UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

008106

SEP 25 1990

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Methyl Parathion--Special 13-Week Feeding Study in Dogs

TO: J. Talarico PM 74
Special Review and Registration Division (H7508C)

FROM: K. Clark Swentzel K. Clark Swentzel 9/18/90
Section Head
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HED (H7509C)

THRU: Marcia van Gemert, Ph.D. Marcia van Gemert 9/20/90
Branch Chief
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EPA ID No. 4787-4
MRID No. 41335401
Caswell No. 372
Project No. 0-0605

Background.

The subject study was designed to supplement a previous 90-day feeding study in dogs (Acc. no. 524-68) with methyl parathion (MP) at dosages levels of 0.3, 1.0 and 3.0 mg/kg/day and a 1-year feeding (Study no. 7830) with MP dosages of 0.03, 0.1 and 0.3 mg/kg/day. Mild RBC and plasma cholinesterase (ChE) inhibition was observed at 0.3 mg/kg in the 1-year study and at 1 mg/kg in the 90-day study; brain ChE was inhibited at 3 mg/kg in the 90-day study. The toxicology reviewer concluded that an LEL was not established in the 1-year study so it was classified Core-supplementary. Subsequently, a protocol for a 90-day feeding study in dogs, which would provide bridging data, was submitted for TB evaluation (memorandum, Swentzel to Edwards, October 30, 1987). The objectives stated in the protocol were: 1) to establish a NOEL/LEL for plasma, erythrocyte and brain ChE activity, 2) to define a dose-response curve for the inhibitory effect of methyl parathion on ChE activity in the dog and 3) to detect and evaluate functional impairment of the eye that might precede histological changes.

Summary of current 13-week feeding study in dogs

The subject study was reviewed by Dynamac Corp. and can be summarized as follows. Methyl parathion (technical) was administered to beagle dogs (8/sex/group) for 13 weeks at dietary doses of 0, 0.03, 0.3 or 3 mg/kg/day. The highest dosage resulted in significant depression of plasma and erythrocyte cholinesterase activity at weeks 6 and 13 and brain cholinesterase activity (pons and cerebellum) at week 13. Following 4 weeks of recovery, each category of cholinesterase activity was comparable between

test and concurrent control males and females. No compound-induced mortalities were observed. Mean body weight gains of high dose males and females were depressed throughout the dosing period even though food consumption was not affected. There was no evidence of functional or morphologic impairment of the eyes as a result of treatment. No compound-related histologic lesions were seen. Based on cholinesterase inhibition, considered the most sensitive parameter, the LEL and NOEL were 3.0 and 0.3 mg/kg/day, respectively.

Classification: Core-supplementary. This study does not satisfy Subdivision F Guideline criteria for a subchronic feeding study in dogs (82-1), however, this is considered a bridging study which achieved the intended objectives indicated above.

Conclusion

A LEL, based on cholinesterase inhibition, was established in the subject feeding study in dogs for methyl parathion. This represents the bridging data necessary to upgrade the Core-classification of the 1-year feeding study in dogs with methyl parathion (Study no. 7830) to Core-minimum.

008106

EPA No.: 68D80056
DYNAMAC No.: 302-A
TASK No.: 3-02A
September 14, 1990

DATA EVALUATION RECORD

METHYL PARATHION

Subchronic Oral Toxicity Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L McLellan for

Date: 9/12/90

EPA No.: 68D80056
DYNAMAC No.: 302-A
TASK No.: 3-02A
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DATA EVALUATION RECORD

METHYL PARATHION

Subchronic Oral Toxicity Study in Dogs

REVIEWED BY:

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Signature: K. Clark Swentzel
Date: 9/19/90

DATA EVALUATION RECORD

GUIDELINE § 82-1

STUDY TYPE: Subchronic oral toxicity study in dogs.

MRID NUMBER: 413354-01.

TEST MATERIAL: Methyl parathion.

SYNONYM(S): N/A.

STUDY NUMBER: Project No. 87-3209.

SPONSOR: Mobay Corporation, Stilwell, KS.

TESTING FACILITY: Biodynamics, Inc. East Millstone, NJ, and A/S Cheminova Lemvig, Denmark.

TITLE OF REPORT: A 13-Week Subchronic Toxicity Study of Methyl Parathion in Dogs via the Diet Followed by a One-Month Recovery Period.

