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

OFFICE OF PESTICIDES AND TOXIC SUBSTANCE

MEMORANDUM DEC 12 1980

SUBJECT: EPA Reg.241-110; 241-208, Malathion; NCI Cancer Bioassays of Malathion and Malaoxon. CASWELL#535; Accession:242903

FROM: William Dykstra, Toxicologist
Toxicology Brancn, HED (TS-769)

TO: William Miller (15)
Registration Division (TS-767)

Recommendations:

1) Malathion was not considered carcinogenic to Osborne-Mendel rats, F344 rats or female B6C3F1 mice in the studies reported. Because of questionable liver findings in the male mice, another study in mice is required.

2) Malaoxon was not considered carcinogenic to F344 rats B6C3F1 mice in the study reported.

Review:

1) Bioassay of Malathion for Possible Carcinogenicity (NCI Carcinogenesis Technical Report Series No. 24, 1978; CAS#121-75-5; NCI-CG-TR-24)

A bioassay of technical-grade malathion for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and B6C3F1 mice. Groups of 50 rats of each sex were administered malathion at one of two doses for 80 weeks, then observed for 33 weeks. Time-weighed average doses were 4,700 and 8,150 ppm. Matched controls consisted of groups of 15 untreated rats of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 108-113 weeks.

Groups of 50 mice of each sex were administered malathion at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 14 or 15 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 94 or 95 weeks.

Results:

Mortality in either rats or mice was not significantly related to the administration of malathion. Sufficient numbers of animals were at risk in the dosed and control groups of rats and mice of each sex for development of late-appearing tumors.

In female rats, three follicular-cell carcinomas and one follicular-cell adenoma of the thyroid occurred in the high-dose group, and three follicular-cell hyperplasias occurred in the low-dose group.

The incidence of three tumors showed a statistically significant (P = 0.026) dose-related trend; however, the results of the Fischer exact test for direct comparison between the dosed and control groups were not significant. More dosed males than females had either tumors or hyperplasia of the follicular cells of the thyroid; however, because of the higher incidence of tumors among the male controls, none of the results of the statistical tests were significant. These thyroid tumors were not considered to be associated with the administration of malathion.

In male mice, hepatocellular carcinoma occurred at the following incidences: matched controls 2/10, pooled controls 5/49, low-dose 7/48, high-dose 11/49. In addition, neoplastic nodules occurred in 3/49 pooled-controls and 6/49 high-dose animals. When the combined incidence of these neoplasms in the dosed animals was compared with that of the pooled controls, the dose-related trend was P = 0.019 and the direct comparison of the high-dose group with the control group was P = 0.031. Thus, when NCI compared this high dose group with the control group using Bonferrone criteria, the difference was not significant since a P of 0.025 was required. Although NCI did not consider these liver tumors to be associated with the administration of malathion, the Agency has concerns about acceptability of this study as a negative study to fulfill our registration requirement for a mouse oncogenic study. Since there was a dose-related trend (P = 0.019) and an increase of tumors at the high dose (P = .031) at levels which the Agency normally considers to be significant, we believe that there is sufficient justification to require another mouse oncogenic study.

Conclusion:

Under the conditions of this bioassay, there was no clear evidence of the association of the tumor incidence with the administration of malathion to Osborne-Mendel rats or B6C3F1 mice. However the questionable increases of liver tumors limit its usefulness as a negative oncogenic study to fulfill a regulatory requirement.

Classification: Core-Minimum Data

2. Bioassay of Malaoxon for Possible Carcinogenicity (NCI Technical Report Series No. 135, 1979; Cas. No. 1634-78-2; NCI-CG-TR-135)

A bioassay of malaoxon, the oxygen analog of malathion, for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were fed diets containing 500 or 1,000 ppm malaoxon for 103 weeks and were then observed for up to an additional 2 weeks. Matched controls consisted of groups of 50 untreated rats and 50 untreated mice of each sex. All surviving animals were killed at 103 to 105 weeks.

Results:

The only effects that could be related to administration of malaoxon at the doses used were increased mortality among male mice, decreased mean body weights of female mice, gastric ulcers in male and female rats, and possibly C-cell adenomas or carcinomas of the thyroid among treated female rats. The incidence of C-cell adenomas or carcinomas among historical controls, however, precluded relating the administration of the chemical to the incidence of these tumors.

Conclusion:

Under the conditions of this bioassay, malaoxon was not carcinogenic for F344 rats or B6C3F1 mice.

Classification: Core-Minimum Data

3. Bioassay of Malathion for Possible Carcinogenicity (NCI Carcinogenesis Technical Report Series No. 192, 1979; Cas. No. 121-75-5; NCI-CG-TR-192)

A bioassay of malathion for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats.

Groups of 49 to 50 rats of each sex were fed diets containing 2,000 or 4,000 ppm malathion for 103 weeks and were then observed for an additional 2 or 3 weeks. Matched controls consisted of 50 untreated rats of each sex. All surviving rats were killed at 105 or 106 weeks.

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Results:

No tumors occurred in the dosed groups of rats of either sex at incidences that could be related clearly to administration of the test chemical. Compound-related toxic effects were not observed in female rats at the doses used, but in males decreased mean body weights, increased mortality, gastritis, and gastric ulcers were dosed related.

Conclusion:

Under the conditions of this bioassay, malathion was not carcinogenic in male or female rats, but the females may not have received a maximum tolerated dose.

Classification: Core-Minimum Data

TS-769:th:LCHITLIK:12-2-80

12-2 R.HMiss (3)

Reviewed by: William Dykstra

Secondary reviewer: Laurence Chitlik

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity bioassay TOX. CHEM. NO.: 535

ACCESSION NUMBER: 242903 MRIL NO.: 31087
43268

TEST MATERIAL: Malathion, technical (95% purity)

SYNONYMS: (S-[1,2-bis(ethoxycarbonyl)-ethyl]O,O-dimethylphosphorodithioate); O,O-dimethylphosphorodithoate of diethylmercaptosuccinate; malathion; mercaptothion; carbofos; mercaptotion; maldison; Calmathion; Celthion; Cythion; Emmatos; For-Mal; Fyfanon; Hilthion; Karbofos; Kop-Thion; Kypfos; Malaspray; Malamar; Malatol; Maltox; Sumitox; Vegfru Malatox; Zithiol

STUDY NUMBER(S): NTIS No. PB278527

SPONSOR: National Cancer Institute, Bethesda, MD

TESTING FACILITY: Gulf South Research Institute, New Iberia, LA

TITLE OF REPORT: Bioassay of malathion for possible carcinogenicity

AUTHOR(S): M. Steinberg, et al.; report prepared at Tracor Jitco under NCI direction

REPORT ISSUED: December 1977

CONCLUSIONS:

Classification: core - minimum

A) Osborne-Mendel rat: under the condition of the test procedure there was no clear evidence of a tumorigenic response to malathion administration in the rat. The Dykstra review concurred with NCI conclusions. The findings were also affirmed by the NTP reexamination. (BAD-0001)

B) B6C3F1 mouse: in contrast with the NCI assessment that there was not a positive oncogenic response, the TB review considered the increased incidence of hepatocellular carcinoma in dosed groups as sufficiently persuasive to preclude accepting the study as negative for regulatory purposes. The reviewer required another study in mice.

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Comment (Dementi):

The TB review neglects to acknowledge a reasonable point NCI employed in rationalizing their conclusions; namely, that historical control incidence for hepatocellular carcinoma in this strain of mouse often is higher than that observed in the high dose group seen in this particular study. (NCI, p.viii)

Review has been examined and found acceptable with comment. (Brian Dementi, 6/1/87).

Brian Dementi 7/6/87

6

6

82-2
RT

Reviewed by: William Dykstra

Secondary reviewer: Laurence Chitlik

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity bioassay TOX. CHEM. NO.: 535

ACCESSION NUMBER: None MRID NO.: 31086
43269

TEST MATERIAL: Malathion, technical (95% purity)

SYNONYMS: (S-[1,2-bis(ethoxycarbonyl)-ethyl]O,O-dimethylphosphorodithioate); O,O-dimethylphosphorodithoate of diethylmercaptosuccinate; malathion; mercaptothion; carbofos; mercaptotion; maldison; Calmathion; Celthion; Cythion; Emmatos; For-Mal; Fyfanon; Hilthion; Karbofos; Kop-Thion; Kypfos; Malaspray; Malamar; Malatol; Maltox; Sumitox; Vegfru Malatox; Zithiol

STUDY NUMBER(S): NTIS No. PB300301

SPONSOR: National Cancer Institute, Bethesda, MD

TESTING FACILITY: Gulf South Research Institute, New Iberia, LA

TITLE OF REPORT: Bioassay of malathion for possible carcinogenicity

AUTHOR(S): C.R. Angel, et al. of Tracor Jitco

REPORT ISSUED: January 1979

CONCLUSIONS:

Classification: core - minimum

NCI concluded and the TB reviewer concurred that under the conditions of testing, malathion was not carcinogenic in male or female F344 rats. However, the MTD may not have been achieved in the case of females.

Comments (Dementi):

The high dose (4000 ppm) employed in this study was only one-half the high dose employed by NCI in a similar study using the Osborne-Mendel rat. (MRID 00043268)

As commented upon in the NTP reexamination (BAD-0001), the following neoplasms were increased in males of the low dose

group: A) pheochromocytoma (adrenal): 5/49 (control), 10/48 (low dose) and 6/46 (high dose); B) leukemia: 13/50 (control), 20/50 (low dose) and 8/49 (high dose). The authors concluded that these incidences do not demonstrate an oncogenic response in the F344 rat.

Review has been examined and found acceptable with comment as indicated. (Brian Dementi, 6/2/87)

Brian Dementi 7/22/87

Study was reviewed and reported in IARC Monographs, Vol. 30, Jan. 1983 IARC supported a negative finding.

8 I-2
Rat
17-82

Reviewed by:: William Dykstra
Toxicology Branch (TS-769C)
Secondary Reviewer: Lawrence Chitlik
Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Oncogenicity bioassay

Tox. Chem. No.: 53

Accession Number: 242903

MRID No.: 43270

Test Material: malaoxon, > 95% purity

Synonyms: O,O-dimethyl S-1,2-bis(ethoxycarbonyl)ethyl phosphorothioate

Study Number(s): NTIS No. PB299858

Sponsor: National Cancer Institute, Bethesda, MD

Testing Facility: Gulf South Research Institute, New Iberia, LA

Title of Report: Bioassay of malaoxon for possible carcinogenicity

Author(s): C. R. Angel, et al. of Tracor Jitco

Report Issued: July 1979

Conclusions:

Classification: core - minimum

NCI concluded in this study that under the conditions of the bioassay, malaoxon was not carcinogenic for F344 rats or B6C3F1 mice. There was a possible increase in C-cell adenomas and carcinomas of the thyroid among treated female rats. However, consideration of historical control data precluded a positive finding. Also noted were increased gastric ulcers in mice and female rats (p. V). The review by W. Dykstra affirmed NCI conclusions.

According to an NTP reexamination of this study [July 14, 1984 letter of E.E. McConnell to John Moore (BAD-0002)], "There was equivocal evidence of carcinogenicity of malaoxon in male and female rats based on C-cell lesions of the thyroid".

Comments (Dementi):

1) In female rats there was a significant ($p < 0.05$) increase

in mammary gland fibroadenoma in the low dose group: 2/50 (control), 9/50 (low dose) and 1/50 (high dose). This was not considered associated with malaoxon in the diet since it was not seen in the high dose group and the incidence in concurrent controls was unusually low. (NCI, p.23)

2) Percent survival at week 90 of the study for control, low dose and high dose groups were, respectively, male: 80%, 82% and 64%; females: 82%, 90% and 80%. (NCI, p.19). NCI indicates (p.21) that sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors. It is uncertain whether NCI evaluated or corrected for the higher mortality in the male high dose group in like manner with the approach taken in TB (survival analysis). However, in the NTP rereview of the data, statistical comparisons were made by survival-adjusted methods and by the Fisher exact test and the Cochran-Armitage trend test (BAD-0001, p. 162).

Review has been examined and commented upon. (Brian Dementi 6/9/87)

Brian Dementi 7/28/87

7/28/87

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

002504

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: William H. Miller, PM #16
Registration Division (TS-767)

THRU: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

SUVJECT: CYTHION® Malathion, EPA Reg. No. 241-208;
Two-Year Rat Feeding Study. Acc. No. 76252
CASWELL #535

Registrant:

American Cyanamid Company
Agricultural Research Division
Princeton, N.J. 08540

The Evaluation of the Chronic Toxicity Effects of Cythion Administered in the Diet to Sprague-Dawley Rats for 24 Consecutive Months by G. Rucci, P.J. Becci and R.A. Parent, Food and Drug Research Laboratories, Inc., Study No. 5436, May 13, 1980; Acc. No. 76252

Test Material:

Cythion Tech., Lot #W70225-1, 92.1% (The Premium Grade Malathion).

