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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID# 057701 - Malathion: Review of a Series 82-4 Subchronic (13-Week) Inhalation Study

Tox Chem No.:	535
MRID No:	432666-01
Submission No.:	S469811
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FROM: Brian Dementi, Ph.D., D.A.B.T. Brian Dementi 4/21/95
Review Section III
Toxicology Branch-I
Health Effects Division (7509C)

TO: Larry Schnaubelt, PM Team 72
Reregistration Branch
Special Review and Reregistration Division (7508W)

THRU: Karl Baetcke, Ph.D.
Chief, Toxicology Branch-I
Health Effects Division (7509C) Karl D. Baetcke 4/24/95

I. Conclusions

The series 82-4 Subchronic (13-Week) Inhalation Study (MRID No. 432666-01, Bio-Research Labs # 90729, March 16, 1994) was reviewed and determined to be CORE MINIMUM.

The study employed groups of 15 male and 15 female rats which were exposed in whole body exposure cambers to malathion aerosols at concentrations of 0, 0.1, 0.45 or 2.01 mg/L, 6 hr/day, 5 days/week for 13 weeks.

Clinical signs such as urogenital staining, excess salivation and ungroomed fur were seen mostly at 2.01 mg/L, but occurred sporadically also at 0.45 and 0.1 mg/L in both sexes, findings consistent with cholinesterase inhibition. A dose-related increase in cholesterol levels, ranging from 5% to 33%, was seen in both sexes.

This study is classified as CORE MINIMUM, because it was generally well conducted. It does not satisfy all Guideline requirements for a subchronic inhalation study (82-4) in rodents, because a NOEL was not established for microscopic lesions of the nose and larynx (both sexes), nor for RBC and plasma cholinesterase inhibition among females. TB1 is deferring to the HED RfD Committee the question of whether further testing will be required to determine a NOEL for the microscopic lesions, or whether an additional uncertainty factor (i.e., beyond 100) would satisfy in lieu of further study. The same issue, no NOEL for cholinesterase inhibition, is also being deferred to the RfD Committee. The question of carcinogenicity as it may relate to the microscopic lesions of the nose and larynx will be addressed in a separate memorandum.

DATA EVALUATION REPORT

MALATHION

Study Type: SUBCHRONIC INHALATION - RAT (82-4)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 94-31

Primary Reviewer:
Rosmarie A. Faust, Ph.D.

Signature: Rosmarie A. Faust
Date: 3/2/95

Secondary Reviewers:
Robert H. Ross, M.S., Group Leader

Signature: R H. R
Date: 3/2/95

Secondary Reviewers:
Carol S. Forsyth, Ph.D.

Signature: Carol S Forsyth
Date: 3/2/95

Quality Assurance:
Susan Chang, M.S.

Signature: S S Chang
Date: 3/2/95

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

[MALATHION] Subchronic Inhalation Study (82-4)

EPA Reviewer: B.A. Dementi, Ph.D.
Review Section III, Toxicology Branch I (7509C)
EPA Section Head: M. Copley, D.V.M., D.A.B.T.
Review Section IV, Toxicology Branch I (7509C)

Brian Dementi Date: 4/14/95

Myron Copley Date: 4/20/95

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Inhalation – Rat (82-4)

C11516

TOX. CHEM. NO: 535

P.C.CODE.: 057701

MRID NO.: 432666-01

TEST MATERIAL: Fyfanon technical (Malathion)

SYNONYMS: [(Dimethoxyphosphinothioyl)thio]butanedioic acid diethyl ester; mercaptosuccinic acid diethyl ester; S-ester with *0,0*-dimethyl phosphorothioate; S-(1,2-dicarbethoxyethyl) *0,0*-dimethyldithiophosphate; *0,0*-dimethyl dithiophosphate of diethyl mercaptosuccinate; insecticide No. 4049; carbofos; mercaptothion; phosphothion; ENT 17034; Cythion; Derbac-M; Malamar 50; Malaspray; Organoderm; Prioderm; Suleo-M

STUDY NUMBER: 90729

SPONSOR: Cheminova Agro A/S, Lemvig, Denmark

TESTING FACILITY: Bio-Research Laboratories Ltd., Product Safety Assessment, 87 Senneville Road, Senneville, Quebec H9X 3R3, Canada

TITLE OF REPORT: A 13-Week Toxicity Study of Aerosolized Malathion Administered by Whole Body Inhalation Exposure to the Albino Rat.

