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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dimethoate - Review of a Chronic Toxicity and Carcinogenicity Study in Mice

Tox. Chem No.: ~~716A~~ 358

FROM: Yiannakis M. Ioannou, Ph.D.
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5/13/87

Registrant: American Cyanamid Company, Princeton, NJ

Accession Numbers: 265362-265364

Toxicology Branch has reviewed a chronic toxicity and oncogenicity study in mice, submitted by the registrant in response to a Data Call-In Notice for dimethoate.

The study was classified as Core-Minimum due mainly to the failure of the registrant to provide the Agency with the appropriate stability data on dimethoate.

Reviewed by: Y.M. Ioannou
Section VII, Toxicology Branch (TS-769C)
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DATA EVALUATION RECORD

Study Type: Chronic Toxicity and Carcinogenicity.(Mouse)

Accession Nos.: 265362-265364 Tox. Chem. No.: 358
MRID # 00163800

Test Material: Dimethoate

Synonyms: Rogor; Cygon

Project No.: 75CO326/8242

Sponsor: American Cyanamid Company, Princeton, NJ

Testing Facility: BASF, Ludwigshafen/Rhein, FRG (West Germany)

Title of Report: Study of the Toxicity of Dimethoate in Mice After 78-Week Administration in the Diet

Authors: J. Hellwig, K. Deckardt, D. Mirea, and B. Hildebrand

Report Issued: September 24, 1986

Conclusions:

The oncogenic NOEL of dimethoate in male and female B6C3F1 mice was found to be 100 ppm (MDT) while the LEL for both sexes was 200 ppm (HDT). The NOEL and LEL for systemic toxicity were found to be lower than 25 ppm (LDT).

Classification: Core-Minimum.

Materials and Methods:

The test material dimethoate, a solid crystalline, grayish-white substance, with a reported chemical purity of 96.71% or greater (Batch No. 611A) was used in this study. Male and female B6C3F1 CrIBr mice (obtained from Charles River U.K. Ltd., Margate, Kent) 38 days old and weighing approximately 20 g for males and 18 g for females were used throughout the study. Upon arrival all animals were checked for clinical signs of disease and only healthy animals were used for the study. The animals were uniquely identified by ear tatoo or ear tatoo and toe amputation, and housed individually in type I Makrolon cages which were placed in air-conditioned rooms with a temperature of 20 °C to 24 °C, a relative humidity of 30 percent to 70 percent, and a 12-hour light/dark cycle (number of air changes per hour not reported). All cages were arranged on racks assuring uniform ventilation and light. Feed (ground Kliba 343) and tap water were available to all animals ad libitum. All animals were acclimated to laboratory conditions for 8 days prior to initiation of the study.

Study Design:

A total of 240 male and 240 female mice were used for this study. The mice were allocated to four groups/sex based on their weight as follows:

Test Group	Dose (ppm)	Number of Animals/Group	
		Male	Female
1. Control	0	50[1] (10)[2]	50 (10)
2. Dimethoate (LDT)	25	50 (10)	50 (10)
3. Dimethoate (MDT)	100	50 (10)	50 (10)
4. Dimethoate (HDT)	200	50 (10)	50 (10)

1/Main study; sacrificed at 78 weeks.
2/Satellite groups; sacrificed at 52 weeks.

For the preparation of the test diets, dimethoate was dissolved in distilled water and then mixed with diet using a Braun MX 32 mixer. This premix was then diluted by mixing with appropriate quantities of diet so that the desired concentrations in the diet (25, 100, or 200 ppm) were obtained. Test diets were prepared fresh every 7 days and stored at ambient temperature. Samples of test diets (five samples/dose) were analyzed for stability, concentration, and homogeneity at 4 and 12 weeks of the study and then every 3 months for the remainder of the study.

X		X		X	
X	Ileum*		Urogenital	X	Adrenals*
X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*		Parathyroids*
XX	Liver*		Epididymides	X	Thyroids*
	Digestive system (cont'd)	X	Prostate		Other
X	Gallbladder*		Seminal vesicle	X	Bone*
X	Pancreas*	XX	Ovaries	X	Skeletal muscle*
	Respiratory	X	Uterus*	X	Skin
X	Trachea*			X	All gross lesions and masses
X	Lung*				

*Recommended by Subdivision F (Oct. 1982) Guidelines for chronic studies.

Statistical Analysis [Abstracted from the original report]:

o Clinical examinations

For the statistical evaluation, means and standard deviations were calculated for the variables (food consumption, body weight, body weight change, and intake of test substance).