Test Animals:

Male and female Sprague-Dawley rats weighing 50-75 gm.

002504

Experimental Design:

Animals were acclimated for ten days at FDRL and assigned to 4 treatment groups each of 50 males and 50 females. Cythion was blended with the basal laboratory diet to obtain concentrations of 0, 100, 1000 and 5,000 ppm cythion. These diets and tap water were provided ad libitum to respective treatment groups throughout the study. Weekly diet samples were taken for cythion feed retention analysis.

All animals were observed daily for gross signs of toxicity and palpated weekly for tissue masses. Individual body weights and food consumption were recorded at the end of weeks 1 through 13, 24, 53, 79 and 103 of the study. Blood samples were collected from 5 rats/sex/group during weeks 12, 26, 53 and 104 of the study for hematological and cholinesterase determinations. Urine was collected during the same time periods for analysis.

All animals that died or were sacrificed moribund during the study and all survivors at termination underwent a complete gross necropsy. A variety of tissues and organs were stored in 10% neutral buffered formalin. Fresh organ weights were recorded for the adrenal glands, brain, gonads (testes or ovaries), heart, kidneys, liver, pituitary, spleen, and thyroid (including parathyroids).

Results:

Daily observations (appearance, behavior, absence of overt signs of toxicity) and the incidence of palpable tissue masses of cythion treated rats were comparable to the respective controls. Mortality/survivability were unaffected by dose.

Mean body weight of males were significantly suppressed at the 1000ppm dose level at weeks 3-8, 13, 79 and 103, and were consistently lower than controls at all other intervals. Females at the 1000 ppm dose level had significantly suppressed mean body weights only at weeks 79 and 103. Mean body weights of males and females at the 5000 ppm dose level were significantly suppressed throughout the study. There was no dose-related trend or pattern of food consumption among dosage groups; however, several sporadic significant differences in mean weekly food consumption did occur.

Erythrocyte cholinesterase was significantly depressed in both males and females at 1000 ppm and above. Other hematologic and urinalysis parameters and blood chemistry (BUN, SGOT, SGPT, glucose) indicate no effects of cythion in treated animals at any dose level.

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Significant increases were observed in mean liver weight and mean relative liver weight of males at the 5000 ppm dose level. However, microscopic examination revealed no differences in tissue structure compared to controls. Other selected organs were of normal weight.

Gross and microscopic examination of tissues and organs revealed no definite morphologic effects attributable to the presence of dietary cythion at dosage levels up to 5000 ppm. Lesions, both neoplastic and non-neoplastic, that did occur were equally distributed among all dose levels and sexes and considered to be naturally occurring and age-associated but not unusual for this strain of rat.

Conclusion:

There were sufficient numbers of animals (found dead, sacrificed moribund and survivors) examined to evaluate the oncogenic potential. In this study Cythion was not an oncogen in rats.

Chronic NOEL = 100 ppm. Mean body weight gain and RBC ChE significantly depressed at 1000 ppm.

Oncogenic NOEL $\geq$ 5000 ppm

Classification:

Core-Guideline

George W. Robinson 2/17/83
George W. Robinson, D.V.M
Review Section #1
Toxicology Branch/HED (TS-769)

TS-769:ROBINSON:sll:X73710:2/10/83 card 4

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004208

EPA: 68-01-6561
TASK: 55
November 7, 1984

Cassette 5 35

DATA EVALUATION RECORD

CYTHION

Chronic Toxicity/Oncogenicity-Rats

CITATION: Rucci, G., Becci, P.J., Parent, R.A. The evaluation of the chronic toxicity effects of Cythion administered in the diet to Sprague-Dawley Rats for 24 consecutive months. An unpublished study (No. 5436) prepared by Food and Drug Research Laboratories, Inc. for Agricultural Division, American Cyanamid Co. Princeton, N.J. Dated May 13, 1980.

REVIEWED BY:

Paul Wennerberg, D.V.M., M.S.
Project Scientist
Dynamac Corporation

Signature: [signature]
Date: 11-7-84

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APPROVED BY:

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EPA Scientist

Signature: [signature]
Date: 1/16/85

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity/Oncogenicity-Rats.

CITATION: Rucci, G., Becci, P.J., Parent, R.A. The evaluation of the chronic toxicity effects of Cythion administered in the diet to Sprague-Dawley Rats for 24 consecutive months. An unpublished study (No. 5436) prepared by Food and Drug Research Laboratories, Inc. for Agricultural Division, American Cyanamid Co. Princeton, N.J. Dated May 13, 1980.

ACCESSION NUMBER: 248179.

LABORATORY: Food and Drug Research Laboratories, Inc.

QUALITY ASSURANCE STATEMENT: Present, signed, and dated May 13, 1982.

TEST MATERIAL: Technical Cythion (malathion), Lot No. W70225-1, 92.1 percent purity, inert ingredients 7.9 percent, supplied by American Cyanamid Co.

METHODS:

1. Sprague-Dawley rats (source: Blue Spruce Farms, Altamont NY.) acclimated to laboratory conditions for 10 days, were used in the study. At study initiation, the mean weights of males and females were 76 and 71 g, respectively. Cythion was administered at levels of 0, 100, 1000, or 5000 ppm in the diet to groups of 50 male and 50 female rats. The rats were individually housed in wire mesh bottom cages. They were maintained in an environmentally controlled room at a temperature of 70 ± 3° F and a 12 hour light-dark cycle. Feed and fresh tapwater were provided ad libitum.

2. A premix of test compound in basal feed (Charles River RHM3200 meal) was prepared fresh weekly by grinding test compound with powdered feed in a mortar. Diets containing the required concentration of the test material were prepared by mixing appropriate amounts of the premix and basal feed in a Hobart mixer. Samples of the prepared diets were collected weekly for analysis by the sponsor, and samples were taken quarterly for analysis of stability and homogeneity of the diets.

3. Although it was stated in the report that clinical observations for signs of toxicity were made daily and palpations of all animals were performed weekly throughout the study, there were no individual animal data presented in the report to support this statement. Body weights and food consumption were measured at week 1 through 13 and at the end of weeks 24, 53, 79, and 103.

4. Hematology, urinalysis, and cholinesterase determinations were conducted on 5 males and 5 females in each group at 3, 6, 12, and 24 months. The hematologic parameters measured were: hemoglobin, hematocrit, erythrocyte and platelet counts, and total and differential leukocyte counts. Cholinesterase activity of red cells and plasma were determined. Urinalyses included observation of appearance, color, and microscopic sediment and measurement of specific gravity, pH, glucose, and semiquantitative determination of albumin. Blood urea nitrogen, glucose, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined at 104 weeks only.

5. Postmortem examinations were conducted on all animals that died, or were sacrificed moribund and on all animals that survived to study termination. Organ weights were determined at study termination (24 months) for the following organs: liver, adrenal, spleen, thyroid, kidney, pituitary, heart, testes/ovaries, and brain.

6. Hematoxylin-eosin stained slides were prepared and the following tissues were examined histologically:

lungs	large intestine	mammary glands
spleen	urinary bladder	sternum
pancreas	salivary glands	eyes
thymus	lymph nodes (M & C)	trachea
testes	thyroid & parathyroid	esophagus
prostate	adrenals	ears
seminal vesicles	liver	tongue
heart	kidney	ovaries
aorta	brain (2 levels)	uterus
muscle & nerve	spinal cord	vagina
stomach (gl. & sq.)	pituitary	and all gross
small intestines (3)	skin	lesions

7. Body weight data, food consumption data, hematological, biochemical and urinalysis parameters, organ weights and relative organ weight data were analyzed by the investigator, using one-way completely randomized design analysis of variance. Differences between treatment groups were determined using Tukey's LSD test assuming a two tailed critical region. Survival data and incidence of tissue masses were analyzed using a chi-square test with Yates correction for 2 x 2 contingency tables. Incidence of gross lesions, tumors, and non-neoplastic microscopic lesions were analyzed by this reviewer using the Fisher exact test.

RESULTS:

Observations and Mortality: It was reported that "daily observations revealed no overt test effects of Cythion at any level", however, clinical observations for individual animals were not presented in the report. It

was reported that there was no differences between treated or control animals in the incidence of palpable masses; however, only summary group incidence data were presented for each 13-week interval in the study and no individual animal data on tissue masses were reported. Table 1 presents the investigator's summary of incidences of palpable masses at weeks 78, 91, and 103.

TABLE 1. Percent Incidence of Palpable Tissue Masses at Selected Study Intervals[a]

Group/ppm	Week 78	Week 91	Week 103
Males			
0	2	6	6
100	2	2	3
1000	4	5	7
5000	4	5	5
Females			
0	5	12	15
100	9	18	24
1000	6	11	19
5000	7	8	10

[a]It was not possible for the reviewer to validate incidences or to determine the locaton of masses.

The administration of test compound caused no statistically significant increase in mortality in dosed animals when compared to controls; in fact, survival in high dose females was remarkably higher than in control or mid-dose females. Mortality and percent survival at 18 and 24 months are summarized in Table 2. Survival in all groups ranged from 90 to 98 percent at 18 months; at 24 months, survival ranged from 48 to 88 percent (Table 2).

TABLE 2. Number of Mortalities and Percent Survival in Rats Fed Cythion[a]

	Males Dose level (ppm)				Females Dose level (ppm)			
	0	100	1000	5000	0	100	1000	5000
78 Weeks								
No. of deaths	2	2	5	5	1	2	2	2
Percent survival	96	96	90	90	98	96	96	96
104 Weeks								
No. of deaths	21	21	25	26	13	18	19	6
Percent survival	58	58	50	48	74	59	62	88

[a]Individual animal data presented were validated by this reviewer. Fifty animals per group were initiated in this study.

Body Weight and Food Consumption: Table 3 presents mean body weight data for male and female rats at selected intervals during the study. As

TABLE 3. Mean Body Weights of Rats Fed Cythion at Selected Intervals

Group/dose (ppm)	Mean body weight (grams)				
	Week 0	13	53	79	103
Males					
0	78	429	539	567	534
100	75	436	548	531	513
1000	75	412*	524	529*	482*
5000	76	405*	520	506*	482*
Females					
0	74	254	313	367	365
100	70*	253	316	354	365
1000	71*	251	309	341*	345*
5000	70*	237*	294*	333*	345*

*Statistically different from controls at a p value < 0.05.

stated in the report, there was throughout most of the study a slight but statistically significant decrease in mean body weights of both males and females in the 5000 ppm group when compared to controls and also a decrease in males at 1000 ppm. However, body weights of males at the high dose were only 6 to 11 percent lower than controls and in high dose females 4 to 9 percent lower than controls.

There were no effects of test compound on food consumption. There were sporadic statistically significant increases in females and decreases in males but they were not consistent with time or dose.

Hematology and Clinical Chemistry: There were no test compound-related effects on hematology parameters. Although there was a statistically significant decrease in hemoglobin in 5000 ppm females at 3 months when compared to controls (13.3 vs 14.9 g/100 ml), the decreased value was within the normal range, and values at other time periods were not different from controls. Mean values for SGOT, SGPT, BUN, and glucose were similar in control and dosed groups. Mean data for hematology and clinical chemistry were supported by individual data. However, data were not present for the 18 month test interval for hematology and were present only for the 24 month interval for blood chemistry; the number of animals tested was limited to 5/group/sex.

Cholinesterase Activity: Red cell cholinesterase activity was statistically significantly lower in 5000 ppm males than in controls at 3, 6, and 12 months but not at 24 months when some recovery of activity was apparent (Table 4). There was a 42% or less depression of activity in 1000 ppm males. In females at 5000 ppm there was a statistically significant maximum depression of activity of 71% at 6 months, but by 24 months some recovery was apparent as indicated by a depression of activity of only 36% in comparison to controls; in 1000 ppm females there was a 45% or less depression of activity. There were no compound-related changes in levels of plasma cholinesterase when dosed animals were compared to controls. Analysis of brain cholinesterase activity was not reported.

TABLE 4. Red Cell Cholinesterase Activity as Percent of Control at Various Study Intervals[a]

Group/dose (ppm)	Months			
	3	6	12	24
Males				
1000	75	73	58*	72
5000	34*	18*	25*	55
Females				
1000	80	64	55*	64*
5000	51*	29*	34*	64*

[a]Mean percent values of 5 animals/group/sex.

*Statistically different from control, $p < 0.05$.

Urinalysis: There were no compound-related effects on the urinalysis parameters reported (pH, specific gravity, albumin and glucose). The results were based on five animals per group per sex.

