MEMORANDUM

SUBJECT: Ethyl Parathion, Review of Chronic/Oncogenic Rat Study

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

FROM: Robert P. Zendzian PhD
Pharmacologist

William Burnam, Chief
Toxicology Branch

Compound Ethyl Parathion

Registration #524-27, 132 & 144

Accession # 252702, 703, 704 & 705

Action Requested

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

Conclusion

The toxic effects seen in this study were:

1) Tremors in 43 to 55% of the females and some of the males at 50ppm during the first three months of the study with a NOEL of 5ppm.

2) Abnormal gait in the hind legs of up to 21% of the females at 50ppm starting at week 21 with a NOEL of 5ppm.

3) Retinal degeneration observed by direct observation in several females at 50ppm at the 24 and 28 month observations with a NOEL of 5ppm.

4) Depression in all RBC parameters (Hct, Hgb & count) in the females at 50ppm at 6, 12, 18 and 25 months with a NOEL of 5ppm.
5) Depression of plasma cholinesterase activity in both sexes at 5 and 50ppm with a NOEL of 0.5ppm.

6) Depression of brain cholinesterase activity at 50ppm with a NOEL of 5ppm.

7) Retinal atrophy observed during histopathology in 43 percent of the 50ppm females compared with 17 percent of the control females with a NOEL of 5ppm.

8) Follicular adenomas in the thyroid gland in 9% of the 50ppm males compared with 2% of the controls. The validity of this observation compared to historical controls could not be determined.

9) Increased loss and/or degeneration of myelinated nerve fibers in the sciatic nerve and its branches at 50ppm compared with controls. A NOEL could not be clearly determined.

Of these compound-related effects, the retinal atrophy and the degeneration of the sciatic nerve must be considered extremely serious.

**Recommendations**

- The registrant must provide an experimental approach to determine a NOEL for the effect of the compound on the sciatic nerve.

- The registrant must provide historical control data on the incidence of follicular adenomas of the thyroid gland in male rats of this strain.
Compound: Ethyl Parathion

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

Reviewed by: Robert P Zendzian PhD Pharmacologist

Core Classification Supplementary
Additional information is required to clarify the toxic effects on the sciatic nerves and on the follicular adenomas of the thyroid gland.

Conclusion

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8) Follicular adenomas in the thyroid gland in 9% of the 50ppm males compared with 2% of the controls. The validity of this observation compared to historical controls could not be determined.
9) Increased loss and/or degeneration of myelinated nerve fibers in the sciatic nerve and its branches at 50ppm compared with controls. A NOEL could not be clearly determined.

Of these compound-related effects, the retinal atrophy and the degeneration of the sciatic nerve must be considered extremely serious.

Materials

Ethyl parathion 95.11% (Lot AK 11 44), slightly viscous amber liquid.

Experimental animals: Male and female Sprague-Dawley CD® weanling rats from Charles River.
Methods

Animals were assigned randomly to four experimental groups as shown in the experimental outline from the report.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ppm)</th>
<th>Init. M/F</th>
<th>25 and term M/F</th>
<th>Mo. 24d Term M/F</th>
<th>Histopathology M/Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.0</td>
<td>60</td>
<td>10</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>II</td>
<td>0.5</td>
<td>60</td>
<td>10</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>III</td>
<td>5.0</td>
<td>60</td>
<td>10</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>IV</td>
<td>50.0</td>
<td>60</td>
<td>10</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

a) Represents males and females.
b) Ten animals/sex were bled and sacrificed prior to initiation of test substance administration for pretest clinical laboratory studies.
c) Terminal cholinesterase evaluations were conducted for the males during Month 26 and for the females during Month 28.
d) Five animals/sex/group were sacrificed at approximately 25 months for qualitative and quantitative evaluations of neurotoxicity.
e) Tissues from all animals including those dying spontaneously, accidentally or sacrificed in a moribund condition were examined.

Animals were observed twice daily for behavior and signs of toxicity and examined weekly for signs of local or systemic toxicity, pharmacologic effect and palpation for tissue masses.

Ophthalmoscopic examinations were performed pretest and at 3, 12 and 24 months, and at termination (females only).

Body weight was determined twice pretest, weekly through 14th week, biweekly, 16 through 110 and 120 weeks for males and females, respectively, and terminally.

Food consumption was determined pretest, weekly through 14 weeks, biweekly 16 through 110 and 120 weeks for males and females, respectively.