The statistical analysis of body weight and body weight change was carried out using an analysis of variance (ANOVA) followed by Dunnett's test.

Significances resulting from this test have been indicated in the Tables (* for S $\geq$ 95%, ** for S $\geq$ 99%).

o Clinical chemistry and hematology

o Blood and plasma examinations

After statistical correction (NALIMOV criterion), means and standard errors were calculated and tabulated together with the individual values.

In order to test significances, the individual dose groups were compared with the control group using the t test.

Significances resulting from the t test have been indicated in the tables (* for S > 95% and ** for S > 99%).

o Pathology

o Organ weights

Study	Sex	Mortality 0	25 ppm	100 ppm	200 ppm
Main-At 78 weeks	M	3/50 (6)*	2/50 (4)	2/50 (4)	1/50 (2)
	F	1/50 (2)	1/50 (2)	5/50 (10)	3/50 (6)
Satellite-At 52 weeks	M	0/10 (0)	1/10 (10)	0/10 (0)	0/10 (0)
	F	0/10 (0)	0/10 (0)	1/10 (10)	1/10 (10)

*Numbers in parentheses denote percent mortality.

Body weight gains were statistically significantly lower in all treated groups of male mice as compared with controls for at least the first 5 weeks on study with, in most cases, a dose-related trend. These differences persisted for the high-dose group up to 50 weeks (day 350) on study while in the lower dose groups such differences were observed but not consistently. Body weight gains appeared to be similar between treated and control groups up to day 420 on study. From this point on, animals of the low- and high-dose groups had body weight gains slightly higher than controls while animals of the mid-dose group had body weight gains statistically significantly higher than controls (persisting until termination of study). For female mice of the main study statistically significantly lower body weight gains were observed in the high-dose group mainly in the first 5 weeks on study. Subsequently, starting with day 63 and continuing until the end of the study, body weight gains were statistically significantly higher than controls in the mid- and high-dose groups while in the low-dose group significantly higher body weight gains were seen from day 168 until the end of the study. Treated male and female animals of the satellite groups had body weight gains comparable to the controls with the exception of a few isolated instances.

Food consumption appeared to be similar between treated and control groups in both sexes throughout the study. However, according to the authors, "isolated animals spilled major amounts of feed especially during the first months of the study." Since the amount of feed spilled was not measured, it is difficult to draw any conclusions concerning food consumption/food efficiency. Based on food consumption, the authors calculated the approximate intake of the test substance (in mg/kg body weight) by all treated groups at different time intervals (3, 6, 12, or 18 months post-treatment). These results show that females (of all treated groups) had a higher test article intake than males at all time points examined, as follows:

Dose (ppm)	Percent Higher Intake of Dimethoate in Females than Males 3 months	6 months	12 months	18 months
25	50	37	47	34
100	49	39	24	15
200	17	12	16	9

A variety of hematology parameters were found to be statistically significantly different between the treated and control groups in both sexes and are shown in Table 2. However, these differences do not appear to be biologically meaningful (no dose response was seen) and they rather reflect normal biological variability.

Table 2

Parameter	Sex	Time 1/ of Sampling	Dose (ppm) 25	100	200
Hemoglobin	M	Week 51			↑**
	F	Week 78	↑***	↑**	
Erythrocytes	F	Week 78	↑**		
Hematocrit	M	Week 78			↓**
MCH	M	Week 78			↑**
	F	Week 78		↑***	↑***
MCHC	F	Week 78		↑***	↑***
Platelets	M	Week 78		↑**	
Leukocytes	M	Week 78		↑**	↑**
	F	Week 51		↓***	↓**
	F	Week 78			↑**

1/ Week 51 = Satellite group; week 78 = main group.
↑** Statistically significantly higher than controls; $p < 0.05$
↑*** Statistically significantly higher than controls; $p < 0.01$
↓** Statistically significantly lower than controls; $p < 0.05$
↓*** Statistically significantly lower than controls; $p < 0.01$

No urinalysis measurements were carried out in either the satellite groups or the main study.

