9-4-84

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003949

9/4/84

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Ethyl Parathion and Methyl Parathion, Toxic Effects on the Retina of Rats in Two-Year Feeding Studies.

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

FROM: Robert P. Zendzian PhD, Acting Head
Review Section III
Toxicology Branch
HED (TS-769)

8/29/84

THROUGH: William Burnam, Chief
Toxicology Branch

WSB 9-4-84

Compound Ethyl Parathion Tox Chem #637

Registration #524-27, 132 & 144 Registrant Monsanto

Accession #252702, 703, 704 & 705

Action Requested

Review of a two-year rat chronic/oncogenicity feeding study.

Compound Methyl Parathion Tox Chem #372

Registration #524-68, 126 & 144 Registrant Monsanto

Accession #252501, 502 & 503, 253346, 253372, 373 & 374

Action Requested

Review of a two-year rat chronic/oncogenicity feeding study.

Recommendation

Two-year chronic feeding studies in rats on methyl and ethyl parathion have been received by the Agency and reviewed by Toxicology Branch. The studies have demonstrated bilateral retinal degeneration by direct opthalmological and histopathological examination in the respective high dose groups (5ppm in each study). Toxic effects on the eye by organo-

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phosphate pesticides has been observed in Japan where extensive human poisoning produced a syndrome of effects on vision ranging in severity from myopia to congestion or atropy of the optic nerve. The Japanese researchers were able to duplicate the effects on refraction in an experimental study on dogs utilizing the organophosphate ethylthiometon. Experimental studies with the organophosphate fenthion on rats by another Japanese scientist demonstrated a syndrome of toxic effects on the eye beginning with functional abnormalities in electrical activity and columinating in retinal degeneration following chronic dosing. Considering the seriousness of the effects demonstrated by ethyl and methyl parathion in the rat feeding studies and the history of human effects of organophosphate pesticides from Japan, it is recommended that these two compounds be refered to Special Review and the existing uses of the compounds' be reconsidered in light of their irreversable occular toxicity.

Background

In Japan agriculture is a labor intensive process designed to produce high quality produce from small fields closely interspaced with living areas. In the early 1950s organophosphate pesticides were introduced to Japanese agriculture and their use rapidly increased until the quantity applied per unit area was the highest used in the world, five to ten times that used in Europe and the United States. Starting in 1957, an increase in the incidence of myopia was observed in Japanese school children. This myopia could not be corrected with eye glasses. The increase showed peaks of incidence in 1964-1965, 1969 and 1973, particularly in junior high school girls. Ishikawa and his coworkers performed a series of medical and epidemiological studies in the agricultural region of Saku district which showed a strong positive correlation between the incidence of the ocular syndrome and the massive application of organophosphate pesticides (Ishikawa and Miyata 1980). The ocular syndrome was not typical of myopia, being generally more severe, accompanied by vertical astigmatism, concentric narrowing of the visual field, and abnormal eye movements. It was not correctable. Additional observations included lowered activity of serum cholinesterase, neurological abnormalities cnaractoristic of anticholiiesterase poisoning and relatively high levels of organophosphate insecticides in the blood of the patients compared with normal individuals from other areas.

The Japanese Ministry of Health and Welfare investigated the toxic effects of organophosphate insecticides on the general population (Shikano 1972), and identified the same ocular syndrome in several farming areas of Japan in adults as well as children.

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Ishikawa and his coworkers were able to produce myopia with vertical astigmatism in a two-year study of ethylthiometon in dogs. This effect was detected by measuring the shape of the refractive surface of the eye. The results of the study are summerized briefly in Ishikawa and Miyata (1980). A reduction in erythrocyte acetylcholinesterase activity was the only additional sign of toxicity. Histopathology revealed a dose-related destruction of the cilliary muscle. Doses ranged from 5 to 15mg/kg/day.

Imai reported a series of studies on the effect of fenthion on the retina of the male rat (1974, 1975 & 1977). A single subcutanious dose as low as 0.005mg/kg produced detectable changes on the electroretinogram (ERG). Administration for one year of 50mg/kg, subcutaniously, twice weekly extinguished the ERG and produced gross and histological abnormalities in the retina.

In the first study (1974) male rats were given a single SC dose of 0.005, 0.05, 0.5, 5.0, 25, 50, 100 or 500mg/kg. On the fourth day after dosing, ERG recordings were made and cholinesterase activity in the retina and brain determined. All animals in the highest dose died before the tests could be made. Compound induced changes in the ERG were seen at all doses. Electrical activity was faciltated in doses up to 5.0mg/kg and depressed in the higher doses. Dose related decreases in cholinesterase activity was observed in retina and brain beginning at 0.5mg/kg and activity was essentially nil at 100mg/kg.

