5/18/82

DRAFT 001882

MEMORANDUM

TO: Jay Ellenberger
PM #12
Registration Division (TS-767)

SUBJECT: Data Reviews - Methyl Parathion (524-68, 524-128, 524-144)

Requested action: Data reviews for

A) 90 day feeding study in dogs
B) 3 month feeding study in rats
C) 3 month feeding study in mice

Recommendation: All three studies are valid and can be used to support registration action on methyl parathion (MP). The NOEL's and LEL's are listed below under "conclusions".

Conclusions:

A) The Lowest Effect Level (LEL) for cholinesterase (ChE) depression in dogs fed methyl parathion (technical) for 90 days was the following: plasma and RBC ChE - 1 mg/kg/day; brain ChE-3 mg/kg/day. The NOEL was as follows: plasma and RBC ChE - 0.3 mg/kd/day; brain ChE - 1.0 mg/kg/day. Core Classification is Core Guidelines.

B) The LEL for rats fed MP (technical) for 3 months was 25 ppm (converts to 2.5 mg/kg/day by FDA guidelines). Effects observed were lowered hemoglobin levels, elevated serum alkaline phosphatase and urine specific gravity, and depressed RBC, brain, and plasma ChE. The NOEL was 2.5 ppm (converts to 0.25 mg/kg/day). Core Classification is Core Guidelines.

C) The LEL for mice fed MP (technical) for 3 months was 10 ppm (lowest dose tested , converts to 1.5 mkd). Decreased testes weight (with no accompanying abnormalities on histopathological examination) was observed at this dose level (10 ppm). Although depression of ChE activity may have been expected since MP is an organophosphate, ChE determinations were unfortunately not performed. Core Classification is Core minimum. These test results do not alter the existing ADI.

Christine F. Chaisson, Ph.D.
Toxicology Branch
Hazard Evaluation Division

C.F. Chaisson
5/20

TS-769:JBrantner:gjd: Rm. 816:557-3710: 5/18/82

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

DATA EVALUATION RECORD

1. CHEMICAL: Methyl Parathion (MP)

2. FORMULATION: Technical (MP)

3. CITATION: 3-Month Feeding Study with Methyl Parathion in Mice (Information in support of the registration of methyl parathion), a report submitted by Monsanto Agricultural Products Company, prepared by Bio/Dynamics Inc., 1980.

4. REVIEWED BY: Gerald M. Marquardt, Ph.D.
Pharmacologist, EPA
Signature GMarquardt
Date: 2/82

5. APPROVED BY: Christine F. Chaisson, Ph.D.
Section Head
Signature CFChaisson
Date: 3/82

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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.

7. CONCLUSION: Charles River CD-1 mice (15 animals/sex/treatment group) were fed diets containing 0, 10, 30, or 60 ppm MP for 3 months. All animals survived this treatment and there were no MP-related deaths. The body weight of high-dose animals was 4-20% lower than control and the body weight of high-dose males was 37% lower than control during the first five weeks of treatment; the body weights of all mid- and low-dose animals were comparable to control. Food consumption was not altered by the administration of MP and there were no physical observations in any of the animals. Slight increases (not statistically significant) were observed in the brain weights of all high-dose animals. These increases were accompanied by a 6% decrease in the terminal body weight for high-dose animals. The testes weights were decreased in all treated males; the ovary weights were decreased in mid and high-dose females. Histopathological examination of the brain, testes and the ovaries of all treated animals revealed no abnormalities.

No drug-related lesions were observed in any of the treated animals. Histopathological examination of tissues from treated animals revealed no compound-related effects.

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8. MATERIALS AND METHODS:

Organism: Charles River CD-1 mice (15 animals/sex/treatment group; 39 days of age approximately 18.5 g in weight) were fed diets containing 0, 10, 30, or 60 ppm MP for 3 months.

Test Substance: Technical (93.65% A.I.) was mixed with standard laboratory feed weekly for administration to test animals.

Housing: Animals were housed individually in elevated animal cages throughout this study.

Food and Water: Standard laboratory diet Purina Lab Chow was administered ad libitum throughout this study.

Experimental Procedure: Mice were examined twice daily for possible mortality and gross signs of pharmacologic or toxicologic effects; detailed physical examinations for signs of local or systemic toxicity, pharmacologic effects, and palpitations for tissue masses were performed weekly. Animals were weighed pre-test, weekly during treatment and terminally.

