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BIOASSAY OF

MALATHION

FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
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Bethesda, Maryland 20205

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FOREWORD: This report presents the results of the bioassay of malathion conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of malathion was conducted by the Gulf South Research Institute (GSRI), New Iberia, Louisiana, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design for this bioassay is based on guidelines for carcinogen bioassays in small animals that have been established by NCI (1). The doses for the chronic study were selected by Drs. E. E. Storrs (2) and O. G. Fitzhugh (3,4), and the principal investigator was Mr. R. J. Wheeler (2). Animal treatment and observations were supervised by Dr. W. E. Greer (2), with the assistance of Ms. D. H. Monceaux (2). Histopathology for the rats was performed by Dr. R. A. Ball (2) and for the mice by Dr. E. Bernal (2), and the diagnoses in this report represent the interpretations of these pathologists.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (5). Statistical analyses were performed by Dr. J. R. Joiner (3), using methods selected for the bioassay program by Dr. J. J. Gart (6). Upon completion of the bioassay, the test material was reanalyzed at Midwest Research Institute under the direction of Dr. T. Woodhouse (7). The chemicals and dosed feed mixtures used in this bioassay were analyzed at GSRI under the direction of Mr. Wheeler (2). Analyses of the feed mixtures were performed by Mr. M. Billedeau (2). The results of the analyses were reviewed by Dr. C. W. Jameson (3,8).

This report was prepared at Tracor Jitco (3) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens (9), toxicologist; Dr. R. L. Schueler, pathologist; Ms. L. A. Owen and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry Mahar, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

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

Groups of 49 or 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.

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 dose related.

It was concluded that 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.

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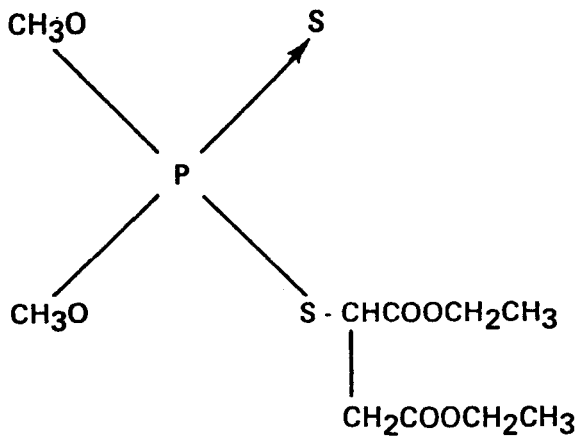
I. INTRODUCTION



MALATHION

Malathion (CAS 121-75-5; NCI C00215), S-(1,2-bis(ethoxycarbonyl)-ethyl)) 0,0-dimethylphosphorodithioate, is an organophosphate insecticide considered to be suitable as a substitute for certain uses of DDT (Environmental Protection Agency, 1975). U.S. consumption in 1974 was 16 million pounds, surpassing that of all other organophosphate insecticides except methyl parathion (Ayers and Johnson, 1976). Household applications accounted for approximately 10% of that volume (Ayers and Johnson, 1976). When malathion was used as directed in homes, on crops, and to control insects of public health importance, the incidence of adverse effects was low among workers and persons living in treated communities (Environmental Protection Agency, 1975). There have been reports, however, of toxic effects among

field workers who were inadequately trained in the handling of this pesticide (Baker et al., 1978).

Registered applications for malathion include use on edible grains, raw agricultural products, forage crops, cotton, tobacco, berries, fruits, nuts, and ornamental plants (Environmental Protection Agency, 1975; Code of Federal Regulations, 1977). Malathion is used as an ectoparasiticide on livestock and domestic animals and is sprayed in and around livestock barns and poultry houses, dairies, food processing plants, slaughter-houses, grain elevators and other food storage facilities. It is also an ingredient of household sprays and garden pesticides. The World Health Organization recommends malathion for use by public health programs to control mosquitoes, (World Health Organization, Division of Malaria and Parasitic Diseases, 1973), and it has been employed as a delousing agent for humans (Harvey, 1975; Hayes et al., 1960).

Although many of these applications produce environmental contamination, malathion and its degradation product malaoxon (formed photochemically and biochemically) have half-lives in soil of one week or less (Paschal and Neville, 1976).

Malathion is classified as an organophosphorus pesticide and induces toxicity mainly by inhibition of cholinesterase. Oral LD_{50}'s of

malathion have been reported to be 5,843 mg/kg body weight in male rats, strain unspecified, and 4,059 mg/kg in male mice, strain unspecified (Hazleton and Holland, 1953). When malathion was administered in the diet at 38 or 75 mg/kg body weight to CFY rats for 90 days (Desi et al., 1976) or at 1,000, 5,000, or 20,000 ppm to rats of unspecified strain for 2 years (NIOSH, 1976), there was reduced cholinesterase activity in the cerebral cortex, erythrocytes, and plasma, depending on the dose. Oral intubation of 8-day-old Wistar rats with malathion at 500 mg/kg body weight reduced brain cholinesterase activity within 0.5 hour. The toxicity of malathion to mammals is lower than that of many other organophosphate insecticides because the ethyl carboxylic acid ester groupings in malathion are hydrolyzed by mammalian carboxyesterases to products that do not inhibit cholinesterase (Norton, 1975; Murphy, 1975). Carboxyesterase activity is low, however, in susceptible insects and is the basis for the selective toxicity of malathion to insects (Eto, 1974).

Malathion was not found to be carcinogenic in an earlier study conducted by the National Cancer Institute using Osborne-Mendel rats and B6C3F1 mice (National Cancer Institute, 1978). It was retested in the F344 (Fischer) rat to examine the sensitivity of this strain to malathion and to compare the effects of malathion with those of its metabolite malaoxon (National Cancer Institute, 1979) which is known to be formed in vivo by oxidative desulfurization (Eto, 1974).

II. MATERIALS AND METHODS

A. Chemical

Malathion was obtained in four different batches from the American Cyanamid Company, Princeton, New Jersey. Batch 01 (manufacturer's assay, 99.7%) was used only as a reference standard. Batches 02 (technical grade) and 03 (manufacturer's assay, 95%) were used in subchronic studies. Batch 04 (Lot No. SPS-10127; manufacturer's assay, 95%) was used in chronic studies. Analysis of the different batches at Gulf South Research Institute included elemental analysis, boiling point, thin-layer and gas-liquid chromatography, and infrared and nuclear magnetic resonance spectometry (Appendix C). The results confirmed the identity of the test chemical and were consistent with the manufacturer's assays. No attempt was made to identify or quantitate impurities. The chemical used for the chronic study was stored in the original container at approximately 25°C. Additional analysis of this batch of malathion at Midwest Research Institute, after completion of the bioassay, indicated that the material had not changed.