Imai's second study (1975) showed that the effect of a single dose of fenthion on the eye could last for at least 66 days. Male rats were given a single SC dose of 5, 25 or 50mg/kg and the ERG taken periodically for up to 66 days. Effects were seen at all doses, but only the animals dosed at 5mg/kg showed recovery from the compound effect at 30-49 days after dosing.

In the third study (1977) male rats were given a SC dose of 50mg/kg twice weekly for one year. ERGs were taken periodically during the study. The ERG was severely depressed after three months treatment and by 12 months no activity was recordable. Visable chances in the ocular fundus were observed by the ninth month and at 12 months severe degenerative changes were apparant. Treated animals sacrificed at three months showed no abnormalities under the light microscope but at 12 months degenerative changes were clearly visable.

New Data

1) Ethyl Parathion.

A two-year chronic study of ethyl parathion was performed in rats. The compound was supplied in the feed at concentrations of 0.5, 5.0 and 50ppm. At the 24 and 28 month observations, retinal degeneration was observed by the veterinary opthalmologist in a total of seven females at the high dose. Five of these were bilateral and two unilateral. At the same time four females in the control group showed retinal degeneration, two bilateral and two unilateral. The percent incidence, combining observation periods, was 12.5% controls and 22.6% high dose The effect was not observed in the low and intermediate dose females nor in any males.

Histopathology revealed a much higher incidence of retinal degeneration/atropy in the female rats at the high dose as shown in table 2 from the DER. The histopathology detected 2.5 times as many lesions in the controls and 3 times as many in the high dose females as well as detecting lesions in the low and intermediate dose females. Percent incidence was 17% controls, 8% 0.5ppm, 10% 5ppm and 43% 50ppm. Lesions were observed in the males but there was no compound-related effect.

Table 2. Ethyl parathion. Retinal degeneration/atropy by histopathology in female rats. (from the report page 19 and appendix L-5)

Group	I	II	III	IV
# of rats examined	54	50	52	49
Retinal atropy, unilateral	5	4	3	9
*Percent	9	8	6	18
Retinal atropy, bilateral	4	0	2	12
*Percent	7	0	4	24
TOTAL	9	4	5	21
*Percent	17	8	10	43

*calculated by the reviewer

2) Methyl Parathion

A two-year chronic study of methyl parathion, identical to that performed with ethyl parathion, was performed in rats. The compound was supplied in the feed at concentrations of 0.5, 5.0 and 50ppm. At the 24 month observation, retinal degeneration was observed by the veterinary opthalmologist in 19 females at the high dose. At month 28, 10 females showed retinal degeneration. No additional females showed the effect at 28 months so that 15 was the total number detected for an incidence of 43% (15/35). No effect was observed in the males.

Histopathology revealed a higher incidence of retinal degeneration/atropy in the female rats at the high dose as shown in the table from the DER. Histopathology detected lesions in the controls, low and intermediate dose females which were not detected by the opthalmologist and an additional five high dose females. Percent incidence was 5% controls, 5% 0.5ppm, 5% 5ppm and 36% 50ppm. Lesions were not observed in the males.

Table. Methyl parathion. Retinal degeneration/atropy by histopathology in female rats. (from the report page 20)

Group	I	II	III	IV
#of rats examined	59	60	60	55
Retinal atropy, unilateral	3	1	1	2
*Percent	5	2	2	4
Retinal atropy, bilateral	0	2	0	18
*Percent	0	3	0	33
TOTAL	3	3	1	20
*Percent	5	5	2	36

*calculated by the reviewer

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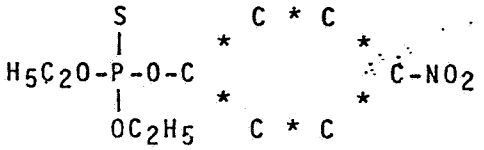
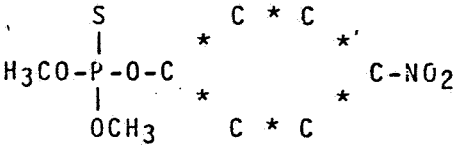
Discussion

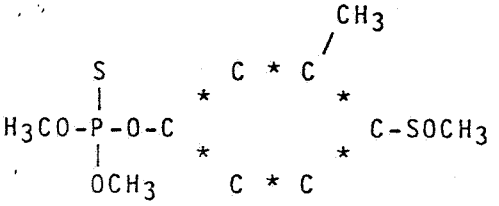
The structural formulas presented below are for the two parathion compounds for which chronic studies are reviewed herein and the two compounds studied in detail for toxic eye effects by the Japanese researchers. Structural relationships are obvious in the first three compounds but even in ethylthiometon the $-CH_2CH_2SC_2H_5$ group can assume a quazi-ring structure under certian solvent conditions.



