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*Review before*  
*releasing*

16 JUN 1991

OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

MEMORANDUM:

SUBJECT: Peer Review of a Document Entitled "Ocular Effects of Organophosphates"

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The RfD/Peer Review Committee convened on February 21, 1991 to discuss the possible association between ocular effects and organophosphate pesticides. Material available for review by the Committee included a document entitled "Ocular Effects of Organophosphates" prepared by Dr. Brian Dementi addressing the association between exposure to organophosphate pesticides and ocular effects observed in population/clinical and animal studies from the Japanese literature, and other animal studies from data submissions.

Members of the Peer Review Committee felt that the report by Dr. Dementi accomplished the objective of characterizing the body of published works in question.

The Committee agreed that:

1) the population studies, though not satisfactorily conforming to contemporary standards in epidemiology, were helpful in establishing a plausible association between eye effects observed in humans and exposure to organophosphates.

2) the population data, though lacking much information that would be necessary to properly characterize the ocular effects and correlate doses with such effects, when viewed as a whole, are adequate to establish, at least, a working hypothesis, that is, exposure of humans to organophosphates may produce ocular toxicity.

3) the animal studies provided conclusive evidence of the potential of organophosphates to produce adverse ocular effects in mammals, and conclusively demonstrate that both lenticular changes and other serious eye damage (e.g. retinal degeneration) will follow exposure to organophosphates under defined circumstances of dosing. It was recognized, therefore, that data from well-designed animal studies are essential to further assess the above hypothesis.

The report is essentially in agreement with several other previous evaluations by Dr. Robert Zendzian of the Health Effects Division, addressing the same subject.

The Committee concluded that the combined toxicological data from epidemiology studies and from bioassay demonstrate the potential for organophosphates to produce a wide range of ophthalmological effects, and hence support the necessity to establish ocular testing as a registration requirement for this class of chemicals for the purpose of hazard characterization and risk assessment.

Individuals in Attendance:

1. RfD/Peer Review Committee Members and Associates (signatures indicate concurrence with the peer review report unless otherwise stated).

Penny Fenner-Crisp

Penny A. Fenner-Crisp

William Burnam

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Karl Baetcke

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Reto Engler

Henry Spencer

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George Ghali

G. Ghali

Rick Whiting

Rick Whiting

2. RfD/Peer Review Members in Absentia (committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the committee).

Marcia Van Gemert

Marcia Van Gemert

3. Scientific Observers (Non-committee members; signatures indicate concurrence with the peer review unless otherwise stated).

Robert Zendzian

Robert Zendzian 4/16/91

William Sette

William Sette 6/11/91

Jerome Blondell

Jerome Blondell 6/11/91

4. Scientific Reviewers (committee or non-committee members responsible for data preparation and presentation of data; signatures indicate technical accuracy of panel report).

Brian Dementi

Brian Dementi 4/16/91

George Ghali

George Ghali

cc: R. Cool  
A. Allan  
F. Chow

Ocular Effects of Organophosphates

Prepared by Brian Dementi, Ph.D., D.A.B.T.

Date: March 27, 1991

## SUMMARY

Many publications in the Japanese literature report findings in human populations of an increased incidence of a visual disease syndrome (Saku disease) which reportedly correlated with increasing use of organophosphate pesticides in agriculture. Follow-up studies in animals using such agents as ethylthiometon, fenthion, and fenitrothion confirmed the ocular effects findings. The eye effects in question, which are manifold, are dose dependent ranging in severity from lenticular changes to the more serious histopathologic changes in such tissues as the ciliary body and retina. Similar episodes of this syndrome in human populations have not been reported outside of Japan. However, we are not aware of any other study involving systematic investigations of human populations (community wide, bystander) for ocular effects in other countries, including the U.S.A. Given that the incidences would be exposure dependent and that organophosphates were heavily used in Japan, it is possible that incidences elsewhere have not risen so high as in Japan (2/1000) to have been identified and followed by scientific inquiry. It is also possible that within the organophosphate class, one particular agent or the spectrum of agents used in Japan was uniquely inducive of ocular effects.