Absolute and relative organ to body weight ratios were found in some cases to be statistically significantly different between treated and control groups in male and female mice at the final as well as at the interim kill. As can be seen from Table 3, absolute liver weight for the mid- and high-dose groups in both sexes of the main study was statistically significantly higher than controls while relative liver weight was statistically significantly higher than controls in the mid- and high-dose groups of male mice and statistically significantly lower than controls in all groups of female mice. For the interim kill (satellite group) the absolute weight of liver in male mice of the mid- and high-dose groups was statistically significantly higher than controls while relative liver weight in all groups of female mice was lower than controls. The absolute and relative weight of the ovaries (main study) in all treated groups was statistically significantly lower than controls with an apparent dose response trend. The relative weight of brain, heart, kidneys,

Table 4

Summary of Macroscopical Observations - Main Study

Macroscopical Observation	Sex	Dose (ppm) 0	25	100	200
Liver - Pale dots, foci	M	10/50[1]/	8/50	12/50	19/50
	F	4/50	9/80	5/50	12/50
- Nodules/Nodes	F	0/50	2/50	3/50	3/50
Spleen - Enlarged/Nodules	M	2/50	2/50	3/50	6/50
Kidneys - Cyst	M	1/50	0/50	0/50	3/50
Bulbourethral gland	M	1/50	1/50	1/50	6/50
Uterine horns - Cyst	F	1/50	0/50	1/50	3/50
Adipose - Yellowish formation/Cyst	F	0/50	1/50	8/50	5/50

1/ Number of mice with specified observations/total number of tissues examined.

Histopathological examination revealed a variety of non-neoplastic and neoplastic lesions in several tissues of male and female mice. A summary of the incidence of major lesions is shown in Table 5. Non-meoplastic lesions: Vacuolization of hepatic cells, mainly of grade 1 (minimal) appears to be dose-dependent resulting in an incidence of 6/50, 16/50, 30/50, and 33/50 in control, low-, and high-dose groups, respectively, in female mice (Table 5). The combined incidence (from minimal-grade 1 to severe-grade 4) of hepatocytic vacuolization in female mice (7/50, 19/50, 36/50, and 38/50 for control, low-, mid-, and high-dose groups, respectively), was statistically significantly higher than control in all treated groups and also shows a dose-response relationship. The incidence of extramedullary hematopoiesis in the spleen was relatively higher in the mid- and high-dose groups, compared to controls for both sexes (especially males - Table 5).

Neoplastic lesions: A higher incidence in the treated groups of male and female mice as compared to controls was observed in several tissues as shown in Table 5. Thus, the incidence of hepatocellular adenomas was higher in the high-dose group females compared to controls (5/50 versus 8/50 for controls and high-dose groups, respectively). The combined incidence of hepatocellular adenomas and carcinomas was considerably higher in the high-dose group females than the controls (5/50 versus 10/50 for controls and high-dose groups, respectively). In males, the incidence of hepatocellular adenomas/carcinomas was comparable between treated and control groups. Similarly, the combined incidence of lung adenomas and adenocarcinomas in male mice was higher in the high-dose group, compared to controls (2/50 versus

7/50 for control and high-dose group, respectively). When the neoplastic lesions of the hemolymphoreticular system of male mice are combined (reticulosarcoma + lymphoma + leukemia - Table 5) a statistically significantly higher incidence is seen with the high-dose group compared to controls (1/50 versus 7/50 for the control and high-dose groups, respectively).

Discussion:

The present study has investigated the oncogenic potential of dimethoate in male and female $B6C3F_1$ mice. The authors reported that the selection of the dose levels used in this study (25, 100, and 200 ppm) was based on the results obtained from two previous studies. The first study was a carcinogenicity study conducted by the National Cancer Institute (NCI-CG-TR #4; 1977 CAS No. 60-51-5) which was negative for oncogenicity under the test conditions in male and female $B6C3F_1$ mice using dimethoate at 250 or 500 ppm in the feed, for up to 80 weeks. Both dose levels resulted in some toxicity to the mice including tremors, alopecia, distended abdomens, and generally poor health. The second study was a range-finding study carried out by the sponsor (BASF) in male and female Wistar rats using 5, 25, or 75 ppm of dimethoate in the diet for 28 days. All three dose levels resulted in significant reduction of plasma, erythrocyte, and brain cholinesterase activities in both sexes.

Review of the analytical data presented in this study indicates that: The purity of dimethoate throughout the study was $\geq$ 96.7 percent; test article concentrations in the diet were approximately the same as the target concentrations; and that the test article was homogeneously distributed in the diet for all dose levels. The stability of dimethoate in the diet at different time intervals (at ambient temperatures) was not carried out in this study but, according to the authors, was investigated in an earlier 28-day range-finding study in rats. We request that the authors provide the Agency with the appropriate stability data on dimethoate.

Clinical signs of toxicity and mortality were approximately the same between the treated and control groups.