Organ Weights: There was a statistically significant increase in the means of both liver weights and percent liver weights relative to body weight in 5000 ppm males when compared to controls: 15.6 g (3.1%) in controls and 17.8 (3.9%) in the 5000 ppm group. There were slight increases (statistically significant) in kidney weights relative to body weights but not in the absolute kidney weights of the 1000 and 5000 ppm males and 5000 ppm females as compared to controls. Mean brain weights in males and females at 1000 and 5000 ppm were slightly lower than controls, but there were no changes in relative brain weights. It was noted that in calculating mean values for pituitary weights, several individual data points were excluded (see Discussion).

Gross pathology: Several statistically significant gross findings were reported and are included in Table 5. These include color alterations in males of the cecum at 5000 ppm and kidneys at 1000 ppm.

There were significantly increased incidence of size alterations in mesenteric lymph nodes of the 100 and 1000 ppm males; thyroid of the 1000 ppm males; and ovaries of the 100 ppm group. No other statistically significant gross lesions were noted. There were other gross lesions observed (Table 5) for which the incidence suggested a positive trend; however, these findings were not statistically significant and are not considered by this reviewer to be compound-related.

Histopathology: In the females the incidence of mammary fibroepithelial tumors (adenomas, fibromas, fibroadenomas, and papillary cystadenomas) in the 1000 ppm group and uterine polyps in the 100 and 5000 ppm groups were significantly higher than in controls. An increased incidence of accumulation of pus within the uterus (pyometra) was also noted in the low and mid dose groups. No other statistically significant increase in tumors was detected. Data on neoplasms are summarized in Table 6.

Significant incidences of non-tumor pathology are presented in Table 7. Tissues of dosed animals showing significantly increased incidence of lesions as compared to controls are: heart in males at 5000 ppm (chronic inflammation); kidneys in males at 5000 ppm (both glomerulesclerosis and casts); liver in males at 5000 ppm (sinusoidal dilation); lung in females at 100 ppm (bronchopneumonia with bronchiectasis); lymph nodes in males at 5000 ppm (reticuloendothelial hyperplasia); spleen in females at 5000 ppm (extrahepatic hematopoiesis); and the uterus at 100 ppm (pyometra). Table 7 also presents the incidence of other lesions suggestive of a positive trend; however, these findings were not statistically significant and are not considered by this reviewer to be compound related.

Table 8 was compiled by this reviewer to assess the major lesions associated with illness and premature death. There was no statistically significant compound related lesion in these animals.

TABLE 5. Incidence of Selected Gross Findings in Rats Fed Cythion[a]

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
I. Color alterations:								
brain	6	10	11	14	1	4	1	3
cecum	1	7	3	9*	3	0	4	2
small intestine	4	6	4	9	3	2	1	1
kidneys	5	8	14*	10	6	6	4	2
liver	21	29	23	28	18	22	14	18
II. Size alterations								
liver	5	8	13	17	2	1	0	4
kidney	1	2	3	6	0	0	0	0
mes. lymph nodes	6	16*	16*	11	12	10	18	19
mammary gland	3	0	0	0	0	5	1	1
ovaries					0	6*	3	1
thyroid	2	5	7*	6	2	1	1	0

[a] This table presents only findings that were significant by the Fisher Exact Test or nonsignificant findings for data in either sex suggestive of a positive trend. The table was prepared by this reviewer based on reported information for 50 animals/ sex/group.

* Significant by Fisher Exact Test, $p < 0.05$; analysis by this reviewer.

TABLE 6. Tumor Incidence in Rats Fed Cythion[a]

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
Adrenals	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
adenoma	1	0	0	2	1	0	1	1
pheochromocytoma	0	2	1	0	0	0	1	1
Mammary gland	(49)	(48)	(49)	(47)	(50)	(50)	(50)	(50)
fibroadenoma[d]	0	0	1	3	9	13	15*	6
adenocarcinoma	0	0	0	1	1	0	3	1
Pancreas	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
adenoma	3	3	4	2	2	1	0	2
Pituitary	(49)	(48)	(45)	(49)	(49)	(46)	(48)	(49)
chromophobe adenoma	18	11[c]	11[c]	10[c]	29	27	30[c]	25
Skin	(50)	(5)	(50)	(50)	(50)	(50)	(50)	(50)
papilloma	0	1	1	1	0	0	0	0
histocytoma	0	0	0	1	0	0	1	1
Thyroid	(50)	(49)	(50)	(50)	(50)	(48)	(50)	(50)
C-Cell carcinoma	1	1	0	1	2	5	2	0
Uterus					(50)	(50)	(50)	(50)
polyps					3	10*	9	10*
other benign tumors					1	1	2	0

[a] This table was compiled by this reviewer and does not include sites where tumor incidence in treated groups are equal to or less than the control group.

[b] Number of tissues examined.

[c] "One animal has a tumor which histologically is a carcinoma" (as stated by applicant).

[d] Combined adenomas, fibromas, fibroadenomas, and papillary cystadenomas.

* Significant by Fisher Exact Test, $p < 0.05$; analysis by this reviewer.

TABLE 7. Non-Neoplastic Lesions in Rats Fed Cythion[a]

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
	(no. examined = 50 unless noted)							
Bone marrow hyperplasia	2	5	4	5	1	6	2	2
Heart chronic inflamation	4	11	9	13[c]	5	3	5	5
Large intestine inflamation	1	1	(49) 4	4	3	1	(49) 1	2
Kidneys	(49)							
glomerulesclerosis	0	3	6	7[c]	3	0	2	3
hydropelvis	1	1	3	4	6	3	5	3
acute inflammation	7	4	1	5	0	0	2	3
tubular casts	9	19[c]	13	15	3	7	7	10[c]
tubular dilation	6	9	9	13	4	2	5	8
Liver	(49)							
cytoplasmic vacuolization	10	6	7	7	6	9	10	10
sinusoidal dilation	1	3	1	10[b]	3	0	1	1
Lung								
acute bronchitis with bronchiectasis	5	10	11	0	0	(49) 4	1	4
bronchopneumonia with bronchiectasis	5	9	12	13	6	15[c]	10	1
Lymph nodes								
inflamation, chronic	24	31	29	29	28	29	(49) 30	32
lymphoid hyperplasia	4	17	17	9	16	16	15	22
reticuloendothelia hyperplasia	7	10	12	18[c]	11	17	11	11
Ovaries								
cysts					17	25	19	18
Pancreas								
duct dilation	0	3	1	4	0	1	1	4
Pituitary	(49)	(48)	(45)	(49)	(49)	(46)	(48)	(49)
cyst	1	2	3	5[c]	3	0	1	2

TABLE 7. Non-Neoplastic Lesions in Rats Fed Cythion[a] (Continued)

	Dose level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
Prostate calcification	8	10	(48) 12	16[c]				
Spleen extramedullary hematopoiesis	6	7	7	8	13	(49) 17	18	26[b]
Thymus cysts	(39) 2	(46) 3	(49) 1	(48) 3	(48) 1	(42) 5	(46) 3	(48) 6
Uterus pyometra					0	7[c]	4	0

[a] This table was compiled by this reivewer and presents only histologic lesions which had a statistically increased incidence in a dosed group or the data appeared suggestive of a positive trend.

[b] Significant by Fisher Exact Test, $p < 0.05$; analysis by applicant.

[c] Significant by Fisher Exact Test, $p < 0.05$; analysis by reviewer.

TABLE 8. Percent Incidence of Lesions Associated with Animals that Died or were Moribund Sacrificed[a]

	Dose Level (ppm)							
	Males				Females			
	0	100	1000	5000	0	100	1000	5000
Ear	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
inflamation of middle or inner ear	4	14	12	11	14	13	11	0
Lungs	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
bronchiectasis, abcesses	9	5	20	15	14	13	21	17
bronchitis, acute	9	0	4	4	0	0	5	17
bronchopneumonia, acute	22	48	32	48	14	26	21	17
Mammary gland	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
fibroadenoma	0	0	0	0	0	4	11	0
Pituitary	(23)	(21)	(25)	(27)	(14)	(23)	(19)	(6)
adenoma	22	5	4	11	21	26	47	33

[a] The numbers in parenthesis are the number of animals whose tissues were examined. The results are presented as the percent of animals with the lesions in the specified group. A few of the animals that died or were moribund sacrificed were not examined (see No. of deaths, Table 2).

DISCUSSION:

1. The results of the present study suggest that the 5,000 ppm dose of Cythion approximated a maximum tolerated dose. That is:

   a. There were significant decreases in body weight in males and females in the mid and high-doses; the final body weights at the high-dose were 6 and 11 percent lower than controls in females and males, respectively (Table 2).

   b. There was marked inhibition of erythrocyte cholinesterase in the high dose animals particularly during the first year of the study. Cholinergic toxic signs were not reported and there was no inhibition of plasma cholinesterase noted. Adaptation and recovery from inhibition in erythrocyte cholinesterase activity was evident, particularly in high-dose animals (Table 4). Data on the levels of brain cholinesterase could have facilitated interpretation of the importance of these effects; however, brain analyses data were not available.

2. The authors of the report concluded that "there were no biologically or toxicologically important test material related histopathologic effects or tumorigenic effects." In this study, there were statistically significant ($p < 0.05$) increases in fibroadenomas of the mammary gland (9/50 in controls versus 15/50 in the 1000 ppm females). This was dismissed in the report on the basis that there were comparable incidences of mammary tumors in "control and high-dose females similar to what would be expected in rats of this strain and age." Similarly, benign tumors of the uterus were statistically significant ($p < 0.05$) when the 100 and 5000 ppm groups were compared to control using the Fisher exact test. These were not considered meaningful in the report since they "occurred at the expected incidence typical of rats of this age and strain." While it is generally recognized that both of these types of tumors are characteristic of aging control rats, historical tumor incidence for this strain and laboratory is essential and needs to be provided for proper evaluation of the data.

3. The following ambiguities in the classification of tumors were noted:

   a. "All focal proliferative lesions of chromaffin cells were diagnosed as pheochromocytomas."

   b. "C-cell tumors of the thyroid appearing cytologically benign were considered malignant because of the tendency of progressive growth characteristic of thyroid tumors."

   c. Some pituitary tumors with areas appearing malignant microscopically were classified as adenomas "because of the tendency of pituitary tumors to remain localized."

Based on such ambiguities, it is considered that the histopathology of endocrine tissues needs further clarification and the slides of these tissues should be re-examined.

4. Although the report noted that extramedullary hemopoiesis of the spleen in high dose females was statistically different from control, this was "not considered due to compound administration since it is a common finding in aging rats." Similarly, other non-neoplastic lesions were noted but were not considered important in the investigator's conclusions. Many of these lesions are generally present in aged rats, and a relationship to test chemical cannot be definitely established. However, many of them were found on our analyses to be statistically significant in the high dose animals when compared to controls and are indicated in Table 7.

   It is considered that the dismissal of statistically significant effects on the basis of a common finding in aging animals must be supported by specific laboratory historical control data.

5. The study has several deficiencies that limit its usefulness for evaluating chronic toxicity:

   a. Blood chemistry determinations, hematology, and urinalyses were not made at the required number of test intervals, only five animals/sex/group were examined, and their estimating of the platelet number alone was not a sufficient examination of the clotting potential of the blood.

   b. Cholinesterase determinations were performed on insufficient numbers of animals to give adequate values for analyses; the lack of an early effect on plasma cholinesterase followed by recovery may have been missed since the earliest interval for analysis was at 3 months; no analyses were reported at 18 months.

   c. Although there was a concluding statement in the report that there were "no overt test effects of Cythion," there were no clinical observations or tissue mass palpation data reported. Clinical observation would have been useful to assess possible cholinergic toxic signs.

6. There were minor errors in recording data. These were noted in comparing tables of pathology incidence and tables correlating gross and histologic findings. In calculating organ weight means, several values were not reported: For example, for mean pituitary weights for females of the 5000 ppm group, weights of 22 pituitaries were omitted; 20 of these were diagnosed as having adenomas. Inclusion of these values did not reveal any differences in mean weights in dosed animals when compared to controls due to the large variance of the population.

CONCLUSIONS:

As noted above in items 2-6 of the Discussion section, several deficiencies involving histopathology diagnoses and interpretation, clinical observations, and blood chemistry limit the usefulness of these data for the assessment of chronic toxicity and oncogenic potential. Despite such

deficiencies, however, statistically increased incidences in female rats of uterine polyps and mammary gland fibroadenomas were observed. These tumorigenic responses were all benign in nature, exhibed no dose-response relationships, and displayed no decrease in latency compared to control animals. Thus, a definite relationship between test material and the increased incidences of tumors cannot be conclusively established. The historical control data is necessary; it should be from the same laboratory and from as many experiments as possible, but not less than from five consecutively performed studies.

