074299 ? CTOX (Liver).

001882

3/31/1982

EPA Reg. Nos.: 524-68, 524-128, 524-144

005095

DATA EVALUATION RECORD

1. CHEMICAL: Methyl Parathion (MP)

2. FORMULATION: Technical MP

3. CITATION: Three Month Feeding Study In Rats (Information in support of the registration of Methyl Parathion), a report submitted by Monsanto Agricultural Products Company, prepared by Bio/Dynamics, Inc., 1981.

4. REVIEWED BY: Gerald M. Marquardt, Ph.D. Signature: CFC. for G Marquardt
Pharmacologist, EPA
Date: 3/5 2

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Section Head, EPA
Date: 3/5/82

6. TOPIC: This study has information pertinent to Discipline Toxicology, topic: Subchronic Oral Toxicity. This study relates to the Proposed Guidelines data requirement 163.82-1.

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7. CONCLUSION: Sprague-Dawley CD rats (20 animals of each sex/treatment group) were administered 0.0, 2.5, 25.0, or 75.0 ppm MP in the diet for 3 months. Hematology, clinical chemistry, and urinalysis evaluations were conducted on one-half of these animals at one and three months; cholinesterase (ChE) determinations were conducted at 1, 2, and 3 months. Fourteen female rats and 1 male rat receiving 75.0 ppm MP died within the first four weeks of treatment. All female and some male animals receiving 75.0 ppm MP exhibited tremors, emaciation, and staining of the anal-genital area. The mean body weights of high-dose animals were significantly reduced during all weeks of treatment. Food consumption was generally greater for the high-dose animals during weeks 4-13 of this study. High-dose females had lower mean RBC counts and hemoglobin levels at 3 months and lower hematocrits at 1 and 3 months. Hemoglobin levels of the mid- and high-dose animals at 1 month, hemoglobin levels of the high-dose males at 3 months, and the hematocrits of high-dose male animals at 1 month were lower than control.

Serum glutamic oxaloacetic transaminase (SGOT) in high-dose females (at 1 month), serum alkaline phosphatase (SAP) in high-dose animals (at 1 and 3 months), SAP in mid-dose females (at 1 month), and blood urea nitrogen (BUN) in high-dose females (at 1 and 3 months) were greater than the values observed in control animals. The plasma levels of

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glucose and total protein, albumin (A) and globulin (G) were lower in high-dose females (at 1 and 3 months); similar decreases in the total protein and G levels and in the glucose levels were observed in high-dose male animals (at 1 and 3 months, respectively).

RBC ChE levels were depressed in the low-, mid-, and high-dose males at 1 month, in the mid- and high-dose males at 2 and 3 months and in the mid- and high-dose females at 1, 2, and 3 months. Although the reductions in the RBC ChE levels in mid- and high-dose animals at 3 months were not statistically significant, they did appear to be dose-related. Plasma ChE levels were decreased in mid- and high-dose males (at 2 and 3 months) and in mid- and high-dose females (at 1, 2, and 3 months). Brain ChE levels were decreased in mid- and high-dose females and high-dose males at 3 months.

The specific gravity of the urine was elevated in high-dose animals (at 1 and 3 months) and in mid-dose females (at 3 months). The greater urinary specific gravity in high-dose males (at 1 and 3 months) was associated with positive urinary protein determinations (100 ug/dl were greater in most of these animals).

Organ weights were reduced in high-dose animals; organ weights in female animals were generally decreased to a greater extent than were the organ weights in male animals. Organ/body weight ratios were generally increased due to decreased body weights.

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Some of the high-dose animals had gross lesions in the stomach. The latter lesions concentrated on the nonglandular mucosa, consisted of discolored areas/foci, raised white areas, and abrasions. A few of these rats had "black/brown tar-like gastric contents"; postmortem examination of these animals revealed microscopic evidence of acute ulcerative gastritis, lymphoid depletion and necrosis of the submaxillary glands and hypocellularity of the bone marrow. The latter changes were considered to be directly related to the administration of MP or secondary to stress (induced by the ingestion of MP).