Evidence of the unique effects of organophosphates upon the visual system as reported in the Japanese population studies cannot be viewed as conclusive, due in part to the lack of reliable information as to the intensity, duration and extent of exposure in the human studies. Yet, when this evidence is considered in the light of the more definitive findings in animal studies, a much more compelling case exists that the association could be real.

Animal studies by Japanese authors and certain studies now being submitted by Registrants to OPP conclusively reveal remarkable effects of organophosphates upon the visual system, e.g. myopia, astigmatism, narrowing of the visual field, reduced vision, histopathologic evidence of degeneration of extraocular muscle, ciliary muscle, and retina, and other ocular tissues. The Japanese literature indicates that certain visual parameters (e.g. pupillary responses and ERG's) respond to organophosphates at doses below those which measurably inhibit retinal and brain cholinesterases, and are responses possibly of a cholinergic nature that could go undetected when OPP's current testing procedures are used.

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While animal studies clearly show that some organophosphates elicit ocular toxicity, there are many knowledge gaps, with regard to effects in humans and the ocular toxicity in general, e.g. time and dose dependency, cholinesterase inhibition vs ocular effects, and effects of routes of exposure. Consequently, we are unable at this time to incorporate hazard assessment data with exposure assessment data and to perform risk assessments on organophosphates based on the ocular toxicity potential of this class of chemicals.

## CONTENTS

- I Population/clinical studies - 1
  
- II Animal Studies - 10
  - Ethylthiometon - 10
  - Fenthion - 15
  - Fenitrothion - 20
  - Mevinphos/malathion - 21
  - DEF - 22
  - Parathion - 23
  
- III Cholinesterases in ocular tissues - 25
  
- IV Case study - 28

## I. Population/clinical studies

Many publications primarily by Japanese investigators claim an association between an increased incidence of persons experiencing adverse effects of the visual system and the agricultural use of organophosphates in Japan. Before citing the findings from various studies, we would consolidate here the principal reasons the authors use to draw the association. Many patients in Japan were from the farming belt where pesticides were increasingly used, there being virtually no findings of this disease in mountainous areas; the incidence of the eye disease increased from 1965 when large amounts of organophosphate pesticides were increasingly used; incidences of persons with visual problems were significantly correlated with agricultural organophosphate dispersions; the eye and attendant structures are richly endowed with cholinesterases and symptoms in many cases were those anticipated to follow cholinesterase inhibitions; symptomatology among affected citizens was in many ways characteristic of that following intoxication by organophosphates used medicinally in ophthalmology; the symptomatology was experimentally produced in animals (dog, rat); visual symptoms were often attended by the presence of organophosphate residues in the blood and urine of patients; in many cases there were reductions in the activity of erythrocyte AChE; symptoms were quite typically responsive in varying degrees to therapeutic agents used in the treatment of organophosphate intoxication, such as 2-PAM.

As indicated, there are many publications by Japanese authors which address this issue. These studies vary in quality from very deficient in certain aspects to very good in other aspects. Often the papers are difficult to follow, perhaps as a result of problems in translation and style of writing. Taken as a whole, this literature serves to establish a reasonable assumption of an association between the use of organophosphates in Japan and an increased incidence of adverse ocular effects among the citizenry.

1. a) Ishikawa, S. and Miyata, M. (1980). Development of myopia following chronic organophosphate pesticide intoxication; an epidemiological and experimental study. In, Neurotoxicity of the Visual System; Merigan, W. and Weiss, B., eds., Raven Press, NY, pp. 233-254.

b) Ishikawa, S. (1973). Chronic optico-neuropathy due to environmental exposure of organophosphate pesticides (Saku disease) - clinical and experimental study. Nippon Ganka Gakkai Sashhi, 77, 1835-1886. (TR-80-0787).