Administration of Cythion in the diet to rats for two years caused a slight decrease in weight gain in males and females at 1,000 and 5,000 ppm, a transient decrease (inhibition) of cholinesterase at 1,000 and 5,000 ppm in males and at 5000 ppm in females, and an increase in liver weights in males at 5,000 ppm which was accompanied by an increased incidence of sinusoidal dilation. No adverse effects appeared to be associated with administration of the chemical at a dose of 100 ppm. However, due to the absence of data on clinical observations for toxic signs, the limited blood chemistry analyses in terms of time frequency of sampling and the number of animals and parameters tested, and the limited hematology, urinalyses, and cholinesterase determinations performed, a definitive NOEL or LEL cannot be established.

See p. 7

lymph nodes
ovaries
uterus } 10x

CORE CLASSIFICATION: Supplementary.

8 I-1
Rat

Reviewed by: [signature] 7/17/87

Secondary reviewer: N/A

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity

TOX. CHEM. NO.: 535

ACCESSION NUMBER: 76252

MRID NO.: 110562

TEST MATERIAL: Cythion Malathion

SYNONYMS: (S-[1,2-bis(ethoxycarbonyl)-ethyl]0,0-dimethylphosphordiethioate); 0,0-dimethylphosphorodithoate of diethylmercaptosuccinate; malathion; mercaptothion; carbofos; mercaptotion; maldison; Calmathion; Celthion; Cythion; Emmatos; For-Mal; Fyfanon; Hilthion; Karbofos; Kop-Thion; Kypfos; Malaspray; Malamar; Malatol; Maltox; Sumitox; Vegfru Malatox; Zithiol

STUDY NUMBER(S): 4123-013-01

SPONSOR: American Cyanamid Co., Princeton, NJ

TESTING FACILITY: Food and Drug Research Labs., Inc.

TITLE OF REPORT: The Evaluation of the chronic toxicity effects of Cythion administered in the diet to Sprague-Dawley rats for 24 consecutive months

AUTHOR(S): George Rucci, Peter Becci and Richard Parent

REPORT ISSUED: 5/13/80

CONCLUSIONS:

Clasification: Invalid

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FDRL TWO YEAR SPRAGUE-DAWLEY RAT STUDY (1980)

This study has been reviewed twice since it was submitted to the Agency; originally in 1983 by Dr. G. Robinson, and then in 1984 by Dynamac. In 1983 it was classified as CORE:Guideline, NOEL = 100 ppm and not oncogenic. In 1984 it was classified Supplementary, no NOEL, and insufficient data to conclude its oncogenic potential.

As a consequence of these divergent opinions of the same study results, it has been reviewed yet a third time in conjunction with the Registration Standard for malathion. Detailed results of this third evaluation will not be formally presented due to reporting ambiguities and other dificiencies in the study. The Dynamac review identifies many of these and is a reasonably comprehensive summary of the study design and observations. Nonetheless, even in this review there are notable discrepancies. For example, Table 6, footnote "a" states that this table was compiled by the reviewer and does not include sites where tumor incidence in treated groups are equal to or less than the control group. However, more than 50% of the examples listed in Table 6 clearly illustrate the opposite, viz. that tumor incidences in control groups were greater than or equal to treated groups (e.g. pituitary, pancreas, adrenal, thyroid). Furthermore, it is not obvious that either of the two previous reviews examined the individual pathology sheets to confirm the incidences reported in the summary tables provided by FDRL. In fact, independent examination of these data revealed notable differences between these separate data sets (e.g. prostate calcification, mammary gland adenoma, tubular casts (protein) in the kidney, etc.).

I am not convinced, however, that the individual animal pathology sheets clarify the ambiguities identified by Dynamac or permit any valid conclusions to be advanced concerning the toxicity of malathion in this study for reasons noted below:

- The summary tables do not distinguish between animals sacrificed at term and animals found dead, sacrificed moribund or accidental deaths. The summary tables lump all under one category.

- Pathology slides were not read "blind" but each pathologist had prior knowledge of the dose level administered to each animal.

- There is no indication of the numerical rating for the degree or severity of change observed by each pathologist. The only notation given was "slight, mild, moderate, and marked", but these were not uniformly applied. It is therefore impossible to establish any dose-related gradation in response due to chemical insult (e.g. kidney glomerulosclerosis).

o There were several pathologists involved in reading the slides: William C. Luft, M.D., Donald R. Weaver, M.D., Joseph T. King; M.D. - these gentlemen reviewed 75% of the slides; the remaining slides were examined by: Daniel G. Branstetter, D.V.M., Peter J. Goldblatt, M.D. and Peter J. Becci, Ph.D. "Each pathologist received slides from every fourth or fifth animal, plus information concerning test treatment, dose level, fate, number of weeks on test, and all gross necropsy findings for those animals."(pg. 712-713). Furthermore, these consultants' reports were reviewed in detail by William D. Johnson, Ph.D. (staff pathologist) for discrepancies between gross and microscopic findings, unusual pathological changes, etc. "During the review of the consultants' reports, microscopic findings were added or deleted as necessary. In some cases, the names for some neoplastic and non-neoplastic diseases were altered to achieve greater uniformity of nomenclature. In addition, some findings were described as not being significant in order to achieve consistency in readings and allow for accurate tabulation and calculation of percent incidence." (pg 713) [NOTE: The late Peter Becci. Ph. D. indicated in this report to be a "staff pathologist" is known <u>not</u> to have been a pathologist].

This whole procedure raises substantial concern for "consistency" and "uniformity", not the least of which is the apparent altering of findings. The extent of this practice and its impact on the findings is unclear. It is known to have effected at least one finding, by Dr.Becci's own admission in the report. "The increase in the high level test group over control numbers in this study most likely resulted more from individual variation among readers as to what amount of extramedullary hematopoeisis is considered normal background, rather than there being an actual biologically significant test material effect. In fact, six animals in the high dose group were diagnosed as having minimal extramedullary hematopoeisis, which could probably have just as well been considered normal background." (pg 723)

o Animals which died, sacrificed moribund or accidental deaths were not examined in a manner to preclude autolysis of tissue. Accidental deaths were attributed to "blood drawing", yet in the majority of cases there was substantial autolysis. No explanation given. Further, in the majority of animals which died, even those which died as late as 104 weeks, a substantial degree of autolysis of tissue was noted. A question of GLP is at issue here.

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o Animals in all groups suffered from a substantial degree of lung disease, kidney degenerative changes, generalized calcification, lymphadenitis and inflammation of the RE system, significant incidence of pituitary chromophobe adenoma (particularly females), and an increase in medial otitis of the ear. The chronic nature and advanced stages of these diseases illustrate the geriatric changes the were prevalent in all groups. The sigificant degree of bronchopneumonia and acute/chronic lung disease could have been caused by pathogens as much as by age, yet no attempt to identify such pathogens was apparently performed. Much of the early deaths in this study were attributed to lung disease. A question of good animal husbandry is at issue here.

o The substantial amount of geriatric changes in all groups makes it extremely difficult to separate or identify normal ageing processes from compound-related effects. This is exaccerabated by the apparent non-uniformity in pathological evaluation distributed among seven pathologists of varied experience and training (e.g. M.D., D.V.M., Ph.D.).

o Kidney, pituitary, adrenals and thyroid weights were selectively screened and eliminated from the organ weight and organ-to-body weight comparisons if they were above or below pre-selected values. This obviously biases the results, as a minimum, aside from being an unnacceptable practice.

o Hematology and urinalysis measurements were performed at 3, 6, 12, and 24 months, often in different animals. Clinical chemistry parameters were examined at 24 months only. Brain cholinesterase activity was not examined. Therefore, sufficient information for examining biochemical and clinical effects in a chronic bioassay were not obtained. Our biostatisticians analyzed the rbc ChE data and their evaluation is appended to this. No significant differences were obtained between control and low dose (100 ppm) groups, although the number of animals utilized may limit the usefullness of these data for extrapolation to man.

o Use of chloroform to euthanize animals at termination of the study is not presently a common practice among contract labs, primarily because of the use of a suspect carcinogen in the lab. Aside from that, there are potential adverse effects on the kidney (mouse), lung, and liver (rat) dependent upon the amount administered and the length of time administered. Some cellular biochemical changes in the liver are known to occur after 30 minutes of administration. While it would be difficult to see anything of substance from the use of chloroform on such geriatric animals, the use of this substance in the lab is not presently an acceptable practice.

In conclusion, an in-depth reevaluation of this study has revealed several substantial faults in study design, conduct and reporting which warrrant a classification of INVALID. As a minimum, Toxicology Branch requires the following:

- o Historical control data for the S-D rat. These must be assembled from studies conducted during the same time period at FDRL and identified according to date performed, pathologist(s) involved, duration of study, etc. The data should also be presented in such a way that animals sacrificed at term are listed separately from those sacrificed moribund or dying prior to term. Data assembled from other labs or at other times should be listed separately. These data must also include non-neoplastic, as well as, neoplastic incidences.

- o A complete and separate report from each pathologist involved in this study must be provided. Each report must include the animals examined (identified by # and sex), the organs/tissues examined, the information available to each pathologist concerning the history and fate of each animal (i.e. dose level, day of death, necropsy findings, etc.), the finding in every case and any conclusions. These reports must be signed in each case by the pathologist performing the histopathological examination. Furthermore, the corrections made to these reports by William D. Johnson ("staff pathologist") must also be identified and included. The C.V.'s for all "staff pathologists" must be provided.

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: MALATHION Mouse Study - Qualitative and Quantitative Risk Assessment of Combined Toxicity and Oncogenicity Study in Rats. Caswell #535

FROM: C.J. Nelson, Statistician
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THRU: Richard Levy, M.P.H.,Leader-Biostatistics Team
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SUMMARY:

In a chronic toxicity/oncogenicity study rats were administered Malathion (Cythion) in the diet for 24 months. Twenty-two lesions, one which was a malignant were statistically evaluated. Red blood cell cholinesterase was also analyzed. There was a teratology study where pregnant female rabbits were fed malathion. Weight gain from day 0 to day 29, and weight gain from day 6 to day 12 were tested by t-test since only summary data was available.

Twenty-two lesions were tested by the Fisher's Exact test for pairwise comparisons and by the Cochran-Armitage Test for a positive dose-response trend. The following lesions had a significant trend and both the high and mid dose was significantly different from controls: swollen liver and kidney glomerulosclerosis in male rats. Prostate calcification, liver sinusoidal dilation, lymph node reticuloendothelial hyperplasia in male rats and kidney tubular casts, spleen extramedullary hematopoiesis in female rats had a significant trend and the high dose was significantly different from control. Heart inflammation in male rats had a significant trend and both the high and low dose groups were different from controls. Thymus cyst and uterus pyometra in female rats had a significant trend and the low dose group was significantly different from the controls. The following lesions had a significant trend and no

pairwise comparison differences: pituitary cyst in male rats and kidney tubular dilation, pancreas duct dilation, thyroid parafollicular cell malignant tumor in female rats. Uterus polyps in female rats had no significant trend but both high and low dose groups were significantly different from controls. The following lesions had no significant trend or pairwise comparisons: kidney tubular casts, kidney tubular dilation, pancreas duct dilation in male rats and lymphoid reactive hyperplasia, mammary gland fibro adenoma, mammary gland fibro adenoma and/or carcinoma in female rats.

Red blood cell cholinesterase (RBC-C) was significantly reduced in the mid and high dose from the controls using Duncan's test for pairwise comparisons in the analysis of variance procedure from SAS. There was also a linear dose-response trend with higher dose animals having reduced RBC-C. Time effects were also observed in this study.

In the teratology study, high and mid dose rabbits had significantly reduced weight gain from day 6 to day 12.

Background:

This was a chronic toxicity/oncogenicity study and rats were administered Cythion in the diet for 24 consecutive months. The study was conducted by Food and Drug Research Laboratories, Inc. for Agricultural Division, American Cyanamid Company. An unpublished report was prepared May 13, 1980 (number 5436). Cythion was administered at levels of 0, 100, 1000, or 5000 ppm in the diet to groups of 50 male and 50 female rats.

The data analyzed in this report was provided by the principal investigator. The non-neoplastic lesions reported in tables 1-22 were from the DER and not from the individual animal pathology reports. Data to examine mortality was not provided, although the DER states " . . . test compound caused no statistically significant increase in mortality in dosed animals when compared to controls;".

Analysis:

The data in the following tables was extracted from summary reports. They have not been adjusted for differences in mortality or verified from the pathology sheets. The data was analyzed using the Fisher's exact test to compare each dosed group to the controls and the Cochran-Armitage Trend test to perform a one sided Chi square test of significance.

Table 1-Prostate Calcification in male rats showed a significant increase (p=.05) at the high dose and a significant positive trend (p=.032).

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Table 2-Lymphoid Reactive Hyperplasia in female rats had no significant pairwise comparisons or trend.

Table 3-Heart Inflammation in male rats showed a significant pairwise comparison at the 100 and 5000 ppm dose (p=.045 and .016 respectively. There was a significant positive trend (p=.048).