AUTHOR: G. Beattie

REPORT ISSUED: March 16, 1994 (Study completion date)

EXECUTIVE SUMMARY: In a subchronic inhalation study, groups of 15 male and 15 female Sprague-Dawley rats were exposed by inhalation in whole body exposure chambers to malathion aerosols at concentrations of 0, 0.1, 0.45, or 2.01 mg/L, 6 hr/day, 5 days/week for 13 weeks. The mass median aerodynamic diameters (MMAD) of the malathion particles were 1.6 μm at 0.1 mg/L and 1.7 μm at 0.45 and 2.01 mg/L.

Clinical signs such as urogenital staining, excess salivation, and ungroomed fur were seen mostly at 2.01 mg/L, but occurred sporadically also at 0.45 and 0.1 mg/L in both sexes. These clinical signs were consistent with decreases in cholinesterase activity. A dose-related increase in cholesterol levels, ranging from 5% to 33%, was seen in both sexes. Microscopic lesions of the nasal cavity and larynx, classified as slight to moderate, were observed in most animals

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of both sexes at all three exposure concentrations. **Based on microscopic lesions of the nose and larynx in both sexes, this study provided a LOEL of 0.1 mg/L; a NOEL could not be established.**

After 13 weeks, dose-related decreases in cholinesterase activity were seen in both sexes. The decreases attained statistical significance for plasma cholinesterase activity at 2.01 mg/L in females; for RBC cholinesterase activity at 0.45 and 2.01 mg/L in both sexes; and for brain cholinesterase activity at 2.01 mg/L in both sexes. Compared with controls, cholinesterase activity decreases at 0.1, 0.45, or 2.01 mg/L, respectively, were as follows: plasma, 2%, 7%, or 18% (males) and 16%, 30%, or 70% (females); RBC, 9%, 22%, or 43% (males) and 11%, 27%, or 44% (females); brain, 5%, 3%, or 17% (males) and 4%, 8%, or 41%, females). **Based on inhibition of cholinesterase activity in RBC and plasma in female rats (>10%), this study provided a LOEL of 0.1 mg/L; a NOEL for cholinesterase inhibition could not be established.**

This study is classified as **Core-Minimum**, because it was generally well conducted. It does not satisfy all guideline requirements for a subchronic inhalation study (82-4) in rodents, because a NOEL was not established for microscopic lesions of the nose and larynx (both sexes), nor for RBC and plasma cholinesterase inhibition among females. TB1 is deferring to the HED RfD Committee the question of whether further testing will be required to determine a NOEL for the microscopic lesions, or whether an additional uncertainty factor (i.e., beyond 100) would satisfy in lieu of further study. The same issue, no NOEL for Cholinesterase, is also being deferred to the RfD Committee. The question of carcinogenicity as it may relate to the microscopic lesions of the nose and larynx will be addressed in a separate memorandum.

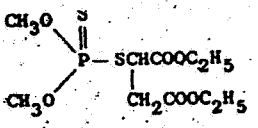
Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Fyfanon technical (Malathion)

C11516

Description: amber liquid
Lot/Batch #: 11029-01
Purity: 96.4 ± 0.3% a.i.
Stability of compound: stable for at least 2 years when stored at ambient temperature in darkness.
CAS #: 121-75-5
Structure:



2. Vehicle and/or positive control

Air; positive controls not employed

3. Test animals

Species: rat
Strain: Sprague-Dawley CD [Crl:CD®(SD)BR]
Age and weight at study initiation: 27-29 days; 177.5-224.0 g (males), 136.4-176.6 g (females)
Source: Charles River Canada, St. Constant, Quebec, Canada
Housing: individually in stainless-steel wire mesh-bottomed cages
Environmental conditions:
Temperature: 22 ± 3°C
Humidity: 50 ± 20%
Air changes: 12-15/hr
Photoperiod: 12 hr light/dark cycle
Acclimation period: 13 days

B. STUDY DESIGN

1. Animal assignment

Animals were assigned randomly to the test groups (15 animals/exposure group/sex) listed in Table 1.

