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Malathion: 2-Week (Range-finding) Inhalation Study in Sprague-Dawley Rats (MRID 44554301)

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DATA EVALUATION RECORD

Study Type: 2-Week (Range-Finding) Inhalation Study in Sprague-Dawley Rats

DP Barcode: D246738

Submission No.: S544067

PC Code: 05 7701

Tox. Chem. No.: 535

Case: 81 8961

Test Material (Purity): Malathion (96.4% a.i.)

Synonyms: Fyfanon technical: O,O-Dimethyl-S-[1,2-di(ethoxycarbonyl)-ethyl]
phosphorodithioate

Citation: Beattie, G. (1993) "A 2-Week Toxicity Study of Aerosolized Malathion Administered by Whole-body Inhalation Exposure to the Albino Rat." Product Safety Assessment, Bio-Research Laboratories Ltd., 87 Senneville Road, Senneville, Quebec H9X 3R3 Canada and Product Stability and Characterization, Cheminova Agro A/S, DK-7620 Lemvig, Denmark. Laboratory Project ID 90557. Study completed July 20, 1993 MRID No. 44554301.

Sponsor: Cheminova Agro A/S, Lemvig, Denmark.

Executive Summary: This 2-week range-finding study completed in July 1993 was conducted in pursuit of dose (concentration) selection for the required Guideline Subchronic Inhalation study in the rat. The concentrations of malathion technical (96.4% a.i.) in air employed in the study were 0 (air), 0.5, 1.5 and 4.5 mg/L. In this brief study there is evidence of considerable attention to GLP principles and FIFRA Guideline testing requirements. The parameters evaluated - clinical signs, body weight, food consumption, complete clinical chemistry including cholinesterase inhibition (plasma, erythrocyte, brain), hematology, urinalysis, organ weights, macro- and microscopic pathology - attest to an exceptional and well-performed study for a range-finding study. It satisfies many Guideline testing requirements, a chief drawback with respect to which being the few animals (5/sex/group) employed as compared to the minimum (10/sex/group) in Guideline testing.

Principal findings include nasal and laryngeal effects at all doses. In the nasal cavity "loss of goblet cells and/or cilia, respiratory epithelium" was reported for all male and female rats in all dose groups. "Hyperplasia of the respiratory epithelium was identified in 4/5 males and 3/5

females in Group 2 and in all animals of both sexes in groups 3 and 4.

In the larynx 3/5, 4/5 and 5/5 male rats, respectively, in Groups 2, 3 and 4 and all female rats in all dose groups exhibited epithelial hyperplasia. The nasal and laryngeal effects were not observed in controls. There were no other remarkable histopathologic findings. It should be noted that in the two animals sacrificed early, i. e., one group 4 male and one group 3 female, sacrificed on days 10 and 9, respectively, the nasal and laryngeal effects were evident.

Male rats exhibited a slight, dosing related decrease in body weight gain at all doses, an effect seen in females only at the highest dose level.

Males consumed less food, in a dosing-related manner across all doses, while in females there was a slight reduction only in the high dose group.

Evidence of cholinesterase inhibition was seen in all doses in both sexes for erythrocyte cholinesterase. Plasma cholinesterase was inhibited in females in all doses and in males at the mid and high dose levels. Brain cholinesterase was clearly inhibited at the highest dose in both sexes and possibly so in females at all doses. It was clear that the enzyme in at least one of its forms was inhibited at all doses in both sexes. There were some cholinergic clinical signs of toxicity in males at all dose levels and in females at the mid and high dose level.

Based on organ weight changes, possible target organs were liver (both sexes) at the top two doses and kidney (males) at possibly all doses. More data would be needed to confirm these and certain other findings, notably those of spleen and thymus among females.

The principal findings in this study were the early onset of nasal and laryngeal epithelial effects that signal the need to determine the time course and dose relatedness of these effects. There was no NOEL for the effects after only 2 weeks of treatment. There was also no NOEL for cholinesterase inhibition. The question of these NOELs was not settled in the subchronic inhibition study that followed this study.