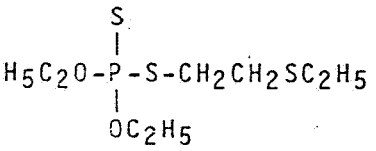
Ethyparathion



Methylparathion



Fenthion



Ethylthiometon

The critical consideration is whether the parathions will produce structural and functional effects at lower doses then those which produced retinal atropy in the chronic rat studies.

Fenthion produced retinal atropy, detected by histopathology, in 100% of the treated male rats at a dose of 50mg/kg twice weekly for one year. Considering that this compound is an irreversable cholinesterase inhibitor and assuming that the retinal effect is secondary to cholinesterase inhibition one may estimate that this dose regimen is equilivant to a daily dose of 14.25mg/kg.

Ethyl and methyl parathion produced retinal atropy, detected by histopathology, in 43% and 36% respectively of the female rats treated for 2 years at 50ppm or 2.5mg/kg/day. They did not produce the effect in the males indicating that the males are the least sensitive sex.

Considering this information, a dose of 14.25mg/kg.day producing 100% effect in a year in the least sensitive sex is not much larger biologically then a dose of 2.5mg/kg/day producing 36 to 43% effect in two years in the most sensitive sex. Adding the close structural relationship of fenthion and the parathions one can reasonably assume that lower doses of the parathions will produce the same effects on electrical activity (functional activity) as the lower doses of fenthion.

As to the effect on the shape of the eye which produces the myopio and astigmatism, this effect was the common factor of the organophosphate toxicity observed in Japan and it is reasonable to expect that the parathions will produce this effect.

The information available strongly indicates that ethyl and methyl parathion are capable of producing a spectrum of serious effects on the eye columinating in the retinal atropy which was detected in the two chronic rat studies submitted. The low dose effects are subtle and not easily distinguished from common abnormalities of the human visual system.

Note. An extensive literature on these toxic effects in Japan is available in the Japanese scientific literature. Copies of many of these papers, in Japanese and in translation, are on hand in the Toxicology Branch and are familar to the author of this memo.

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Imai, H., Toxicity of Organophosphorus Pesticide (Fenthion) on the Retina. Electroretinographic and Biochemical Study. Acta Soc. Ophthalm. Jap. 78 (4):163-172, 1974

Imai, H., Research on the Ocular Toxicity of Organophosphorus Agents. Report 2. Residue Properties on the Rat After One Time Administration of Baytex (Low Toxicity Organophosphorus Agent) (Especially ERG Changes and Changes Over Time in the Serum, Liver, Retina Cholinesterase lActivity) Acta Soc. Ophthalm. Jap. 79(8):1067-1076, 1975

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DERs

Two-Year Chronic Feeding Study of Ethyl Parathion in Rats. I.W. Daley & G.K. Hogen, Biodynamics Incorporated, Project # D B 78-005, Study #: 77-20-55 Jan. 23, 1984

A Two-Year Chronic Feeding Study of Methyl Parathion in Rats. I.W. Daley, Biodynamics Incorporated, Project # 77-2060 Dec. 22, 1983

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 2-year methyl parathion rat study

TO: Robert Zendzian, Ph.D.
Toxicology Branch
HED (TS-769)

FROM: Byron T. Backus
Toxicology Branch
HED (TS-769)

Byron T Backus
07-12-84

Tox Chem # 372

Registrant: Monsanto

Action

The registrant has submitted a 2-year rat feeding study.

Conclusions:

1. As an oncogenicity study this is core minimum data. Under the study conditions there was no evidence of oncogenicity in rats at levels up to 50 ppm (HDT).

2. As a 2-year feeding study the classification is core supplementary data. A NOEL for neurologic changes was not defined.

3. The neurologic effects seen in this study are particularly pronounced at the HDT (50 ppm). A small sampling of females from this level showed a significantly lower proportion of normal nerve fibers relative to controls. Sciatic nerve preparations from high-dose males showed loss of myelinated nerve fibers, and "more myelin sheath degeneration and Schwann cell proliferation."

   The retinal atrophy and related effects (posterior subcapsular cataract) seen in high-dose females are of particular concern.

4. Because of the neurologic effects, this material is recommended for special review.

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5. The RBC ChE NOEL is set at 0.5 ppm, with an LEL of 5.0 ppm.

6. Except for neurologic changes the NOEL = 0.5 ppm, with an LEL = 5.0 ppm (abnormal gait in one female, slight to moderate decreases in mean hemoglobin, hematocrit and erythrocyte levels in males at 24 months, slight RBC ChE depression in both sexes).

Discussion:

Under the conditions of this study there was no evidence of oncogenicity in Sprague-Dawley CD® rats of either sex as a result of dietary exposure to the test material at levels of up to 50 ppm.

As a 2-year feeding study a NOEL is not defined for neurologic changes. Paraffin-embedded sciatic nerve preparations from 1/5 male rats in the low-dose group and 1/5 in the median-dose group are reported as showing moderate degenerative changes.