B. Dietary Preparation

All diets were formulated using finely ground Wayne® Lab Blox (Allied Mills, Chicago, Illinois) to which was added the required amount of malathion for each dietary concentration. The test compound was first dissolved in a small amount of acetone (Mallinckrodt Chemicals, St. Louis, Mo.), which was then added to the feed. Corn oil (Lou Ana®, Opelousas Refinery, Opelousas, Louisiana) was also added to the feed, primarily as a dust suppressant, and the diets were mixed mechanically for not less than 25 minutes to assure homogeneity and to allow for evaporation of the acetone. Final diets, including those for the control groups of animals, contained corn oil equal to 2% of the final weight of feed. Formulated diets were stored at room temperature until used, but not longer than 1 week.

Stability of malathion in feed was tested by determining the concentration of the compound in formulated diets containing 4,000 and 2,000 ppm at intervals over a 7-day period. No significant changes in concentration on standing at ambient temperature were found for this period. As a quality control test on the accuracy of preparation of the diets, the concentration of malathion was determined in randomly selected batches of formulated diets at 8-week intervals during the chronic study. The results are summarized in Appendix D. At each dietary concentration, the mean

of the analytical concentrations for the samples tested was within 1.6% of the theoretical concentration, and the coefficient of variation was 4.3%.

C. Animals

F344 rats of each sex were obtained from the NCI Frederick Cancer Research Center (Frederick, Md.). Animals were acclimated within the test facility for 2 weeks, and when 6 weeks of age were assigned to dosed or control groups.

D. Animal Maintenance

Rats were housed individually in hanging galvanized steel mesh cages (Hoeltge, Inc., Cincinnati, Ohio). Cages and racks were washed every 2 weeks in an industrial washer at 82°C with Acclaim® Detergent (Economics Laboratory, Inc., St. Paul, Minn.) and then rinsed. Absorbent Kimpak® cage liners (Kimberly Clark Corp., Nenah, Wis.) were placed under the rat cages and were changed twice per week. Feed jars and water bottles as well as sipper tubes and stoppers were washed twice per week in a Vulcan Autosan Washer (Louisville, Ky.) at 82°C, using Acclaim Detergent®, and then rinsed.

Cage racks were rotated to a new position in the room once per week; at the same time, each cage was moved to a different row within the same column of a rack. Rats receiving malathion and their respective controls were housed in the same room. No animals receiving other test chemicals were housed in the room with the animals receiving malathion.

Animal rooms were maintained at 22 to 24°C, and relative humidity was 40 to 70%. Fresh air was filtered through air maze filters (Air Maze Incom International, Cleveland, Ohio), at a rate to allow 10 to 12 changes per hour. Fluorescent lighting provided illumination 10 hours per day. Food and tap water were available ad libitum. Excess remaining feed was discarded and fresh feed was provided twice a week.

E. Subchronic Studies

Subchronic feeding studies were conducted to determine the two concentrations used in the chronic studies (referred to in this report as "low" and "high" doses). Groups of 10 rats of each sex were fed diets containing malathion at one of several doses for 13 weeks, and groups of 10 control animals of each sex were fed basal diet only. The diets were stored at room temperature and fresh feed

was provided twice a week. Animals were weighed each week. Table 1 shows doses fed, the survival of animals in each dosed group at the end of the study, and the mean body weight of the dosed animals at week 13, expressed as percentages of mean body weights of controls. At the end of the 13-week period, the animals were killed and necropsied.

As shown in table 1, 5 out of 10 male rats fed 16,000 ppm died by week 9, and 9 out of 10 females fed the same dose died by week 5. Mean weights decreased in the males fed 16,000 ppm and in the females fed 8,000 ppm.

When malathion was fed to Osborne-Mendel rats in a previous chronic study (National Cancer Institute, 1978), the initial low-dose was 8,000 ppm and the initial high-dose was 12,000 ppm. Due to toxic effects, these doses were lowered at weeks 3 and 14 to 4,000 and 8,000 ppm for the remainder of the study.

The low and high doses for the rats were set at 2,000 and 4,000 ppm for the present chronic study.

Table 1. Doses, Survival, and Mean Body Weights of Rats Fed Malathion in the Diet for 13 Weeks

	Male		Female	
Dose(a) (ppm)	Survival (b)	Mean Weight at Week 13 as % of Control	Survival (b)	Mean Weight at Week 13 as % of Control
0	10/10	100	10/10	100
1,000	10/10	96	10/10	98
2,000	10/10	106	10/10	97
4,000	10/10	101	10/10	97
8,000	10/10	96	10/10	86
16,000	5/10(c)	82	1/10(d)	50

(a) Necropsies were performed on animals at all doses and their respective controls. No gross pathologic changes ascribable to malathion were observed.

(b) Number surviving/number in group.

(c) Five male rats in the group receiving 16,000 ppm died by week 9.

(d) Nine female rats in the group receiving 16,000 ppm died by week 5, and administration of malathion to this group was then discontinued.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in table 2.

G. Clinical Examinations and Pathology

All animals were observed twice per day for signs of toxicity, weighed every 2 weeks, and palpated for masses at each weighing. Animals that were moribund at the time of clinical examination and those that survived to the end of the bioassay were killed using pentobarbitol and necropsied.

Pathology consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, gallbladder (mice), pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% neutral buffered formalin,

Table 2. Experimental Design for Chronic Malathion Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	Malathion Doses (b) (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	50	0		105-106
Low-Dose	50	2,000	103	2
High-Dose	49	4,000	103	2
Female				
Matched-Control	50	0		105-106
Low-Dose	50	2,000	103	2-3
High-Dose	50	4,000	103	2-3

(a) Rats were 6 weeks of age when placed on study.

(b) Test and control diets were provided *ad libitum*.

embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized as necessary.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals may have been missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Data on this experiment were recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

These data were analyzed using the appropriate statistical techniques described in this section. Those analyses of the experimental

results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple

sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the

exact interval on the odds ratio (Gart, 1971). The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS

A. Body Weights and Clinical Signs

Mean body weights of the low- and high-dose male rats were lower than those of the controls in a dose-related manner after about 50 weeks on study (figure 1). Mean body weights of the female rats were essentially unaffected by the test chemical throughout the bioassay. A temporary depression in mean body weights of all groups at week 78 was due to an unexplained rejection of feed; recovery of weight occurred when freshly-mixed control diet was administered for 4 days.

A variety of clinical signs, including rough hair coat, alopecia, dermatitis, anemia, tachypnea, dark urine, loose stools, and in the females, vaginal discharge and bleeding, occurred with increasing incidence in both the dosed and control groups during the second year of the bioassay.