Hematology:

- hemoglobin
- hematocrit
- erythrocyte count
- erythrocyte morphology
- platelets
- total and differential leukocytes
- reticulocytes**

** Reticulocytes were evaluated for females at months 6, 12, 18 and 25 and for the males at month 25.
Clinical Chemistry:

- Serum glutamic oxaloacetic transaminase
- Serum glutamic pyruvic transaminase
- Alkaline phosphatase
- Lactic acid dehydrogenase
- Blood urea nitrogen
- Fasting glucose
- Cholesterol
- Total protein
- Albumin
- Globulin
- A/G ratio
- Potassium
- Calcium
- Total bilirubin
- Direct bilirubin

Cholinesterase:

- Plasma
- Red blood cells
- Brain (pretest and termination only)

Tissues Preserved:

- 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 cortexa and basal ganglia, parietal cortex and thalamus and cerebellum and pons)*
- Esophagus
- Eye (2-with optic nerve and contiguous Harderian glands)
- Head (to include nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx and middle ear)
- Heart*
- Intestine
- Cecum
- Colon
- Duodenum
- Ileum
- Jejunum
- Kidney (2)*
- Liver (2 sections)*
- Lungs (with mainstem bronchi and trachea)
- 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 tissue)  
tissue masses or suspect neoplasms and regional lymph nodes  
*organs weighed

Special neurological examination:

Five animals per sex per dose were selected for special evaluation by the Experimental Pathology Laboratories. Animals were selected randomly from all groups except the high dose females. The high dose females were those which showed hind limb paralysis. The animals were anesthetized with Na pentobarbital and perfused through the heart with 2.5% gluteraldehyde. The sciatic nerves and branches, brain and cervical spinal cord were collected from each animal. Brain, spinal cord and one sciatic nerve were prepared with hematoxylin and eosin (H&E) or luxol fast blue (LFB). Sections of the remaining sciatic nerve were embedded in Epon and three cross and three longitudinal sections prepared for qualitative evaluation and quantitative measurements of the myelinated fiber spectrum. Sections of the tibial or sural nerve were used for teased nerve preparations. Fifty fibers were utilized from the control and high dose animals and ten from the low and mid dose animals.

Statistical analysis was performed on appropriate numerical data.

Results

In the males excessive mortality occurred in group III between months seven and 23 but total mortality was comparable in all groups at termination. In the female excessive mortality was observed in group IV from month 2 through 19 but was subsequently comparable in all groups. The effect cannot be clearly attributed to the compound.

Weight gain was depressed in both sexes in group IV throughout the study and can be considered compound related.

Slight tremor was observed in 33 high-dose females and one male during week two. Tremors were observed frequently in high-dose females during the first three months and then only sporadically in a few during the rest of the study. Sporadically, slight tremors were noted in high dose males during the study.
Beginning in week 21, several high-dose females exhibited an abnormal gait in the hind limbs. The incidence of this observation, tremors and survival of females with time are shown in data taken from table 1, page 12.

<table>
<thead>
<tr>
<th>Week</th>
<th>Group</th>
<th>Altered Gait</th>
<th>IV(%)</th>
<th>Tremors</th>
<th>IV(%)</th>
<th>Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>43(77)</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>55(98)</td>
</tr>
<tr>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8(14)</td>
</tr>
<tr>
<td>39</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4(7)</td>
</tr>
<tr>
<td>52</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3(6)</td>
</tr>
<tr>
<td>69</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(4)</td>
</tr>
<tr>
<td>82</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(4)</td>
</tr>
<tr>
<td>105</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4(14)</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(8)</td>
</tr>
</tbody>
</table>

% Percent of survivors showing toxic sign

Retinal degeneration/atrophy was observed in the female rats by direct examination. The incidence is shown in the table below.

Table 1. Retinal Degeneration by direct observation in female rats.

Abstracted from consultant veterinary ophthalmologist's report of April 7, 1982
(month 24)

Group I
248L 257B
(32)

Group II
(34)
(16)

Group III
(29)
(16)

Group IV
807R 808B 821R 806B 817B
844B 856B
(31) (11)

B=both eyes, R=right eye, L=left eye

Note the consultant veterinary ophthalmologist did not indicate the number of rats examined either total or per group. () number of rats examined taken from table on page 13 of the report.

Hematology:
Mean hemoglobin, hematocrit, and erythrocyte count were depressed, compared to concurrent controls, in the 50ppm females at 6, 12, 18 and 25 months. The depression in mean hemoglobin and hematocrit was statistically significant at 6, 12 and 18 months.
Clinical Chemistry:
Mean values for alkaline phosphatase and blood urea nitrogen were significantly elevated in the high dose females at 6, 12 and 18 months compared to concurrent controls. At 25 months the numerical values of the treated animals were similar to those at the previous determinations but the concurrent control values had increased so that the differences were no longer significant.

Cholinesterase:
Plasma cholinesterase activity was decreased in both sexes at 5 and 50ppm for all determinations during the treatment period. The decrease was dose-related.

Erythrocyte cholinesterase activity varied from control in the treated animals but not significantly nor in a dose-related manner.

Brain cholinesterase activity at termination was significantly depressed in both sexes of the high dose animals compared with concurrent controls.

Urinalysis:
No compound-related effect was observed.

Terminal organ weight:
No compound-related effect was observed.

Gross Necropsy:
No compound-related effects were observed.