Additionally, while nerve preparations from females in low and mid-dose groups did not appear to differ from controls, no fiber spectra were prepared and numbers of fibers were too low for statistical analysis. The effect at 50 ppm was well defined, as there was a significantly lower proportion of normal nerve fibers from high-dose females than from controls, and the fiber distribution peaked at 11-12 "mm" (presumably this is a typographical error for um, or microns) compared to 8-9 "mm" in controls.

Pre-exposure cholinesterase measurements were taken only from a group of 10M, 10F rats which were then sacrificed. This one group supplied an initial reading which was used for each of the other four groups (as a result, time zero ChE activities for all 4 groups are the same). No pre-exposure ChE activities were actually obtained from the rats subsequently used. Also, no ChE activities were taken during the first four months of the study when high-dose females were frequently showing tremors. It is likely that this was the period when the greatest amount of ChE inhibition occurred.

However, after examining the data it is this reviewer's conclusion that there is sufficient information to set the plasma and brain ChE NOEL's at 5.0 ppm, and the LEL's at 50 ppm. One animal (#412) among the low-dose females had an extremely low brain ChE value at termination; as now low values were observed in any of the mid-dose rats, and as the plasma activity in #412 at termination was normal, it is doubtful that this low brain ChE activity was a result of exposure to the test material.

For RBC ChE, in males at months 6, 12 and at term average activities were: low-dose group > mid-dose group > high-dose group. At 6 and 18 months averages for controls were somewhat greater (but this was not statistically significant) than averages for the low-dose group. At 12 months and at term averages for controls and the low-dose groups were essentially identical. Average RBC ChE activities in females at 6, 12 and dose group. Female control RBC ChE averages were lower than those of the low-dose group at 6 and 12 months; as no pre-exposure RBC ChE values were obtained from these specific subjects, the possibility exists that the control group had a lower "normal" average RBC ChE activity than did any of the other groups, and this might have been detected by a pre-exposure reading. Because of the trends seen in the data, the RBC ChE LEL must be set at 5.0 ppm, and the NOEL at 0.5 ppm.

There is a minor discrepancy relating to the bilateral retinal degeneration in high-dose females. The report dated May 15, 1982 from Lionel F. Rubin V.M.D. identifies 15 subjects as showing bilateral retinal degeneration, including #826 and #832. However, the individual data (Appendix L, table III) do not indicate this condition was found in the individuals designated #4527 (=826) or #4533 (=832) although in the latter the gross postmortem observations note that the right cornea appeared destroyed. However, it makes little difference whether 18 or 20 female rats in the high-dose group showed this effect, as in either case its occurrence is clearly dose-related.

Except for neurologic changes the NOEL = 0.5 ppm. The LEL is 5.0 ppm, on the basis of abnormal gait in one female, slight to moderate increases in mean hemoglobin, hematocrit and erythrocyte levels in males at 24 months, slight RBC ChE depression in both sexes.

Effects at 50 ppm not seen at 5 ppm include greater incidence of alopecia (particularly in females), bilateral retinal degeneration (females only), marked brain and plasma ChE inhibition (both sexes), occasional irritability (both sexes), body tremors (almost exclusively in females), greater incidence of yellow stains in the ano-genital area, reduced incidence of chromodacryorrhea, soft stools (particularly near termination), higher food consumption on a body weight basis (in males from weeks 2 through 16, in females throughout the study after week 2).

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Data Evaluation Report

Compound:

Methyl parathion

Citation:

Daly, I. W. A Two Year Chronic Feeding Study of Methyl Parathion in Rats. Project No. 77-2060, dated 12-22-83. Study conducted at Bio/dynamics Inc. P.O. Box 43, East Millstone, NJ 08873. Received at EPA 2-23-84; in Acc. 252501, 252502, and 252503. Additional material was received at EPA 5-22-84, and is in Acc. 253346, 253372, 253373, and 253374.

Reviewed by:

Byron T. Backus
Toxicologist

Byron T. Backus 07-10-84 OK 7/11/84

Core Classification:

As an oncogenicity study: Core Minimum Data
As a 2 year feeding study: Core Supplementary Data

Product Classification:

N/A

Conclusions:

1. The study is acceptable as demonstrating there is no oncogenicity at 50 ppm (HDT).

2. The study does not define a NOEL for neurologic changes.

3. Preexposure cholinesterase readings were taken only from a group of 10M and 10F rats which were then sacrificed. This one group supplied an initial reading which was used for each of the 4 other groups. No preexposure ChE activities were actually made on the rats which were subsequently used.

   No ChE activities were measured during the first four months of the study when high-dose females were frequently showing tremors.

   Because of these factors, as well as trends observed in the data, the RBC ChE NOEL is set at 0.5 ppm and the LEL is set at 5.0 ppm.