B. Survival

Estimates of the probabilities of survival for male and female rats administered malathion in the diet at the doses of this bioassay,

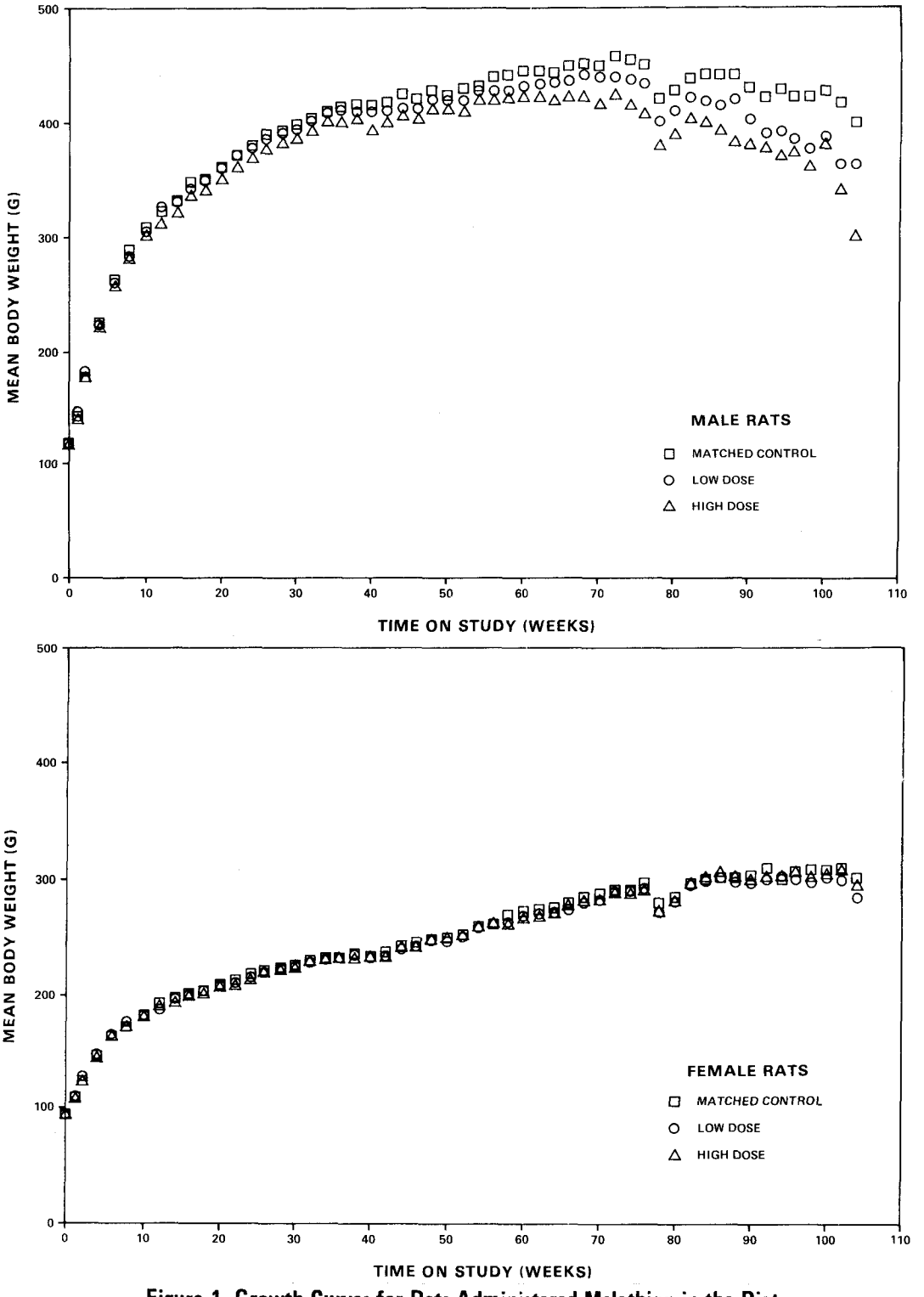


Figure 1. Growth Curves for Rats Administered Malathion in the Diet

together with those of the matched controls, are shown by the Kaplan and Meier curves in figure 2. The result of the Tarone test for positive dose-related trend in mortality is significant for male rats (P less than 0.001) but is not significant for the females.

In male rats, 39/49 (80%) of the high-dose group, 43/50 (86%) of the low-dose group, and 44/50 (88%) of the control group were alive at week 78 on study. In the females, 45/50 (90%) of the high-dose group, 49/50 (98%) of the low-dose group, and 47/50 (94%) of the control group were alive at week 78.

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix B, tables B1 and B2.

A variety of neoplasms were observed in both control and dosed groups of animals which, with the possible exception of pheochromocytomas of the adrenal in the male rats, were not believed to be

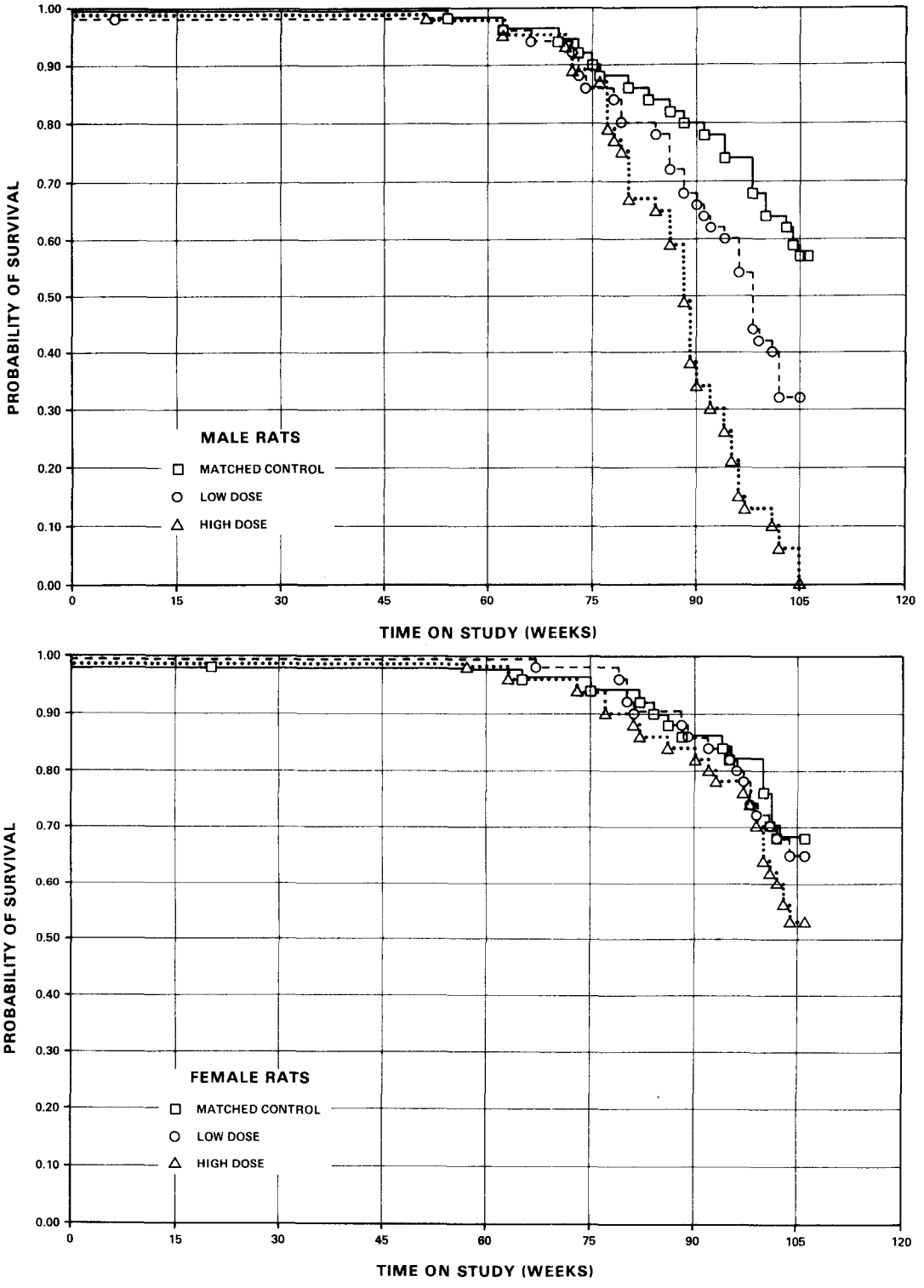


Figure 2. Survival Curves for Rats Administered Malathion in the Diet

compound-related. The incidences of adrenal pheochromocytomas in the males, 2/49 (4%) in the controls, 11/48 (23%) in the low-dose, and 6/49 (12%) in the high-dose groups, were not considered to be related to the administration of the test compound.