AUTHOR: Daly, Ira W.

REPORT ISSUED: November 20, 1989.

CONCLUSIONS:

The 13-week dietary administration of methyl parathion to male and female beagle dogs at dose levels of 0.03, 0.3 or 3.0 mg/kg/day resulted in significant depression of plasma and erythrocyte cholinesterase activity at study weeks 6 and 13 and brain cholinesterase activity (pons and cerebellum) at study week 13 in animals fed 3.0 mg/kg/day. Following 4 weeks of recovery, erythrocyte, plasma and brain cholinesterase activity of males and females were comparable to concurrent control activity. No compound-related deaths occurred. Mean body weight gains of males and females fed the highest dose were depressed throughout the dosing period. Food consumption was not affected. Two high-dose animals appeared dehydrated and emaciated during the final 2 weeks of dosing; one low-dose animal appeared thin during the last quarter of the dosing period and during recovery. There was no functional or morphologic impairment of the eyes as a result of dosing. There were no compound-related histologic lesions. The inhibition of cholinesterase was considered to be the most sensitive parameter of dosing. The LOEL is 3.0 mg/kg/day and the NOEL is 0.3 mg methyl parathion/kg/day in the beagle dog.

Classification: Core Supplementary. This study does not meet guideline requirements for a subchronic toxicity study in dogs; however, the parameters of interest for the study were completed (see Reviewers' Discussion and Interpretation of Results).

A. MATERIALS:

1. Test Compound: Methyl parathion; description: brown liquid; lot No.: 233690479; purity: 94.9%.

2. Test Animals: Species: dog; strain: beagle; age: approximately 5½-6 months at study initiation; weight: males--6.1-9.2 kg, females--5.1-7.0 kg.; source: Marshall Farms, USA, Inc., North Rose, NY.

B. STUDY DESIGN:

1. Animal Assignment: Following a 7-week acclimation period, animals were ranked by body weight and assigned to the following test groups:

Test group	Dose in diet (mg/kg/day)	Main study[a] (13 weeks) Males	Main study[a] (13 weeks) Females	Recovery (4 weeks) Males	Recovery (4 weeks) Females
1 Control	0	8	8	4	4
2 Low (LDT)	0.03	8	8	4	4
3 Mid (MDT)	0.30	8	8	4	4
4 High (HDT)	3.00	8	8	4	4

[a]Four animals/sex/dose were sacrificed following the 13-week dosing period and four animals/sex/dose were sacrificed following the 4-week recovery period.

Dogs were vaccinated by the supplier for canine distemper, hepatitis, leptospirosis, parvovirus and/or corona virus, bordetella-parainfluenza, papillomas, rabies, and adenovirus type 2, and treated for intestinal parasites. The study laboratory vaccinated the dogs for canine distemper, adenovirus type 2, hepatitis, parainfluenza, parvovirus, Leptospira canicola, and Leptospira icterohaemorrhagiae during the acclimation period.

Animals were housed individually in an environmentally controlled room (temperature, 58 to 78°F; humidity, 8 to 89%) with a 12-hour light/dark cycle. The low humidity level was reported to occur during the acclimation period and remained low for 3 days; this deviation from the desired humidity range was not considered to have an adverse effect on the study.

2. Diet Preparation: The test diets were prepared on a weekly basis and stored frozen. Appropriate amounts of the test compound were mixed with the basal diet to equate nominal concentrations of 0.03, 0.30, or 3.00 mg/kg/day. Individual doses were adjusted by most recent body weight data. Control animals received the standard basal diet. Diets were offered to the dogs daily for 4.5 hours, 7 days/week for 13 weeks.

On study day 14, crystallization of the test material was observed; concentration analysis of a 0.5-g sample was reported to be 98.5% of nominal. A sample of the test material was forwarded to the sponsor at study termination for stability analysis. Concentration analyses were performed on dietary samples extracted at weeks 1, 2, 3, 4,

8, and 13. Homogeneity and stability analyses were conducted prior to study initiation on mock batches of control feed and low- and high-dose test mixtures.

Results: The range of variation of homogeneity of the mock batches of the 0.03- and 3.00-mg/kg test diets was between -15% and +18%; mean concentrations of the test compound from three samples from the top, middle, and bottom of the dose mixture ranged from 85.6 to 118% and from 89.4 to 104% of nominal for the low- and high-dose preparations, respectively. Two original sample results for the low-dose diet (82.9 and 120% of nominal) were replaced by four additional samples, which were extracted and analyzed.