These publications disclose an increased incidence of persons with myopia and other visual abnormalities in Japan during a period of increased use of organophosphates.

An initial investigation evaluated the incidence of myopia among some 40,000 students of the primary and junior high schools in Tokushima Prefecture during the period 1957-1973. According to the study authors, until 1971 the major organophosphate compounds used were malathion, EPN and ethyl and methyl parathions. These were replaced by fenthion, dipterex, fenitrothion and diazinon after the latter group were considered to be less toxic to humans. This study revealed that the increased myopia incidence correlated with the increasing use of agricultural pesticides in general, and specifically so with the organophosphate component. Tamura and Mitsui (Jap. J. Ophthalmol., 19, 250-253, 1975) disclose a more compelling statistical argument that the amount of organophosphorus used significantly correlated with the incidence of myopia.

Another investigation was conducted in the same area of Japan during the years 1960-1972. In this study, the incidence of patients (youths) with visual problems (Saku disease, to be characterized subsequently) was evaluated against the application of pesticides by helicopter on mixed agricultural and residential areas. (see attached bar graph) Pesticides applied by helicopter during this period included two organophosphates, malathion and vamidothion, and two carbamates, hopcide and BPMC. The bar graph shows that relatively little of the carbamates were used and their use occurred following the period when visual effects were first recorded. Hence, this appears to be essentially an organophosphate study. Malathion alone was applied via helicopter from 1960-1967, with the other pesticides being introduced and used along with malathion from 1968. The first patients were found in 1966. Their number increased as the area sprayed by helicopter increased. The incidences from among a Saku population of approximately 310,000 increased from about 5 patients in 1966 to approximately 117 patients/year during 1970-1971. It is noteworthy that during the 1966-1972 study period, in addition to the 321 child patients who are represented by this study, there were 255 adult cases.

The authors advise that individuals in the Saku district were not limited to organophosphate exposure via helicopter. The pesticidal use of parathion and malathion was extensive in the area by other than aerial means. However, they advise that patients were more often found in the area where the chemicals were sprayed by helicopter. We should note that the association between the youthful subjects with this disease and organophosphates as indicated in this study is not a proof of causation.

Another study, initiated in 1969, which in essence characterizes Saku disease, involved the careful examination of some 71 children from the Saku district. These children, ages 4-16 years, were evidently chosen for the study, as they presented with