Increased incidences of toxic gastric and hepatic lesions in dosed animals of each sex are summarized in the following table:

	MALE			FEMALE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
STOMACH						
Number of Animals with Tissues Examined Microscopically	49	46	47	50	44	47
Chronic Inflammation	2	6	11	0	2	4
Ulcer	1	9	15	1	2	2
LIVER						
Number of Animals with Tissues Examined Microscopically	49	50	49	50	50	48
Fatty metamorphosis	1	3	2	0	6	9

The gastric lesions were usually focal and singular. The ulcers were chronic in nature. The pathologic examination indicates that under the conditions of this bioassay malathion was not carcinogenic for F344 rats.

D. Statistical Analyses of Results

Tables 3 and 4 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In female rats, the results of the Cochran-Armitage test for dose-related trend in the incidences of tumors and the results of the Fisher exact test comparing the incidences of tumors in the control group with those in each dosed group are not significant.

In male rats, the result of the Fisher exact test comparing the incidence of pheochromocytomas of the adrenal between the low-dose and control groups is significant ($P = 0.006$), but the incidence in the high-dose group is not significant. The result of the Cochran-Armitage also is not significant. The historical-control data for adrenal pheochromocytomas in untreated male F344 rats at this laboratory show an incidence of 8/275 (3%), compared with 2/49 (4%) in the control group, 11/48 (23%) in the low-dose group and 6/49 (12%) in the high-dose group of this study.

Significant results in the negative direction are observed in the incidence of leukemia and in the incidence of carcinomas of the

pituitary in male rats, which may be accounted for by the shorter survival of the dosed animals as compared with that of the control animals.

In each of the 95% confidence intervals for relative risk shown in the tables, except for the incidence of pheochromocytomas of the adrenal in low-dose male rats, the value of one or less than one is included: this indicates the absence of significant positive results. It should also be noted that each of the intervals, except for the incidence of carcinomas of the pituitary in high-dose male rats, has an upper limit greater than one, indicating the theoretical possibility of tumor induction by malathion, which could not be detected under the conditions of this test.

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Malathion in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	0/49 (0)	3/50 (6)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.590	0.054
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	84	94
Hematopoietic System: Undifferentiated Leukemia (b)	9/49 (18)	8/50 (16)	2/49 (4)
P Values (c,d)	P = 0.025 (N)	N.S.	P = 0.025 (N)
Relative Risk (f)		0.871	0.222
Lower Limit		0.319	0.024
Upper Limit		2.333	1.004
Weeks to First Observed Tumor	76	72	101

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Malathion in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS (b)	6/44 (14)	2/40 (5)	0/45 (0)
P Values (c,d)	P = 0.008 (N)	N.S.	P = 0.012 (N)
Relative Risk (f)		0.367	0.000
Lower Limit		0.038	0.000
Upper Limit		1.910	0.609
Weeks to First Observed Tumor	88	86	--
Pituitary: Carcinoma, NOS or Adenoma, NOS (b)	16/44 (36)	12/40 (30)	9/45 (20)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.825	0.550
Lower Limit		0.409	0.242
Upper Limit		1.615	1.173
Weeks to First Observed Tumor	62	78	76

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Malathion in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	2/49 (4)	11/48 (23)	6/49 (12)
P Values (c,d)	N.S.	P = 0.006	N.S.
Departure from Linear Trend (e)	P = 0.013		
Relative Risk (f)		5.615	3.000
Lower Limit		1.316	0.569
Upper Limit		49.840	29.224
Weeks to First Observed Tumor	83	73	86
Thyroid: C-cell Adenoma (b)	3/47 (6)	2/46 (4)	0/44 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.681	0.000
Lower Limit		0.059	0.000
Upper Limit		5.670	1.769
Weeks to First Observed Tumor	86	73	---

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Malathion in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Adenoma (b)	7/49 (14)	3/48 (6)	4/48 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.438	0.583
Lower Limit		0.077	0.133
Upper Limit		1.791	2.137
Weeks to First Observed Tumor	100	79	77
Testis: Interstitial-cell Tumor (b)	41/49 (84)	44/49 (90)	43/48 (90)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.073	1.071
Lower Limit		0.903	0.899
Upper Limit		1.242	1.241
Weeks to First Observed Tumor	76	72	71

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Malathion in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Ear Canal: Squamous-cell Carcinoma (b)	1/49 (2)	0/50 (0)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	3.000
Lower Limit		0.000	0.251
Upper Limit		18.285	154.197
Weeks to First Observed Tumor	73	--	62

(a) Dosed groups received 2,000 or 4,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Malathion in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Undifferentiated Leukemia (b)	10/50 (20)	5/50 (10)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.500	0.600
Lower Limit		0.144	0.194
Upper Limit		1.482	1.676
Weeks to First Observed Tumor	75	80	97
Pituitary: Carcinoma, NOS (b)	9/48 (19)	5/50 (10)	4/46 (9)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.533	0.464
Lower Limit		0.151	0.111
Upper Limit		1.638	1.534
Weeks to First Observed Tumor	86	79	100

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Malathion in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS or Adenoma, NOS (b)	35/48 (73)	39/50 (78)	28/46 (61)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.070	0.835
Lower Limit		0.836	0.617
Upper Limit		1.359	1.142
Weeks to First Observed Tumor	65	67	77
Thyroid: C-cell Adenoma (b)	3/46 (7)	2/49 (4)	5/49 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.626	1.565
Lower Limit		0.054	0.324
Upper Limit		5.220	9.581
Weeks to First Observed Tumor	106	105	73

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Malathion in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma, NOS (b)	2/50 (4)	3/50 (6)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.500	0.500
Lower Limit		0.180	0.009
Upper Limit		17.329	9.290
Weeks to First Observed Tumor	95	102	92
Mammary Gland: Fibroadenoma (b)	7/50 (14)	5/50 (10)	9/50 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.714	1.286
Lower Limit		0.191	0.463
Upper Limit		2.434	3.749
Weeks to First Observed Tumor	82	102	93

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Malathion in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	5/49 (10)	6/49 (12)	4/44 (9)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.891
Lower Limit		0.327	0.188
Upper Limit		4.654	3.871
Weeks to First Observed Tumor	102	105	90

(a) Dosed groups received 2,000 or 4,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

IV. DISCUSSION

Mean body weights of the low- and high-dose male rats were lower than those of the corresponding controls in a dose-related manner after about 50 weeks on study. Mean body weights of female rats were essentially unaffected by the test chemical throughout the bioassay. Female rats may have been able to tolerate a higher dose. Mortality was dose related in male rats but was unaffected in females. Survival was 80% or greater at week 78 on study in the dosed and control groups of rats of each sex. Sufficient numbers of rats were at risk in all groups for the development of late-appearing tumors.