Histopathology:
Retinal degeneration/atrophy was observed in the female rats by histopathological examination. The incidence is shown in the table below.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td># of rats examined</td>
<td>54</td>
<td>50</td>
<td>52</td>
<td>49</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal atrophy, unilateral</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>*Percent</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>18</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal atrophy, bilateral</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>*Percent</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TOTAL</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>21</td>
</tr>
</tbody>
</table>

*Percent               | 17  | 8   | 10  | 43  |

*calculated by the reviewer
Follicular adenomas of the thyroid gland were observed in the treated males in higher incidence than in controls as shown in table 3 from the report (page L-6).

Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td># of rats examined</td>
<td>59</td>
<td>58</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Follicular adenoma #</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>%</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

The report stated that "The incidence of this lesion in untreated control male rats from 14 similar studies conducted at Bio/dynamics, Inc. ranged from 0.0% to 6.5%." 

No other compound-related abnormalities were reported.

Special neurological examination:
- No compound-related abnormalities were observed in the sections from the brain or spinal cord.

H and E sections of the sciatic nerve showed loss of myelinated fibers in the males of group IV. Male rats showed more perivascular Myelin debris and Schwann cell proliferation. The group-related differences were not seen in the sciatic nerves from the high-dose females.

Epon sections of the male rats showed increased degenerative changes compared with the controls. These included cholesterol clits, myelin ovoids, myelin sheath ballooning, loss of myelinated fibers and Schwann cell proliferation. This difference was not seen in the females.

The teased nerve fiber preparation showed significant differences between controls and high dose in both sexes as shown in the table from the text (appendix K page 14) which presents the arithmetic mean (+ standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group II</td>
<td>Group IV</td>
</tr>
<tr>
<td>Total # Fibers Examined</td>
<td>267</td>
<td>269</td>
</tr>
<tr>
<td>Not Remarkable</td>
<td>56±21</td>
<td>19±15**</td>
</tr>
<tr>
<td>Myelin Corrugations</td>
<td>16±17</td>
<td>49±9†</td>
</tr>
<tr>
<td>Myelin Ovoids</td>
<td>19±15</td>
<td>30±14</td>
</tr>
<tr>
<td>Bubbling of Myelin</td>
<td>18±9</td>
<td>17±10</td>
</tr>
<tr>
<td>Demyelinated Lengths</td>
<td>25±5</td>
<td>48±16**</td>
</tr>
<tr>
<td>Remyelinated lengths</td>
<td>4±7</td>
<td>5±4</td>
</tr>
</tbody>
</table>

Student's t-tests, * = P < 0.05, ** = P < 0.01, † = P < 0.005
In the males the morphometric evaluation showed a flattening of the distribution of myelinated fiber diameters in the group IV animals indicating a relative increase in demyelination and loss of myelinated fibers. The decrease in myelinated fibers was significant at $P < 0.01$). This change was confirmed by the decrease of internodal lengths seen in the longitudinal sections. These changes with treatment were not seen in the female rats.

**Discussion**

The toxic effects seen in this study were:

1) Tremors in 43 to 55% of the females and some of the males at 50ppm during the first three months of the study with a NOEL of 5ppm.

2) Abnormal gait in the hind legs of up to 21% of the females at 50ppm starting at week 21 with a NOEL of 5ppm.

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8) Follicular adenomas in the thyroid gland in 9% of the 50ppm males compared with 2% of the controls. The validity of this observation compared to historical controls could not be determined.

9) Increased loss and/or degeneration of myelinated nerve fibers in the sciatic nerve and its branches of the 50ppm males compared with controls. A NOEL could not be clearly determined.

The depression of cholinesterase activity is expected with this compound and the occurrence of tremors early in the study is a common secondary effect of this biochemical toxicity. Tolerance develops to such effects and can account for the general decrease in tremor incidence over the
course of the study.

The appearance of abnormal gait in the females led to the special studies on the brain, spinal cord and sciatic nerve. The observation of degenerative changes in the sciatic nerves of male but not female rats poses a serious problem of cause and effect relationship. One could expect a nerve effect of the compound leading to functional abnormalities and/or degeneration in the hind legs as a cause of the abnormal gait in the females but the nerve degeneration appeared only in the males which did not show the toxic sign. This becomes more confusing when it is remembered that the females selected for special testing at the high dose were said to be showing the toxic signs of abnormal gait. This quandry must be resolved in future studies.

The appearance of retinal degeneration is particularly disturbing. It is important to remember that functional changes can occur in the eyes that would not be detected with the direct and histopathological procedures used although histopathology was a more sensitive indicator than direct observation. Studies on the effect of ethyl parathion on refraction and electrical activity of the eye are necessary to evaluate functional effects.

The depression of all RBC parameters in the high-dose females is unexpected with this organophosphate but the existence of a clearcut NOEL makes it a more easily handled toxicity.

The appearance of follicular adenomas in the thyroid gland must be further evaluated by comparing the incidence with the incidence in historic controls.