TABLE 1. STUDY DESIGN[a]

Test Group	Nominal Concentration (mg/L)	Analytical Concentration (mg/L)	MMAD[a] (μm)	Rats/Sex
1 Control	0	0	NA	15
2 Low (LTD)	0.24	0.1	1.6	15
3 Mid (MTD)	1.10	0.45	1.7	15
4 High (HTD)	4.94	2.01	1.7	15

Data taken from p. 23, MRID No. 432666-01.
[a]MMAD = mass median aerodynamic diameter
NA = not applicable

Dose selection rationale – The exposure concentrations used in this study were based on the results of a 2-week range-finding study of malathion administered by whole-body inhalation exposure conducted at Bio-Research (Bio-Research Project No. 90557 – A Two-Week Toxicity Study of Aerosolized Malathion Administered by Whole-Body Inhalation Exposure in the Albino Rat.) Experimental protocol and results were not provided.

2. Generation of the test atmosphere and description of the chamber

The test atmospheres were generated by aerosolizing the test material with six-jet atomizers supplied with pre-dried compressed air at regulated pressure. To achieve the increasing test material concentrations, increasing numbers of jets and decreasing rates of dilution air flow were employed. The resultant aerosols were delivered to the chamber air inlets through stainless steel tubing designed to decrease the MMAD. At the chamber air inlet, the aerosols were mixed with filtered, conditioned air and were drawn into the exposure chamber. Time to equilibrium was 15 minutes.

Each group of animals (15 males and 15 females) was exposed in one of four whole body exposure chambers made of stainless steel and glass (internal volume, 650 L) and divided by wire mesh partitions. The chambers were operated under slight negative pressure to prevent outward leakage of the test atmosphere. Chamber airflow was monitored in the exhaust line as the differential pressure across an orifice plate by means of a precalibrated gauge. Chamber airflow, temperature, and relative humidity were recorded hourly during exposure. Temperature and relative humidity (Table 2) were generally in the acceptable range of 22 ± 2°C and 40-60%, respectively. The

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air flow through the inhalation chambers was set at levels which provided a minimum of 12 air changes per hour and an oxygen level of at least 19% prior to test material introduction.

The animals were subjected to whole-body exposure for 6 hr per day, 5 days per week for 13 weeks. Controls were handled identically, but were exposed to room air only. To compensate for local variations in chamber atmosphere concentrations, the animals' position within each chamber was rotated daily starting on day 18.

TABLE 2. CHAMBER TEMPERATURE AND RELATIVE HUMIDITY [a]

Test group	Temperature (°C)	Relative humidity (%)
Control	24±1.02	52.9±4.02
0.1 mg/L	23.8±0.68	54.5±6.05
0.45 mg/L	23.3±0.68	50.0±3.30
2.01 mg/L	22.5±0.73	51.3±3.56

Data taken from Table 5, pp. 68 and 71, MRID No. 432666-01
[a]Mean of daily means and standard deviation

Test atmosphere concentration – Test atmosphere concentrations are presented in Table 1. Test concentrations were determined by gravimetric analysis and confirmed by gas chromatographic analysis. Samples were collected hourly in each chamber using glass fiber filters situated at the animal breathing zone. Nominal chamber concentrations were determined in each chamber by dividing the amount of test material used by the generation system by the total volume of air drawn through the chamber during the exposure period. Analytical concentrations were determined by chemical analysis of the deposit on the gravimetric filters collected once during the pretreatment period and weekly during animal exposures. The concentrations of malathion determined by gas chromatographic analysis were 100.5%, 101.1%, or 101.3%, respectively, of the gravimetric concentrations for the low-, mid-, and high-exposure groups.

The homogeneity of the test material concentrations was determined prior to study initiation at each exposure concentration in duplicate across the horizontal plane of the animal level. The results of this evaluation were considered acceptable.