No tumors occurred in the dosed groups of rats of either sex at incidences that could be clearly related to administration of the test chemical. The incidence of pheochromocytomas of the adrenal in the low-dose male F344 rats was not supported by the incidence in the high-dose group or by a dose-related trend.

In previous studies, albino Carworth Farms rats or rats of unspecified strain were administered doses of 100, 1,000, 5,000, or 20,000 ppm of malathion in 2-year feeding studies (Hazleton and Holland, 1953; NIOSH, 1976). In the male or female Carworth Farms

rats fed 5,000 ppm, food intake was reduced; in the males fed 5,000 ppm, growth was retarded; and all males fed 20,000 ppm died within 20 days. No lesions were reported, however, from gross and microscopic tissue examination. The 5,000-ppm dose approximates the high dose used in the present bioassay. General toxicological examination of CFY rats administered malathion at 38 or 75 mg/kg body weight for 90 days was reported to show no significant changes in liver, kidney, or body weight (Desi et al., 1976).

Evidence of possible association of administration of malathion with promotion of tumors was reported by Okey (1972). In these studies, female Sprague-Dawley rats given single doses of 15 mg of dimethylbenzanthracene by gavage had mammary tumors at a higher incidence with shorter induction time when the animals were fed a diet containing 250 ppm of malathion (1.31 $\pm$ 0.18 tumors/rat; 20 days to first tumor) than when they were fed a control diet (1.07 $\pm$ 0.15 tumors/rat; 25 days to first tumor).

A carcinogenesis bioassay of malathion using Osborne-Mendel rats and a bioassay of malaoxon using F344 rats have been conducted at the same laboratory as the present bioassay (National Cancer Institute, 1978, 1979). No clear evidence was obtained to associate the occurrence of tumors at any site with the administration of the time-weighted average concentrations of 4,700 and 8,150 ppm of

malathion or with the administration of 500 and 1,000 ppm of malaoxon in the diet. The results obtained in the present bioassay using F344 rats are, therefore, consistent with those previously obtained using Osborne-Mendel rats and with those for malaoxon using F344 rats. However, gastric nonneoplastic lesions were found in F344 rats administered malathion or malaoxon but were not detected in Osborne-Mendel rats administered malathion.

Under the conditions of this bioassay, malathion was not carcinogenic for F344 rats of either sex; however, the females may not have received a maximum tolerated dose.

V. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Ayers, J. H. and Johnson, O. H., Insecticides. In: Chemical Economics Handbook, Stanford Research Institute, Menlo Park, Calif., 1976, sec. 573.3007G-H.

Baker, E. L., Jr., Warren, M., Zack, M., Dobbin, R. D., Miles, J. W., Miller, S., Alderman, L., Teeters, W. R., Epidemic malathion poisoning in Pakistan malaria workers. Lancet 1(8054): 31-34, 1978.

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel of Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Burchfield, H. F. and Johnson, D. E., Guide to The Analysis of Pesticide Residues, Vol. II, U. S. Department of Health, Education, and Welfare, Washington D.C., 1965.

Code of Federal Regulations, 40 CFR 180.111, 1977.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B34:187-220, 1972.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp. 48-52.

Desi, I., Dura, G., Gonczi, L., Kneffel, Z., Strohmayer, A., and Szabo, Z., Toxicity of malathion to mammals, aquatic organisms and tissue culture cells. Arch. Environ. Contem. Toxicol. 3(4): 410-425, 1976.

Environmental Protection Agency, Initial Scientific and Mini-economic Review of Malathion, U.S. Environmental Protection Agency, Office of Pesticide Programs, Criteria and Evaluation Division, Washington, D. C., 1975, pp iii, 17, 33, 35-36, 49, 83-84, 87, 91-92, 97-98 and 104.

Eto, M., Organophosphorus Pesticides: Organic and Biological Chemistry, CRC Press, Inc., Cleveland, Ohio, 1974, pp. 162-163, 196-201, 254-255.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification., Rev. Int. Stat. Inst. 39:148-196, 1971.

Harvey, S. C., Antiseptics and disinfectants; fungicides; ectoparasiticides. In: The Pharmacological Basis of Therapeutics, Goodman, L. S. and Gilman, A., eds., Macmillan Publishing Co., Inc., New York, 1975, pp. 987 and 1015.

Hayes, W. J., Jr., Mattson, A. M., Short, J. G., and Witter, R. T., Safety of malathion dusting powder for louse control. Bull Wld. Htlh. Org. 22:503-514, 1960.

Hazleton, L. W. and Holland, E. G., Toxicity of malathion. AMA Arch. Ind. Hyg. Occup. Med. 8:399-405, 1953.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53:457-481, 1958.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Mendoza, C. E., Toxicity and effects of malathion on esterases of suckling albino rats. Toxicol. Appl. Pharmacol. 35(2):229-238, 1976.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Murphy, S. D., Pesticides. In: Toxicology - The Basic Science of Poisons, Casarett, L. J. and Doull, J., eds., Macmillan Publishing Co., Inc., New York, 1975, p. 421-422.

National Cancer Institute, Bioassay of Malathion for Possible Carcinogenicity, Technical Report No. 24, DHEW Publication No. (NIH) 78-824, U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, Md., 1978.

National Cancer Institute, Bioassay of Malaoxon for Possible Carcinogenicity, Technical Report No. 135, DHEW Publication No. (NIH) 79-1390, U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, Md., 1979.

NIOSH, Occupational Exposure to Malathion, HEW Publication No. (NIOSH) 76-2055, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1976. Citing work of: Clyne, R. M. and Shaffer, C.B., Toxicological Information -- Cyanamid Organophosphate Pesticides, Edition 3, American Cyanamid Co., Princeton, N.J.

Norton, T. R., Metabolism of toxic substances. In: Toxicology - The Basic Science of Poisons, Casarett, L. J. and Doull, J., eds., Macmillan Publishing Co., Inc. New York, 1975, pp. 107-108.

Okey, A. B., Dimethylbenzanthracene - induced mammary tumors in rats: inhibition by DDT. Life Sciences 11:833-843, 1972.

Paschal, D. C. and Neville, M. E., Chemical and microbial degradation of malathion in an Illinois soil. J. Environ. Qual. 5 (4):441-443, 1976.

Sunshine, J., editor, CRC Handbook of Analytical Toxicology, The Chemical Rubber Co., Cleveland, Ohio, 1969.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62:679-682, 1975.

