A SAFETY STUDY ON RAT'S EYE AFTER 13-WEEK ORAL ADMINISTRATION WITH FENITROTHION

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ABSTRACT — The potential of ocular toxicity of fenitrothion (O,O-dimethyl O-4-nitrom-tolyl phosphorothioate) was assessed in Sprague-Dawley (Crl:CD) rats of both sexes receiving a diet containing the test compound at concentrations of 0, 2.5, 5, 10, or 30 ppm for 13 weeks. The animals were observed daily for clinical signs and their body weights and food consumption were measured weekly during the study. At termination of treatment, surviving animals were subjected to ophthalmoscopy, electroretinography, and biochemical analyses of plasma, erythrocyte, and brain cholinesterase (ChE). Histopathological examinations of ocular tissues were performed on all animals by light microscopy and on two animals/sex/dose by electron microscopy. There were no treatment-related changes in clinical signs, body weights, and food consumption. A significant inhibition of ChE activity was observed in males (plasma and erythrocyte ChE) and females (plasma, erythrocyte, and brain ChE) at 30 ppm and in females (plasma ChE) at 10 ppm. Ophthalmological and histopathological examinations revealed neither functional nor morphological alterations in the visual system at any dose level. Under the conditions of the present study, there was no evidence of ocular toxicity of fenitrothion for male and female rats at dose levels up to 30 ppm (1.70 mg/kg/day for males and 1.96 mg/kg/day for females) where distinct inhibition of ChE activity was observed.

KEY WORDS : Organophosphate, fenitrothion, Ocular toxicity, Rats, Cholinesterase (ChE), Electroretinogram (ERG), Ultrastructure

INTRODUCTION

Fenitrothion, O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate, is an organophosphorus insecticide used worldwide for control of harmful agricultural insects, flies, mosquitoes, and cockroaches. This compound has a high insecticidal potency but lower mammalian toxicity as compared to other organophosphorus insecticides such as EPN, ethylthiometon, and parathion. The toxic effects of this chemical are primarily attributed to its excessive cholinergic activity (Miyamoto et al., 1963, Misu et al., 1966, Ecobichon et al., 1980, Yamanaka et al., 1993), but no treatment-related histological lesions have been demonstrated by routine histopathological examinations in rats from oral chronic (Ecobichon et al., 1980, Kanoh et al., 1982), oral subchronic (Misu et al., 1966, Durham et al., 1982), and inhalation (Breckenridge et al., 1982) toxicity studies. After repeated oral administration of fenitrothion to dogs at 2 mg/kg/day for 379 days, no significant changes were observed in ophthalmological examination of ocular fundus or ocular tension, but inhibition of cholinesterase activity was observed (Ogata, 1972). However, Ishikawa and Miyata (1980) reported the development of
myopia and degeneration of the ciliary muscle in
dogs after repeated long-term oral
administration of fenitrothion. In addition, the
possibility of adverse effects of organophos-
phate on the human visual system has been
pointed out recently by Dementi (1994) in his
review article. Therefore, we have conducted a
further 13-week feeding study to assess the
potential of ocular toxicity of fenitrothion in
rats. This paper describes the results of ophthal-
moscopy, electroretinography and histopatho-
logical examinations of ocular tissues by light
and electron microscopy.

MATERIALS AND METHODS

Test compound

Fenitrothion, O,O-dimethyl O-4-nitro-m-
tolyl phosphorothioate (Sumithion®, Fig.1), was
obtained from Sumitomo Chemical Co., Ltd.
( Osaka, Japan) and administered orally by
dietary admixture for 13 weeks. The stability,
homogeneity, and concentration of the com-
pound in test diets were verified for each dose
level.

Animals

Specific pathogen-free (SPF) Sprague-
Dawley (Crj:CD) rats of both sexes at 4 weeks
of age were purchased from Charles River Japan
Inc. ( Kanagawa, Japan) and acclimatized to the
testing environment for 9 days. These animals
were housed 3 per cage (width 310 mm × depth
440 mm × height 230 mm) in an animal room
with automatically controlled temperature
(24°C), relative humidity (55%), and illumina-
tion (12 hour light/dark cycle). They were
allowed free access to water and laboratory diet
(Oriental Yeast Co., Tokyo, Japan) with low
residual levels (<0.05 ppm) of organophospho-
rus contaminants.