Particle size determination – The particle sizes (MMAD) of the test material are presented in Table 1. The aerosol particle size distribution was determined gravimetrically for each dose level before initiation of animal exposure and daily during exposure using an Anderson Cascade Impactor. A breakdown of the particle size distribution showed that only 15% of particles in the low-dose group and 10% of particles in the mid- and high-dose groups were smaller than 1 μm. The study author estimated that 25% of particles were 1.1 μm in the low-dose group and 1.2 μm in the mid- and high-dose groups. The Standard Evaluation Procedure (SEP) for Inhalation Toxicity Testing (1988) states that 25% of the particles should be less than 1 μm; therefore, this requirement was not met. However, 75% of particles were in the 1 to 3.2 μm range, an aerodynamic diameter that could deposit throughout the rodent respiratory tract (see Pesticide Regestristration Rejection Rate Analysis - Toxicology, 1993).

3. Diet

PMI Certified Rodent Chow No. 5002 (PMI Feeds, Inc.) and tap water (softened, purified, and sterilized) were available *ad libitum* except during exposures and prior to necropsy (food).

4. Statistics

The data were analyzed for homogeneity of variance using Bartlett's test. Homogeneous data were analyzed using Analysis of Variance and the significance of differences between the control and treated groups was assessed using Dunnett's t test. Heterogeneous data were analyzed using Kruskal-Wallis test and the significance of differences between the control and treated groups was assessed using Dunn's test.

5. Signed and dated GLP and quality assurance statements were present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality.

Results – No deaths occurred during the 13-week exposure period.

Clinical signs considered related to malathion exposure included red staining of the urogenital areas, ungroomed (oily) fur, and excessive salivation in males and females. Urogenital staining and ungroomed fur were seen in most animals exposed to 2.01 mg/L and in a few animals exposed to 0.45 mg/L or 0.1 mg/L (oily fur), but not in controls. Specific data for urogenital staining were not presented in the summary table (Table 6, MRID No. 432666-01) of recurring clinical signs. Excessive salivation after exposure was frequently seen at the highest concentration and occasionally at the mid and low concentration. Excess salivation, first noted during week 5 (males) and week 4 (females), occurred with greater frequency from week 6 on. Oily fur, seen primarily from week 5 to 11, was observed in both sexes exposed to 2.01 mg/L and in a few animals exposed to 0.45 mg/L.

In addition, there was a high incidence of recurring red staining of the muzzle and lower jaw that was generally concentration-related, and at lower frequency, red staining of the forelimbs and cranial, periorbital, cervical, and abdominal or ventral thoracic areas. Most of these effects were noted during the first week of the study and continued to the end of the treatment period. The study author noted that the staining effects are consistent with known effects of other organophosphate pesticides, but they are also commonly observed non-specific effects of exposure to high concentrations of aerosols. Therefore, these effects were not necessarily related to treatment with malathion.

2. Body weight

Animals were weighed on the day of randomization (week -1) and weekly during the 13-week exposure period. In addition, body weights were recorded for fasted animals prior to necropsy (week 14).

Results – Weekly mean body weights are presented in Table 3. There were no statistically significant decreases in body weight at any time during the study period in male or female rats. Compared with controls, slight decreases in body weights were seen in males, but the difference was less than 10%. For males, the overall net weight gain during the study was 96, 94, and 94% of controls for the low-, mid-, and high-dose groups, respectively. The weight gain in females was 97, 98, or 101% of controls for the respective dose groups. The decreased body weights in males were not considered related to exposure.

TABLE 3. GROUP MEAN BODY WEIGHTS (g) AT WEEKLY INTERVALS OF MALE AND FEMALE RATS EXPOSED TO MALATHION BY INHALATION FOR 13 WEEKS

Week of study	Treatment group/exposure level (mg/L)							
	Males				Females			
	0	0.10	0.45	2.01	0	0.10	0.45	2.01
-1	137.5	138.0	137.0	137.6	124.6	125.6	125.3	125.1
1	197.3	196.1	194.2	194.9	156.5	156.1	154.5	157.9
2	246.8	243.5	239.5	236.3	178.0	176.4	177.6	178.8
3	301.0	295.4	291.2	285.1	204.3	202.0	204.5	203
4	346.5	338.3	331.4	325.9	224.8	222.4	226.1	222.1
5	382.9	370.5	365.5	359.8	244.5	240.3	244.3	245.2
6	411.6	401.1	389.3	387.1	260.9	254.9	257.6	259.6
7	435.8	424.1	414.3	413.1	272.4	270.6	272.3	271.2
8	461.6	445.9	437.3	434.8	283.5	282.5	282.4	282.7
9	481.9	466.3	458.9	457.3	292.0	295.0	294.5	290.4
10	502.6	481.2	476.9	472.3	300.6	299.8	299.6	302.7
11	518.4	500.8	494.1	485.2	308.0	308.9	308.4	313.5
12	532.3	516.4	504.7	500.5	319.6	314.1	316.8	320.1
13	531.5	514.5	508.0	503.7	320.9	313.3	315.6	320.4
14	537.4	520.2	511.0	512.2	324.0	319.6	320.0	326.3
Body weight gain[a]	399.9	382.2 (96)	374.0 (94)	374.6 (94)	199.4	194.0 (97)	194.7 (98)	201.2 (101)