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After examination under ether anesthesia, various tissues [brain (with entire brain stem), heart, kidneys, liver, ovaries and testes] were excised, weighed and organ/body weight and organ/brain weight ratios were calculated. Certain tissues [adrenal glands, blood smears, bone and bone marrow (costachandral junctions), brain, esophagus, eyes (with optic nerve), gall gladder, heart, cecum, duodenum, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and mediastinal), ovaries, parathyroid gland, pituitary, prostate, salivary gland (mandibular), skeletal muscle (right bicep femoris), spleen, stomach, testes (with epididymus), thymus, thyroid, urinary bladder, uterus, gross lesions and tissue masses] were removed from some animals at random from the high-dose groups; animals from the low-dose and mid-dose groups were examined (if indicated by findings in the high-dose group), fixed in Bouin's Solution, stained with Hematoxylin and Eosin and examined microscopically.

Statistical Analysis: Body weights, food consumption, organ weights and organ/body weight and organ/brain weight ratios were analyzed by accepted procedures.

9. Results: All animals survived this experiment and there were no physical observations noted which were considered to be related to the administration of MP. The body weight of high-dose males was 4-20% lower than control during all weeks of this study; the body weight of mid-dose males was 3-7% lower than control during the first five weeks of treatment, but the body weight was comparable to control thereafter. The body weight of high-dose females was 4-11% lower than control during all weeks of treatment. Body weights of low and mid-dose males were 3-20% higher than control throughout this study.

Brain weights of high-dose animals were slightly increased by MP treatment; the testes weights of all treated males and the ovary weights of mid- and high-dose females were slightly decreased but there were no histopatological findings in the brains, testes, or ovaries of these animals which would account for these changes in organ weights.

No gross compound-related lesions were observed in any of the treated animals.

10. Discussion: There were no compound-related deaths or physical observations in any of the animals in this test.

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Body weights of high-dose animals were lower than control during this study; the body weights of low and mid-dose animals were quite variable but the mean values were comparable to control. The food consumption of low-dose males and all treated females were 3-20% higher than control; food consumption values were quite variable for all animals.

Slight increases (not statistically significant) in the brain weights and brain/body weight ratios were observed in all high-dose animals; the latter increases were at least partially accounted for by decreased body weights (e.g., the terminal body weight of high-dose animals was 6% lower than control). The testes weights and the ovary weights were decreased in all treated males and in mid-, high-dose females, respectively.

Histopathological examinations of the brain, testes, and the ovaries of all animals revealed no abnormalities.

There were no compound-related lesions in any of the animals; histopathological examinations of the testes from all male animals revealed no abnormalities. One serious fault with this study was the absence of ChE determinations in various tissues of the animals. The reason for this omission should have been stated.

11. Score - CORE Minimum

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

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: 3/82

5. APPROVED BY: Christine F. Chaisson, Ph.D. Signature: C.F. Chaisson
Section Head, EPA
Date: 3/82

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 hemogoblin 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

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.

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).

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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 micropscopic evidence of acute ulcerated gastritis, lymphoid depletion, necrosis (lymph nodes, spleen, thymus), necrosis of the submaxillary salivary glands and hypocellularity of the bone marrow.

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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

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

DATA EVALUATION RECORD

1. CHEMICAL: Methyl Parathion (MP)

2. FORMULATION: MP (94.32% A. I.)

3. CITATION: Feeding Study in Dogs (Information in support of the registration of Methyl Parathion). Submitted by Monsanto Agricultural Products Company, prepared by Pharmaceutical Research Laboratories, Inc., 1981.

4. REVIEWED BY: Gerald M. Marquardt, Ph.D.
Pharmacologist, EPA
Signature: CFC for G. Marquardt
Date: 2/82

5. APPROVED BY: Christine F. Chaisson, Ph.D
Section Head, EPA
Signature: CF Chaisson
Date: 3/82

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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.

7. CONCLUSION: Beagle dogs (four animals/sex/treatment group) were fed 0.0, 0.3, 1.0 or 3.0 mg/kg/d X 90 days MP in this study. All dogs survived this experiment. The food consumption and the body weight of the treated animals were not significantly different from those of control. Fasting blood sugar (FBS), blood urea nitrogen (BUN), Serum Glutamic Oxaloecetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Gamma Glutamyl Transpeptidase (GGTP), and Serum Alkaline Phosphatase (SAP) had unremarkable activities at various times in the different treatment groups.

Plasma ChE levels were significantly lower than control for high-dose animals (at 13 weeks). Red blood cell (RBC) ChE activities were significantly reduced in high-dose animals (at 6 and 13 weeks) and in mid-dose animals (at 13 weeks). Brain ChE levels were significantly reduced in high-dose animals (at 13 weeks).

RBC counts, hemoglobin (HGB) levels, hematocrits (HMCT), white blood cell (WBC) counts, WBC differential counts, RBC and WBC morphologies, and platelet estimates were similar in all of the animals throughout this study.

The appearance, specific gravity, pH, protein content, sugar content, RBC/h.p.f., and WBC/h.p.f. were similar in the urines of control and treated animals at all times.