Experimental design

Animals at 6 weeks of age were randomly
divided into 5 groups of 12 animals/sex and fed
a diet containing fenitrothion at dose levels of 0,
2.5, 5, 10, or 30 ppm for 13 weeks. The dose
levels were selected on the basis of the results of
a 2-week preliminary study in which brain ChE
activities of males and females treated with this
compound at 30 ppm were inhibited by 94 and
79% of the corresponding controls, respectively.
The cages in the racks were equally allocated
among groups and periodically rotated to equal-
ize the conditions of light exposure to animals.

All animals were observed daily for clinical
signs, and their body weights and food con-
sumption were recorded weekly during the treat-
ment period. Chemical intakes were calculated
from body weights, food consumption, and tar-
gent concentrations of the test compound in test
diets. In consequence of these calculations, the
average group mean chemical intakes for an-
imals treated at dose levels of 2.5, 5, 10, and 30
ppm were determined to be 0.140, 0.282, 0.570,
and 1.70 mg/kg/day for males and 0.169, 0.331,
0.648, and 1.96 mg/kg/day for females, respec-
tively.

Ophthalmoscopic examination for the conjunc-
tiva, cornea, iris, lens, vitreous body, and
retina was performed on all animals prior to ini-
tiation of treatment and on all surviving animals
(11 or 12 animals/dose/sex) at week 13 using a
halogen ophthalmoscope BX-13 (Neitz
Instruments Co., Ltd., Tokyo, Japan). Animals
showing any ophthalmological abnormality pri-
or to initiation of treatment were not subjected
to the study.

Electroretinogram (ERG) was recorded for
each animal prior to initiation of treatment and
for all surviving animals (11 or 12 ani-
mals/dose/sex) at week 13 using a Model ERG-
50 (Kowa Co., Ltd., Tokyo, Japan). The record-
ing procedures were as follows:

1. A mydriatic, 0.5% tropicamide (Santen
Pharmaceutical Co., Ltd., Osaka, Japan)
was applied to the eyes of animals that
had received a dark adaptation for 3 hr.

2. The animals were deeply anesthetized with
an intraperitoneal injection of 30-35 mg/kg
sodium pentobarbital (Pitman-Moore Inc.,
New Jersey, U.S.A.) and placed in a sealed
3. A topical anesthetic, 0.4% benoxynate hydrochloride (Santen Pharmaceutical Co., Ltd.) was instilled in the right eye of each animal.

4. After the sterilization of electrodes with ethanol, an earth electrode and a reference electrode were fitted to the left auricle and parietal regions of the animals, respectively.

5. Scopisol® 15 (Senju Pharmaceutical Co., Ltd., Osaka, Japan) was placed on the cornea of the right eye as a corneal protector, and an electrode of contact-lens type (Nihonkoden Tokyo Co., Ltd., Tokyo, Japan) was brought in contact with the cornea.

6. A flash stimulator (Kowa Co., Ltd, Tokyo, Japan) was positioned at a distance of 20 cm from the top of the cornea.

7. Flash stimulation of 2.5 joule was provided.

8. All records of the amplitude and latency time of the a- and b-waves using a Model ERG-50 were made with a sweep time of 10 msec/division, a time constant of 0.3 sec, and sensitivity of 200 $\mu$V/division.

9. After 5 min of the recording of the a- and b-waves, the records of the oscillatory potentials were made with a sweep time of 10 msec/division, a time constant of 0.003 sec, and sensitivity of 25 and 50 $\mu$V/division.

10. After the completion of the recording of ERG, each electrode was removed and a miotic, 2% pilocarpine hydrochloride (Santen Pharmaceutical Co., Ltd.) was applied to the eye.

The amplitude and peak latency time of the a- and b-waves as well as the first and second peak latency times of the oscillatory potentials were determined by the procedures shown in Figs. 2 and 3.