Body weight gains were statistically significantly lower in all treated groups of male mice compared to controls at the initial stages of the study (up to week 5). The high-dose group had significantly lower body weight gains throughout the first year of study. In all cases, however, body weight gains in treated groups approached that of controls and subsequently exceeded control body weight gains. In females, only the high-dose group had a statistically significantly lower body weight gain in the first 5 weeks on study. As was the case with male mice, body weight gains of the treated groups reached that of the

dose-response relationship and the absence of correlation with specific histopathological lesions suggest that these findings are of no biological significance (i.e., no effect of dimethoate on the hemopoietic system).

The evaluation of data on organ weights indicates that the only finding of some toxicological significance was the statistically significant increase in absolute weight of the liver in male and female mice of the mid- and high-dose groups (Table 3). Although this increase can be partially explained by the increase in body weight in these groups, it also correlates to a great extent with the increase in non-neoplastic/neoplastic lesions in the livers of the same groups of male and female animals (Table 5). Thus, we considered this effect to be compound-related. The statistically significant difference between treated and control groups in absolute/relative weights of a number of other organs in male and female animals does not appear to be of biological significance since there is no evidence of dose-response and/or direct correlation with histopathological changes.

The following points can be made concerning the oncogenic potential of dimethoate in $B6C3F_1$ male and female mice.

1. There was a statistically significant increased total incidence in hepatocytic vacuolization with a highly significant linear trend in female mice (7, 19, 36, and 38 animals with this lesion for the control, low-, mid-, and high-dose groups, respectively). Most of the increase was due to the "minimal" (grade 1) increase in vacuolization. This increase in hepatocytic vacuolization correlates with the statistically significant increase in absolute liver weight in the same treated groups. Although this is a non-neoplastic reversible lesion (fatty degeneration) it is considered to be a biologically significant lesion. In male mice the incidence of hepatocytic vacuolization in the treated groups (mid- and high-dose) was only slightly higher than controls.

2. There was slight increase in the incidence of hepatocellular adenomas in the high-dose group of female mice as compared to controls (5 versus 8 tumors for the control and high-dose groups, respectively). The incidence of hepatocellular carcinomas was also higher in the mid- and high-dose groups (female mice) compared to controls (0, 1, and 2 tumors for the control, mid- and high-dose groups, respectively). The combined incidence of hepatocellular adenomas and carcinomas in female mice was considerably higher but not statistically significant in the high-dose group compared to controls (5 versus 10 tumors for control and high-dose groups, respectively). The incidence of hepatocellular adenomas/carcinomas in male mice was comparable between treated and control groups.

The presence of liver tumors in the current study was as follows:

Tumor/Dose	0	25	100	200
Adenoma	5/50 (10%)	4/50 (8%)	3/50 (6%)	8/50 (16%)
Carcinoma	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Combined	5/50 (10%)	4/50 (8%)	4/50 (8%)	10/50 (20%)

The total incidence of non-neoplastic lesions for liver was as follows:

0	25	100	200
7/50 (14%)	19/50 (38%)	36/50 (72%)	38/50 (76%)

In a feeding study of 104 weeks in the same strain of mice, under the same laboratory conditions and evaluated by the same pathologist which was partly run in parallel with this experiment, the incidences of liver tumors in controls for this experiment were as follows:

Adenoma	6/55 (11%)
Carcinoma	6/55 (11%)
Combined	12/55 (22%)

HEMOLYMPHORETICULAR SYSTEM (HLRS) - MALES

The average mean value and range for naturally occurring tumors of the hemolymphoreticular system presented in the attachment as hisorical control data by the registrant were as follows:

Mean %	Range %	N (# of Studies)
11.7	2-33	N = 7
12.2	8-14	N = 7
7.2	0-14	N = 7
11.8	0-28	N = 22
12.0	0-20	N = 11
Avg. = 11.2	0-33	54

The number of tumors of the HLRS in the current study was as follows:

Tumor/Dose	0	25	100	200
Reticulo-sarcoma	0/50 (0%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Lymphoma	1/50 (2%)	0/50 (0%)	1/50 (2%)	5/50 (10%)
Leukemia	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Combined	1/50 (2%)	1/50 (2%)	1/50 (2%)	7/50*(14%)

*Statistically significant

In a nearly parallel study, tumors of the HLRS in controls (Appendix III) was reported as follows for males:

HLRS
(Lymphoma-leukemia) 5/55 (9.1%)

Although historical control data suggest that the incidence of spontaneous tumors in the present study was unusually low, the fact still remains that in evaluating this study more weight should be given to the concurrent rather than the historical control data.

Dimethoate toxicology review

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