Table 4-Swollen Liver in male rats had significant pairwise comparisons at the 1000 and 5000 ppm doses (p=.033 and .004 respectively. There was a highly significant trend (p=.0025).

Table 5-Mammary Gland Fibro Adenoma in female rats had no significant pairwise comparisons and no trend. There were increases up to 1000 ppm but the 5000 ppm dose was lower than the control.

Table 6-Mammary Gland Fibro Adenoma and/or Carcinoma in female rats had the same results as table 5.

Table 7-Uterus Polyps in female rats had significant pairwise comparisons at the 100ppm and 5000ppm doses (p=.036 for both) but no significant linear trend.

Table 8-Kidney Glomerulosclerosis in male rats had significant pairwise comparisons at the 1000 and 5000ppm doses (p=.014 and .007 respectively) and a significant positive trend.

Table 9-Kidney Tubular Casts in male rats had a significant pairwise comparison at 100 ppm (p=.025) but no significant trend.

Table 10-Kidney Tubular Casts in female rats had a significant pairwise comparison at 5000 ppm (p=.036) and a significant positive trend (p=.047).

Table 11-Kidney Tubular Dilation in male rats had no significant pairwise comparisons and no significant trend. There was an increasing trend and 5000ppm was nearly different.

Table 12- Kidney Tubular Dilation in female rats had no significant pairwise comparisons. There was a significant linear trend (p=.028).

Table 13-Liver Sinusoidal Dilation in male rats had a significant pairwise comparison at 5000 ppm (p=.004) and a significant positive trend (p<.0001).

Table 14-Lymph Node Reticuloendothelial Hyperplasia in male rats had a significant pairwise comparison at 5000 ppm (p=.01) and a significant linear trend (p=.006).

Table 15-Pancreas Duct Dilation in male rats had no significant pairwise comparisons and no linear trend.

Table 16-Pancreas Duct Dilation in female rats had no significant pairwise comparisons. There was a significant positive linear trend (p=.008).

Table 17-Pituitary Cyst in male rats had no significant pairwise comparisons. There was a significant positive linear trend (p=.046).

Table 18-Spleen Extramedullary Hematopoiesis in female rats had a significant pairwise comparison at 5000 ppm (p=.007) and a significant positive linear trend (p=.005).

Table 19-Thymus Cyst in female rats had a significant pairwise comparison at 100 ppm (p=.001) and no positive linear trend. There was a significant departure from trend (p<.001).

Table 20-Uterus Pyometra in female rats had a significant pairwise comparison at 100 ppm (p=.006), a significant positive trend (p=.034) and a significant departure from trend (p=.007).

Table 21-Brain Color Alteration in male rats had a significant pairwise comparison at 5000 ppm (p=.039) and a significant positive linear trend (p=.048).

Table 22-Thyroid Parafollicular Cell Malignant Tumor in female rats had no significant pairwise comparisons but had a significant positive linear trend (p=.03).

Red cell cholinesterase activity was measured in five rats of both sexes from all dose levels at 3, 6, 12, and 24 months. The rats at the first 3 time periods were the same animal. Hence a repeated measure analysis of variance was run on this data set. Since the animals at 24 months were a different set of animals, a repeated measure analysis of variance could not be done and a 2 factor factorial analysis of variance was performed.

For the repeated measure analysis of variance, dose effects and time effects were significantly different for female rats. Using Duncan's multiple range to test pair wise comparisons, the control and low dose groups were not significantly different. The 1000 ppm and 5000 ppm dose groups were significantly differently from the controls and each other. There was a significant positive increasing trend of cholinesterase activity with increasing dose. The 6 month cholinesterase activity measure was significantly less than the 3 and 12 month activity.

In the repeated measure analysis of variance, dose effects and time effects were significantly different for male rats. The control and low dose groups were not significantly different. The 1000 ppm and 5000 ppm dose groups were significantly differently from the controls and each other. There was a

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7-

B) Females

Parameter	Dose Group	Number Rats With Effect FDRL	Dynamac	Dementi	Animal I.D. No. - Dementi
Kidney, tubular casts	C	3	3	3	207, 221, 242
	L	7	7	7	251, 263, 267, 269, 278, 282, 288
	M	7	7	7	302, 307, 309, 315, 332, 338, 344
	H	10	10	10	357, 370, 371, 373, 386, 387, 390, 391, 397, 400
Kidney, tubular dilation	C	4	4	4	211, 223, 227, 249
	L	2	2	3(2)	269, 275, (284?)
	M	5	5	5	305, 309, 336, 342, 350
	H	8	8	8	354, 359, 376, 385, 391, 394, 397, 398
Mammary gland, fibroadenoma	C	9	9	10(9)	202, 211, 212, 214, 215, 220, 227, 229, 247, (210-"probable adenoma")
	L	13	13	13	259, 261, 266, 267, 268, 276, 280, 286, 288, 289, 292, 293, 299
	M	15	15	16	302, 303, 308, 311, 312, 313, 320, 321, 325, 326, 328, 331, 335, 336, 346, 348
	H	6	6	6	351, 359, 363, 382, 394, 397
Mammary gland, carcinoma	C	1	1	1	250
	L	0	0	0(1)	267-"possible carcinoma within a fibroadenoma"
	M	3	3	3	305, 343, 347
	H	1	1	1	383
Pancreas, duct dilation	C	0	0	0	
	L	1	1	1	297
	M	1	1	1	
	H	4	4	2	367, 388
Spleen, extramedullary hematopoiesis	C	13	13	13	209, 214, 221, 224, 225, 229, 232, 233, 236, 240, 242, 247, 250
	L	17	17	15	256, 261, 262, 263, 270, 273, 276, 278, 282, 284, 287, 288, 295, 296, 299
	M	18	18	18	302, 303, 304, 306, 308, 314, 315, 320, 322, 324, 327, 328, 331, 332, 343, 344, 347, 348
	H	26	26	26	351, 352, 355, 357, 360, 361, 362, 364, 365, 369, 370, 371, 372, 373, 377, 378, 381, 383, 386, 387, 388, 390, 391, 395, 397, 400

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Females, con't

Parameter	Dose Group	Number Rats With Effect FDRL	Dynamac	Dementi	Animal I.D. No. - Dementi
Thymus, cyst	C	1	1	1	234
	L	13	5	5	254, 259, 264, 285, 297,
	M	3	3	4	304, 316, 317, (340-"cystic")
	H	6	6	5	372, 377, 386, 394, 399
Uterus, polyps	C	3	3	3	222, 234, 241
	L	10	10	10	253, 254, 259, 262, 264, 271, 276, 285, 287, 298
	M	9	9	9	303, 304, 305, 312, 317, 318, 319, 324, 328
	H	10	10	10	356, 359, 363, 372, 374, 380, 381, 384, 397, 399
Uterus, pyometra	C	0	0	0	
	L	7	7	7	251, 263, 270, 278, 288, 292, (254-"pyometrium")
	M	4	4	3	302, 328, 331
	H	0	0	0	
Extra	C	2		2	203, 233
	L	5(6)		6	258, 264, 272, 273, 289, 300
Thyroid, C-cell carcinoma	M	2		2	316, 341
	H	0		0	

significant positive increasing trend of cholinesterase activity with increasing dose. The 12 month cholinesterase activity measure was significantly more than the 3 and 6 month activity.

The results of the factorial analysis of variance on the entire data set was essentially the same for females. However in the males there was a significant dose*time interaction. This finding precludes interpretation of the dose means or the time mean by themselves, rather they must be looked at together. The graph (Figure 1 for females and figure 2 for males) for males indicate that the control and 100 ppm rats responded much the same across all time periods, however the 1000 ppm rats showed little or no change across the time periods and the 5000 ppm rats decreased from 3 to 6 months and then gradually increased near the level of the 1000 ppm group at 24 months.

In summary, the control and low dose groups were not significantly different. The mid and high dose groups were significantly different from the controls and from each other. There was a linear decrease of RBC cholinesterase with increasing doses of malathion. The above statements are true regardless of which analysis was used. However, at the 24 month sacrifice the high dose animals RBC cholinesterase increased. The increase was enough to cause a dose by time interaction when the 24 month sacrifice was included in the analysis.

There was also teratology data from a different study. Female rabbit weights were summarized (means and stand deviations, I believe) for weight gain during days 0-29 and weight gain from day 6 to day 12 (table 23). The individual animal weights were missing from the report, hence a t-test was the only test that could be performed. The low, medium, and high dose groups were compared to the controls. There was no significant differences for the 0-29 day weight gains. For the 6-12 day weight gains, both the medium and high dose groups were significantly different from the controls ($p=.021$ and $.013$) respectively.

It seems odd that the 0-29 day weight gain standard deviations is as large as the mean. This implies that the data is not normally distributed. For instance, the control mean is 230 grams and the standard deviation is 220 grams, if the data was normally distributed we would expect some weights below zero and some around 770 grams (mean plus or minus 2 standard deviations). Is it possible that non-pregnant females were included? In the 6-12 days weights, the control females gained an average of 190 grams in one week. Is this a reasonable one week gain for this type of rabbit in this time period?

Table 1-Prostate Calcification in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	8	10	12	16
(%)	(16)	(20)	(25)	(32)
# On Test	50	50	48	50
Fisher's Exact p		.398	.197	.050 *

Cochran-Armitage Trend Test

	χ^2	p
Trend (Ho: no trend)	3.432	.032
Non-linearity (Ho: linear) df= 2	0.566	.754

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Table 2-Lymphoid Reactive Hyperplasia in Female Rats Fed Mal

Dose Level ppm	0	100	1000	5000
# Responding	16	16	15	22
(%)	(32)	(32)	(31)	(44)
# On Test	50	50	49	50
Fisher's Exact p		.585	.527(n)	.151

Cochran-Armitage Trend Test

	χ^2	p
Trend (Ho: no trend)	2.374	.062
Non-linearity (Ho: linear) df= 2	0.218	.897

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Table 3-Heart Inflammation in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	4	11	9	13
(%)	(8)	(22)	(18)	(26)
# On Test	50	50	50	50
Fisher's Exact p		.045 *	.117	.016 *

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	2.769	.048 *
Non-linearity (Ho: linear) df= 2	3.167	.205

Table 4-Swollen Liver in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	5	8	13	17
(%)	(10)	(16)	(26)	(34)
# On Test	50	50	50	50
Fisher's Exact p		.277	.033 *	.004 **

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	7.830	.0025 *
Non-linearity (Ho: linear) df= 2	2.213	.331

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Table 5-Mammary Gland Fibro Adenoma in Female Rats Fed Malat

Dose Level ppm	0	100	1000	5000
# Responding	9	13	15	6
(%)	(18)	(26)	(30)	(12)
# On Test	50	50	50	50
Fisher's Exact p		.235	.121	.288(n)

Cochran-Armitage Trend Test		χ^2	p
Trend (Ho: no trend)		2.635	.0525
Non-linearity (Ho: linear)	df= 2	3.142	.208

Table 6-Mammary Gland Fibro Adenoma and/or Carcinoma Fem Mal

Dose Level ppm	0	100	1000	5000
# Responding	10	13	18	7
(%)	(20)	(26)	(36)	(14)
# On Test	50	50	50	50
Fisher's Exact p		.318	.059	.298(n)

Cochran-Armitage Trend Test		χ^2	p
Trend (Ho: no trend)		2.344	.063
Non-linearity (Ho: linear)	df= 2	4.893	.087

Table 7-Uterus Polyps in Female Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	3	10	9	10
(%)	(6)	(20)	(18)	(20)
# On Test	50	50	50	50
Fisher's Exact p		.036 *	.061	.036 *

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	1.117	.146
Non-linearity (Ho: linear) df= 2	3.942	.139

Table 8-Kidney Glomerulosclerosis in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	0	3	6	7
(%)	(0)	(6)	(12)	(14)
# On Test	49	50	50	50
Fisher's Exact p		.125	.014 *	.007 **

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	4.570	.016 *
Non-linearity (Ho: linear) df= 2	3.458	.177

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Table 9-Kidney Tubular Casts in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	9	19	13	15
(%)	(18)	(38)	(26)	(30)
# On Test	49	50	50	50
Fisher's Exact p		.025 *	.251	.132

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	0.098	.377
Non-linearity (Ho: linear) df= 2	4.818	.090

Table 10-Kidney Tubular Casts in Female Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	3	7	7	10
(%)	(6)	(14)	(14)	(20)
# On Test	50	50	50	50
Fisher's Exact p		.159	.159	.036 *

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	2.799	.047
Non-linearity (Ho: linear) df= 2	1.440	.487

Table 11-Kidney Tubular Dilation in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	6	9	9	13
(%)	(12)	(18)	(18)	(26)
# On Test	49	50	50	50
Fisher's Exact p		.303	.303	.068

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	2.625	.0625
Non-linearity (Ho: linear) df= 2	0.516	.773