World Health Organization, Division of Malaria and Parasitic Diseases, Manual on Larval Control Operations in Malaria Programmes, 1973.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED MALATHION IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED MALATHION IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
SQUAMOUS CELL PAPILLOMA			1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	1 (2%)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(49)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
#LUNG	(49)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(49)
UNDIFFERENTIATED LEUKEMIA	9 (18%)	7 (14%)	2 (4%)
#SPLEEN	(49)	(49)	(49)
HEMANGIOSARCOMA		2 (4%)	
#LIVER	(49)	(50)	(49)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIP	(49)	(50)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#SALIVARY GLAND	(49)	(48)	(46)
MYOEPITHELIOMA		1 (2%)	
#LIVER	(49)	(50)	(49)
HEPATOCELLULAR CARCINOMA		2 (4%)	
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(49)
TUBULAR-CELL ADENOMA		1 (2%)	
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
#URINARY BLADDER	(46)	(43)	(48)
TRANSITIONAL-CELL PAPILLOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(40)	(45)
CARCINOMA,NOS	6 (14%)	2 (5%)	
ADENOMA, NOS	10 (23%)	10 (25%)	9 (20%)
CRANIOPHARYNGIOMA		1 (3%)	
#ADRENAL	(49)	(48)	(49)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA	2 (4%)	11 (23%)	6 (12%)
#ADRENAL MEDULLA	(49)	(48)	(49)
NEUROBLASTOMA			1 (2%)
#THYROID	(47)	(46)	(44)
PAPILLARY CARCINOMA	1 (2%)		
PAPILLARY ADENOMA	1 (2%)	1 (2%)	
C-CELL ADENOMA	3 (6%)	2 (4%)	
#PANCREATIC ISLETS	(49)	(48)	(48)
ISLET-CELL ADENOMA	7 (14%)	3 (6%)	4 (8%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(49)
ADENOCARCINOMA, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
*PREPUTIAL GLAND	(49)	(50)	(49)
CARCINOMA,NOS			1 (2%)
ADENOMA, NOS		1 (2%)	
#TESTIS	(49)	(49)	(48)
INTERSTITIAL-CELL TUMOR	41 (84%)	44 (90%)	43 (90%)
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(48)
GRANULAR-CELL TUMOR, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
*EAR CANAL	(49)	(50)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		3 (6%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERICARDIUM	(49)	(50)	(49)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
ALL OTHER SYSTEMS			
CONNECTIVE TISSUE			
FIBROMA	2		
FIBROSARCOMA			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	3	1
MORIBUND SACRIFICE	19	31	46
SCHEDULED SACRIFICE	2	2	2
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	27	14	
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	48	45	48
TOTAL PRIMARY TUMORS	86	96	77
TOTAL ANIMALS WITH BENIGN TUMORS	45	45	47
TOTAL BENIGN TUMORS	66	77	66
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	16	10
TOTAL MALIGNANT TUMORS	19	18	11
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	1	
TOTAL UNCERTAIN TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED MALATHION IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS			1 (2%)
FIBROSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(49)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
UNDIFFERENTIATED LEUKEMIA	10 (20%)	5 (10%)	6 (12%)
#THYMUS	(1)		
THYMOMA, MALIGNANT	1 (100%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(48)
NEOPLASTIC NODULE	2 (4%)		1 (2%)
ANGIOMA			1 (2%)
#COLON	(44)	(47)	(43)
ADENOCA IN ADENOMATOUS POLYP	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#URINARY BLADDER	(50)	(47)	(46)
TRANSITIONAL-CELL CARCINOMA	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(50)	(46)
CARCINOMA,NOS	9 (19%)	5 (10%)	4 (9%)
ADENOMA, NOS	26 (54%)	34 (68%)	24 (52%)
#ADRENAL	(49)	(49)	(49)
PHEOCHROMOCYTOMA		2 (4%)	2 (4%)
#THYROID	(46)	(49)	(49)
PAPILLARY ADENOMA			2 (4%)
C-CELL ADENOMA	3 (7%)	2 (4%)	5 (10%)
#PANCREATIC ISLETS	(49)	(50)	(48)
ISLET-CELL ADENOMA	2 (4%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	2 (4%)	3 (6%)	1 (2%)
FIBROADENOMA	7 (14%)	5 (10%)	9 (18%)
#UTERUS	(49)	(49)	(44)
SARCOMA, NOS			1 (2%)
ENDOMETRIAL STROMAL POLYP	5 (10%)	6 (12%)	4 (9%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)		
HEMANGIOSARCOMA		1 (2%)	
#OVARY	(47)	(50)	(46)
GRANULOSA-CELL TUMOR			1 (2%)
LIPOMA			2 (4%)
HEMANGIOSARCOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
CARCINOMA, NOS, INVASIVE		1 (2%)	