4. Except for neurologic changes the NOEL = 0.5 ppm. Effects observed at 5.0 ppm include abnormal gait in one female, slight to moderate decreases in mean hemoglobin, hematocrit and erythrocyte levels in males at 24 months, slight RBC ChE depression in boths sexes.

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5. Effects at 50 ppm not seen at 5 ppm include greater incidence of alopecia (particularly in females), bilateral retinal degeneration (females only), marked brain and plasma ChE inhibition (both sexes), reduction in mean body weight (both sexes), occasional irritability (both sexes), body tremors (mostly in females), greater incidence of yellow stains in the ano-genital area, reduced incidence of chromodacryorrhea, soft stools (particularly near termination), higher food consumption on a body weight basis (in males from weeks 2 through 16, in females throughout the study after week 2).

Materials:

Sprague-Dawley CD® Rats (obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts), 4 weeks old when received; 6 weeks old at initiation treatment

Methyl Parathion - identified as lot AK 0911; 93.65%.

Procedure:

Rats were assigned randomly to four groups, each consisting of 60 males and 60 females. Groups received 0, 0.5, 5 or 50 ppm methyl parathion in the diet until termination (month 26 for males, and month 28 for females).

Two additional groups, each consisting of 10 males and 10 females, were bled and sacrificed for 1) pretest clinical laboratory measurements and 2) determinations of cholinesterase (plasma, RBC and brain) activities.

Animals were observed twice daily and examined weekly. During pre-exposure individual body weights were determined twice and food consumption was determined once. During exposure weights and food consumption were determined weekly through week 14 and biweekly thereafter. Ophthalmoscopic examinations were conducted on all animals pretest, at 3, 12 and 24 months and on females only at one week before termination (28 months). Blood samples were taken via venipuncture of the orbital sinus under light ether anesthesia from 10 animals/sex/group at 6, 12, 18 and 24 months; insofar as was possible the same individuals were used for all blood samplings. Urine samples were taken from these same animals 1-8 days before blood was taken. The following observations were made:

Hematology:

- Hemoglobin
- Hematocrit
- Erythrocytes (count and morphology)
- Platelets
- Total and differential leukocytes
- Reticulocytes

Clinical Chemistry:

glutamic oxaloacetic transaminase	blood urea nitrogen	globulin
glutamic pyruvic transaminase	fasting glucose	A/G ratio
alkaline phosphatase	cholesterol	potassium
lactic acid dehydrogenase	total protein	calcium
total and direct bilirubin	albumin	

Urinalysis:

gross appearance	glucose	occult blood
specific gravity	ketones	urobilinogen
pH	bilirubin	microscopic analysis
protein		

Preexposure plasma, RBC and brain ChE activities were made on 10 rats/sex which were sacrificed. Plasma and RBC ChE activities were determined for 10 rats /sex/group at months 6, 12, 18 and at termination. Brain ChE activities were determined at termination. No preexposure ChE measurements were made on these subjects. Except at termination when some groups had fewer than 20 rats/sex, samples were normally taken from different animals than those from which blood was taken for hematology.

All animals dying spontaneously, accidentally, sacrificed in a moribund condition, or at termination were given a "complete gross postmortem examination."

The following organs were preserved; those marked with an asterisk were also weighed. Organ/body and organ/brain weight ratios were calculated (except when masses, cysts, or morphologic abnormalities were present):

adrenal(2)*
aorta (abdominal)
blood smear (taken from all animals; examined only if anemia, enlarged thymus, lymphadenopathy or hepatosplenomegaly was present).
bone and bone marrow (costochondral junction).
brain (3 sections including frontal cortex and basal ganglia, parietal cortex and thalamus and cerebellum and pons).*
esophagus
eye (2-with optic nerve and contiguous Harderian glands).
head (with nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx and middle ear).
heart*
intestine (with cecum, colon, duodenum, ileum, and jejunum).
kidney (2)*
liver (2 sections)*
lungs
lymph node (mediastinal and mesenteric)
mammary gland (right inguinal)
nerve (right sciatic)
ovary (2)*
pancreas
parathyroid
pituitary*
prostate
salivary gland (mandibular)
seminal vesicles
skeletal muscle (right biceps femoris)
skin (with mammary gland)
spinal cord (cervical, lumbar)
spleen
stomach
testis (2-with epididymis)*
thymus
thyroid
urinary bladder
uterus (body and cervix)
gross lesions (including a section of normal-appearing portion of same tissues).
tissue masses or suspect neoplasms and regional lymph nodes

To evaluate possible neurotoxic effects, 5 animals/sex/group were sacrificed during week 106 and specially treated for examination of brain, spinal cord and sciatic nerve. Five sections of brain, cervical and lumbosacral spinal cord, as well as cross and longitudinal sections of sciatic nerve, were prepared from each animal.