Table 12-Kidney Tubular Dilation in Female Rats Fed Malathio

Dose Level ppm	0	100	1000	5000
# Responding	4	2	5	8
(%)	(8)	(4)	(10)	(16)
# On Test	50	50	50	50
Fisher's Exact p		.339(n)	.500	.178

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	3.665	.027
Non-linearity (Ho: linear) df= 2	0.696	.706

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Table 13-Liver Sinusoidal Dilation in Male Rats Fed Malathio

Dose Level ppm	0	100	1000	5000
# Responding	1	3	1	10
(%)	(2)	(6)	(2)	(20)
# On Test	49	50	50	50
Fisher's Exact p		.316	.747(n)	.004 **

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	13.804	<.000 **
Non-linearity (Ho: linear) df= 2	1.832	.40

Table 14-Lymph Node Reticuloendothelial Hyperplasia Male Mal

Dose Level ppm	0	100	1000	5000
# Responding	7	10	12	18
(%)	(14)	(20)	(24)	(36)
# On Test	50	50	50	50
Fisher's Exact p		.298	.154	.010 **

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	6.534	.006 **
Non-linearity (Ho: linear) df= 2	0.670	.715

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Table 15-Pancreas Duct Dilation in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	0	3	1	4
(%)	(0)	(6)	(2)	(8)
# On Test	50	50	50	50
Fisher's Exact p		.121	.500	.059

Cochran-Armitage Trend Test			
		χ^2	p
Trend (Ho: no trend)		2.581	.054
Non-linearity (Ho: linear)	df= 2	2.627	.269

Table 16-Pancreas Duct Dilation in Female Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	0	1	1	4
(%)	(0)	(2)	(2)	(8)
# On Test	50	50	50	50
Fisher's Exact p		.500	.500	.059

Cochran-Armitage Trend Test			
		χ^2	p
Trend (Ho: no trend)		5.874	.009
Non-linearity (Ho: linear)	df= 2	0.311	.856

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Table 17-Pituitary Cyst in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	1	2	3	5
(%)	(2)	(4)	(7)	(10)
# On Test	49	48	45	49
Fisher's Exact p		.492	.277	.102

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	2.845	.046 *
Non-linearity (Ho: linear) df= 2	0.479	.787

Table 18-Spleen Extramedullary Hematopoiesis Female Malathio

Dose Level ppm	0	100	1000	5000
# Responding	13	17	18	26
(%)	(26)	(35)	(36)	(52)
# On Test	50	49	50	50
Fisher's Exact p		.235	.194	.007 **

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	6.777	.005 **
Non-linearity (Ho: linear) df= 2	0.759	.634

Table 19-Thymus Cyst in Female Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	1	13	3	6
(%)	(2)	(31)	(7)	(13)
# On Test	48	42	46	48
Fisher's Exact p		<.001 **	.292	.056

Cochran-Armitage Trend Test	X^2	p
Trend (Ho: no trend)	0.045	.416
Non-linearity (Ho: linear) df= 2	19.295	<.000

Table 20-Uterus Pyometra in Female Rats Fed Malathion

Dose Level 0	0	100	1000	5000
# Responding	0	7	4	0
(%)	(0)	(14)	(8)	(0)
# On Test	50	50	50	50
Fisher's Exact p		.006 **	.059	1.000

Cochran-Armitage Trend Test	X^2	p
Trend (Ho: no trend)	3.358	.034
Non-linearity (Ho: linear) df= 2	10.014	<.007

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Table 21-Brain Color Alteration in Male Rats Fed Malathion

Dose Level ppm	0	100	1000	5000
# Responding	6	10	11	14
(%)	(12)	(20)	(22)	(28)
# On Test	50	50	50	50
Fisher's Exact p		.207	.143	.039 *

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	2.786	.048 *
Non-linearity (Ho: linear) df= 2	1.233	.540

Table 22-Thyroid Parafollicular Cell Malignant Tumor Malath

Dose Level ppm	0	100	1000	5000
# Responding	2	5	2	0
(%)	(4)	(10)	(4)	(0)
# On Test	50	48	50	50
Fisher's Exact p		.201	.691	.247(n)

Cochran-Armitage Trend Test	χ^2	p
Trend (Ho: no trend)	3.575	.03 *
Non-linearity (Ho: linear) df= 2	2.688	.26

Table 23 - Mean body weight gains, (Standard Deviations) and number of Pregnant Female Rabbits

Weight Gain (kg) during days 0-29

Control	Low	Medium	High
0.23(.22) 16	0.20(.19) 14	0.14(.16) 13	0.11(.21) 14

Weight Gain (kg) during days 6-12

Control	Low	Medium	High
0.19(.21) 17	0.06(.33) 15	-0.03(.30) 15	-0.03(.27) 16

Note:
The repeated measures model used for the RBC cholinesterase analysis is follows. This model assumes that the same rat is measured at every time point.

RBC-C = M + D + R(D) + T + D*T + error [T*R(D)]

RBC-C is the independent variable or cholinesterase value. M is the overall mean, D is the dose effects, R is the individual rat identifier, and T is the time effect. The dose effects are tested by the between rat effects [R(D)] if the R(D) effect is significant. Otherwise test all effects by the error term.

The factorial model is similar and the same designations will be used.

RBC-C = M + D + T + T*D + error.

The two error terms are pooled together if the assumption is made that only one measure is made on each rat.

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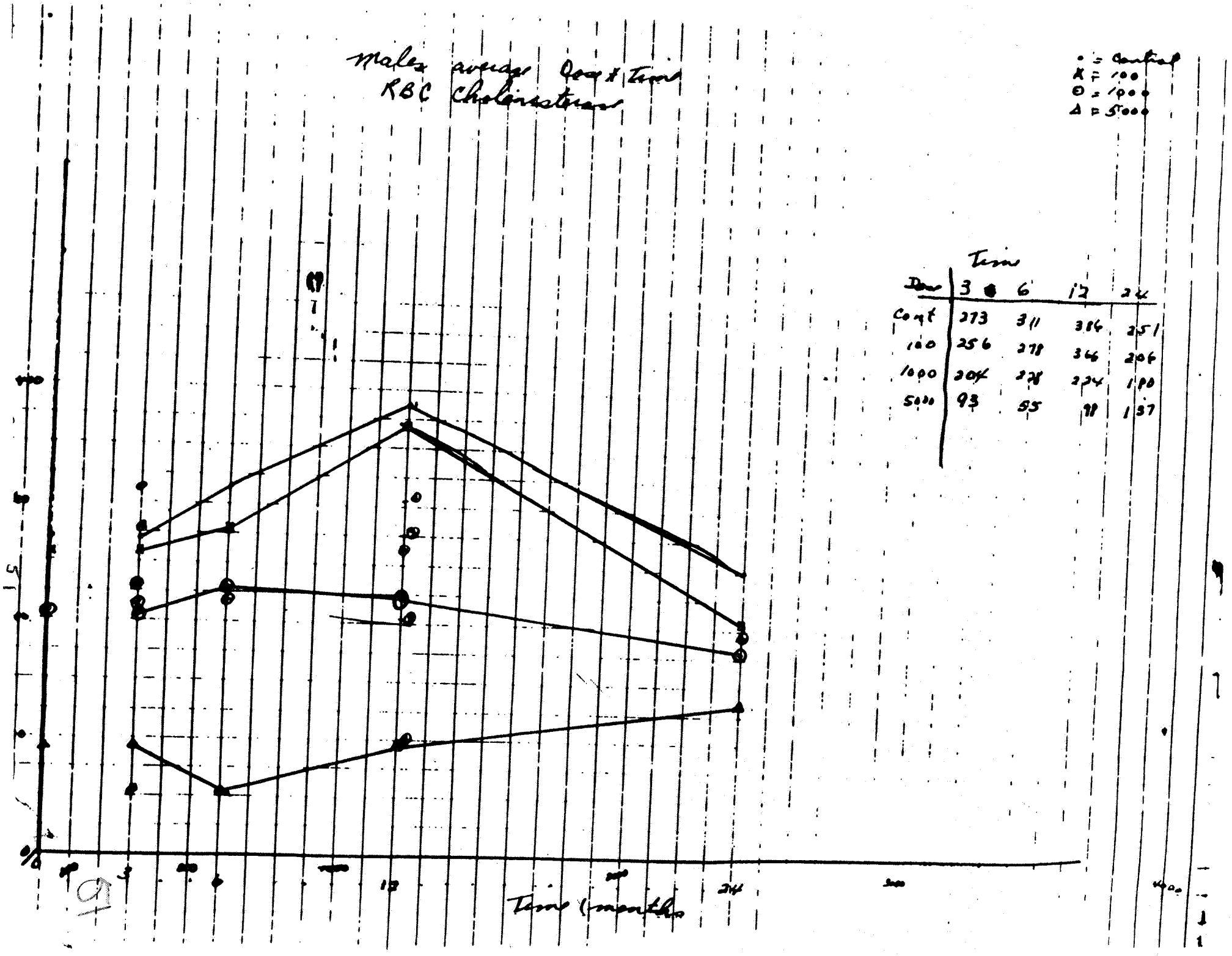
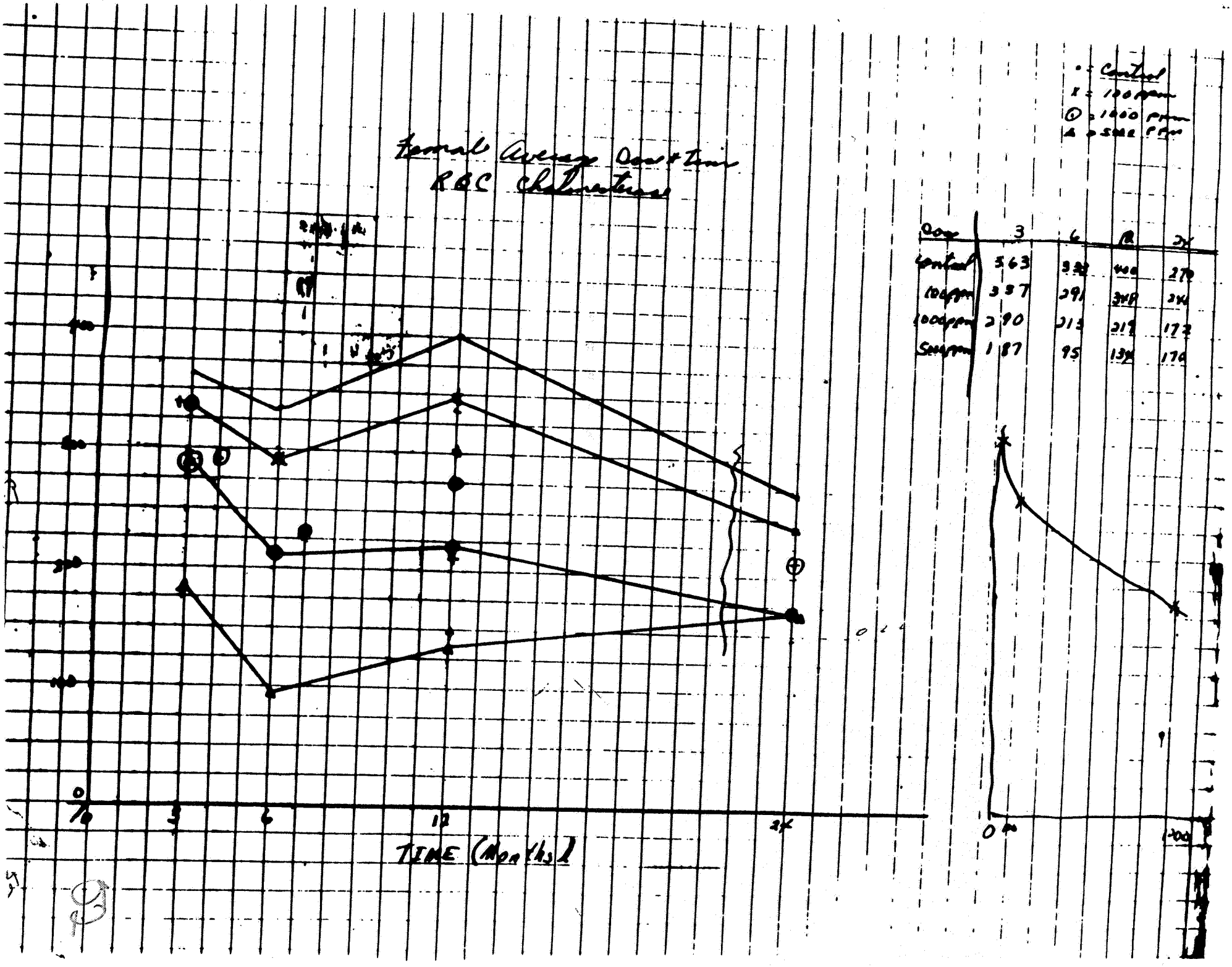
Males average Dose & Time
RBC Cholinesterase

• = Control
× = 100
○ = 1000
Δ = 5000

Dose	Time 3	6	12	24
Cont	273	311	386	251
100	256	278	346	206
1000	204	228	224	190
5000	93	55	98	137




Normal Average Dose + Time
RBC Cholinesterase
TIME (Months)
Control
100 ppm
1000 ppm
5000 ppm
Dose
3
6
12
24

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Lab Audit for Malathion Rat Feeding Study at FDRL (3/11-13/86)

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TSD-769)

10/27/87

Theodore M. Farber 10/27/87

TO: Mrs. Joanne Edwards
PM #16 Team
Insecticide/Rodenticide Branch
Registration Division (TS-767)

The following information summarizes the concerns and position of Toxicology Branch relative to the Malathion 2 year feeding study in rats, conducted at FDRL and issued 5/13/80. EPA audited this study at FDRL, Waverly, New York, on 3/11-13'86. However, the results were not forwarded to BUD (Al Jennings) until 7/31/87 and subsequently to the Registration Division and Toxicology Branch until 10/21/87. We have a difficult time understanding why it takes 18 months for the results of a lab audit to reach the ultimate user of this information.