The test material was stable in the diet at room temperature for 4 days and frozen for 8 days. After storage at room temperature for 4 days in open feeder jars, concentrations of the test material in low- and high-dose preparations were 91 ± 4.9 and 94.3 ± 2.3% of nominal, respectively. After frozen storage for 8 days in open feeder jars, concentrations were 93.5 ± 12 and 91.8 ± 7.3% of nominal for low- and high-dose preparations, respectively. The high-dose preparations appeared to be more stable than those at the low dose.

Greater than 10% deviation from target concentration was found in test diets; test compound concentrations at six intervals of analysis ranged from 83.5 to 122%, from 86.9 to 112%, and from 88.4 to 104% of target for the low-, mid-, and high-dose preparations, respectively.

3. Food and Water Consumption: Animals received food (400 grams of Purina Certified Canine Diet No. 5007) daily for 4.5 hours and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights, food consumption, and % depression of cholinesterase data from controls were analyzed statistically. Bartlett's test was performed to test for variance homogeneity. If the data followed a normal distribution, analyses of variance using the F distribution and Dunnett's test were performed to assess significance. The Kruskal-Wallis test and Dunn's test (summed rank test) were performed on nonparametric data. Standard regression with a test for trend and lack of fit were used to test for trend on parametric data; Jonckheere's test for monotonic trend was performed on nonparametric data.

5. Quality Assurance: A signed quality assurance statement was dated December 15, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of morbidity and mortality. Animals received detailed physical examinations prior to study initiation and weekly thereafter.

   Results: No deaths occurred as a result of dosing. One mid-dose male (animal No. 3007) and one high-dose female (animal No. 4501) died during the study (study days 83 and 70, respectively) as a result of anesthetic overdose during or following electroretinogram evaluations.

   Individual data on clinical observations were not reported. Two high-dose animals (1/8 males, 1/8 females) exhibited emaciation, dehydration, and thin appearance from study weeks 11 to 13; in addition, one low-dose male appeared thin from study weeks 9 to 13 and throughout the recovery period. These findings were considered to be related to dosing. One high-dose male (animal No. 4005) exhibited paraphimosis, pale gums, and hypothermia; the paraphimosis was corrected surgically. One to two high-dose females exhibited otitis from study weeks 7 to 13. These latter observations were considered to be normally encountered in beagle dogs and were not attributed to dosing.

2. Body Weight: Dogs were weighed for 6 weeks prior to study initiation and weekly during the dosing and recovery periods.

   Results: Representative data on mean body weights and body weight gains are summarized in Tables 1 and 2. Mean body weights of high-dose males and females were slightly but not significantly depressed (10 to 15% depression in males, 4 to 6% depression in females) from study week 2 to termination of dosing (Week 13). Body weight gains of high-dose males and females were depressed throughout the dosing period; mean body weight gains of high-dose males were 0.15 and 0.64 kg as compared with 1.41 and 2.0 kg for controls from weeks 0 to 7 and 0 to 13 respectively. The depression in body weight gains of dosed males from weeks 0 to 13 was dose related. Mean body weight gains of high-dose females were 0.14 and 0.63 kg as compared with 0.70 and 0.96 kg for controls from weeks 0 to 7 and 0 to 13, respectively. Body weight gains in control females were lower than in mid- and high-dose females throughout the study because of the depressed weight gain of one control (animal No. 1508). Body weights of dosed males and females were similar to controls following recovery.

TABLE 1. Mean Body Weights at Selected Intervals for Dogs Fed Methyl Parathion for 13 Weeks

Dose (mg/kg/day)	Mean Body Weight (kg ± S.D.) at Week: 0	6	12	18 (recovery)
		Males		
0	7.4 ± 0.6	8.6 ± 1.3	9.3 ± 1.5	10.5 ± 1.4
0.03	7.3 ± 1.1	8.3 ± 1.5	8.9 ± 2.0	9.6 ± 2.6
0.30	7.5 ± 0.9	8.6 ± 1.3	8.7 ± 1.9	8.9 ± 1.2
3.00	7.4 ± 0.9	7.5 ± 1.6	8.0 ± 2.2	9.3 ± 1.4
		Females		
0	6.0 ± 6.4	6.7 ± 0.9	6.9 ± 0.8	7.3 ± 1.2
0.03	6.1 ± 0.6	7.0 ± 0.7	7.5 ± 0.8	7.3 ± 0.5
0.30	6.1 ± 0.5	7.0 ± 0.7	7.3 ± 0.9	7.7 ± 1.4
3.00	6.1 ± 0.6	6.1 ± 0.9	6.7 ± 1.2	8.1 ± 0.7

