group. Maturation delays in the kidney leading to hydronephrosis are usually permanent if there is persistent dilation of the renal pelvis beyond day 21 in weanlings. In the F_1 generation dilation of the renal pelvis up to day 21 (and in a few cases beyond day 21) was reported in 6/7 controls, 4/5 at the low dose 9/9 at the mid dose and 15/15 at the high dose. In the F_2 generation, dilated renal pelvises were reported up to and beyond day 21 in 4/5 controls, 16/16 at the low dose, 9/10 at the mid dose and 50/52 at the high dose.

The results of this study demonstrate a parental NOEL of < 150 ppm and the LOEL of 150 ppm based on decreases in cholinesterase activity (plasma, erythrocyte and brain) in both generations. In F_0 females in the high doses group, chronic pneumonia was diagnosed by microscopic examination. At 1750 ppm, in both sexes in the F_1 generation, there were decreases in body weights, and renal (hydronephrosis and mineralization) and pulmonary (chronic pneumonia) lesions that were associated with the administration of the test material. Chronic pneumonia and kidney mineralization were also noted in the mid dose females in the F_1 generation.

The reproductive NOEL was 500 ppm and the LOEL was 1750 ppm based on decreased pup weights during lactation days 7 and 21 and the presence of dilated renal pelvises.

The study is classified as core guideline and satisfies the requirement for a two generation reproduction study (83-4).

TABLE I
Homogeneity, Stability and Concentration

Homogeneity

Layer	Position	100 ppm	2500 ppm
Top	1	100	2050
	2	93.1*	2390
	3	117	2285
Middle	4	106	2629
	5	79*	2520
	6	105	2598
Bottom	7	110	2158
	8	94.9	2826
	9	96.7	2415
Mean % Nominal		97	97

Stability

Day (room Temp.)	100 ppm		2500 ppm	
	Conc.	% rel.	Conc.	% rel.
0	96.8	100	2567	100
7	90.6	94	2482	97
14	66.7	69	2059	80
17	81.6	84	2170	85
Day (-23°C)				
0	97.8	100	2603	100
7	96.8	99	2567	99
14	111.0	113	2332	90
21	84.7	87	2636	101

Concentration Analysis

	150 ppm	**500 ppm**	**1750 ppm**
Mean conc.(based on 5 samples)	135	489	1885
% Nominal	90	98	108

* = average of two samples
Data taken from pp 88-96 of report.

TABLE II
Reproductive Parameters and Litter Data for F_0 Adults

Parameters	Dose group (ppm)			
	0	150	500	1750
Mating index	100	100	100	93
fertility index	90	90	93	96
gestation index	100	100	100	93
Birth index	94	93	93	86
Gest. length (d)	21.7	21.6	21.8	21.6
mean # implants	15	15	15	14
pup gender (% M)	56	49	51	56
Litter size	14	14	14	13
Live count (day 4)	14	14	14	11
% viability (day 4)	98	99	98	93
% viab. (day 21)	100	100	100	97*
Avg. pup weight (g)				
Day 0	6.3	6.2	6.3	6.0
Day 4	9.2	9.0	9.6	8.9
Day 7	15.1	14.8	15.5	13.6*
Day 14	31.1	31.6	31.3	25.5*
Day 21	49.8	49.2	49.6	36.8*

* = $p < 0.05$

TABLE III

Reproductive Parameters and Litter Data for F_1 Adults

Parameters	Dose group (ppm)			
	0	150	500	1750
Mating index	100	97	100	97
fertility index	72	93	70	90
gestation index	100	96	95	96
Birth index	95	85*	92	83*
Gest. length (d)	21.6	21.6	21.7	21.8
mean # implants	15	14	14	14
pup gender (% M)	52	50	47	53
Litter size	15	13*	14	12*
Live count (day 4)	14	13	14	11
% viability (day 4)	98	98	98	90
% viab. (day 21)	99	99	98	91
Avg. pup weight (g)				
Day 0	6.1	6.3	6.2	6.1
Day 4	9.2	9.8	9.2	8.6
Day 7	14.8	15.8	14.9	13.7
Day 14	29.6	30.8	30.1	26.1*
Day 21	46.6	48.3	45.6	37.6*

* = $p < 0.05$

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HED File Code	**13000 Tox Reviews**
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