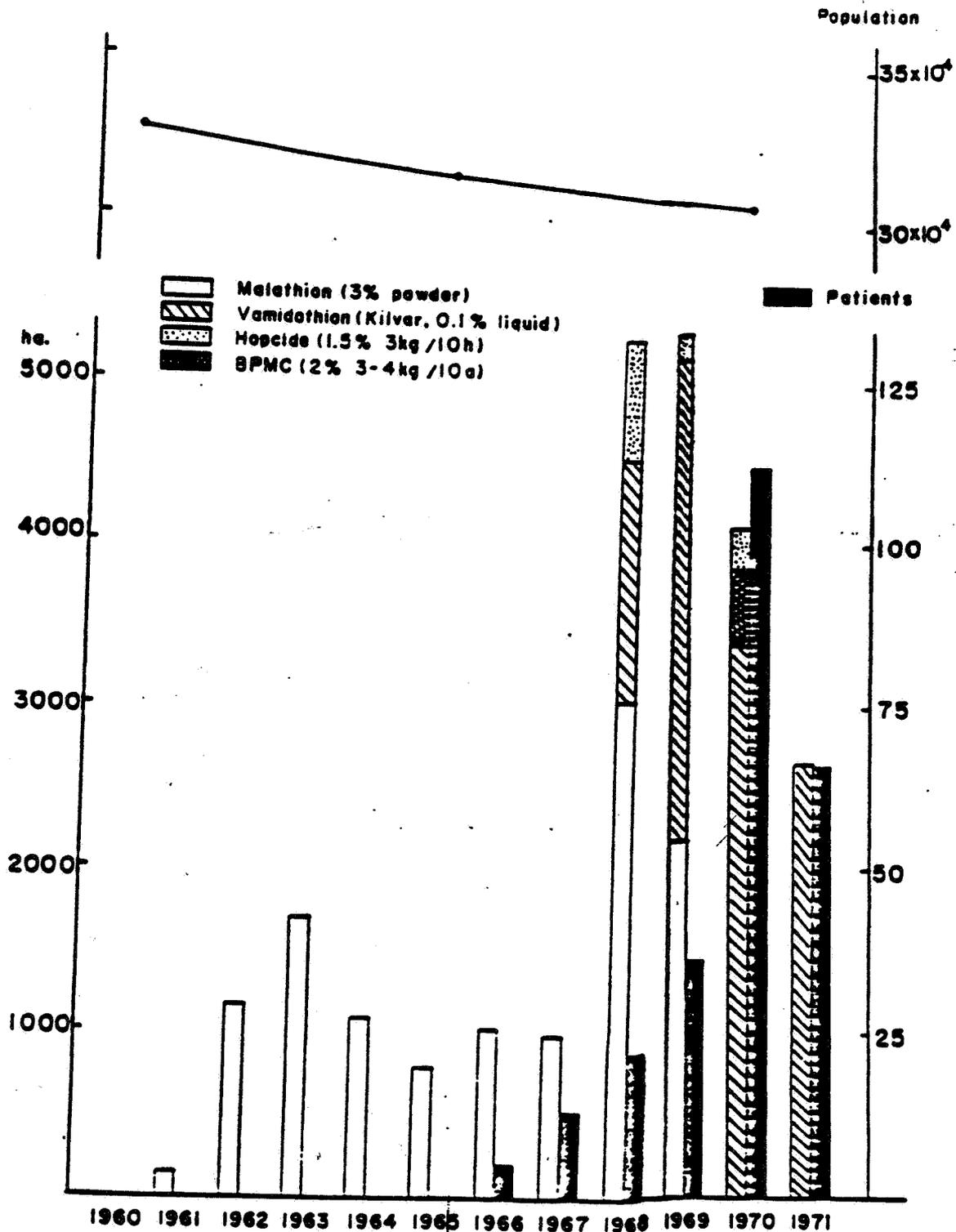


Fig. 1. Use of organophosphate pesticides (malathion and vamidothion) and carbamyl esters Hopcide and BPMC, at Saku district estimated from the sprayed area in hectare ha., by the helicopter over years is shown. Black histograms denote the occurrence of the new children's patients examined at Asama hospital. Upper curve indicates the population during these period. The patients were first seen in 1966 and gradually increased up to 1970. The warning to the government about this disastrous events was made in the April of 1970. The use of the pesticide decreased in 1971. The patients also reduced. The data do not show the massive use of the pesticides at the farms made by each farmer.

signs of reduced visual activity, narrowing of the visual fields and optic neuritis. Control patients consisted of 100 children of similar age from Tokyo who had no known direct exposure to organophosphates. The incidences of clinical findings exhibited by the Saku children are summarized as follows: 98% had reduced visual acuity, 50% of whom could not be corrected with lenses because of high vertical astigmatism; 88% had refractive anomalies; 98% exhibited a narrowing of visual field (10-20 degrees smaller than controls); 53% had abnormal eye movements (a biopsy of the lateral rectus muscle of several of these patients revealed total inhibition of cholinesterase activity); abnormal pupillary responses were observed in 52% of subjects; 49% exhibited abnormalities of the optic nerve (optic neuritis, pallor of the optic disc and/or optic atrophy; 33% had lowered serum cholinesterase (degree of lowering not indicated); 71% exhibited one or more positive neurological findings such as sensorial neuropathy, dysmetria, abnormal EEG, etc.; organophosphates detected in the blood of all subjects examined.