CORE CLASSIFICATION: Subchronic Oral Toxicity

8. MATERIALS AND METHODS:

Test Substance: MP (93.65% A.I.) was mixed with animal feed and fed to the test animals for 3 months.

Organism: Sprague-Dawley CD rats (20 animals/sex/treatment group; 42 days of age; approximately 150-200g) were fed 0.0, 2.5, 25.0, or 75.0 ppm MP for 3 months.

Experimental Procedure: Food consumption (recorded weekly during testing); body weight (recorded weekly starting 1 week prior to testing); general observation for mortality and gross signs of toxicologic or pharmacologic effects (recorded twice daily during testing); physical examination for signs of local or systemic toxicity, pharmacologic effects, and palpitation

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for tissue masses (recorded weekly during testing); hemoanalysis* (performed/recorded before and at 1 month, and 3 months); ChE determinations** (performed/recorded before testing and at 1, 2, and 3 months); urinalyses*** (performed/recorded at months 1 and 3); and postmortem examination **** (performed on all surviving animals at the end of 3 months) were performed/reported at the appropriate time.

* Blood was obtained via venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anesthesia. Rats were fasted overnight prior to blood collection. Samples (0.5 ml) were analyzed for hemoglobin, hematocrit, RBC's, platelets, total and differential leukocytes, SGOT levels, glutamic pyrubic transaminase, SAP, BUN, fasting blood glucose, total protein, A, G, A/G ratio, cholesterol, potassium, calcium, total bilirubin, and lactic dehydrogenase analyzed by standard methods.

** Aliquots of the blood samples for hemoanalysis were used to determine the Che activity in the plasma and RBC's by accepted procedures; brain samples (brain tissue from ten animals/sex/treatment group taken before testing, at 1 month, at 2 months, and at 3 months) were analyzed for ChE activity by standard methods.

*** Urinalyses (performed at months 1 and 3 on urine samples from 10 animals/sex/treatment group) involved the determination of specific gravity, pH, total protein, glucose, ketones, bilirubin, and urobilinogen as well as standard and microscopic analyses; all parameters were examined by established procedures.

**** The animals that died spontaneously and sacrified animals (exsanguination under ether anesthesia) were necropsied, various organs [brain (with entire brain stem), gonads, heart, kidneys, and liver] were removed, weighed, and preserved (in Bouin's Solution) and certain tissues [adrenals, bone marrow (sternum), brain (two sections), epididymis, esophagus, eyes (with optic nerves), Harderian glands, heart, intestinal sections (Cecum, colon, duodunum, ileum, and jejunum), kindeys, liver, lungs, lymph nodes (mesenteric mediastinal), pancreas, pituitary, prostate, salivary glands (mandibular), seminal vesicles, skeletal muscle (right bicep femoris), spleen, stomach, testes, thymus, thyroid/parathyroid glands, trachea, urinary bladder, uterus, tissue masses, and gross lesions] were fixed in Bouin's Solution, stained with Hematoxylin and Eosin, and examined for integrity [these tissues were also examined histopathologically for animals receiving either 0.0 or 75.0 ppm MP]; kidneys, liver, heart, and any tissues with gross alterations were examined histopathologically for all animals.

Statistical Analysis: The mean values, the range of individual values, and the standard deviations were calculated/reported by accepted procedures.

9. REPORTED RESULTS:

Gross Observations: High-dose females exhibited tremors during all weeks of feeding MP and became increasingly emaciated as the study progressed; 14 of these animals died spontaneously or were sacrificed in a moribund condition during the first four weeks of this study. Five high-dose males exhibited tremors during one or more weeks of MP administration; one of these animals was sacrificed in a moribund condition during the first four weeks of this study. Most high-dose females and some high-dose male animals exhibited staining of the anal-genital area.

Body Weight: Body weights of high-dose animals were significantly lower than the body weights of control animals during all weeks of MP administration (e.g., 186.0-284.0g = final body weight of animals receiving 75.0 ppm MP compared to 358.0-459.0g = final body weight of control animals).