This 2-week inhalation study in rats is classified as **Acceptable (non-Guideline)**. It does not satisfy the Guideline requirement for a subchronic inhalation study (82-4) because it was conducted as a rangefinding study for purposes of dose selection for the conduct of the full subchronic inhalation Guideline study.

Compliance: Signed and dated GLP, Quality Assurance and Data Non-confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Malathion
Description: Liquid (amber)
Test Substance C.A.S Number: 121-75-5
Batch No.: 1102901
Purity: 96.4%
Stability of compound: 2 years at least when stored at ambient temperature (<25 degrees C) in darkness.
2. Vehicle: None. The test material was atomized in air.
3. Test Animals: Species: Rat.
Strain: Sprague-Dawley
Age and Weight at Study Initiation: age not provided at time of study;
weight: males: 205.1-230.8 grams; females: 153.5-180.0 grams.
Source: Charles River Canada, St. Constant, Quebec, Canada.
Housing: individually following randomization.
Diet: PMI Certified Rodent Chow No. 5002; PMI Foods, Inc., available ad libitum.
Water: Softened, purified by reverse osmosis and stabilized by U.V. light, available ad libitum.
Environmental condition: 12-15 air changes/hour; 22± 3 degrees C temperature; 50±20% humidity; and 12-hour light/dark light cycle.
Acclimation period: 2 weeks.

B. STUDY DESIGN:

1. Animal Assignment

“One week before treatment commenced, all rats were weighed and assigned to treatment groups using a computer-based randomization procedure which ensured homogeneity of group means and variances for body weight. Males and females were randomized separately. Five males and 5 females were assigned to each group. Rats in poor health or at the extremes of the body weight range were not assigned to study groups.

Animals were randomized into the following groups:

Group/ Identification	Targeted Aerosol Conc. (Mg/L)*	Animal Numbers	
		Males	
1 Air Control	-	1001- 1005	1501-1505
2 Malathion, Low dose	0.5	2001- 2005	2501-2505
3 Malathion, Intermediate dose	1.5	3001- 3005	3501-3505
4 Malathion, High dose	4.5	4001- 4005	4501-4505
* Based on the maximum practical aerosol concentrations which could be achieved while maintaining particle size distributions which approached those recommended in the Hazard Evaluation Division Standard Evaluation Procedure - Inhalation Toxicity Testing by the U.S. EPA (August, 1988) (pp. 13-14 of Study Report)			

2. Test Material Preparation:

The following are selected passages as quoted from pages 15-17 of the Study Report.

“Following selection of the aerosol generation/delivery system and target aerosol concentrations, atmospheres approximating each of the target levels were established and were characterized by gravimetric, chemical and particle size analysis. In addition, the homogeneity of chamber atmosphere distribution was evaluated at each dose level, in duplicate, across the horizontal plane of the animal level prior to the start of treatment. The results of these evaluations were considered acceptable.

“The test atmospheres were generated by aerosolizing the test article with six-jet atomizers (source: TSI Inc., St. Paul, MN, U.S.A.) supplied with pre-dried compressed air at regulated pressure. To achieve the increasing test article 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 configured to decrease the mass median aerodynamic diameter. At the chamber air inlet, the aerosols were mixed with filtered, conditioned air and were drawn into the exposure chambers.

"The animals were subjected to whole-body exposure for 6 hours per day, 5 days a week, for two weeks. Group 1 animals were handled and restrained identically to animals exposed to the test article but, when housed within the inhalation chamber, were exposed to room air only.

"During the treatment period, the animals of each group were loaded into the compartmentalized cage within the chamber, and the generation system turned on. The test atmospheres were continuously generated for 6 hours - a 15-minute equilibration period (calculated to exceed the t_{25} of the system - the point in time at which approximately 95% of the desired concentration has been established within the chamber) followed by 5 hours and 45 minutes of continuous operation. The system was then turned off and the animals removed from the chamber 15 minutes later.