According to the authors, the patients with myopia differed from ordinary myopes in having attendant mild clinical complications of the central, peripheral and autonomic nervous systems. The unique symptomatology for Saku disease led the authors to coin the synonym, "optico-autonomic-peripheral neuropathy", for this disease.

2. Ishikawa, S. and Ohto, K. (1972). Optico-neuropathy induced by environmental exposure to organophosphate pesticides. Acta Proc. 5th Afro-Asian Cong. Opthal., Session VI, Refraction, Toxicology, BEHCET, Orbit and others. Kyurinsha, Tokyo, 434-443+.

This publication offers additional information on the 71-child Saku study. Almost all children in the cohort exhibited more or less neurological abnormalities. The parameters in question are many and while relatively small percentages of individuals in the cohort exhibited any one symptom, it was evident that neurologic symptoms were more frequently expressed in the study cohort than in the control group. Typical nicotinic and muscarinic reactions characteristic of acute intoxication were not observed in these patients. As indicated above, 33% of the study group had reduced plasma pseudo ChE. The reduction in activity was most apparent immediately after helicopter spraying and recovered several days afterward. When cholinesterase activity was assayed for the entire cohort of 71 children and the mean compared to that for 99 control subjects, plasma pseudo ChE was reduced about 22% and erythrocyte AChE reduced about 24%. Both reportedly were statistically significant. We should note that, as indicated previously, cholinesterase activity of the lateral rectus muscle was totally inhibited in those several subjects evaluated. It would be desirable to have on an individual basis both the lateral rectus muscle and blood cholinesterase data in order to see clearly the relative inhibitions of the enzymes. Failing this, the

findings nevertheless suggest that cholinesterase of the lateral rectus muscle may be more responsive than blood cholinesterases to the influence of organophosphates.

According to Ishikawa and Ohto, the amount of narrowing of the visual fields correlated with the reduction of plasma pseudo ChE. Principal changes were noted in the optic disc progressing in the extreme to bilateral atrophy. In the full blown condition, patients displayed retinochoroidal atrophy and abnormalities of retinal pigment. These authors indicate that individuals who had large amounts of organophosphate residue in the blood were clinically very severe in terms of both ocular and neurologic symptoms. Usually vision improved a few months after therapy, with the rate of improvement paralleling the rates of recovery of cholinesterase activity. Refractive changes due to corneal deformity are not improved with treatment. Strong myopic astigmatism was the most characteristic clinical finding in the study cohort.

3. Ishikawa, S. (1971). Eye injury by organic phosphorus insecticides. (Preliminary report) Jap. J. of Ophthal., 15, 60-68.

The author in this publication provides the following additional perspective on the 71 child study. Of the 71 cases, 35 had neurological signs. From this statement it would appear that the effects on the visual system do not necessarily fall within an overall umbrella of neurologic signs, i.e. one cannot presume that the ocular symptomatology must be accompanied by other neurologic signs. Among 56 cases that received optic nerve examinations, optic neuritis was observed in 14 cases, temporal pallor of the disc in 18 cases, atrophy in 5 cases and there were 19 cases which appeared normal.

4. Ishikawa, S. (1978). Effects of pesticides on eyes and/or vision. Sci. Am., 1, 68-82. (TR-81-0244).

In this publication, the author indicates that myopia in the case of Saku disease is characteristically different from the usual form of myopia. Results are presented of an investigation into the nature of myopia in some 39 Saku patients (4-15 years old) vs. that in 98 subjects of essentially the same age range with myopia who lived in Tokyo. Myopia in Saku patients was attributed to excessive vertical as opposed to horizontal refraction of the lens, which according to the author identifies myopic direct astigmatism. Furthermore, the Saku patients exhibited corneal astigmatism as well.

5. Oto, K. (1977). Eye diseases induced by organophosphorus insecticides. Adult cases. Nippon Ganka Gakkai, Zasshi, 75, 1944-1951. (TR-81-0029).