Food Consumption: Food consumption for the high-dose males was comparable to that of controls during the first three weeks of this study while that of high-dose females was slightly-significantly lower than that of controls during this same period. Food consumption of the high-dose animals was significantly greater than that of controls during weeks 4-13.

Hematology: RBC counts at 3 months, hemoglobin levels at 3 months, and hematocrit values at 1 and 3 months in high-dose female animals were slightly (but not significantly) lower than control. Hemoglobin levels in the mid- and high-dose male animals (at 1 month) and in the high-dose male animals (at 3 months) were significantly lower than control. The hematocrit of high-dose males was significantly lower than control at 1 month, but not at 3 months. Platelet and leukocyte counts were not altered by MP treatment.

Clinical Chemistry: SGOT levels were slightly increased in high-dose females (at 1 month). SAP activities were increased in high-dose animals (at 1 and 3 months) and in mid-dose females (at 1 month).

BUN levels were increased and glucose, total protein, A, and G levels were decreased in the high-dose females (at 1 and 3 months).

Other changes in clinical chemistry parameters (some statistically significant) were not dose-related or consistent over time and were not, therefore, considered to be related to the administration of MP.

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ChE Determinations: RBC ChE activities were decreased in the low-, mid-, and high-dose males (at 1 month), in the mid- and high-dose males (at 2 and 3 months), and in the mid- and high-dose females (at 1, and 2, and 3 months). RBC ChE activities in the mid- and high-dose animals (at 1 month), in the mid- and high-dose males (at 2 and 3 months), and in the mid- and high dose females (at 1, 2, and 3 months) were lower than control. RBC ChE activities in the mid- and high-dose animals (at 3 months) were not statistically significant but were dose-related. See the table below for the actual RBC ChE activities.

Plasma ChE activities were decreased in the mid- and high-dose males (at 2 and 3 months) and in the mid- and high-dose females (at 1, 2, and 3 months). Actual values of plasma ChE activities for the various treatment groups are given in the table below.

Brain ChE activities were decreased in the mid- and high-dose females and in the high-dose males (see table below).

ChE Activity*

[MP]	Sex	Plasma^0	RBC^0	Brain^0	Plasma^1	RBC^1	Plasma^2	RBC^2	Plasma^3	RBC^3	Brain^3
0.0	M	1.4	3.9	2.2	1.1	2.8	1.5	2.7	1.3	2.0	12.1
2.5	M	2.5	-	-	1.3	2.7	1.4	2.5	1.2	1.9	13.3**
25.0	M	-	-	-	1.2	1.7**	1.3	1.6***	1.0	1.7	11.5
75.0	M	-	-	-	0.9**	2.6	1.1***	1.4***	1.0	1.6	3.1***
0.0	F	1.7	3.7	2.3	2.4	3.6	2.7	2.7	2.8	2.4	14.0
2.5	F	-	-	-	2.7	3.5	2.6	2.6	3.0	2.5	13.8
25.0	F	-	-	-	1.7***	2.4**	1.8**	1.7***	1.7***	2.1	9.5***
75.0	F	-	-	-	1.1***	3.4	1.1***	1.0***	0.9***	1.8	4.9***

* ChE activities are expressed in terms of um/ml/min (for plasma and RBC ChE activities) or um/g/min (for brain ChE activities) and represent the mean of 10 samples obtained before testing (0), at one month (1), at two months (2), or at three months (3).

** Statistically different from control ($p \leq 0.05$).

*** Statistically different from control ($p \leq 0.01$).

Urinalysis: The specific gravity of urine samples from high-dose animals (at 1 and 3 months) and mid-dose females (at 3 months) was slightly (but not significantly) elevated. The increased specific gravities of urine samples from high-dose males (at 1 and 3 months) were associated with urinary protein levels of 100 ug/dl or greater in most animals.

Organ and Body Weights: Body weights were reduced in high-dose males and females (17% and 27%, respectively, at 3 months) and organ weights were reduced in high-dose males and females (5-50% and 12-26%, respetively, at 3 months). These decreases in organ weights were accompanied by decreases in the organ/brain weight ratios. Organ/body weight ratios were generally increased due to reduced body weights.