"Analytical chamber concentrations were determined by chemical analysis of the deposit on the gravimetric filters collected once during the pretreatment period and twice weekly during animal exposure.

"The mass median aerodynamic diameter and geometric standard deviation (MMAD \pm GSD) and the aerodynamic particle sizes corresponding to 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90% of mass collected are presented in the Study Report.

"Airflow through the inhalation chamber was set at levels which provided a minimum of 12 air changes per hour." (pp. 15-17 of Study Report)

Results: mean gravimetric concentrations of Malathion were 0.56, 1.58 and 4.23 mg/L, respectively, for groups of 2, 3 and 4. Gravimetric results were confirmed by chemical analysis during the first week of exposure. As disclosed in Table 4 of the Study Report (pp. 36-38), the MMAD was 1.7 μ m for both groups 2 and 3, and was 1.9 μ m for group 4. Twenty-five percent of the particles in groups 2 and 3 were estimated to be smaller than 1.2 μ m and in group 4 was estimated to be less than 1.3 μ m. The Study Report advises that "Although the atmospheres generated during this study did not contain 25% submicron particles by mass, the mean mass median aerodynamic

diameters were 1.7 um for Groups 2 and 3 and 1.9 um for Group 4 and fell within the 1-4 um range recommended by the Inhalation Specialty Section of the Society of Toxicology." (reference provided, p. 22).

3. Statistics:

"Numerical data obtained during the conduct of the study were subjected to calculation of group mean values and standard deviations." (P. 20 of Study Report)

C. METHODS:

1. Experimental Procedures: As indicated 5 male and 5 female rats/group were exposed via the inhalation route to Malathion aerosols in air, control being treated in like manner without Malathion present in the air.
2. Body Weight: Measured on day of randomization and twice weekly during the treatment period.
3. Food Consumption: Measured weekly.
4. Laboratory Investigations: (The following passages are quoted with slight modification from pp. 18-19 of the Study Report.)

"Hematological and biochemical analyses were performed on all surviving animals at study termination. Blood samples were collected from the abdominal aorta of all animals at necropsy following an overnight fast.

Urine samples were collected during the second week of exposure from individual animals placed in metabolism cages overnight, during which time they were deprived of food.

a. Clinical chemistry:

	ELECTROLYTES		OTHER
x	Calcium	x	Albumin
x	Chloride	x	Blood creatinine
	Magnesium	x	Blood urea nitrogen

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x	Phosphorus	x	Total cholesterol
x	Potassium	x	Globulin (calculated)
x	Sodium	x	Glucose
	ENZYMES		Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)
x	Cholinesterase (ChE)	x	Triglycerides
	Creatine phosphokinase		Serum protein
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (SGPT)		
x	Serum aspartate amino-transferase (SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

b. Hematology:

x	Hematocrit (HCT)	x	Leukocyte differential count
x	Hemoglobin (HGB)	x	Mean corpuscular HGT (MCH)

x	Leukocyte count (WBC)		Mean corpusc. HGB conc. (MCHC)
x	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
x	Platelet count	x	Reticulocyte count
x	Mean platelet volume (MPV)	x	Blood cell morphology
x	Blood clotting measurements (Partial thromboplastin time) (Clotting time) (Prothrombin time)		

c. Urinalysis

x	color and appearance	x	bile pigment (bilirubin)
x	pH	x	urobilinogen
x	glucose	x	protein
x	ketones	x	nitrite
x	blood (hemoglobin)	x	microscopy of
x	volume	x	centrifuged deposit
x	specific gravity		

5. Sacrifice and Pathology:

Animals were sacrificed by exsanguination under pentobarbital anesthesia. All animals had been fasted overnight before sacrifice, and terminal body weight was recorded at the time of necropsy. The exsanguinated animals were necropsied and assessed by gross pathology. The weights of adrenals, brain, heart, kidneys,

liver, lungs with trachea, ovaries/testes, pituitary (after fixation), prostate, spleen, thymus, thyroid lobes and parathyroids (weighed together) and uterus were measured and recorded. The following tissues were fixed in neutral buffered 10% formalin: heart, kidneys, larynx, liver (sample of 2 lobes), lungs (all lobes), and nasal cavities and sinuses (3 levels - decalcified prior to sectioning). Additional tissues were taken at the request of the supervising pathologist to elucidate abnormal findings.