A thorough examination of this lab audit report did not resolve many of the concerns raised by Tox Branch (R.B.Jaeger, 7/17/87) regarding the conduct of the study and reporting of the data. We note that Dr. A. Gross was the EPA inspector who examined the toxicology and pathology phases of the study. Questions identified by Dr. Gross (3/11-13/86) regarding the histopathology, and similarly identified by R.B. Jaeger, have not been adequately resolved. Dr. Gross re-examined the microscopic slides from 23/50 control females and 3/50 high dose females, although it is not clear that all tissues for these animals were re-examined. Multiple slides were identified for only a select few of the 23 control females in Dr. Gross's report. He stated that, with one or two exceptions, his re-examination concurred with the "original" diagnosis. However, by his own admission, and due to subsequent pen/ink changes in the pathology sheets, it is difficult to reconstruct what the "original" diagnosis was or why some were changed in many instances.

Toxicology Branch remains of the opinion that there are sufficient concerns regarding the preparation of slides, pathology

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diagnosis and presentation of results to warrant an independent reevaluation of the microscopic slides from this study. These need not include all tissues, but certainly must include all doses and both sexes. A "select" listing of tissues might therefore include the following:

liver, lung, kidney, pituitary, spleen, lymph nodes, mammary gland, reproductive organs, adrenal gland;

possibly also: skin/ear, pancreas, thyroid, and heart

The above reevaluation must include a grading of the severity of identified changes. Further, Tox Branch recommends that the slides be reread "blind", without prior knowledge of dose level, although obviously sex must be identified. Toxicology Branch recommends the sponsoring company be notified that this reevaluation of the microscopic slides is required and further that Tox Branch be informed of their notification and response. Toxicology Branch will be available to discuss, with the sponsor, the exact number of tissues which will need to be reevaluated.

In the interim and until a thorough reevaluation of these slides has been completed by an independent pathologist (e.g. Dr. Busey, EPL), Toxicology Branch does not believe the lab audit removes the substantial "doubts" regarding the conduct of the study and reporting of findings. We have confirmed this with Dr. A. Gross (personal conversation with R.B. Jaeger, 10/26/87) who supports our position relative to the multiple number of pathologists involved and the possible non-uniformity of their respective diagnoses. Toxicology Branch will reexamine its position relative to malathion after receiving and evaluating the findings of the independent pathologist.

cc: Dr. A. Gross

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

Rec'd 11-16
10/21/87

SEP 20

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Food and Drug Lab. Inc. Malathion

TO: Herbert Harrison, Cheif
Insecticide-Rodenticide Branch (TS-767)

FROM: Owen F. Reeder
Lab Audit Coordinator
Project Coordination Section
Registration Support and
Emergency Response Branch (TS-767C)

I have enclosed the lab audit from the Food and Drug Lab. Inc, of Waverly, N.Y. on the subject chemical/product Malathion for handling and distribution. Please review and return. Our current records show this chemical is assigned to PM 16.

Attachment

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

Received in TC 10/21/87 RJ

JUL 31 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: GLP Inspection/Study Audit Report; Request for Regulatory Review

FROM: Dexter S. Goldman, Director dsg
Laboratory Data Integrity Assurance Division (EN-342)

TO: Allen Jennings, Director
Benefits and Use Division (TS 768C)

Attached please find a copy of the report of the data audits conducted at Food & Drug Research Laboratories, Inc., Waverly, NY during the period of March 11-13, 1986.

The following studies were audited:

1. Malathion; Two-year Feeding Study in Rats; Lab. Study No. 5436

2. CGA-12223; Six-month Subchronic Study in Beagle Dogs; Lab. Study No. 7121-5

The inspector's report is enclosed, and some of the major findings are highlighted in the following paragraphs.

Please note that these studies were conducted prior to the publication of the Good Laboratory Practice Regulations under FIFRA. During the audit the following general deviations from GLPs were noted:

1. Corrections were not properly documented.

2. There is not an adequate chain of custody for tissues between trimming and histology.

3. Animal identification was inadequate - this has already been corrected.

4. The statistical procedures used in one study may not have been adequate.

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The data audit findings are summarized below:

In the Malathion study,

1. Corrections in the raw data were not properly documented.
2. There was no documentation on the preparation of extra slides.
3. There was no chain of custody for tissues between necropsy and histology.
4. The test animals were not adequately identified.
5. QA did not inspect all critical phases of the study.
6. There appears to have been an excessive number of pathologists reading the slides for this study; it is not clear how uniformity was maintained.
7. Some pathology reports were not signed and dated.
8. Changes in diagnoses were not properly documented.
9. An excessive amount of time passed between the slide reading and the pathology report signoff.
10. Tissues first noted as missing reappeared about a year later. No explanation.
11. The statistical method used in histopathology was questioned. It is not clear that this statistical procedure will give an adequate picture of the significance of lesions.
12. The terminal GGT determinations, required by the protocol, were not conducted or, if they were, the data were lost.
13. In the high dose diet preparation corn oil was not used until 9/9/77 (5 months after the study start date).

In the CGA 12223 study,

1. Corrections in the raw data were not documented.
2. There was no documentation on the preparation of extra slides.

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3. There was no chain of custody for tissues between necropsy and histology.

4. There was no SOP for the QAU on critical phase inspections.

For both studies these findings are indicative of a need for tighter managerial control over the processes but were not considered sufficient to question the validity of either study.

This report was submitted to the laboratory and their response is attached as an addendum to the report.

Please provide me, within 90 days, with a regulatory review of this report.

Along with the report I have enclosed background material and exhibits resulting from this inspection. This material is for your archives, but should be available to us if there is further need.

Attachment

cc: A. E. Conroy II (Memo only)
Douglas Campt (Memo only)

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V. CLOSING CONFERENCE

At the conclusion of the data audit on March 13, 1986, an exit interview was held. FDRL was represented by John Biesimeier, president; Michael Kangiser, vice president and Frederick Paul, manager QA. Members of the EPA audit team present were Francisca Liem, inspector; Adrian Gross, pathologist; Willa Garner, chemist, and Linda Plankenhorn, data inspector.

→I thanked the laboratory for their cooperation. I presented our findings in general, while specific findings were presented by each auditor.

Reported findings were summarized and presented as follows:

The studies audited had all been conducted prior to the publication of the EPA GLP regulations and prior to the last FDA/EPA inspection conducted on 1/9/85 and therefore did not comply to the GLP regulations in some areas and did not reflect comments concerning GLP requirements and data audit made by the 1985 inspection team. However, the laboratory management and staff appeared to be committed to full compliance with GLP regulations.

I. General

1. Tissues should not be rinsed in water.

2. Lungs were overinflated.

II. Cythion study

1. Improper correction procedures were practiced.

2. Documentation on the preparation of extra slides was not available.

3. There was no paper trail on the custody of the tissues between the trimming technician and the histologist.

4. A few missing and unexamined tissues were noted.

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5. Tissue list (histology and necropsy sheet) is incomplete.

6. The method of animal identification was inadequate.

7. Quality Assurance:

   a. A SOP for critical phases to be inspected should be established.

   b. Not all critical phases on this study were inspected.

   c. The QA statement should list the dates of inspection and dates findings were reported to the study director and to management.

8. Paraffin blocks should be kept in a temperature-controlled area.

9. There were too many pathologists reading the slides.

10. Some of the pathology reports for individual animals were not signed and dated by the examining pathologist. In some cases, there were more than one examiner.

11. Changes of diagnosis were given on animals, with no reasons, initials and dates indicated. In some cases the lesions were downgraded.

12. Some individual pathology reports were signed off more than seven months after the examination of the slides.

13. Some of the changes made on the histopathology findings were questionable.

14. Tissues which first were missing, suddenly were evaluated one year later.

15. The statistical method used for histopathology was inadequate.

16. Protocol deviations were noted:

   a. GGT determination at termination was either not conducted or data were lost.

   b. Diet preparation: corn oil was not used in the high dose mix (until 9/9/77).

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Inspection at Food and Drug Research Laboratories March 11th-13th, 1986.-

Further notes on pathology operations by M. Adrian Gross

The evaluation of chronic toxicity effects of Cythion (24 months study) in rats American Cyanamid - report No. 5436 dated May the 13th, 1980.

The histopathology examinations of the sections collected from the animals in this study were carried out by no less than six pathologists:- Drs. Luft, Weaver, King, Brandstetter, Goldblatt, and Becci, with a seventh one, Dr. William D. Johnson having written the pathology report.

Examination of the raw data on those examinations revealed a number of problems:

a) some of the reports made for individual animals were undated and unsigned by the person having made such examinations; as an example of these see the report for control male animal no. 36040 dead on 11/27/78, attached here as Appendix 1;

b) the reports for two animals, No. 36051, said to have been "sacrificed in a moribund state" on 9/29/78 from the 100 ppm experimental group and No. 36030, a control male animal dead on 3/6/79, had tissues examined by one person, Dr. Becci; as the two Appendices 2 and 3 show, however, the handwriting in those two reports could hardly have been more different and this applies to the signature itself; also note in Appendix 2 that a finding made allegedly by Dr. Becci for the lung had been struck through and a new finding was substituted by Dr. Johnson more than two months after the original initialling of this report.

c) the report for female animal No. 36333 at 1000 ppm, given as Appendix 4 here, contains two handwritings but only one signature;

d) the reports for animals 36397 and 36184, respectively a female and male at 5000 ppm are signed by one person but the two handwritings could hardly be more different; see Appendices 5 and 6 here;

e) Appendix 6 also indicates several strike-throughs unexplained, undated, and uninitialled; upon enquiry we were told by Dr. Johnson that he made this kind of "editorial changes" himself, to make those reports (written, as mentioned initially here, by one of half a dozen different pathologists) sound more "consistent with each other"; Dr. Johnson also claims that such changes, deletions and additions as he made to such reports were all approved by the author of each report, though there is no written evidence of this.

f) Appendices 7 and 8 indicate two reports allegedly by Dr. Luft which were presumably transcribed (although indicated as having been dictated) by Mary Smith on 10/19/79; each of those reports were not initialled or signed off by Dr. Luft until May or June the following year. The question here is how could Dr. Luft have assured himself that his dictation was accurately transcribed more than seven months after he had presumably examined those slides (assuming, of course, that he had carried out such examinations on the very day the results were initially transcribed from his dictation). Also note in Appendix 7 an

undated and uninitialled change from a certain type of lesion in the adrenal gland to a "softer" one and the "downgrading" of a malignant tumor of the thyroid gland to another one of nonspecified malignancy, another change also undated and uninitialled, but both changes having probably been made by Dr. Johnson.

g) Appendix 9 represents a change made by Dr. Johnson where he apparently made a second tumor in the skin to vanish entirely after briefly considering this to be a normal structure (the bulbourethral gland) but then rejecting such idea. Perhaps Dr. Weaver, a pathologist trained in human material, is unfamiliar with this particular structure in rats. For the thymus, an initial impression of hyperplasia (exaggerated growth) has been changed into one of no growth whatsoever (atrophy);

h) Appendix 10 represents a change in characterization of an adrenal gland tumor by someone where it is unclear who the initial examiner was since there is no signature or initial for the original results;

i) Appendix 11 is an example of a situation similar to that described for Appendix 7;

j) Appendix 12 is yet another example similar to Appendix 11;

k) Appendix 13 represents the reverse kind of change:- an initially perceived "not significant" change is now characterized to be a malignant tumor;

l) Appendix 14 indicates three separate instances where a tumor of the uterus has been downgraded:- in the first example a blood-vascular tumor had metamorphosed into a polyp; furthermore, although the transcription of the dictated results was carried out on 11/..0/79, Dr. Weaver apparently signed this report some 9 days earlier. Also note that Dr. Weaver apparently changed his mind the following month. Question - who or what induced him to re-examine the uterus section ? The other two examples here refer to a smooth muscle tumor of the uterus which was changed to no tumor at all a month later with the same problem of Dr. Weaver having signed off on the typed results before they were typed.