Pathology: One and 20 male and female animals, respectively, died spontaneously or were sacrificed in a moribund condition during this study. A number of lesions were observed in the stomachs of high-dose animals. The lesions, concentrated on the nonglandular mucosa, consisted of discolored area/foci, raised white areas, and abrasions. Some of these rats had black/brown tar-like gastric contents. Postmortem examination of these animals revealed microscopic evidence of acute ulcerated gastritis, lymphoid depletion, necrosis (lymph nodes, spleen, thymus), necrosis of the submaxillary salivary glands and hypocellularity of the bone marrow.

10. DISCUSSION: Fourteen female and 1 male high-dose animals died spontaneously or were sacrificed in the moribund condition during the first four weeks of this study. These deaths appeared to be directly related to the ingestion of 75.0 ppm MP for 3 months. All high-dose females and some high-dose males exhibited tremors during this study. Many high-dose females and some high-dose males became increasingly emaciated as the study progressed and exhibited staining of the anal-genital area. The body weights of high-dose animals were significantly reduced during all weeks of MP administration.

Food consumption of high-dose females was lower than control during the first three weeks of this study; food consumption of high-dose animals was significantly greater than control during weeks 4-13.

RBC counts and hemoglobin levels were reduced in the high-dose females (at 3 months); hemoglobin levels in mid- and high-dose males (at 1 month), hemoglobin levels in high-dose males (at 3 months), and the hematocrit of high-dose males (at 1 month) were significantly reduced. Platelet and leukoycte counts were similar in all animals.

SGOT and SAP activities were increased in high-dose females (at 1 month), in high-dose animals (at 1 and 3 months) and in mid-dose females (at 1 month). BUN levels were increased in high-dose females (at 1 and 3 months); glucose, total protein, A and G levels were decreased in high-dose females (at 1 and 3 months). Total protein and G levels were decreased in the

high-dose males (at 1 month). Glucose levels similarly decreased in high-dose males (at 3 months). Other changes in clinical chemistry parameters (in high-dose males) (some of which were statistically significant) were not considered to be related to the administraiton of MP because these changes were not dose-related or they were not consistent over time.

RBC ChE levels decreased in the low-, mid-, and high-dose males (at 1 month), in the mid- and high-dose males (at 2 and 3 months), and in the mid- and high-dose females (at 1, 2, and 3 months). Although RBC ChE activities were not significantly reduced in mid- and high-dose animals (at 3 months), the reductions did appear to be dose-related. Plasma ChE levels were reduced in mid- and high-dose males (at 2 and 3 months) and in the high-dose femals (at 1, 2, and 3 months). Brain ChE levels were depressed in mid- and high-dose females (at 3 months) and in high-dose males (at 3 months).

The specific gravity of urine samples from high-dose animals (at 1 and 3 months) and mid-dose females (at 3 months) was greater than control; the observed increases in urinary specific gravity may be related to protein in the urine ($\leq$ 100 ug/dl) in high-dose males (at 1 and 3 months). Although the latter hypothesis is plausible, it must remain a speculation since no evidence is presented in this study to support this.

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Terminal body weights were reduced in high-dose males (17%) and high-dose females (27%). Organ weights were reduced 5-26% in high-dose animals; organ weights were reduced to a greater degree in female animals. These reductions in absolute organ weights were accompanied by decreases in organ/brain weight ratios. Organ/body weight ratios were elevated in treated animals (probably due to decreased body weights).

Fourteen females and 1 male animals, receiving 75.0 ppm MP, died spontaneously or were sacrificed in a moribund condition during this study. Some high-dose animals had stomach lesions (postmorten examination of these animals revealed microscopic evidence of acute ulcerated gastritis, lymphoid depletion, necrosis (lymph nodes, spleen, thymus), necrosis of the submaxillary salivary glands, and hypocellularity of the bone marrow. All of these effects were directly related to the administration of MP or secondary to stress induced by MP ingestion.

11. Score . CORE GUIDELINE