11/7/1985   005588

DATA EVALUATION REPORT

A. <u>Compound</u>:

Methyl Parathion; (0,0-dimethyl O-p-nitrophenyl phosphorothioate)

$$(CH_3O)_2 \underset{\underset{O}{\|}}{P}O \langle o \rangle NO_2$$
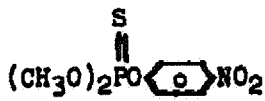
B. <u>Study Report Citation</u>:

Title: "Methyl Parathion: One Year Dog Study"

Testing Facility: Pharmacopathics Research Laboratories, Inc.
Laurel, MD 20810

Report Numbers: R.D. #393; Special Report MSL 1967;
Testing Facility's Report No. 7830;
Sponsor's Report No. PRL 77-118

Date: December 22, 1981

Submitted to EPA by: Monsanto Agricultural Products Co.
St. Louis, MO 63167

Authors: Ahmed, F.E., Sagartz, J.W., Tegeris, A.S., <u>et al</u>.

Compiled by: L.A. Suba

C. <u>Reviewed By</u>: Alan C. Katz, M.S., D.A.B.T
Toxicologist   (Signature)
Toxicology Branch
Hazard Evaluation Division (TS-769C)   (Date) 10/2/85

D. <u>Secondary Review By</u>: Robert P. Zendzian, Ph.D.
Acting Head, Review Section IV (Signature)
(Date) 11/5/85

E. <u>Classification</u>:

Supplementary

F. <u>Conclusions</u>:

This study did not include adequate ophthalmologic evaluation.

Details of clinical observations were not sufficiently presented.

There appeared to be no attempt to set the high dose level at or near the MTD.

The cholinesterase activity determinations were too variable to be used for a substantive evaluation. It is apparent, however, that methyl parathion administration at a dose of 0.3 mg/kg/day caused mild inhibition of plasma and

F. Conclusions (Cont'd):

RBC cholinesterase. It is also possible that meaningful cholinesterase changes at lower doses were masked by the variability of the data.

G. Materials:

Test compound: Methyl parathion, lot no. AK 0911
    Purity: 93.65% a.i.

Animals: 64 purebred Beagle dogs (8/sex/group)
    Supplier: Hazleton Laboratories, Vienna, VA
    Age: 4-5 months when received at laboratory;
    6-7 months old at initiation of dosing.

Basal feed: Wayne Dog Food (meal)

H. Methods:

Animals were assigned to dose groups (8/sex/group) such that there were no significant group mean body weight differences at initiation, and no littermates of the same sex were assigned to the same dose group. The dogs were given diet containing methyl parathion at dietary concentrations calculated to provide doses of 0.03, 0.1 or 0.3 mg/kg body weight/day. A control group was given the basal feed only.

Feed (350 g/animal/day) was available for 1 hour daily. Fresh diets were prepared weekly. Water was available ad libitum. Environmental controls provided 10 air changes/hour, 40-70% humidity, with temperature at approximately $73^{+}_{-}2$ F and a 12-hour on/12-hour off light cycle. The type of artificial lighting was not specified in the report. The animals were housed individually in suspended stainless steel cages. Males and females were housed in separate rooms.

The animals were observed daily for clinical signs. Body weights were recorded weekly. Food consumption was determined daily.

The following tests were performed on blood and urine samples:

hematology (Prior to initiation of dosing; monthly thereafter)

Hematocrit	Total leukocyte count
Hemoglobin	Differential leukocyte count
Reticulocyte count	Platelet estimate
Erythrocyte count	Erythrocyte morphology

Clinical Chemistry (Prior to initiation of dosing; monthly thereafter)

Fasting blood sugar	Total bilirubin
Blood urea nitrogen	Direct bilirubin
Total protein	Cholesterol
Albumin	Sodium
Globulin	Potassium
Serum glutamic oxaloacetic transaminase	Calcium
Serum glutamic pyruvic transaminase	Serum lactic dehydrogenase
Serum alkaline phosphatase	Gamma glutamyl transpeptidase

005583

Special Chemistry

Plasma cholinesterase (Prior to initiation of dosing, and 2, 4 and 12 months)
RBC cholinesterase   (Prior to initiation of dosing, and 2, 4 and 12 months)
Brain cholinesterase (12 months)