Body weight, food consumption, hematology values, clinical chemistry values, terminal organ and body weights, organ/body and organ/brain weight ratios were analyzed by a variety of statistical procedures.

Results:

Physical observations for low- and mid-dose males were unremarkable throughout the study. Tremors occurred in eight high-dose males during week 3, in one during week 6, and in one near study termination. Tremors occurred frequently in all high-dose females during the first 4 months of exposure, but thereafter only sporadically.

Alopecia was observed in all groups, occurring most frequently in high-dose females. The following incidences were calculated from animals showing the symptom/animals alive at that date:

		WEEK				
		26	52	78	100	average 4-term
Males:	control	0.15	0.179	0.222	0.375	0.169
	low	0.121	0.107	0.154	0.2	0.152
	median	0.119	0.158	0.268	0.4	0.202
	high-dose	0.233	0.2	0.317	0.429	0.247
Females:	control	0.067	0.068	0.115	0.326	0.121
	low	0.117	0.067	0.070	0.459	0.150
	median	0.117	0.083	0.074	0.108	0.098
	high-dose	0.533	0.500	0.509	0.705	0.478

Yellow ano-genital staining was consistently more frequent in high-dose males and females than in any other group.

Abnormal gait was observed during weeks 22-26 in a few females in the high-dose group, and then again in this same group after week 48. The incidence in this group was consistently above 20% during weeks 87 through 108. It also is reported as occurring in one female of the median-dose group from week 87 through termination. Among males the symptom was rarely seen and did not occur outside the high-dose group.

Irritability was occasionally observed in a few high-dose males and females, but not in any other group.

Chromodacryorrhea occurred less frequently among high-dose males and females, and to some extent among median-dose females, than among controls. It is assumed that it occurs as a normal (protective?) response to irritation, and its lower incidence in these exposure groups is of interest because of the occurrence of retinal atrophy in high-dose females at termination.

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Increased survival to termination was observed for both males and females in the high-dose group. In the case of females this increased survival is probably significant, possibly correlating with lower body weight.

High-dose males consistently averaged less weight than other groups after exposure began. This was significant with respect to controls from weeks 1 through 50 (with the exception of week 26 when number of animals decreased, due to exclusion of rats which were used for blood sampling). Average weight for high-dose females was also consistently lower than other groups; this was significant with respect to controls during weeks 1-22 and sporadically thereafter. There was no significant difference in average weights for males and females of the two lower exposure groups with respect to their corresponding controls.

Food consumption averages (on a body weight basis) for high-dose males were consistently greater than those of the other 3 groups from weeks 1 through 16, but after week 20 there was no significant difference between the 4 groups. For females, food consumption (on a body weight basis) in the high-dose group was, after the first two weeks, consistently above that of any of the other 3 groups, and it was frequently significantly so. Group IV females frequently averaged more food consumption during weeks 3-40 than the other 3 groups. During the first two weeks food consumption in group IV females was considerably below that observed in the other 3 groups, both in quantity and on a body weight basis.

On ophthalmoscopic examination there was no evidence of compound-related eye effects at months 3 or 12. At month 24 15 (out of 35 alive at that date) group IV females showed bilateral retinal degeneration. One week before termination 10 (out of 24 alive at that date) group IV females showed bilateral degeneration; and 10 (5 of which did not show bilateral retinal degeneration) had posterior subcapsular cataract in one or both eyes.

Hematology: High-dose females consistently showed significantly (usually $p \leq 0.01$) lower averages for hemoglobin and hematocrit than did controls. Except at 12 months, high-dose females averaged significantly lower RBC counts than controls. High and mid-dose males at 24 months averaged significantly lower HCT and RBC than did controls but this was not statistically significant. Effects were most pronounced near study termination.

Averages with respect to controls:

	High-dose females			Mid-dose males			High-dose males		
	HGB	HCT	RBC	HGB	HCT	RBC	HGB	HCT	RBC
6 months	-8.0%§	-9.3%§	-5.1%§	+1.9%	+2.3%	+1.5%	-1.3%	0.0%	+0.5%
12 months	-7.2%§	-7.1%*	-4.3%	0.0%	0.0%	-0.5%	+1.3%	0.0%	+1.1%
18 months	-13.0%§	-13.0%§	-10.6%§	+1.9%	+2.3%	+4.9%	-1.9%	-2.3%	-1.0%
24 months	-25.9%§	-27.9%§	-27.6%§	-14.8%	-16.7%*	-17.3%*	-16.1%	-16.7%*	-17.2%*

*significant at $p \leq 0.05$

§significant at $p \leq 0.01$

Clinical Chemistry: High-dose males showed the following differences with respect to control values: at 6 months glucose was lower; at 12 months BUN was lower. High-dose females average significantly lower albumin, albumin/globulin and total bilirubin with respect to control values. Findings were significant at $p \leq 0.05$.