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EAR CANAL	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		2 (4%)
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
RHABDOMYOSARCOMA			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
ANGIOSARCOMA			1 (2%)
CONNECTIVE TISSUE			
FIBROSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@		5	4
MORIBUND SACRIFICE	16	12	19
SCHEDULED SACRIFICE	2	2	2
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	32	31	25
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	43	45	45
TOTAL PRIMARY TUMORS	73	65	71
TOTAL ANIMALS WITH BENIGN TUMORS	31	40	35
TOTAL BENIGN TUMORS	43	50	50
TOTAL ANIMALS WITH MALIGNANT TUMORS	25	15	18
TOTAL MALIGNANT TUMORS	28	15	19
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2		2
TOTAL UNCERTAIN TUMORS	2		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED MALATHION IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED MALATHION IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(49)
INFLAMMATION, NOS	2 (4%)	3 (6%)	1 (2%)
INFLAMMATION, GRANULOMATOUS			1 (2%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(49)	(49)
CONGESTION, NOS		3 (6%)	3 (6%)
SCAR	2 (4%)	1 (2%)	
ATROPHY, NOS	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
HEMATOPOIESIS		1 (2%)	
#MANDIBULAR L. NODE	(46)	(45)	(46)
CYST, NOS			1 (2%)
#RENAL LYMPH NODE	(46)	(45)	(46)
EOSINOPHILIC INFILTRATE	1 (2%)		
CIRCULATORY SYSTEM			
#HEART	(48)	(49)	(49)
BLOOD CLOT, POSTMORTEM		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#AURICULAR APPENDAGE	(48)	(49)	(49)
SCAR			1 (2%)
#LEFT VENTRICLE	(48)	(49)	(49)
SCAR	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(49)
METAMORPHOSIS FATTY	1 (2%)	3 (6%)	2 (4%)
FOCAL CELLULAR CHANGE	9 (18%)	6 (12%)	4 (8%)
#BILE DUCT	(49)	(50)	(49)
HYPERPLASIA, NOS	20 (41%)	7 (14%)	3 (6%)
#PANCREAS	(49)	(48)	(48)
PERIARTERITIS	2 (4%)		
ATROPHY, NOS	2 (4%)	1 (2%)	
#STOMACH	(49)	(46)	(47)
INFLAMMATION, CHRONIC	2 (4%)	6 (13%)	11 (23%)
ULCER, CHRONIC	1 (2%)	9 (20%)	15 (32%)
CALCIFICATION, NOS	2 (4%)		
HYPERPLASIA, NOS			2 (4%)
HYPERKERATOSIS		2 (4%)	
#GASTRIC MUCOSA	(49)	(46)	(47)
HYPERPLASIA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(49)
INFLAMMATION, CHRONIC	36 (75%)	42 (84%)	42 (86%)
#URINARY BLADDER	(46)	(43)	(48)
HEMORRHAGE			1 (2%)
METAPLASIA, SQUAMOUS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(40)	(45)
CYST, NOS	2 (5%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS		2 (5%)	1 (2%)
HYPERPLASIA, FOCAL	5 (11%)	1 (3%)	2 (4%)
#ADRENAL	(49)	(48)	(49)
LIPOIDOSIS			1 (2%)
#ADRENAL CORTEX	(49)	(48)	(49)
LIPOIDOSIS	1 (2%)		
HYPERPLASIA, NOS			1 (2%)
#ADRENAL MEDULLA	(49)	(48)	(49)
HYPERPLASIA, NOS	4 (8%)	3 (6%)	
#THYROID	(47)	(46)	(44)
HYPERPLASIA, C-CELL	7 (15%)	1 (2%)	1 (2%)
#PARATHYROID	(37)	(35)	(33)
HYPERPLASIA, NOS	4 (11%)	16 (46%)	2 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(49)
GALACTOCELE	1 (2%)		
ABSCESS, NOS		1 (2%)	
LACTATION	2 (4%)	2 (4%)	2 (4%)
#PROSTATE	(46)	(44)	(49)
INFLAMMATION, ACUTE	2 (4%)		2 (4%)
#TESTIS	(49)	(49)	(48)
ATROPHY, NOS	2 (4%)		2 (4%)
*EPIDIDYMIS	(49)	(50)	(49)
STEATITIS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(48)
HYDROCEPHALUS, NOS	2 (4%)	1 (2%)	
INFLAMMATION, NOS	1 (2%)	1 (2%)	
GLIOSIS	1 (2%)		
DEGENERATION, NOS		1 (2%)	
#CEREBELLUM	(49)	(50)	(48)
THROMBOSIS, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFARCT, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(49)	(50)	(49)
CATARACT		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(49)
ABSCESS, NOS		1 (2%)	
CALCIFICATION, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID		2 (4%)	
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY	1		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED MALATHION IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(49)
EMPHYSEMA, ALVEOLAR		1 (2%)	
HEMORRHAGE	1 (2%)	1 (2%)	
INFLAMMATION, NOS		1 (2%)	
GRANULOMA, NOS		1 (2%)	1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(49)	(48)
CONGESTION, NOS		1 (2%)	
INFARCT, NOS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
#MANDIBULAR L. NODE	(36)	(34)	(36)
HYPERPLASIA, NOS		1 (3%)	1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(48)
GRANULOMA, NOS	1 (2%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
METAMORPHOSIS FATTY		6 (12%)	9 (19%)
BASOPHILIC CYTO CHANGE	1 (2%)		
FOCAL CELLULAR CHANGE	7 (14%)	14 (28%)	17 (35%)
HEMATOPOIESIS		1 (2%)	
#BILE DUCT	(50)	(50)	(48)
HYPERPLASIA, NOS	4 (8%)	2 (4%)	2 (4%)
#PANCREAS	(49)	(50)	(48)
ATROPHY, NOS		2 (4%)	2 (4%)
#STOMACH	(50)	(44)	(47)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC		2 (5%)	4 (9%)
ULCER, CHRONIC	1 (2%)	2 (5%)	2 (4%)
CALCIFICATION, NOS		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERKERATOSIS		1 (2%)	
#COLON	(44)	(47)	(43)
NEMATODIASIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
HYDRONEPHROSIS	2 (4%)	1 (2%)	
CYST, NOS	1 (2%)		
INFLAMMATION, CHRONIC	16 (32%)	30 (61%)	31 (63%)
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(50)	(46)
CYST, NOS	2 (4%)		4 (9%)
CONGESTION, NOS	1 (2%)		1 (2%)
HEMORRHAGE			1 (2%)
HEMORRHAGIC CYST	2 (4%)		1 (2%)
HYPERPLASIA, NOS	1 (2%)	3 (6%)	
HYPERPLASIA, FOCAL	3 (6%)		2 (4%)
#ADRENAL	(49)	(49)	(49)
CYST, NOS	1 (2%)		
HEMORRHAGIC CYST	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
LIPOIDOSIS	1 (2%)	8 (16%)	1 (2%)
ANGIECTASIS	1 (2%)		
#ADRENAL CORTEX	(49)	(49)	(49)
LIPOIDOSIS	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		1 (2%)
#ADRENAL MEDULLA	(49)	(49)	(49)
HYPERPLASIA, NOS	2 (4%)		1 (2%)
#THYROID	(46)	(49)	(49)
HYPERPLASIA, C-CELL	7 (15%)	2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ABSCESS, NOS		1 (2%)	
HYPERPLASIA, NOS	1 (2%)		
METAPLASIA, SQUAMOUS	1 (2%)		
LACTATION		1 (2%)	
#UTERUS	(49)	(49)	(44)
STEATITIS			1 (2%)
#UTERUS/ENDOMETRIUM	(49)	(49)	(44)
INFLAMMATION, NOS			1 (2%)
#OVARY	(47)	(50)	(46)
STEATITIS	1 (2%)	2 (4%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
HYDROCEPHALUS, NOS	6 (12%)	6 (12%)	4 (8%)
INFLAMMATION, NOS	1 (2%)		
GRANULOMA, NOS	1 (2%)		
GLIOSIS	1 (2%)		
DEGENERATION, NOS			1 (2%)
*TRIGEMINAL GANGLION	(50)	(50)	(50)
ABSCESS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
STEATITIS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX C

ANALYSIS OF MALATHION

APPENDIX C

Analysis of Malathion

A. Elemental Analysis

Element:		C	H	P	S
Theory:		36.35	5.80	9.38	19.41
Found:	Batch 01	36.58	5.74	9.43	19.68
	Batch 02	36.19	5.65	9.43	19.37

B. Boiling Point

Literature: 157°C at 0.7 mm Hg (Sunshine, 1969).
Found: 156-157°C at 0.7 mm Hg.

C. Thin-Layer Chromatography

Plate used: Alumina coated
Visualization: Ultraviolet light
System: Hexane:Acetone (4:1)
Results: R_f 0.29, with trace at origin.

D. Vapor-Phase Chromatography

Instrument: Hewlett-Packard 7610
Detector: EC at 300°C
Column: 10% DC200 on Chromosorb W, 80/100 mesh, glass at 185°C
Inlet Temp: 250°C
Results: Single homogeneous peak with a retention time of 5.9 minutes.

E. Spectral Data

1. Infrared:	All batches gave infrared absorption spectra (batch 02, figure 3) that were consistent with the structure and with the spectrum reported in literature (Burchfield and Johnson, 1965).
2. Nuclear Magnetic Resonance:	All batches gave nuclear magnetic resonance absorption spectra (figure 4) that were consistent with the structure.