Data taken from Appendix 1, Table 7, p. 80, MRID No. 432666-01.
[a]Calculated by reviewer; numbers in parenthesis are percent of control weight gain.

3. Food consumption

Food consumption was measured weekly during the prestudy and exposure periods beginning on the day of randomization.

Results – Group mean food consumption for all exposure groups was comparable to control values. Food efficiency was not calculated.

4. Ophthalmoscopic examination

Eyes (dilated with 0.5% atropine sulfate) were examined by funduscopic and microscopic examination of the cornea, conjunctiva, sclera, iris, and fundus prior to exposure and during week 13 of exposure.

Results – No ocular lesions attributable to the test compound were noted.

5. Blood was collected from the abdominal aorta of rats for hematology and clinical chemistry analysis from all animals at study termination following an overnight fast. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothombin time)		

*Required for subchronic studies.

Results – Analysis of hematologic parameters did not provide evidence of any treatment-related effects after 13 weeks of exposure. At the high dose, the red cell distribution width (RDW) value in males was significantly ($p<0.01$) increased relative to the control group (13.7% vs 13.0% for controls). Although statistically significant, the increase in RDW was not considered biologically significant because of a lack of other supporting evidence. In high-dose females, the mean WBC count was significantly ($p<0.01$) increased (6.2×10^3 vs 4.6×10^3 for controls). The high WBC count in females was considered incidental because of an outlying value in one animal which was reflected in the group mean value.

b. Clinical chemistry

X	Electrolytes
X	Calcium*
X	Chloride*
	Magnesium*
X	Phosphorus*
X	Potassium*
X	Sodium*

X	Enzymes
X	Alkaline phosphatase (ALK)
X	Cholinesterase (ChE)†
	Creatinine phosphokinase
	Lactic acid dehydrogenase (LDH)*
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*
	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

X	Other
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total bilirubin
X	Total serum protein (TP)*
X	Triglycerides
	Serum protein electrophoresis

* Required for subchronic studies.
† RBC, plasma, and brain activity were measured. Cholinesterase activity was not evaluated for 2 low-dose (1 male and 1 female) and 2 high-dose (1 male and 1 female) animals, most likely as a result of hemolysis.

Results – Pertinent clinical chemistry values are summarized in Table 4. Compared with controls, marked dose-related decreases in plasma, red blood cell (RBC), and brain cholinesterase activity were observed. The decreases of plasma cholinesterase activity attained statistical significance ($p < 0.001$, Dunn's test) only in females at 2.01 mg/L. RBC cholinesterase activity was significantly decreased in males and females at 0.45 and 2.01 mg/L ($p < 0.05$ and $p < 0.01$, respectively, Dunnett's test). Brain cholinesterase activity was significantly ($p < 0.01$, Dunnett' test) decreased in males and females at 2.01 mg/L. There was a dose-related increase in cholesterol levels in both sexes that was statistically significant ($p < 0.01$) only at 2.01 mg/L. The effects on cholinesterase activity and cholesterol levels were considered treatment-related effects. Also seen at 2.01 mg/L was a statistically significant increase of albumin concentrations in males ($p < 0.01$; 3.2 g/dL vs 3.0 g/dL for controls), a statistically significant increase in A/G ratio in females ($p < 0.05$; 0.93 vs 0.98), and minor increases of glucose in females. Deviations from control values for these parameters were not considered toxicologically significant.