Urinalysis

Color	pH
Appearance	Specific gravity
Protein	Bilirubin
Glucose	Urobilinogen
Ketones	Microscopic examination

At termination of the study, all animals were sacrificed and necropsied. The method of sacrifice was not specified in the study report or the protocol. Organs weighed at necropsy were: brain, heart, pituitary, liver, kidneys, adrenals, testes, ovaries and thyroid/parathyroids. The brain, heart, liver, kidneys and testes were weighed fresh; the other (smaller) organs were weighed after fixation in 10% buffered formalin. The following tissues were collected for histopathologic evaluation:

Heart	Ovaries	Trachea
Lungs	Mammary tissue	Skin
Liver	Parathyroids	Mesenteric lymph nodes
Spleen	Tongue	Gallbladder
Kidneys	Salivary gland	Urinary bladder
Adrenals	Pancreas	Eyes
Pituitary	Thymus	Optic nerve
Brain	Esophagus	Sciatic nerve
Thyroid	Stomach	Aorta
Testes	Jejunum	Skeletal muscle
Seminal vesicle	Ileum	Bone
Prostate	Colon	Bone marrow
Uterus	Bronchi	Spinal cord

Although not included among tissues examined microscopically according to the text of the report, the Individual Animal Data Records (Appendix B) indicate that the cecum was also collected at necropsy. Apparently, the duodenum and rectum were NOT collected for microscopic evaluation.

Body weight, feed consumption, hematology, organ weight and routine clinical chemistry data were analyzed using Student's t-test. The cholinesterase data were analyzed by one-way analysis of variance and Dunnett's t-test. The 95% confidence level was used to determine statistical significance for all parameters tested.

Apparently, fecal exams were performed only prior to initiation of the study (and may have been for detection of parasites only) and ophthalmoscopic examinations were not conducted.

I. **Results:**

No deaths occurred during this study. No compound-related effect was apparent with respect to body weight or feed consumption in males or females.

No compound-related toxicity was evident on the basis of >< clinical chemistry results, with the exception of sporadically, mildly reduced plasma and RBC cholinesterase activity, particularly in high dose animals. Although a slight (22%) reduction (not statistically significant) in brain cholinesterase activity was found in high dose females, a surprising, substantial (87%) increase occurred in high dose males. Plasma, RBC and brain cholinesterase activity values are summarized in Table 1.

The LDH values for males and females in month 11 are considered invalid because there does not appear to be a reliable basis for comparison of these data with those of other determination points with respect to potential treatment-related changes. Exclusion of these data does not affect the overall integrity of this study.

It was reported that no differences were found between groups with respect to clinical signs; however, because the daily clinical observations are not presented in the study report, this negative finding cannot be verified.

Urinalysis results revealed no apparent treatment-related changes.

Absolute liver weights were slightly, but significantly (p<0.05), increased in high dose males. Other differences in male organ weights are not considered toxicologically significant. No significant differences were found with respect to absolute organ weights of treated females, or relative (organ:body or organ:brain) organ weights of treated males or females.

No compound-related histomorphological changes were apparent.

Results of assays of blended feed for concentration, stability and homogeneity were within marginally acceptable limits.

J. **Discussion/Recommendations**:

Ophthalmoscopic or equivalent examinations at termination of the in-life phase of the chronic study is mandatory. The importance of this type of evaluation is underscored in this case by the fact that methyl parathion has been shown in a long-term feeding study to cause retinal atrophy in rats. Retinopathy and other ocular changes associated with organophosphate pesticide exposure in humans and laboratory animals have been widely reported in the scientific literature. In the absence of documentation that eyes were carefully examined during the in-life phase by qualified personnel using appropriate instrumentation, Tox Branch must regard this study as "Supplementary."

The registrant is requested to explain why gross diagnoses (listed for individual animals under "Microscopical Findings" in Table #T-4.14.2, "Inventory of Male/Female Dogs") are not included in summary pathology tables (i.e., Table #T-4.14.3, "Pathology Distribution for Male and Female Dogs.")

Details of the method and criteria used for estimation of platelets should be provided.

J. Discussion/Recommendations (Cont'd):

The rationale for selection of doses in this study is not clear, especially since the high dose selected elicited no effect in dogs dosed at this level in a prior 90-day feeding study.