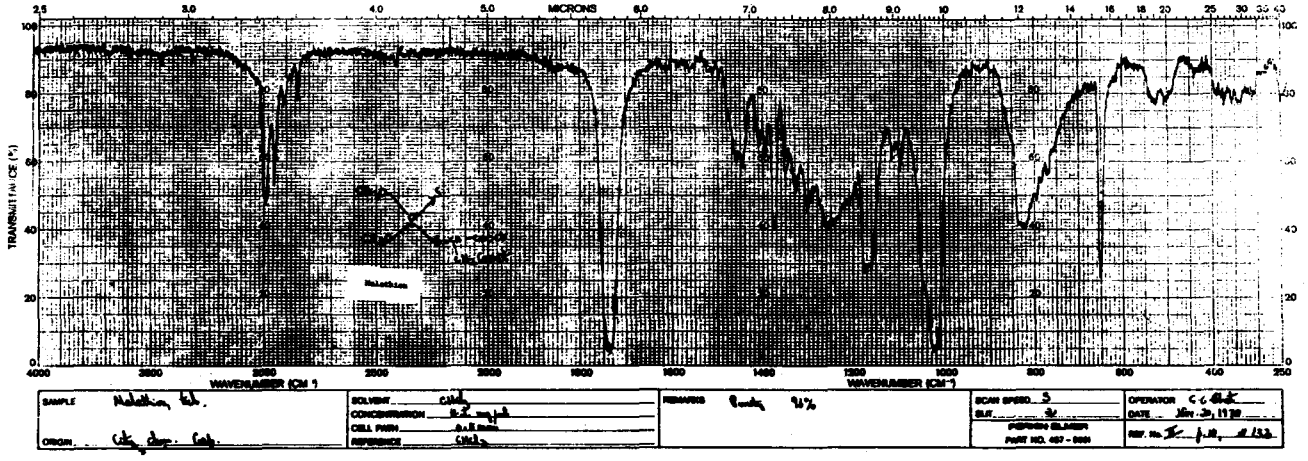


Figure 3. Infrared Absorption Spectrum of Technical-Grade Malathion

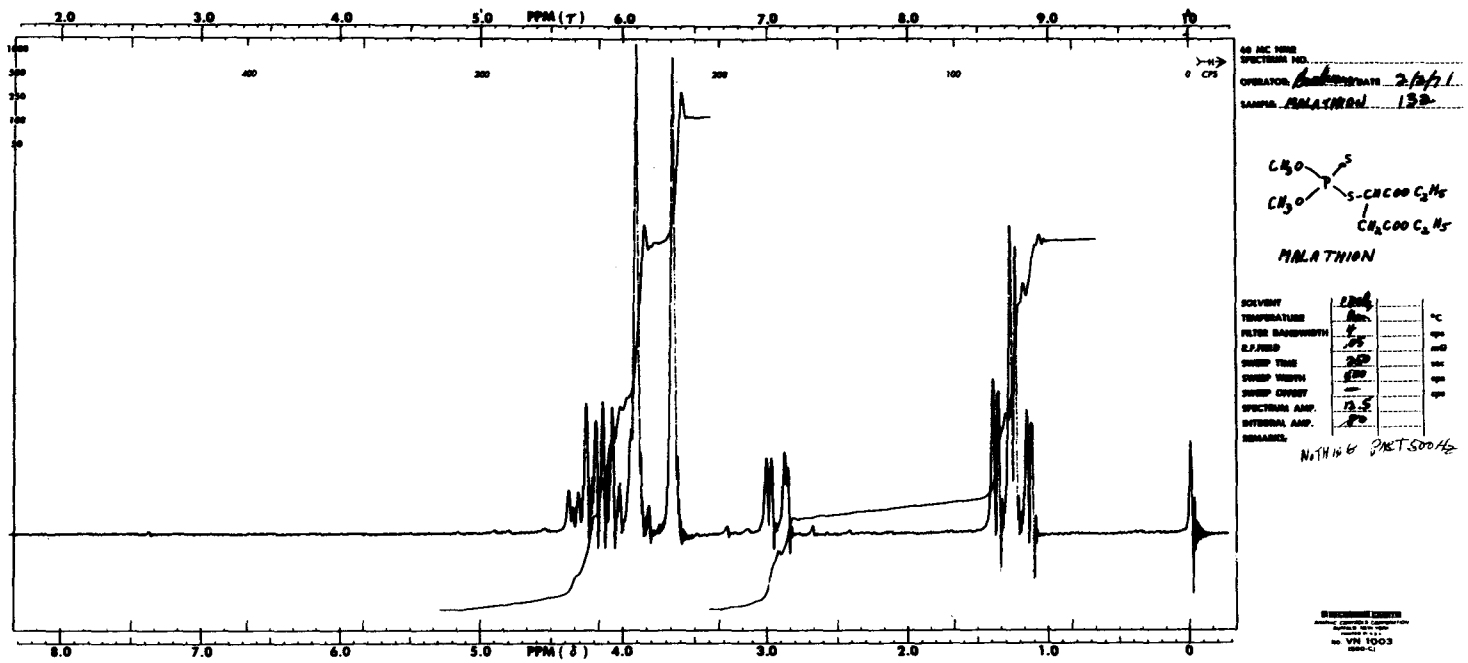


Figure 4. Nuclear Magnetic Resonance Spectrum of Malathion

APPENDIX D

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF MALATHION

Appendix D

Analysis of Formulated Diets for Concentrations of Malathion

A 10-g sample of the formulated diet was shaken with 250 ml of benzene at room temperature for 3 hours on a wrist action shaker. The feed was allowed to settle and a 1-mg aliquot of the benzene extract was removed and quantitatively analyzed for malathion by gas-liquid chromatography (electron capture detector, 10% DC-200 on Gas Chrom Q column at 165°C). Recoveries were checked with malathion-spiked samples carried through the workup and analysis, and external standards were used for calibration.

Theoretical Concentrations in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
2000	17	2032.8	4.3	1855-2190
4000	17	4055.3	4.1	3826-4435

Review of the Bioassay of Malathion* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

May 1, 1979

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute on the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Malathion.

The primary reviewer for the report on the bioassay of Malathion said that the compound was not carcinogenic in Fischer 344 rats, under the conditions of test. These findings confirmed a previous study in which Malathion was "negative" in Osborne-Mendel rats and B6C3F1 mice (Bioassay of Malathion for Possible Carcinogenicity, NCI Technical Report No. 24). After a brief description of the conditions of test, he noted that there was a decrease in the incidence of leukemias and pituitary tumors in treated male rats; possibly associated with weight loss. He also noted that Malathion was not mutagenic in eight tests (A Rational Evaluation of Pesticidal vs. Mutagenic/Carcinogenic Action, ed. R.W. Hart et. al., HEW/NIH 78-1306).

The secondary reviewer had nothing to add to the previous critique. He moved that the report on the bioassay of Malathion be accepted as written. The motion was seconded and approved unanimously.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
David B. Clayson, University of Nebraska Medical Center
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center

Sheldon Samuels, AFL-CIO
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or for other reasons. Thus, certain comments and criticisms reflected in the review may no longer be applicable.

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