TABLE 4. SELECTED CLINICAL CHEMISTRY VALUES IN MALE AND FEMALE RATS EXPOSED TO MALATHION BY INHALATION FOR 13 WEEKS

Parameter	Exposure concentration (mg/L)			
	0	0.1	0.45	2.01
Males				
Cholinesterase				
Plasma (U/L)	404.7±64.97	396.4±116.46 (-2%)[a]	375.5±74.46 (-7%)	330.8±44.55 (-18%)
RBC (U/L-RBC)	2037.2±322.12	1856.4±468.84 (-9%)	1585.7±486.95* (-22%)	1167.4±445.53** (-43%)
Brain (U/g)	8.31±0.810	7.93±0.886 (-5%)	8.09±0.853 (-3%)	6.90±0.939** (-17%)
Cholesterol (mg/dL)	50.9±11.76	53.3±13.99 (+5%)	54.2±15.60 (+6%)	67.6±15.74** (+33%)
Females				
Cholinesterase				
Plasma (U/L)	2979.4±1191.58	2492.2±832.82 (-16%)	2099.1±608.19 (-30%)	906.6±227.95*** (-70%)
RBC (U/L-RBC)	1517.7±473.46	1357.1±490.39 (-11%)	1115.2±332.90* (-27%)	846.8±372.70** (-44%)
Brain (U/g)	8.26±0.857	7.89±0.926 (-4%)	7.61±1.182 (-8%)	4.85±1.306** (-41%)
Cholesterol (mg/dL)	72.1±12.51	76.4±18.82 (+6%)	81.7±18.03 (+13%)	94.8±15.53** (+31%)

Data taken from Table 10, pp. 103, 105, 107, and 109, MRID No. 432666-01.
[a] Numbers in parenthesis are percent less than or greater than control values (calculated by reviewer).
* $p<0.05$ (Dunnett's test)
** $p<0.01$ (Dunnett's test)
*** $p<0.001$ (Dunn's test)

6. Urinalysis*

An overnight sample of urine was collected from fasted animals during week 12 of exposure. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Nitrate (nitrite)
X	Protein	X	Urobilinogen

* Not required for subchronic studies.

Results – Changes in urinalysis parameters were seen primarily at the highest concentration and included decreased pH values (6.0 to 6.5 compared with 7.0 in most controls) and increased incidence of amorphous urate crystals in females (none observed in controls), and increased incidences of white blood cells and epithelial cells in males compared with controls.

7. Sacrifice and pathology

All animals were sacrificed on schedule by exsanguination from the abdominal aorta following intraperitoneal injection of sodium pentobarbital and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination and the (XX) organs, in addition, were weighed.

X	Digestive system
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall Bladder*
X	Pancreas*
	Respiratory
X	Trachea*
XX	Lung*
X	Nose
X	Pharynx
X	Larynx

X	Cardiovasc./Hemat.
X	Aorta*
XX	Heart*
X	Bone marrow*
X	Lymph nodes*
XX	Spleen
XX	Thymus*
	Urogenital
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
X	Epididymides
XX	Prostate
X	Seminal vesicle
XX	Ovaries*+
XX	Uterus*

X	Neurologic
XX	Brain*+
X	Periph. nerve*
X	Spinal cord (3 levels)*
XX	Pituitary*
X	Eye (optic n.)*
	Glandular
XX	Adrenal gland*
	Lacrimal gland
X	Mammary gland*
XX	Parathyroids*
XX	Thyroids*
	Other
X	Bone*
X	Skeletal muscle*
X	Skin*
X	All gross lesions and masses*

* Required for subchronic and chronic studies.
+ Organ weight required in subchronic and chronic studies.

Results -

a. Organ weight - Compared with controls, animals exposed to 2.01 mg/L malathion exhibited a few statistically significant increases in absolute and/or relative organ weights (Table 5). Increased absolute liver weights (14% and 15% for males and females respectively, $p<0.05$); increased relative liver weights (relative to body and brain weight) in both sexes (males, 20% and 16%, respectively, $p<0.01$; females, 14%, $p<0.01$, and 13%, $p<0.05$, respectively); increased absolute kidney weights in females (9%, $p<0.05$); increased relative kidney weights in males (12% relative to body weight, $p<0.01$; 8% relative to brain weight, $p<0.05$); increased relative lung weights in males (9% relative to body weight, $p<0.01$); an increased absolute lung weights in females (8%, $p<0.05$) were considered treatment-related effects. Although not statistically significant, increases in absolute and relative liver and kidney weights were also seen at 0.45 mg/L, suggesting a dose response. Organ weights of animals exposed to 0.1 mg/L were comparable to controls. In the absence of associated histopathological changes, the effects on the liver, kidney, and lung weights at the highest concentration were of uncertain toxicological significance.