This investigator attempted to characterize the nature of Saku disease through clinical investigations of a cohort of 51 adults and 100 children. All of the adults were engaged in farming and had a history of contact with organophosphate and carbamate pesticides. More than 95% of the children had contact with agricultural pesticides as well as household pesticides. Myopia was present in 80% of the children and in 30% of the adults. The authors indicate that findings in adult cases varied with the kind of organophosphate used. For example, congestion of the optic nerve was external in those cases in whom strong organophosphates such as parathion had been used. In those cases of chronic intoxication with such weakly (acute) toxic organophosphates as malathion or sumithion, etc., the principal symptoms were a fading of the optic nerve and finally atrophy. The optic nerve was swollen in 100% of the adult subjects. Approximately 79% of the cases evaluated exhibited abnormalities in responses of the pupil. Serum cholinesterase was somewhat reduced. Urinary p-nitrophenol, a metabolite of certain organophosphates, such as EPN and methyl and ethyl parathion was measured in 14 cases and all were positive, showing values of 10-66 gamma averaging 25.4 gamma. Healthy subjects average 1.75 gamma.

In addition to ocular effects, the study reports a variety of other neurologic findings which would suggest that some patients had been exposed to considerable amounts of cholinesterase inhibiting substances. Nevertheless, there was a substantial number of patients experiencing effects upon the visual system without experiencing other neurologic signs. The author cites the responsiveness of patients to 2-PAM, but indicates that a considerable length of time is required for improvement of the optic nerve symptoms.

6. Ohto, K. (1974). Long-term follow-up of chronic organophosphate pesticide intoxication (Saku Disease) with special reference to retinal pigmentary degeneration, Nippon Ganka Gakkai Zasshi, 78, 237-243. (TR-81-0025).

This investigator conducted a follow-up study on 12 patients diagnosed as having chronic organophosphate intoxication (Saku disease) according to diagnostic criteria of the Japanese Ministry of Health and Welfare. The intent was to evaluate long-term health consequences in a select group who for one reason or another had not received proper attention to their condition. Parameters evaluated included visual acuity, refraction, ERG, erythrocyte AChE, retinal microangiography and fundoscopic examinations of the retina and optic disk.

Most patients exhibited myopia and astigmatism, altered ERGs, varying degrees of retinal pigmentary degeneration, papilledema of the optic disc and constriction of the retinal vasculature. The mean erythrocyte AChE value was 1.84 (range 1.18-2.72), where the lower limit of normality was viewed as 1.80 and, as such, 6 of the

12 cases dropped below this value. The mean organophosphate blood level was 0.101 ppm (range 0.01-0.30 ppm), where all were above the Ministry of Health's diagnostic criteria of 0.01 ppm. Two of the 12 subjects exhibited a non-recordable ERG and displayed degenerative changes of the retina. The author claims that one of these two patients responded to treatment, and that as a result of 3 1/2 years of therapy a "spectacular" recovery was seen in visual acuity. In reference to this recovery, the author says, "In routine retinal pigmentary degeneration and other hereditary retinal degeneration disease, this type of recovery is absolutely impossible."

Conclusions of the author include: 1) the disease is related to prolonged exposure to organophosphates; 2) retinal degeneration does not progress after treatment with 2-PAM and atropine; 3) the disease exhibits a progression, characterized by ever worsening condition of the optic disc, retina and retinal vasculature and ERG responsiveness. This is a well-developed paper, but the selection of patients was based upon criteria which may not necessarily establish an organophosphate etiology.

Information as derived from the references cited above serve to convey the basic nature and findings of investigations conducted in Japan into the etiology of increased incidences of myopia and other visual effects experienced by Japanese citizens during the 1960s and 1970s. There are many more publications by Japanese authors which address the same issue.