There is an apparent error in the study report Summary (1.1-Abstract) in the statement: "Cholinesterase assays (plasma and blood) were performed on all dogs twice prior to initiation, monthly thereafter and at termination." This is contradicted in the Materials and Methods section (3.9-Clinical Chemistry, Special) and in the data tables, where it is indicated that these assays were conducted pretest and at 2 and 4 months and at termination. Indeed, it is unfortunate that cholinesterase activity was not measured more frequently (especially since the dogs were bled monthly for routine clinical chemistry and hematology tests), since the data at hand are so variable as to be difficult to interpret in terms of meaningful treatment-related effects. Nevertheless, it is recognized that all clinical pathology tests on blood samples were performed more frequently in this study than required under FIFRA regulations. Statistically significant ($p < 0.05$) plasma cholinesterase depression occurred in high dose males at 2, 4, and 12 months and in all methyl parathion-treated female groups at 4 months. RBC cholinesterase inhibition was also statistically significant in mid and high dose males at 2 months and in all methyl parathion-treated male and female groups at 12 months. If mere statistical significance were to be relied upon to determine "toxicity" at the various dose levels, this study would have no NOEL; i.e., plasma cholinesterase activity at the lowest dose tested (0.03 mg/kg/day) was depressed in females at 4 months compared to control levels, and RBC cholinesterase activity was reduced in low dose males and females at 12 months. On the other hand, the authors of the study report argue (p.28):

"Even though there appears to be statistically significant differences at different intervals throughout the study between the various mean test cholinesterase values and the corresponding control values for plasma and red blood cell cholinesterase, we do not consider them to be biologically signifciant because all mean values fell within PRL's normal biological range."

Certainly, no clear-cut toxicologic effect was demonstrated at the highest dose, although some degree of RBC and/or plasma cholinesterase depression -- if only transient -- is apparent in high dose males and females. Data borrowed from the subchronic dog study lend support to the proposition that RBC and plasma cholinesterase are not greatly affected by methyl parathion at levels up to and including 0.3 mg/kg body weight/day. However, the possibility cannot be overlooked that the depressed plasma cholinesterase activity in high dose males may reflect subtle hepatic toxicity, with mild liver weight changes in this group offered as corroborative evidence.

TABLE 3 - MEAN CHOLINESTERASE ACTIVITY VALUES* (± S.D.)

Plasma Cholinesterase (IU/1)

Dose (mg/kg/day)	Day 0 Initial	Day 0 Repeat	Month 2	Month 4	Month 12	Brain Cholinesterase (IU/1) Month 12
MALES						
0	1588+226	1590+234	2229+307	1851+241	2068+254	447+54
0.03	1541+277	1559+286	2214+324	1804+244	1830+385	435+103
0.1	2229+778†	2253+774†	2414+244	2003+274	1783+221	482+140
0.3	1759+280	1916+565	1849+257†	1529+178†	1365+260†	837+115†
FEMALES						
0	1705+234	1620+219	2116+235	2356+138	1624+169	717+557
0.03	1916+630	1798+585	2124+339	1898+271†	1675+240	780+310
0.1	1823+161	1715+146	2256+245	1699+278†	1797+213	658+444
0.3	1575+289	1571+339	1835+246	1223+80†	1610+217	562+140

RBC Cholinesterase (IU/1)

Dose (mg/kg/day)	Day 0 Initial	Day 0 Repeat	Month 2	Month 4	Month 12	
MALES						
0	1874+293	1687+236	2284+160	2502+451	2147+380	
0.03	1716+204	1571+349	2205+349	2432+464	1700+263†	
0.1	1997+364	1515+200	1974+187†	2263+360	1465+193†	
0.3	1658+125	1775+363	1877+135†	1990+229	1741+155†	
FEMALES						
0	1697+279	1726+256	2782+266	2225+276	2276+329	
0.03	1860+439	1798+305	2643+313	2089+372	1609+138†	
0.1	1808+213	1947+210	2407+400	2062+284	1616+153†	
0.3	1697+196	1796+301	1832+314	1804+233	1764+196†	

* n= 8
† Statistically different from control value, p<0.05

6
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