m) Appendix 15 illustrates eight instances on how hemorrhages in the adrenal gland (noted by Dr. Luft) were changed to merely dilated vessels; although Dr. Luft had initialled the making of those changes, again, who or what prompted him to review those particular slides again (if indeed he did re-review them) and what was the reason for such re-examination, if any ?

n) Appendix 16 represents an interesting variation on this general theme:- here apparently the typist had left out a (four-letter ?) word for the pituitary gland tumor - perhaps what was meant here is "some" invasion into (the) brain parenchyma. Instead the words congestion and hemorrhage were entered in handwriting (which would be inappropriate as qualifiers for "invasion") but perhaps they are meant to apply for the gross changes noted in the adrenal gland - but if the latter is true, how could such changes be made by anyone not present during the gross examination of the carcass of the animal ?

o) Appendix 17 illustrates a problem in the thyroid gland similar to what was discussed under (f) here and one in the adrenal gland similar to what was discussed under (m) here.

p) Appendix 18 is a report signed by Dr. Branstetter in 1979 where it is said that no tissues whatsoever were present for evaluation. Presumably such tissues were located the following year when they were described by Dr. Johnson. Interestingly, it appears that someone else had entered Dr. Johnson's original initials on March the 4th, 1980. The writing instrument used for this particular animal was not the same and the notation (vascular changes) (cellular alteration) given as they are in parentheses and not clearly referable to any specified tissue are mysterious; they appear to have been made by someone else than the one making the rest of the observation and it is equally puzzling why they appear in the middle of the other observations.

q) Appendix 19 represents an example of extensive changes apparently having been made by Dr. Johnson in a report signed by Dr. Goldblatt.

Appendix 20 represents a problem of a different kind:- it represents pages 9 and 10 of the FDRL report. The last paragraph on page 9 there contains one piece of nonsense and one false statement:- the nonsense is in giving an "expected (parametric) incidence" for endometrial stromal polyps of the uterus as 6%, 20%, 18%, and 20% - but there can be only <u>one</u> expected incidence in any situation. What the writers of the report seem to confuse is <u>observations</u> and <u>expectations</u>, with the former being merely a number of <u>estimates</u> for the latter. The false statement there is that "There is no statistically significant difference in tumor incidence between any of the test groups when compared to the control group, as analyzed by the 2 x 2 contingency test with Yates' correction." In both the low dose and the high dose groups there was an incidence of 10/50 animals with uterine polyps which when contrasted with a control incidence of 3/50 is not only significant at the p = 0.035,654 by Fisher's Exact Test based on the hypergeometric distribution, but also significant at the p = 0.037,204 probability level by the test that gives merely an approximation to the probability issuing from Fisher's Exact Test and the one used by FDRL:- the 2x2 contingency test i.e., the chi square test with Yates' correction for continuity which turned out to be as large as 3.183.

The following tissue sections were examined by me microscopically and my own findings did not differ substantially from the findings listed in the report prepared by FDRL except in one instance for the thyroid gland of No. 203:-

adrenal gland - cortical adenoma - No. 223; brain - glioma - Nos. 208 and 237; small intestine - histiocytoma- No. 203; mammary gland - fibroadenoma - Nos. 210 and 211, - adenocarcinoma - No. 250; ovary - papillary cystadenocarcinoma - No. 213; pancreas - islet cell adenoma - Nos. 217 and 245; for the thyroid gland of No. 203 the FDRL report lists a parafollicular (C cell) tumor which I thought represented no more than a state of hyperplasia but I agreed with the characterization of carcinoma for the thyroid gland of No. 233; uterus - papilloma - No. 236; uterine polyps - Nos. 222, 234 and 241; leiomyosarcoma - No. 209; choroiditis - No. 209, chronic myocarditis - Nos. 207, 217, 242 and 375; kidney - fibrosis - No. 232; glomerulosclerosis - Nos. 202 and 225; tubular atrophy Nos. 210 and 211 (the latter I thought was somewhat questionable);

liver - nodular hyperplasia - No. 367; bile duct hyperplasia - Nos. 208, 226; ovary - tubular adenomatous change - No. 250; pancreas - fibrosis and atrophy - No. 233; islet cell hypertrophy - No. 237; salivary glands - atrophy - Nos. 385, 210, 226, and 248 (the last one somewhat questionable); skeletal muscle - chronic inflammation - No. 241; stomach - ulceration - Nos. 209 and 210; thyroid gland - follicular atrophy - No. 237; tongue - hyperkeratosis - No. 233.

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Six months (26 weeks) Subchronic Toxicity Study of CGA 12223 in Beagle Dogs - Ciba Geigy - Project 7121 - report dated 2/15/83
study initiated 1/12/82 - necropsy 7/13 - 8/10/82

A sample of tissue sections were examined microscopically by me and I found no deviations whatsoever from the findings of the original examining pathologist:-

No. 030 - subcutaneous mass - epidermal inclusion cyst;

- adenohypophysis - cysts with eosinophillic material

No. 032 - mesenteric lymph nodes - slight congestion

- pituitary gland - cyst

No. 035 - large intestine - normal mucosa

- peribronchial lymph nodes - slight congestion

- small intestine - normal with merely slight congestion

- mammary gland - hemorrhage and proliferation of acinar parenchyma and and of the stromal elements

No. 039 - pituitary gland - cyst

- lung - slight subacute pneumonia with some chronic foci of same

No. 041 - all tissues were essentially normal in appearance

No. 044 - parathyroid gland - cyst

- large intestine, small intestine, and mesenteric lymph node - very slight congestion

No. 49 - liver - moderate albuminous degeneration

No. 54 - pitutary gland - cystic

- lung - pneumonitis

- mediastinal lymph nodes - slight congestion

- no visible lesions for uterus, stomach, duodenum, pancreas, urinary bladder, ovary with corpus luteum and corpus albicans, pancreas, salivary glands, liver, heart, cerebellum, left and right eyes, cerebrum, trachea, left and right kidney, spleenm, thymus, bone, bone marrow, left and right adrenal gland, adeno and neurohypophysis, left and right thyroid glands, skin and mammary gland.

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Two Year Chronic Toxicity Study of Malathion in Sprague-Dawley Rats. Food and Drug Research Laboratories, May 13, 1980, Study No. 4123-013-01. Malathion Registration Standard Reviewer's Opinion

FROM: Brian Dementi, Ph.D. Brian Dementi 12/1/87
Review Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Theodore Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

During the development of the toxicology chapter of the Malathion Registration Standard certain data from the referenced study were submitted to the Tox Branch Statistics Team for statistical evaluation. A report of that statistical evaluation appears in the Standard. The histopathology data submitted at that time were taken from summary tables in the subject FDRL study report and from the 1985 Dynamac review of the study. I am herewith submitting as supplementary information my independent counts of the number of rats exhibiting those effects previously identified and considered for statistical evaluation. I am including not only the total counts, but the identifying numbers for those animals considered by me to exhibit the effect. For purposes of comparison I include the counts as reported in the FDRL summary table and in the Dynamac review. I make no claim that my counts are free from error, since it is difficult to identify each finding from among so many parameters on numerous histopathology sheets. The possibility of misinterpretation is also a problem, rendered more difficult by the retirement of Dr. Louis Kaza, Branch pathologist with whom I often consulted for histopathologic opinions. In some cases I have placed question marks or comments beside animal I.D. numbers for which I experienced some uncertainty in including in the total count. In those cases, brackets around the count for that parameter represent an alternative but less likely count, as I perceive it.

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Relatively little difference exists between the number of animals exhibiting the various effects as determined by me and those previously reported. Noteable exceptions being those for prostate calcification in males and thymus cyst in females. As to the latter parameter, 13 as reported by FDRL for the low dose group is remarkably different from 5 as reported by Dynamac and confirmed by me.

It was originally my intention to comment more at length as to the concerns I experience over these various findings often exhibited in a dose-related matter and evident at the lowest dose. However, it has recently come to my attention that this study was audited by EPA and additional independent evaluation of microscopic slides has been recommended. Hence, I will defer to that anticipitated evaluation as the mechanism by which concerns will be resolved.

It is nevertheless my opinion that if the findings were/are to stand they constitute ample justification for a peer review for non-oncogenic effects, and, in concert with other oncogenic studies on malathion and malaoxon discussed in the Registration Standard, constitute justification for a peer review for oncogenic effects.

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A) Males

Parameter	Dose Group*	Number Rats With Effect FDRL	Dynamac	Dementi	Animal I.D. No. - Dementi
Brain color alteration	C	6	6	6	009, 011, 013, 030, 033, 050
	L	10	10	10	053, 059, 062, 066, 073, 077, 088, 090, 091, 092
	M	11	11	11	103, 104, 110, 123, 128, 132, 138, 139, 144, 145, 148
	H	14	14	14	151, 156, 157, 163, 164, 165, 173, 177, 180, 185, 187, 189, 195, 198
Heart inflammation	C	4	4	4	003, 004, 006, 035
chronic prots	L	11	11	11(9)	058, 062, 064, 065, 073, 079 084(?), 092, 093(?), 094, 096
	M	9	9	9(7)	101(?), 102, 110, 111, 118, 121, 137, 144(?), 147
	H	13	13	13	151, 153, 156, 168, 170, 172, 173, 174, 175, 190, 191, 193, 194
Kidney tubular casts	C	9	9	11(10)	001, 005, 006, 010, 018, 020, 028, 031, 032, 038, 045(?)
	L	19	19	19	052, 058, 061, 062, 065, 066, 069, 070, 074, 076, 077, 081, 084, 085, 088, 089, 093, 095, 097
	M	13	13	13	103, 104, 108, 112, 118, 119, 120, 128, 135, 136, 138, 140, 149
	H	15	15	14	155, 156, 161, 162, 165, 171, 174, 180, 183, 187, 191, 193, 196, 197
Kidney tubular dilation	C	6	6	4	009, 020, 039, 044
	L	9	9	7	070, 083, 085, 089, 093, 095, 097
	M	9	9	8	106, 109, 116, 117, 124, 133, 136, 144
	H	13	13	12	153, 159, 161, 164, 165, 174, 176, 177, 180, 189, 196, 199
Kidney glomerulo-sclerosis	C	0	0	0	
	L	3	3	3	070, 085, 097
	M	6	6	7(6)	109, 120, 134, 136(?), 146, 147, 150
	H	7	7	7(6)	155, 156, 165, 166(?), 169, 174, 184

* C (control), L (low dose, 100 ppm), M (mid dose, 1000 ppm), H (high dose, 5000 ppm)

Males, con't

Parameter	Dose Group	Number Rats With Effect FDRL	Dynamac	Dementi	Animal I.D. No. - Dementi
Liver, sinusoidal dilation	C	1	1	1	005
	L	3	3	3	056, 069, 093
	M	1	1	1	103
	H	10	10	10	152, 155, 164, 168, 178, 182 186, 187, 191, 196
Liver, swollen	C	5	5	5	024, 030, 037, 038, 046
	L	8	8	8	055, 057, 059, 062, 083, 092 095, 097
	M	13	13	13	102, 106, 114, 115, 121, 123, 132, 134, 136, 137, 146, 149, 150
	H	17	17	17	151, 160, 162, 168, 171, 172, 174, 175, 176, 180, 185, 191, 193, 194, 196, 197, 198
Lymph node, reticuloendothelial hyperplasia	C	7	7	7	008, 012, 028, 033, 038, 043, 048
	L	10	10	12	056, 063, 067, 071, 073, 076, 078, 086, 089, 092, 093, 099
	M	12	12	12	104, 105, 113, 122, 127, 129, 132, 136, 142, 143, 146, 147
	H	18	18	15(16)	156, 157, 163, 166, 167, 169, 173, 175, 177, 178, 180, 183, 191, 192, 198 - possibly 190
Pancreas, duct dilation	C	0	0	0	
	L	3	3	2	086, 099
	M	1	1	1	147
	H	4	4	4	157, 167, 174, 193
Pituitary, cyst	C	1	1	1	025
	L	2	2	2	078, 083
	M	3	3	4	115, 120, 126, 129
	H	5	5	5	155, 162, 175, 191, 193
Prostate, calcification	C	8	8	8	003, 012, 025, 027, 029, 030, 039, 049
	L	10	10	7	064, 072, 075, 083, 087, 090, 100
	M	12	12	12	102, 111, 117, 118, 123, 124, 130, 133, 137, 144, 145, 148
	H	16	16	15	151, 153, 159, 160, 164, 170, 172, 176, 181, 186, 189, 195, 197, 199, 200

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