Urinalyses: All observations were generally unremarkable.

Cholinesterase: High-dose males and females consistently averaged significantly ($p \leq 0.01$) less plasma cholinesterase activity than controls. Cholinesterase activities (values with respect to controls on these dates):

	High-dose males			High-dose females		
	Plasma	RBC	Brain	Plasma	RBC	Brain
6 months	-71.4%	-13.0%	-	-86.2%	0.0%	-
12 months	-66.7%	-16.1%	-	-87.1%	- 8.5%	-
18 months	-75.0%	-19.8%	-	-88.5%	-14.0%	-
termination	-81.8%	- 9.0%	-75.9%	-82.4%	- 5.0%	-79.1%

mid-dose males at 6 months averaged 8.7% RBC ChE depression.

Histopathology: The only lesion observed considered to be compound-related was present in the eyes of high-dose female rats, and consisted of unilateral and bilateral retinal atrophy (also observed ophthalmoscopically). From the table on p. 20:

Group	I	II	III	IV
# of rats examined	59	60	60	55
retinal atrophy (unilateral)	3	1	1	2
retinal atrophy (bilateral)	0	2	0	18
TOTAL	3	3	1	20

Organ weights: At termination brain weights and brain to body weights for high-dose males averaged higher than control males, but neither was statistically significant. Average liver and heart weights of high-dose females were higher than those of controls, but there was no statistical significance in either case; however, the higher liver and heart to body weight ratios were statistically significant.

Possible neurotoxic effects:

Brain and spinal cord: Findings from microscopic examination of five sections of brain and two sections of spinal cord from each of 10 high-dose rats sacrificed at week 106 were similar to those obtained from controls.

Nervous tissue: "In the paraffin embedded sciatic nerve preparations from the treated male rats, there was loss of myelinated nerve fibers which was most apparent in those from group IV. The male rats in this high dose group also had more myelin sheath degeneration and Schwann cell proliferation. Similar changes of less severity were seen in lower incidence in the mid and low dose males and in the controls with only one animal (Number 337) in the low dose and one in the mid dose (Number 506) having more severe changes than in the controls."

In the teased fiber examination, two high-dose males showed the most evidence of myelin sheath degeneration.

For females, high-dose animals had more fibers with myelin corrugations ($p \leq 0.05$), more mylein ovoids ($p < 0.05$), and fewer normal, not remarkable, fibers (p reported as ≤ 0.005). In high-dose females the fiber spectrum peaked around 11-12 "mm" (presumably this is um, or microns, rather than millimeters) diameter, as opposed to 8 to 9 "mm" in controls.

Discussion:

The more frequent incidence of tremors in high-dose females during the first 4 months of exposure correlates with the higher average amount of compound intake (>36 mg/kg/day) which occurred in this group through week 16.

During the first two weeks group IV males averaged almost as much food consumption (on an absolute basis) as did males of the other 3 groups. However, they showed less weight gain, particularly during week 1:

	Week 1		Week 2	
	Av. food consumption (g)	Av. weight change (g)	Av. food consumption (g)	Av. weight change (g)
Group I	77.3	+45.5	81.0	+45.8
Group II	77.3	+41.6	80.2	+44.6
Group III	77.2	+43.9	80.8	+48.3
Group IV	73.4	+25.9	79.0	+40.3

In females there was less food consumption during weeks 1 and 2, and a dramatically reduced gain in weight:

	Week 1		Week 2	
	Av. food consumption (g)	Av. weight change (g)	Av. food consumption (g)	Av. weight change (g)
Group I	53.4	+17.7	57.6	+19.0
Group II	53.5	+17.5	56.0	+19.7
Group III	53.6	+18.7	55.9	+20.2
Group IV	44.6	- 2.2	45.0	+ 8.1

For initial dietary exposure to methyl parathion, the female data suggest some reduced palatability at 50 ppm; the male data suggest metabolic effects at this exposure level.

The emaciation in 28.3% of the high-dose females at week 4 was presumably related to reduced food consumption and metabolic effects. However, emaciation was not observed in this group at week 8, probably because food consumption in this group had increased. Towards the end of the study, high-dose females showed a slightly greater incidence of emaciation than did controls and rats of the other two exposure levels. A few males also were emaciated near the end of the study, with the condition mostly occurring in the high-dose group.

At termination high-dose males averaged 653 g; controls averaged 728 g, but this was not statistically significant. Brain weights and brain to body weight ratios for high-dose males averaged higher than control males, but

neither was statistically significant. Trends of decrease in average testes and kidney weights, and increase in average heart weights with increased exposure were not statistically significant. No trends or statistically significant average weight differences were observed for pituitary, adrenals or livers.

One probable reason for lack of statistical significance in the "trends" was the comparatively low number of males (9 to 15/group) at termination.