TABLE 5. SELECTED ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE AND FEMALE RATS EXPOSED TO MALATHION BY INHALATION FOR 13 WEEKS

Organ	Exposure concentration (mg/L)			
	0	0.1	0.45	2.01
Males				
Liver	13.059±1.7098[a] 2.597±0.1757[b] 594.467±71.3389[c]	12.672±1.5173 2.591±0.1260 588.527±67.6175	13.092±1.6133 2.729±0.1529 603.541±66.6917	14.848±2.1415* 3.114±0.1928** 687.233±88.7943**
Kidney	3.265±0.2934 0.653±0.0571 148.853±13.6009	3.193±0.2118 0.684±0.0535 148.226±7.5486	3.262±0.2671 0.648±0.0477 150.511±11.2824	3.477±0.4017 0.734±0.0718** 160.917±15.2568*
Lung[d]	1.703±0.1021 0.341±0.0216 77.599±4.2909	1.706±0.1349 0.355±0.0207 79.437±5.7200	1.659±0.1279 0.348±0.0227 76.545±5.3217	1.751±0.1600 0.370±0.0370** 81.113±6.6020
Females				
Liver	7.538±1.4877 2.552±0.1902 381.468±64.8294	7.659±0.9806 2.623±0.1905 387.565±44.3490	8.061±0.7915 2.732±0.2027* 409.020±43.0374	8.679±0.6579* 2.909±0.1637** 432.297±36.2165*
Kidney	1.982±0.2393 0.680±0.0755 100.657±11.5067	1.992±0.1535 0.687±0.0689 100.846±6.4241	2.082±0.1226 0.708±0.0566 105.648±7.3375	2.166±0.1197* 0.729±0.0677 107.834±6.5405
Lung	1.290±0.1414 0.442±0.0430 65.455±6.5377	1.260±0.0901 0.434±0.0395 63.853±4.7853	1.284±0.0900 0.436±0.0342 65.048±4.1173	1.390±0.0823* 0.468±0.0417 69.205±4.4773

Data taken from Tables 11, 12, and 13, pp. 110-127, MRID No. 432666-01.
[a] Absolute organ weight (g)
[b] Relative to body weight (g%)
[c] Relative to brain weight (g%)
[d] Lungs/trachea
* $p<0.05$ (Dunnett's test)
** $p<0.01$ (Dunnett's test)

b. Gross pathology – No treatment-related gross lesions were observed in either male or female rats.

c. Microscopic pathology –

1) Non-neoplastic – With the exception of lesions in the nasal cavity and larynx in both sexes, significant increases of non-neoplastic lesions were not observed in animals of any of the treatment groups. A high incidence of degeneration and/or hyperplasia of the olfactory epithelium of the nasal cavity and hyperplasia of the larynx was seen in all groups exposed to malathion (Table 6). The severity of nasal and laryngeal lesions, rated as slight to moderate, also increased with exposure concentration. Locally extensive, the nasal lesions in the olfactory epithelium affected primarily the nasal septum and dorsal-caudal epithelium. The olfactory-respiratory epithelial junction was severely affected in most animals. The nasal lesions were characterized by an increased basophilia (due to increased numbers of nuclei), disorganization of the nuclear pattern, increased chromatin density in some cells, with occasional individual cells showing nuclear degeneration. The luminal cytoplasmic border of the epithelium was replaced by nuclei and the epithelium appeared thicker in some areas, particularly in level three sections. Microcysts and fine vacuolar changes were seen at the base of the epithelium, and cytoplasmic eosinophilia was observed in a few cells. The lesions in the larynx were characterized as epithelial hyperplasia, with squamous keratinization occurring in some rats.

2) Neoplastic – There was no evidence of neoplastic lesions in any of the treated or control animals.