Analysis of cholinesterase (ChE) activity was performed at termination of treatment (week 13) on the plasma, erythrocytes (RBC), and brains from 9 or 10 animals/dose/sex. The blood samples were taken using heparinized syringes from the posterior vena cava of animals selected in random order and anesthetized with diethyl ether. The plasma samples were separated by centrifugation at 2000 x g for 10 min at 4°C. The RBC samples were obtained by washing the centrifuged sediment once with saline and following centrifugation at 3000 x g for 5 min at 4°C. The lateral hemisphere of the brain was removed from each animal and homogenized in 5 ml of buffer coating physostigmine sulfate with a homogenizer at 4°C. The supernatant obtained from the centrifugation of the homogenate (12000 x g for 10 min at 4°C) was used for the brain ChE analysis. The ChE activities were determined by DTNB procedure using Hitachi 726 (Nissei Sangyo Co., Ltd., Tokyo, Japan) for plasma and brain ChE and Technicon Autoanalyzer II® (Nihon Technicon Kabushiki Kaisha, Tokyo, Japan) for RBC ChE.

For light microscopy, the eye, optic nerve, and musculus rectus medialis from all animals subjected to ChE analysis (9 or 10 ani-
mals/dose/sex) were fixed with phosphate-buffered formaldehyde-glutaraldehyde solution (pH 7.2). Paraffin sections of the following tissues were stained with hematoxylin and eosin and were observed using a light microscope: retina (three sections including one on the sagittal meridian), optic nerve, musculus rectus medialis, ciliary body, and iris.

For electron microscopy, 2 males and 2 females from each group were perfused through the left cardiac ventricle with 2% phosphate-buffered glutaraldehyde (pH 7.2) under anesthesia by an intraperitoneal injection of sodium pentobarbital. Parts of the retina, optic nerve, and musculus rectus medialis were removed from these animals and post-fixed with 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.2) for 1 hr, dehydrated in ethanol, and embedded in epoxy resin. Ultrathin sections of these tissues from the control and high-dose groups were prepared and observed using a transmission electron microscope (JEM-1200EX II, JEOL Ltd., Tokyo, Japan) after staining with 2% uranyl acetate and lead citrate.

Statistical analysis

Dunnett's or Scheffe's method (Gad and Weil, 1989) was applied to determine the significance of the data on body weight, food consumption, electroretinogram, and ChE activities. Fisher's exact probability test (Gad and Weil, 1989) was used for the data of clinical signs, mortality, ophthalmology, and pathology. Significance of the differences between the control group and the treated groups was estimated at 5 and 1% levels of probability.

RESULTS

Clinical signs, body weights, and food consumption

There were no treatment-related abnormalities in clinical signs, mortality, body weights, and food consumption for each group of either sex. In the 2.5 ppm group, two accidental deaths due to fracture of nasal bones and abdominal hemorrhage occurred (one male at week 8 and one female at week 12).

Ophthalmology

No ophthalmologic abnormalities were seen in any testing animal at week 13 except for two control females showing opacity of the right cornea and one male in the 5 ppm group showing incidental hemorrhage of the right vitreous body.

Table 1. Electroretinographic changes in peak latency times and amplitude of a- and b-waves as well as oscillatory potentials in male rats treated with fenitrothion for 13 weeks.

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>No. of animals examined</th>
<th>Week of treatment</th>
<th>a-wave</th>
<th>b-wave</th>
<th>Oscillatory potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak latency time (msec)</td>
<td>Amplitude (μv)</td>
<td>Peak latency time (msec)</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>Pretreatment</td>
<td>11.1±1.1</td>
<td>359±130</td>
<td>49.0±3.0</td>
</tr>
<tr>
<td>2.5</td>
<td>12</td>
<td></td>
<td>12.1±1.6</td>
<td>318±80</td>
<td>48.8±1.8</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td></td>
<td>12.5±1.3</td>
<td>273±53</td>
<td>49.4±2.5</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td></td>
<td>12.0±1.4</td>
<td>345±79</td>
<td>50.2±1.5</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td></td>
<td>11.9±1.2</td>
<td>312±91</td>
<td>50.1±3.0</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>13</td>
<td>10.7±1.1</td>
<td>221±59</td>
<td>46.4±1.9</td>
</tr>
<tr>
<td>2.5</td>
<td>11</td>
<td></td>
<td>10.6±0.7</td>
<td>206±34</td>
<td>45.9±2.3</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td></td>
<td>11.0±1.0</td>
<td>175±37</td>
<td>47.4±1.9</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td></td>
<td>10.9±1.3</td>
<td>207±54</td>
<td>47.5±2.0</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td></td>
<td>10.9±1.4</td>
<td>213±59</td>
<td>48.6±2.2</td>
</tr>
</tbody>
</table>

Mean± Standard deviation.
Recording of ERG

The results of ERG prior to initiation of treatment and at week 13 are summarized in Tables 1 and 2, respectively. The ERG recorded prior to initiation of treatment was found to be normal in all animals. As for the ERGs examined at week 13, there were no statistically significant differences in the peak latency time and amplitude of a- and b-waves as well as in the first and second peak latency times of the oscillatory potentials between the treated groups and the control of either sex.