TABLE 2. Representative Results of Mean Body Weight Gains for Dogs Fed Methyl Parathion for 13 Weeks[a]

Dose (mg/kg/day)	Mean Body Weight Gain (kg/dog ± S.D.) at Weeks: 0-7	7-13	0-13
		Males	
0	1.41 ± 0.93	0.64 ± 0.50	2.05
0.03	1.13 ± 0.76	0.59 ± 0.45	1.72
0.30	1.13 ± 0.88	0.27 ± 0.59	1.40
3.00	0.15 ± 1.23	0.49 ± 0.44	0.64
		Females	
0	0.70 ± 0.64	0.26 ± 0.23	0.96
0.03	1.00 ± 0.31	0.46 ± 0.39	1.46
0.30	0.80 ± 0.58	0.39 ± 0.31	1.19
3.00	0.14 ± 0.57	0.49 ± 0.45	0.63

[a]Body weight gains (mean ± S.D.) calculated by reviewers.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated prior to study initiation and weekly thereafter.

Results: Food consumption results are presented in Table 3. Mean food consumption of dosed animals was similar to that of controls. Compound consumption data were not reported.

4. Ophthalmology: Ophthalmological examinations (including measurement of intraocular pressure and electroretinograms) were performed prior to study initiation, at termination of dosing, and following the recovery period. Photographs of the ocular fundus were taken prior to study initiation for all animals, at completion of dosing in control and high-dose animals, and following recovery in all recovery animals. Due to health problems, an ophthalmoscopic examination and measurement of intraocular pressure were performed on one high-dose male (animal No. 4005) during study week 11.

Results: There were no ophthalmological abnormalities as a result of dosing with methyl parathion. Incidental ocular changes (retinal folds, cellular deposits on lens capsule, focal lens opacity, iritis, small optic disk, suture tip lens opacity, hypertrophy, and prolapse of the gland of nectituns) were considered similar to changes seen prior to dosing or were considered to be common findings in the beagle dog. The intraocular pressure of dosed and control animals decreased over time from pretest evaluation to recovery (Table 4). The intraocular values of dosed animals at study week 13 were similar to those of controls; at recovery the intraocular values of high-dose males and mid-dose females were slightly lower than those of concurrent controls. Intraocular values of high-dose females were equal to those of controls at this time. The study authors were unclear regarding the significance of the decreased recovery values. There was no evidence of retinal functional damage in dosed males or females based on electroretinographic evaluations. One high-dose female (animal No. 4506) exhibited a low amplitude a-wave (photoreceptor function) recording at pretest and study week 13 and no a-wave recording at recovery. However, other response amplitudes of this animal were also low at recovery. Since rod and cone response were present at this time, the electroretinogram was considered to be normal by the study authors. The low response recordings were considered to be due to a deeper than usual level of anesthesia.

TABLE 3. Representative Mean Food Consumption of Dogs Fed Methyl Parathion for 13 Weeks

Dose (mg/kg/day)	Mean Food Consumption (g/kg/day) at Week: 0	6	12	18(recovery)
		Males		
0	35.7 ± 4.9	32.5 ± 4.2	34.3 ± 8.5	27.0 ± 7.8
0.03	36.0 ± 6.1	35.0 ± 7.6	31.5 ± 8.4	31.3 ± 7.1
0.30	38.9 ± 5.4	31.9 ± 6.4	33.9 ± 4.6	33.6 ± 6.1
3.00	39.5 ± 6.7	35.6 ± 8.7	37.3 ± 10.2	34.7 ± 8.4
		Females		
0	41.0 ± 9.1	40.2 ± 8.6	37.1 ± 11.0	42.1 ± 7.8
0.03	40.9 ± 4.8	36.7 ± 4.3	35.2 ± 7.9	31.4 ± 4.4
0.30	38.9 ± 5.0	37.8 ± 7.1	34.7 ± 5.5	36.6 ± 3.8
3.00	38.7 ± 4.9	31.9 ± 8.4	34.6 ± 7.1	34.1 ± 4.2