At termination high-dose females averaged 378 g; controls averaged 410 g; this is reported as not statistically significant. Brain weights and brain to body weight ratios of high-dose females were higher than those of controls, and this was statistically significant. Average liver and heart weights of high-dose cance in either case; however, the higher heart and liver to body weight ratios were statistically significant.

Hematology: The low averages for mid-dose males at month 24 were due to extremely low readings obtained for HGB and HCT from two males of this group, lower than those obtained from any other male in any other group.

At 24 months, 2 males (#507, 509) in the median-dose group and one (#701) in the high-dose group showed high (>50 x $10^3/mm^3$) WBC counts. #507 had a 5.19 x 10^5 WBC/mm^3, mostly (91%) blast cells, suggesting a blood dyscrasia.

At 24 months, 3 males and 2 females in the high-dose group showed elevated platelet counts (>1.6 x $10^6/mm^3$). Values this high had been sporadically seen before, although never in more than one animal/sex/group on any prior date.

Clinical Chemistry: Although there were differences which were significant at $p \leq 0.05$, there was no consistency and no obvious trends. These findings are reported (and can be accepted as) incidental.

Cholinesterase: The most obvious problem is that preexposure readings were derived from a single group of 10M and 10F, and these were then sacrificed. No preexposure readings were taken from the rats which were subsequently used. The importance of baseline (preexposure) data can be best illustrated by examining rankings (1 = highest; 10 = lowest) for plasma ChE activities at different times:
Males - group I (controls)

Animal #	6 months	12 months	18 months
104	9	9	6.5
105	5	6	*
108	5	3	1
119	5	4.5	*
129	7	7	*
132	1.5	1.5	2
146	1.5	1.5	*
151	3	4.5	3.5
154	9	8	*
159	9	10	8.5

*dead at 18 months; another animal used as replacement. Numbers at 18 months reflect rankings among the total group of 10 animals.

Females - group I (controls)

Animal #	6 months	12 months	18 months
209	3.5	2	2
210	9	7	*
216	6.5	6	10
217	2	4.5	4
223	8	4.5	5
230	3.5	3	3
233	6.5	8	8
235	10	9	9
252	5	10	6.5
259	1	1	1

*dead at 18 months; another animal used as replacement. Numbers at 18 months reflect rankings among the total group of 10 animals.

It is obvious in the above that there is a strong consistency in relative rankings with respect to plasma ChE activities for individual animals during the period 6-18 months. Given this, there is a strong possibility that the plasma ChE activities at preexposure would also correlate with subsequent activities.

Histopathology: It is reported that 18/55 females in group IV showed bilateral retinal atrophy. Individual animal numbers (from table III) were 805, 809, 812, 813, 815, 818, 825, 827, 831, 833, 836, 839, 840, 844, 848, 850, 852 and 856. Rats 832 and 835 showed unilateral retinal atrophy. The report of May 15, 1982 from Lionel F. Rubin V.M.D. identifies 15 subjects of group IV as showing bilateral retinal degeneration. In addition to the subjects listed above 826 and 832 are on this list; 805 is not (#809, 818 and 848 are also not listed, but they had died or been used for interim sacrifice before this examination). If 826 and 832 showed bilateral retinal atrophy, this would further raise its incidence. The report of July 21, 1982 from Lionel F. Rubin V.M.D. lists 10 subjects showing (at termination) bilateral retinal degeneration. Although #805 is reported as showing subcapsular cataract in the right eye, this rat is not reported as showing unilateral retinal degeneration.

However, it makes little difference whether 18/55 or 20/55 rats in this group showed the effect. One week before termination 15 (out of 24) group IV females showed retinal degeneration and/or posterior subcapsular cataracts in one or both eyes. These effects are clearly related to administration of the compound.

It is disturbing that the mechanism causing retinal degeneration is not defined, particularly as it may be a manifestation of an effect which occurs at lower exposure levels.

Neurology: In the rats examined at week 106, the signs of myelin sheath degeneration were most pronounced (degree of myelin sheath ballooning, loss of myelinated fibers, segmental myelination, remyelinated fibers, Schwann cell proliferation, myelin phagocytosis and presence of cholesterol clefts) in distal sciatic nerve preparations. One animal (#337) in the low-dose group showed more severe changes than those of controls, but for this rat data indicate changes were only slightly more pronounced (relative to controls) in the proximal sciatic nerve. Distal sciatic nerve changes were essentially the same as those in some

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controls. While the statement is made: "Changes in these two rats [referring to the two males in the low and median-dose groups] are not clearly related to treatment since only five rats were examined in each group." the data do not allow a NOEL for neurologic changes to be defined.

As well-defined neurologic effects did occur as a result of high-dose dietary exposure to this test material, it is particularly disturbing that the data as presented do not allow the setting of a NOEL for such effects.