ChE activity

The activity of ChE analyzed at termination of treatment (week 13) was summarized in Table

Table 2. Electoretinographic changes in peak latency times and amplitude of a- and b-waves as well as oscillatory potentials in female rats treated with fenitrothion for 13 weeks.

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>No. of animals examined</th>
<th>Week of treatment</th>
<th>a-wave</th>
<th>b-wave</th>
<th>Oscillatory potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak latency time (msec)</td>
<td>Amplitude (μ v)</td>
<td>Peak latency time (msec)</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>Pretreatment</td>
<td>12.1 ± 2.2</td>
<td>361 ± 59</td>
<td>49.6 ± 1.9</td>
</tr>
<tr>
<td>2.5</td>
<td>12</td>
<td></td>
<td>12.9 ± 1.5</td>
<td>398 ± 73</td>
<td>50.0 ± 2.1</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td></td>
<td>12.9 ± 1.2</td>
<td>406 ± 66</td>
<td>49.4 ± 1.7</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td></td>
<td>13.2 ± 1.7</td>
<td>400 ± 110</td>
<td>50.0 ± 2.5</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td></td>
<td>12.2 ± 1.1</td>
<td>365 ± 100</td>
<td>48.7 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13</td>
<td>10.9 ± 1.4</td>
<td>263 ± 100</td>
<td>50.8 ± 3.6</td>
</tr>
<tr>
<td>2.5</td>
<td>11</td>
<td></td>
<td>10.8 ± 0.7</td>
<td>280 ± 72</td>
<td>50.3 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td></td>
<td>10.9 ± 1.1</td>
<td>262 ± 57</td>
<td>51.9 ± 3.5</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td></td>
<td>11.1 ± 1.2</td>
<td>256 ± 56</td>
<td>51.5 ± 3.6</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td></td>
<td>10.9 ± 1.6</td>
<td>241 ± 70</td>
<td>51.9 ± 3.4</td>
</tr>
</tbody>
</table>

Mean ± Standard deviation.

Table 3. Effects of fenitrothion on plasma, RBC, and brain cholinesterase activities in rats after 13 weeks of treatment.

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>No. of animals examined</th>
<th>Plasma (unit/ml)</th>
<th>RBC (unit/ml)</th>
<th>Brain (unit/ml)</th>
<th>Plasma (unit/ml)</th>
<th>RBC (unit/ml)</th>
<th>Brain (unit/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0.22 ± 0.06</td>
<td>0.32 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>10</td>
<td>1.95 ± 0.45</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>2.5</td>
<td>9</td>
<td>0.17 ± 0.04</td>
<td>0.32 ± 0.04</td>
<td>0.48 ± 0.03</td>
<td>9</td>
<td>1.40 ± 0.21</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.21 ± 0.04</td>
<td>0.31 ± 0.04</td>
<td>0.50 ± 0.04**</td>
<td>10</td>
<td>1.63 ± 0.39</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0.17 ± 0.03</td>
<td>0.31 ± 0.04</td>
<td>0.48 ± 0.03</td>
<td>10</td>
<td>1.05 ± 0.25*</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>0.13 ± 0.02**</td>
<td>0.21 ± 0.02**</td>
<td>0.45 ± 0.03</td>
<td>10</td>
<td>0.30 ± 0.10**</td>
<td>0.13 ± 0.02**</td>
</tr>
</tbody>
</table>

Mean ± Standard deviation.