TABLE 4. Mean Intraocular Pressure Values of Dogs Fed Methyl Parathion for 13 Weeks[a]

Testing Interval/ Exposure Level (mg/kg/day)	Mean Intraocular Pressure (mmHg)			
	Males		Females	
	Right Eye	Left Eye	Right Eye	Left Eye
Pretest				
0	20 ± 7	21 ± 3	21 ± 3	21 ± 3
0.03	23 ± 3	24 ± 2	21 ± 2	24 ± 3
0.30	21 ± 2	21 ± 4	19 ± 6	23 ± 4
3.00	18 ± 5	23 ± 6	22 ± 4	24 ± 3
Week 13				
0	15 ± 2	17 ± 5	18 ± 4	16 ± 5
0.03	19 ± 8	20 ± 6	19 ± 4	22 ± 4
0.30	19 ± 5	18 ± 3	16 ± 2	18 ± 3
3.00	17 ± 3	18 ± 4	22 ± 10	17 ± 6
Recovery				
0	17 ± 4	19 ± 6	17 ± 3	18 ± 5
0.03	21 ± 5	22 ± 7	21 ± 3	23 ± 5
0.30	16 ± 1	17 ± 5	13 ± 3	15 ± 6
3.00	13 ± 3	14 ± 3	17 ± 8	18 ± 4

[a]A total of four animals/sex/dose were sacrificed following 13 weeks of dosing; the remaining four animals/sex/dose were sacrificed following an additional 4 weeks of recovery.

5. Cholinesterase Activity: Blood was collected from fasted dogs via jugular venipuncture during three separate periods prior to study initiation and at weeks 6, 13 (dosing termination), and 17 (recovery) for analysis of plasma and erythrocyte cholinesterase activity; brain cholinesterase activity was measured at dosing termination and recovery only. (Erythrocyte cholinesterase was calculated based on measured total blood and plasma cholinesterase activity and hematocrit values. Whole-blood cholinesterase and hematocrit values of individual animals were not reported). No other hematology or clinical chemistry parameters were measured.

Results: Erythrocyte and plasma cholinesterase activity of high-dose males and females were significantly ($p < 0.05$, $p < 0.01$) depressed when compared to pretest activity and concurrent controls at study weeks 6 and 13 (Table 5). Plasma cholinesterase activity decreased 54 and 47% in males, and 59 and 53% in females, respectively, at weeks 6 and 13, while erythrocyte cholinesterase activity decreased 22 and 18% in males, and 20 and 23% in females, respectively. In addition, brain cholinesterase activity of the pons (43% and 47% depression in males and females, respectively) and cerebellum (55 and 49% depression in males and females, respectively) was significantly ($p < 0.01$) depressed at study week 13. Following 4 weeks of recovery, erythrocyte, plasma, and brain cholinesterase activities of males and females were comparable to concurrent control activity.

Low- and mid-dose animals were not affected, with the exception of the depressed (19% depression) mean plasma cholinesterase activity of low-dose males at study week 13. The study authors did not consider this depression to be compound related.

6. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination. The eyes, optic nerves, and lateral and superior rectus extraocular muscles of control and dosed animals were examined histopathologically. No other tissues were collected or examined.

a. Organ Weights: Organ weights were not obtained.

b. Gross Pathology: There were no compound-related macroscopic lesions.

TABLE 5. Mean Cholinesterase Values (Mean ± S.D.) in Dogs Fed Methyl Parathion for 13 Weeks