Tissues were prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin. Histopathological examination was performed on all fixed tissues identified above from all animals in all groups.

II. RESULTS

A. OBSERVATIONS:

Two animals were sacrificed during the exposure period due to the presence of excessive signs of cholinesterase inhibition (tumors, respiratory distress and urogenital staining). The animals in question were a high dose (Group 4) male on day 9 and an intermediate dose (Group 3) female on day 10. The symptoms were attributed to effects of the test material.

Based upon inspection of Table 6 (pp. 42-45 of the Study Report), principal clinical signs of concern included recurring muzzle, lower jaw and ventral thoracic and cervical staining. Red staining of the muzzle and lower jaw occurred in all dose groups while ventral thoracic and cervical staining were limited to Groups 3 and 4. In the case of muzzle and lower jaw staining, the phenomenon generally occurred with high incidence in Groups 3 and 4, but was expressed among 3 of the 5 rats of both sexes in Group 2 several times. The Study Report (p. 23) acknowledges such symptoms as being consistent with known effects of organophosphate exposure, but are also common non-specific effects. The concern here is that muzzle and lower jaw staining may be correlates of an adverse effect on nasal tissue due to malathion, and that if so, it occurred early in the study, following the first day of treatment. So an important question is whether malathion exerted an early effect on nasal tissues in this study.

Other clinical signs in this study included excessive salivation (occasionally observed in Group 2, 3 and 4 males and Groups 3 and 4 females) and as claimed in the text (p. 23), though not shown in table 6. "ungroomed fur (observed in all Group 4 animals and with a dose relationship in females)."

B. BODY WEIGHT:

Inspection of table 7 in the study report, supports the conclusions that male rats exhibited a slight, but dosing related decrease in body weight gain at all doses, an effect seen in females only at the highest dose level.

C. FOOD CONSUMPTION:

Inspection of Table 8 showing mean food consumption over the first eight of the 12 day study period indicates that males consumed less food, in a dosing-related manner across all doses, while in females there was a slight reduction only in Group 4. Hence, there was no NOEL for males.

D. CLINICAL CHEMISTRY, HEMATOLOGY AND URINALYSIS:

1. Clinical Chemistry: Inspection of Table 10 discloses the following findings among the parameters listed as evaluated. There were no other remarkable effects. Blood urea nitrogen may have been slightly increased in males at all doses and decreased in females of Group 4. Cholesterol was increased in Group 4 males in possibly all dose group females. Cholinesterases were inhibited: cholinesterase inhibition (% of control) (Groups 2 through 4, respectively) Plasma: males: 93, 80, 50; females: 51, 29, 16. Erythrocyte: males: 82, 67, 42; females: 74, 61, 47. Brain: males; no inhibition, 96, 64; females; 88, 82, 41. While not statistically analyzed in this range finding study the data indicate that among males, erythrocyte cholinesterase was inhibited in all dose groups, plasma cholinesterase was inhibited in Groups 3 and 4 and brain cholinesterase was inhibited only in group 4. In females, on the other hand, all three forms of the enzymes may have been inhibited at all doses, though brain cholinesterase may not have been inhibited in Groups 2 or 3, but the dose response supports an effect at all doses. Five animals per group is too few to make a definitive conclusion.
2. Hematology: Inspection of Table 9 of the study report indicates no remarkable effects on the parameters assessed except: white count may have been increased in Group 3 and 4 males and more clearly so in all female dose groups; segmented neutrophils were decreased in males across all doses and increased (variably) in females across all dosing; % eosinophils were evident in Group 3 and 4 males and in all dose groups of females. It is uncertain how definitive these findings may be or what the toxicologic implications, if any, may be.
3. Urinalysis: Inspection of data in Appendix 6 (pp. 123-126) confirms lower pH in males and females (particularly males) and higher ketone concentrations in males.

in groups 3 and 4. Also, higher incidences of urate crystals, white blood all counts and a lower incidence of phosphate crystals were observed in Groups 3 and 4.