The weights of the thyroid glands, heart, spleen, liver, kidneys, brain, and the gonads were similar for all treated and control animals. The weight of pituitary glands was statistically higher in high-dose females; histopathologic examination of the pituitary glands of these animals revealed no abnormalities. The oragan weights were similar in all control and treated animals.

Gross and microscopic examinations of all animals (at necropsy) revealed no compound-related abnormalities.

8. MATERIALS AND METHODS:

Test Substance: MP (94.32% A.I.) was mixed with Wayne Dog Food (meal form) for administration to test animals. Food consumption was measured daily and water was administered ad libitum.

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Organism: Purebred beagle dogs (4 animals/sex/treatment group; 4.5-8.0 kg in weight; approximately four months of age) were used in this study. Four animals/sex were used in each treatment group; these animals received either 0.0, 0.3, 1.0 or 3.0 mg/kg/d X 90 days MP during this experiment.

Experimental Prodecure: SGPT, BUN, FBS, SAP, and GGTP levels were analyzed on an Abbott Bichromatic Analyzer 100 for all blood samples (the volume and source of these samples were not described) before testing, at 6 weeks, and at the termination of this study. All animals were fasted 24-36 hours prior to the collection of blood samples.

Plasma, RBC and brain ChE activities were determined colorometrically by accepted procedures. ChE activities in plasma and in the RBC samples (the volume and the source of these samples were not described) were determined (after 24-36 hours of fasting) at 0, 6, and 13 weeks. Brain (the source and weight of these samples were not described) ChE activities were determined (after 24-36 hours of fasting) at the termination of this study.

The following hematological parameters were examined for all aniamls at weeks 0, 6 and 13: RBC and WBC counts (determined in a Coulter Counter), WBC Differential Counts (determined in a Coulter Counter), HMCT (determined in an Internaticnal Micro-Hematocit Centrifuge), and HGB levels (determined spectrophotometrically). The volume and the source of these samples were not described.

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Urine samples (volume not specified) were obtained in metabolism cages from all animals at 0, 6 and 13 weeks. Urine samples were examined for color, appearance, pH, specific gravity, protein content, sugar content, RBC/h.p.f. and the WBC/h.p.f. All analyses were done by accepted procedures.

The following organs were removed and weighed at necropsy: thyroid glands, heart, spleen, liver, kidneys, adrenal glands, gonads, pituitary gland, and the brain.

At the termination of this study all dogs were euthanized with Somlethol (i.v. administration) after 24-36 hours of fasting and subsequently necropsied. The skin, eyes, tongue, mammary glands, skeletal muscle (lumbar vertebrae), bone marrow, salivary glands, lymph nodes, thyroid glands, trachea, urinary bladder, esophagus, aorta, thymus, heart, liver, kidney, adrenal glands, spleen, pancreas, stomach, intestines, gall bladder, prostrate, uterus, gonads, pituitary gland, brain, spinal cord, and peripheral nerve (sciatic nerve). The tissues were then embedded in paraffin stained with hematoxylin and Eosin, and were examined microscopically.

Statistical Analysis: Data were analyzed using the Student's t-test.

Results: All dogs survived this study. Some dogs (from all treatment groups) displayed loose stools and some dogs vomited at one time or another. The pulse rate and the pupillary diameter (both determined at the start and end of testing) were similar for all dogs. The pulse rate of high-dose female dogs (at 13 weeks) was, however, significantly ($p \leq 0.05$) reduced; the mean pulse rate of these animals was 121 compared to 161 for control animals. The authors of this study consider this to be of no biological significance; no explanation of this belief is provided.

Food consumption and the body weight of all treated animals were similar to the food consumption and the body weight of contol animals at all times.

The FBS levels of high-dose animals (at week 0) and of high-dose males (at 13 weeks) were significantly lower than control. The authors attribute this to the fact that some dogs were fasted for longer than 24 hours prior to sacrifice; their explanation seems reasonable. The BUN of mid-dose females (at 6 weeks) was significantly higher than control. One of these animals had an extremely elevated BUN (31.5 mg %;) histological examination of the urinary system of this animal, however, yielded normal results. SGOT values in the low-dose and the mid-dose males (at week 0) and the low-dose and the high-dose females (at weeks 0 and 6) were significantly lower than control.

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Several dogs (in all treatment groups) had low SGOT values at 13 weeks; histological examinations revealed no hepatic damage. SGPT values of mid- and high-dose males (at 13 weeks) and the high-dose females (at 13 weeks) were significantly lower than control. GGTP values were similar for all treated and control animals at all times. SAP values were significantly reduced in mid-dose males (at 6 weeks) and in the high-dose females (at 13 weeks).

Plasma ChE activities were significantly lower in mid-dose males (at 13 weeks) and in the high-dose animals (at 6 and 13 weeks) [see table below]. RBC ChE activities were significantly depressed in high-dose animals (at 6 weeks) and in the mid-dose animals (at 13 weeks) [see table below]. Brain ChE activities were significantly reduced in high-dose animals (at 13 weeks) [see table below].