Parameter/ Week	Exposure Level (mg/kg/day)							
	Males				Females			
	0	0.03	0.30	3.00	0	0.03	0.30	3.00
Plasma Cholinesterase (IU/mL)								
Pretest 1	2.147 ± 0.44	1.925 ± 0.225 (10%)*	2.054 ± 0.381 (4%)	2.060 ± 0.333 (4%)	1.952 ± 0.218	1.939 ± 0.202 (1%)	1.955 ± 0.288 (0)	1.720 ± 0.165 (12%)
Pretest 2	1.854 ± 0.313	1.619 ± 0.200 (13%)	1.725 ± 0.305 (7%)	1.747 ± 0.294 (6%)	2.012 ± 0.181	1.969 ± 0.157 (2%)	1.998 ± 0.265 (1%)	1.769 ± 0.194 (12%)
Pretest 3	1.979 ± 0.356	1.781 ± 0.224 (10%)	1.854 ± 0.279 (6%)	1.918 ± 0.305 (3%)	1.977 ± 0.195	1.890 ± 0.212 (4%)	1.916 ± 0.267 (3%)	1.715 ± 0.189 (13%)
Week 6	2.025 ± 0.406	1.661 ± 0.176 (18%)	1.735 ± 0.402 (14%)	0.926 ± 0.126 (54%)**	1.832 ± 0.266	1.751 ± 0.281 (4%)	1.681 ± 0.186 (8%)	0.749 ± 0.156 (59%)**
Week 13	1.857 ± 0.284	1.502 ± 0.227 (19%)*	1.599 ± 0.331 (14%)	0.981 ± 0.168 (47%)**	1.707 ± 0.188	1.681 ± 0.192 (2%)	1.610 ± 0.204 (6%)	0.794 ± 0.118 (53%)**
Week 17	1.793 ± 0.318	1.689 ± 0.115 (6%)	1.641 ± 0.223 (8%)	1.770 ± 0.412 (1%)	1.691 ± 0.108	1.644 ± 0.069 (3%)	1.765 ± 0.179 (0)	1.521 ± 0.251 (10%)
Erythrocyte Cholinesterase (IU/mL)								
Pretest 1	3.9 ± 0.3	4.2 ± 0.4 (0)	3.9 ± 0.3 (0)	4.5 ± 0.3 (0)	4.1 ± 0.4	4.2 ± 0.5 (0)	4.0 ± 0.4 (2%)	4.0 ± 0.4 (2%)
Pretest 2	3.3 ± 0.2	3.6 ± 0.2 (0)	3.7 ± 0.3 (0)	3.6 ± 0.3 (0)	3.4 ± 0.5	3.7 ± 0.6 (0)	3.2 ± 0.4 (6%)	3.3 ± 0.6 (3%)
Pretest 3	4.7 ± 0.9	4.8 ± 0.7 (0)	5.1 ± 0.4 (0)	5.0 ± 0.9 (0)	3.6 ± 0.5	3.8 ± 0.6 (0)	3.6 ± 0.5 (0)	3.6 ± 0.5 (0)

(continued)

TABLE 5. (continued)

Parameter/ Week	Exposure Level (mg/kg/day)							
	Males				Females			
	0	0.03	0.30	3.00	0	0.03	0.30	3.00
Week 6	4.5 ± 0.9	4.5 ± 0.7 (0)	4.4 ± 0.3 (2%)	3.5 ± 0.5 (22%)*	4.4 ± 0.6	4.7 ± 0.7 (0)	4.3 ± 0.5 (2%)	3.5 ± 0.4 (20%)**
Week 13	3.8 ± 1.0	4.0 ± 0.8 (0)	3.8 ± 0.4 (0)	3.1 ± 0.6 (18%)	3.5 ± 0.4	3.9 ± 0.6 (0)	3.2 ± 0.4 (9%)	2.7 ± 0.3 (23%)**
Week 17	3.8 ± 0.3	3.9 ± 0.3 (0)	3.7 ± 0.3 3%)	3.4 ± 0.5 (11%)	4.0 ± 0.5	4.2 ± 0.9 (0)	3.5 ± 0.4 (13%)	3.5 ± 0.2 (13%)
Brain (pons) Cholinesterase (IU/g)[c]								
Week 13	6.9 ± 0.6	7.4 ± 0.7 (0)	7.0 ± 1.3 (0)	3.9 ± 0.6 (43%)**	7.0 ± 0.9	7.4 ± 0.6 (0)	7.8 ± 0.9 (0)	3.7 ± 0.6 (47%)**
Week 17	6.7 ± 0.5	6.7 ± 2.1 (0)	7.0 ± 1.0 (0)	6.5 ± 0.8 (3%)	6.6 ± 0.7	7.0 ± 0.4 (0)	6.6 ± 0.6 (0)	5.9 ± 0.4 (11%)
Brain (cerebellum) Cholinesterase (IU/g)[c,d]								
Week 13	12.7 ± 2.1	12.1 ± 2.5 (5%)	12.5 ± 3.7 (2%)	5.7 ± 0.5 (55%)**	11.6 ± 2.4	11.2 ± 1.5 (3%)	12.8 ± 1.6 (0)	5.9 ± 0.7 (49%)**
Week 17	11.9 ± 0.9	11.9 ± 1.7 (0)	11.5 ± 1.6 (3%)	11.9 ± 0.9 (0)	10.9 ± 2.6	12.2 ± 2.5 (0)	11.3 ± 1.2 (0)	10.3 ± 2.2 (6%)