E. SACRIFICE AND PATHOLOGY:

1. Organ Weights: Inspection of Tables 11-13 which provide absolute, relative to body weight and relative to brain weight organ weight data, supports the following general assessment. Among males and females, liver weight was increased by all modes of expression in Groups 3 and 4. Also, in males, kidney weight was increased in Group 4 (absolute weight) and Groups 2, 3 and 4 (body weight and brain weight bases). Among females, spleen weight was decreased in Groups 3 and 4, both on absolute and body weight bases and in Group 4 on brain weight basis. Also in females, thymus weight tended to be down in Groups 3 and 4 on all bases of expression. In short, organ weight data indicate that target organs may be liver (both sexes), kidney (males), spleen (females) and thymus (females).
2. Gross Pathology: Inspection of Table 14 (pp. 80-81) of the study report does not reveal any remarkable effects in animals of either sex in any dose group.
3. Microscopic Pathology: Inspection of Table 15 (pp. 82 - 85 of the Study Report) discloses in the nasal cavity a "loss of goblet cells and/or cilia, respiratory epithelium" in the case of all male and female rats in all dose groups. Hyperplasia of the respiratory epithelium was identified in 4/5 males and 3/5 females in Group 2 and in all animals of both sexes in Groups 3 and 4.

In the larynx 3/5, 4/5 and 5/5 male rats, respectively, in Groups 2, 3 and 4 and all female rats in all dose groups exhibited epithelial hyperplasia. The nasal and laryngeal effects were not observed in controls. There were no other remarkable histopathologic findings. It should be noted that in the two animals sacrificed early, i.e., one group 4 male and one group 3 female, sacrificed on days 10 and 9 respectively, the nasal and laryngeal effects were evident.

F. DISCUSSION:

In this brief (2-week) range-finding study there is evidence of considerable attention to the GLP principles for subchronic studies.. The study is thorough and of exceptional quality for a range-finding study. Excepting the few number of animals, i.e., 5/sex/group, as contrasted with a minimum of 10/sex/group in Guideline subchronic studies, the study largely conforms to Guideline testing requirements, though not satisfying any Guideline testing requirements.

Two animals were sacrificed early in the study, i.e., at 9 and 10 days, short of the 12 day testing period goal. These animals were exhibiting cholinergic signs. Evidence of cholinesterase inhibition was seen in all doses in both sexes for erythrocyte cholinesterase. Plasma cholinesterase was inhibited in females in all doses and in males at the mid and high dose levels. Brain cholinesterase was clearly inhibited at the highest dose in both sexes and possibly so in females at all doses. It was clear that the enzyme in at least one of its forms was inhibited at all doses in both sexes. There were some cholinergic clinical signs of toxicity in males at all dose levels and in females at the mid and high dose level. There were no other clear dosing-related clinical or hematologic findings. Urine pH was decreased in rats of both sexes in the mid and high dose groups. Based on organ weight changes, possible target organs were liver (both sexes) at the top two doses and kidney (males), possibly at all doses. More data would be needed to compare low dose effects. Among females, spleen and thymus may be target organs at the top two doses.

The most notable histopathologic findings were nasal and laryngeal hyperplasia in all dose groups, virtually all animals, of both sexes. There was no NOEL for these findings after only 2 weeks of testing. Such effects were not seen in control rats.

Malathion

2-Week Range-Findings
Inhalation (82-4)

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