MP Dosage [a]	Sex	Length of Treatment [b]	Plasma ChE Activitie [c]	RBC ChE Activitie [c]	Brain ChE Activitie [c]
0.0	Male	0.4	2,340	2,294	-
0.0	Female	0	2,438	1,893	-
0.0	Male	6	2,440	1,914	-
0.0	Female	6	1,930	1,706	-
0.0	Male	13	2,385	1,977	715
0.0	Female	13	1,898	1,829	800
0.3	Male	0	2,710	2,006	-
0.3	Female	0	2,503	1,529	-
0.3	Male	6	2,114	1,506	-
0.3	Female	6	1,793	1,603	-
0.3	Male	13	2,058	1,524	810
0.3	Female	13	1,743	1,591	910
1.0	Male	0	2,593	1,952	-
1.0	Female	0	2,200	1,872	-
1.0	Male	6	1,929	1,290	-
1.0	Female	6	1,549	1,394	-
1.0	Male	13	1,725 [d]	1,253 [d]	700
1.0	Female	13	1,588	1,176 [d]	785
3.0	Male	0	2,698	2,029	-
3.0	Female	0	2,133	1,735	-
3.0	Male	6	1,301 [d]	442 [d]	-
3.0	Female	6	809 [d]	578 [d]	-
3.0	Male	13	1,080 [d]	526 [d]	255 [d]
3.0	Female	13	710 [d]	461 [d]	353 [d]

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a. MP was adminintered in the diet at 0.0, 0.3, 1.0, or 3.0 mg/kg/d for 13 weeks; ChE activities were determined in plasma, RBC, and brain samples (see the appropriate section of the Materials and Methods section of this DER for details).

b. These values indicate length of time (weeks) during which the animals were fed diets containing the appropriate amount of MP.

c. These values represent the mean (N=4) ChE activities for the appropriate samples [see the appropriate section of this DER for further details]. Results are expressed in terms of IU/ml for plasma and RBC ChE activities or IU/g for brain samples.

d. Significantly different from control ($p \leq 0.05$).

RBC counts were normal for all animals; two male dogs [one control animal and one mid-dose animal (at 13 weeks)] had RBC counts of 4.6-4.9 million cells/mm^3 (control RBC counts were 5.0-8.5 X 10^6 cells/mm^3) were histologically normal, however. HGB concentrations were normal for dogs at 0, 6, and 13 weeks; a couple of high-dose male dogs, 1 low-dose female dog (at 0 weeks) and 1 mid-dose female dog (at 13 weeks) had abnormal HGB concentrations, but this did not appear to be related to the administration of MP. HMCT values were similar for all dogs at 0, 6, 13 weeks. Low-dose males (at 6 weeks), and high-dose females (at 0 weeks) had significantly ($p \leq 0.05$) reduced WBC counts.

Histological examinations revealed no abnormalities. All dogs displayed normal polymorphonuclear leukocyte-lymphocyte (WBC Differential Count greater than 1 at all times).

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RBC morphologies were normal for all dogs; one control dog, one low-dose dog, two mid-dose dogs, and 1 high-dose dog displayed mild hypochronia and anisocytosis at week 0 and the high-dose dogs also displayed these findings at 6 weeks. Platelet examinations revealed no abnormalities for any dogs at any time.

The color, appearance, pH, and specific gravity of urine samples were normal for all dogs at all times. No dogs displayed proteinuria, hematuria, or glucosuria during this study. One high-dose female displayed trace glucosurina and one mid-dose animal (at 0 weeks) and 1 high-dose animal (at 6 weeks) had significantly elevated WBC/h.p.f. values. Histological examinations revealed no abnormalities in any of the treated or control animals.

Organ weights and organ/body weight ratios were similar for all animals; the pituitary gland of high-dose females weighed more than control but histological examination revealed no abnormalities.

Gross and microscopic examinations of all animals were unremarkable.

10. DISCUSSION: MP administration to beagle dogs (0.3, 1.0, or 3.0 mg/kg/d X 90 days) produced significant decreases in plasma, RBC, and brain ChE activities. Plasma ChE activities were significantly reduced in high-dose animals (at 6 and 13 weeks) and mid-dose males (at 13 weeks). RBC ChE activities were significantly depressed in high-dose animals (at 6 and 13 weeks) and mid-dose animals (at 13 weeks). Brain ChE activities were significantly lower than control in high-dose animals (at 13 weeks).

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11. Discussion:

This study demonstrates the following NOEL's:

....for plasma ChE at 13 weeks - 0.3 mg/kg/d
....for RBC ChE at 13 weeks - 0.3 mg/kg/d
....for brain ChE at 13 weeks - 1.0 mg/kg/d

12. CORE score - Core Guideline