[a]The values in parentheses equal % depression from concurrent controls.

[b]Calculation of erythrocyte cholinesterase = $\frac{\text{WB CHE} - [\text{PL CHE } (1-.\text{HCT})]}{.\text{HCT}}$

Abbreviations: CHE = cholinesterase, PL = plasma, WB = whole blood, HCT = hematocrit.

[c]Performed at week 13 and week 17 only.

[d]Individual cerebellum cholinesterase activity values were rounded off in study report for summary tables in appendix and table of % depression of cholinesterase from controls on study page 14. Actual values presented here are without rounding as taken from individual data.

*Significantly different from controls at $p < 0.05$.

**Significantly different from controls at $p < 0.01$.

c. Microscopic Pathology: There were no compound-related microscopic lesions. Retinal folds were observed in one control male, swollen lens fibers were observed in one high-dose female, and lymphoid cell infiltrates of the lateral and superior rectus muscles were observed sporadically in control and dosed animals. The severity of these findings was minimal; the findings were not considered to be related to dosing.

D. STUDY AUTHORS' CONCLUSIONS:

The 13-week dietary administration of methyl parathion to male and female beagle dogs at dose levels of 0, 0.03, 0.3, or 3 mg/kg/day resulted in an inhibition of erythrocyte, plasma, and brain cholinesterase activity in high-dose animals; appearance and body weight gain of these animals were also affected. There was no functional or morphologic impairment of the eyes as a result of dosing. The inhibition of cholinesterase is considered to be the most sensitive parameter of dosing with methyl parathion in the beagle dog. The NOEL is 0.3 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study was reported to be conducted as a 13-week subchronic toxicity study in accordance with Guideline 82-1 of the EPA Pesticide Assessment Guidelines, 1984; however, many requirements of Guideline 82-1 were not satisfied. Hematology and clinical chemistry parameters were not tested with the exception of cholinesterase; macroscopic and microscopic examinations were limited to the eyes and optic nerves and muscles; and organ weights were not measured. Liver, kidneys, testes and thyroid/parathyroids, specified in the guidelines for examination and weights, were not included. The reviewers consider this study to be a specialized study, since the protocol included only the parameters of interest. The purpose of the study was to establish a No-Effect Level for plasma, erythrocyte, and brain cholinesterase activity, and to detect and evaluate any functional impairment of the eye as a result of dosing. The study does not meet the guideline requirements for a subchronic toxicity study in dogs.

In addition, individual brain cerebellum cholinesterase activity values were rounded off for summary tables in Appendix H and the table of percent depression of cholinesterase from controls on study page 14. These values should not have been rounded. For this reason, values for the percent depression of cholinesterase for the cerebellum were incorrectly calculated in the text. Erythrocyte cholinesterase activity was calculated based on the plasma and total blood cholinesterase and

hematocrit (see formula for calculation, footnote b, page 15). Only the derived erythrocyte cholinesterase data were reported; individual animal data for total blood cholinesterase and hematocrit were not presented.

The homogeneity of the mock test diets varied more than 15% (-15% to +18%). The concentration of the test diets deviated more than 10% from the target concentration. These variations are above acceptable limits. However, the high-dose preparations appeared to be more stable than those of the low dose. Actual samples instead of mock samples should have been tested for dietary analysis prior to storage for 4 days for accurate assessment. No individual data were presented for clinical observations.

We agree with the study authors' conclusions that methyl parathion caused depressed plasma, erythrocyte, and brain cholinesterase activity; depressed body weight gains; and emaciation and dehydration in high-dose animals. The LOEL is 3.0 mg/kg/day, and the NOEL is 0.3 mg/kg methyl parathion/day.