* and **: Significantly different from the control at 5 and 1% levels of probability, respectively.
3. In the 30 ppm group, significant inhibition was observed in plasma (59 and 15% of the control in males and females, respectively) and erythrocytes (66% in males and 48% in females) of both sexes and in the brain of females (86%) in the 30 ppm group. In the 10 ppm group, plasma ChE activity was significantly inhibited in females by 54% of the control levels. There were no significant changes in ChE levels in the 5 and 2.5 ppm groups except for the incidental increase in brain ChE activity in males of the 5 ppm group.

**Light and electron microscopical examinations**

There were no microscopical abnormalities relating to the treatment in the various ocular tissues including the retina, optic nerve, musculus rectus medialis, and ciliary body of males and females in each group.

The retinal tissues from males and females in the 30 ppm group subjected to electron microscopy showed no morphological abnormalities in the ganglion cell layer, inner and outer nuclear layers, layer of rods and cones (Photo.1), and pigment. The ultrastructures of the optic nerve (Photo.2) and musculus rectus medialis (Photo.3) of either sex of this group were also similar to those of the controls.

**DISCUSSION**

Abnormal ERG reflecting functional changes of the sensory retina was demonstrated in rats receiving an intramuscular injection of fenthion (Miyata et al., 1973). In that study, prolongation of latency time and decreased amplitudes of a- and b-waves in ERG indicating sub-normal response of the retina were demonstrated at dose levels of 25 mg/kg and above, while a super-normal ERG was recorded even at

![Photo 1](An electron micrograph of ellipsoids (E) and rods (R) in the retina of a male rat in the 30 ppm group. Normal appearance although some artificial products are seen in the organella. ×7500.)
Ocular toxicity of fenitrothion in rats


Photo 3. An electron micrograph of a longitudinal section of musculus rectus medialis of a male rat in the 30 ppm group. Normal appearance. ×10000.
0.005 mg/kg. In their further study (Imai et al., 1983), inhibition of ChE activity in the retina and cerebellum was reported to correlate with the decrease in ERG response, and these biochemical and functional changes appeared before any structural damage could be detected in the retina. Disappearance of ERG in rats was also found in acute intoxication of parathion (Carricaburu and Lacroix, 1973). These findings are, therefore, suggestive of ERG as a sensitive tool for detection of ocular toxicity of organophosphorus compounds as well as a suitable indicator for estimation of the state of their toxicity.

The effect of fenitrothion on ERG was reported in male Wistar rats that received a single intraperitoneal injection at a dose level of 0.05 mmol/kg (equivalent to 13.85 mg/kg, Yoshikawa et al., 1990). The change was observed as a slight and transient decrease in the amplitude of a- and b-waves and recovered within 2 days after the administration at which the brain ChE was clearly inhibited. In the present study, inhibition of brain ChE was also observed in females of the 30 ppm group but neither sub-normal nor super-normal response of the retina was detected in the ERG of animals treated at any dose level. Based on the present results, it is concluded that the retinal function of rats was not affected by the treatment with fenitrothion at dose levels up to 30 ppm for 13 weeks, although distinct inhibition of ChE was observed.

Some histological lesions related to the treatment with organophosphorus chemicals were reported in ocular tissues of experimental animals. The lesions in dogs that received a long-term administration of ethylthiometon were located in the ciliary muscle, optic nerve, and retina (Suzuki and Ishikawa, 1974, Uga et al., 1977). Imai et al. (1983) reported that chronic exposure to fenthion produced funduscopical and light microscopical abnormalities in the rat retina characterized as the decreased number of pigment epithelial cells and photoreceptor, thinning of the outer nuclear layer, pyknotic cells in the inner nuclear layer, and severe atrophy of the choroid. As for the ocular toxicity of fenitrothion, induction of myopia due to edema and/or degeneration of ciliary muscle was only reported in dogs that received oral administration of the compound twice a week for one (Tokoro et al., 1976) or two years (Ishikawa and Miyata, 1980). While in the present study, light and electron microscopical examinations elicited no morphological abnormalities related to the treatment in any components of ocular tissues of rats described above. The absence of histological lesions is well correlated to the results of no changes in ERG, because abnormalities in ERG are likely to precede histological abnormalities in the retina (Imai et al., 1983).

Based on the results of the present study, it is concluded that ChE activity is more susceptible to the toxicity of fenitrothion than ERG, and that no toxic changes in ERG and histopathology for ocular tissues were produced in the animals treated with fenitrothion at 30 ppm where distinct inhibition of ChE activity was induced.

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