TABLE 6. INCIDENCE OF LESIONS OF THE NASAL CAVITY AND LARYNX IN MALE AND FEMALE RATS EXPOSED TO MALATHION BY INHALATION FOR 13 WEEKS[a]

Organ/Lesion	Exposure concentration (mg/L)			
	0	0.1	0.45	2.01
Males				
Nasal cavity, olfactory epithelium/ degeneration and/or hyperplasia	0	13	15	15
Larynx/epithelial hyperplasia	1	15	15	14
Females				
Nasal cavity, olfactory epithelium/ degeneration and/or hyperplasia	0	15	15	15
Larynx/epithelial hyperplasia	1	10	15	14

Data taken from Table 15, pp. 130 and 132, MRID No. 432666-01.
[a] 15 animals/sex/group were examined.

D. DISCUSSION

Male and female Sprague-Dawley rats were exposed by inhalation in whole-body chambers to malathion aerosols at concentrations up to 2.01 mg/L (MMADs ranged from 1.6 to 1.7 μm.) The concentrations for the 13-week study were selected from a 2-week range-finding study with rats conducted in same laboratory. However, details of this study were not provided.

Clinical observations revealed high incidences of red staining of the urogenital areas, ungroomed (oily) fur, and salivation following exposure at 2.01 mg/L, and lower incidences at 0.45 and 0.1 mg/L. These clinical effects were consistent with neurotoxicity from inhibition of cholinesterase. Although the magnitude of the observed cholinesterase inhibition at the low and mid concentrations is not clearly an adverse effect, plasma and RBC cholinesterase levels decreased in a dose-dependent manner in both males and females. At 2.01 mg/L, brain cholinesterase activity was 81% and 59% of control values for males and females, respectively. The authors concluded that the NOEL is 0.1 mg/L for RBC and 0.45 mg/L for brain and plasma cholinesterase inhibition. However, the reduction of plasma and RBC cholinesterase activity at all exposure levels in females was greater than the 10% cut off recommended in guidelines whereas in males the reduction of cholinesterase activity (plasma, RBC, and brain) was less than 10% at 0.1 mg/L. Based on these findings, the reviewer considers 0.1 mg/L, the lowest concentration tested, as the LOEL for cholinesterase inhibition.

Dose-related increases in cholesterol levels, statistically significant ($p < 0.01$) only at 2.01 mg/L (33% for males and 31% for females compared with controls), were seen in both sexes. However, the magnitude of the increases were not biologically significant. Although increased liver weights (absolute and relative to body and brain weight) seen in both sexes at 2.01 mg/L, there were no histopathologic correlates in the liver indicative of a treatment-related hepatic effect. Also observed were increased absolute and relative kidney weights (dose-related in females), increased relative lung weights in males, and increased absolute lung weights in females. No toxicological significance can be attached to the organ weight changes because pathological lesions were not seen in the corresponding organs.

None of the changes in urinalysis parameters appear to be effects of malathion exposure. Precipitation of urate crystals in females is probably a function of higher urine acidity and is not biologically significant. WBC and nitrate levels in urine of males did not correlate with the number of bacteria found, indicating that the urine sat for a considerable time at room temperature before analysis.

Inhalation of malathion produced a high incidence of lesions in the nasal and laryngeal mucosa, most likely due to the regional deposition of the test material. The lesions were graded as slight to moderate in severity, but were seen at all three exposure concentrations. Therefore, 0.1 mg/L can be considered the LOEL for nasal and laryngeal lesions.

Based on the findings of this study, it does not appear that a NOEL was identified. The LOEL is 0.1 mg/L for both cholinesterase inhibition and nasal/laryngeal lesions; the NOEL is < 0.1 mg/kg.

E. STUDY DEFICIENCIES

The study did not provide a NOEL because the lowest concentration tested produced biologically significant effects.

Homogeneity of the test atmosphere was determined and found adequate; however, data were not provided.

Details of the 2-week range-finding study were not given.

Magnesium, lactic acid dehydrogenase, and creatinine phosphokinase were not measured. The first two parameters are omissions according to 82-4 guidelines.

Although urogenital staining was considered a treatment-related effect, the incidence was not provided in the summary table of recurring clinical signs (Table 6, MRID No. 432666-01).