We summarized at the beginning of this section the various reasons the investigators have cited as rational for drawing the association between visual effects and the use of organophosphate pesticides. To the extent that it can be accepted that visual impairment among citizens in the study cohort(s) was due to organophosphate exposure, the findings in these studies would indicate that such observations as cholinesterase inhibition and neurologic symptoms (other than visual system) do not necessarily accompany the visual effects, although, it appears to be true in this literature that visual effects are generally more pronounced when accompanied by the other effects. Nevertheless, the absence of the latter in exposed populations could not provide assurances that visual degeneration would not be occurring. This is born out by evidence in both the findings in human subjects in Japan and by results in animal studies to be discussed subsequently. It is also supported ~~by the~~ following study by Misra, et al (1985) of a pesticide worker cohort.

7. Misra, U.K., et al (1985). Some observations on the macula of pesticide workers. Human Toxicol., 4, 135-145.

This study of interest here consists primarily of the assessment of retinal (macula) changes in a group of fenthion exposed workers. The worker cohort in question consisted of 79

individuals who mainly sprayed fenthion as an aqueous suspension. They also sprayed crude oil and paris green (copper acetoarsenite), but to a much less extent. The authors consider this to be essentially a study of the consequence of fenthion exposure. The subjects worked 5-6 hours per day, did not use protective clothing, face mask or gloves. Control subjects for this study consisted of 100 hospital employees matched for age, sex, nutritional and socioeconomic status, who had no known direct exposure to pesticides. Evaluations included a physical examination incorporating a detailed neurological assessment, visual acuity, refraction, color vision, and detailed examination of fundus oculi. Twenty-two workers and twenty control subjects were evaluated for serum cholinesterase activity.

Subjects in the worker group were exposed to fenthion for a mean duration of 8.6 years. Macula changes were evident in 15 (19%) of the 79 worker cohort as compared with 3 (3%) such cases among the 100 control subjects. The difference was reportedly highly significant ( $P = <0.01$ ). The macula as characterized in the study had such features as perifoveal areas of hypopigmentation of  $1/8$  to  $1/3$  of the disc diameter, irregularity of background pigmentation and a dull foveal reflex. It is noteworthy that "pathological" myopia was not observed in any of the workers. Eight of the 15 workers with macula changes "had no symptoms", (p.139) while visual impairment was reported in the remaining 7 workers. Macula changes were reported as severe in 4 of the 15 workers so affected, characterized by large areas of perifoveal hypopigmentation of  $1/4$  to  $1/3$  of the disc diameter. Other findings among these 4 subjects included reduced visual acuity, abnormal color vision and constriction of visual fields. Among the other 11 workers with macula changes, 3 had poor vision due to unidentified extra-retinal causes. Corneal opacity was present in 2 of the workers and lenticular change in 1 worker. "The corneal opacity in both of these cases was reported to follow accidental falling of fenthion into their eyes while spraying." (p.140)

Of the 3 control subjects with macula changes 1 had bilateral and 2 had unilateral perifoveal hypopigmented spots. Only one complained of poor vision. Their color vision and visual fields were normal.

Serum cholinesterases were reduced by about 25-30% in the worker cohort group assayed (22 subjects). The changes were statistically significant.

This appears to be a well-conducted study which implicates fenthion as having an adverse effect upon the retina with attendant effects upon vision. It is of considerable concern that retinal changes occurred in some cases without the subject's experiencing other symptoms, suggesting an insidious toxicologic effect.

The following citations are representative of those in the literature which do not support the concept of any unique sensitivity of the visual system to organophosphates.

8. Plestina, R. and Piukovic-Plestina, M. (1978). Effect of acetylcholinesterase pesticides on the eye and on vision. *CRC Crit. Rev. Tox.*, 6, 1-23.

This article provides a survey review of many publications dealing with the effects of organophosphates (and other agents) upon the visual system. As to the studies of effects in human populations in Japan as presented in the Japanese literature, the above authors advise that the suggestion by the Japanese literature that the etiology of the disease was connected with exposure to organophosphates was taken for granted. Furthermore, these authors say that evidence of a similar effect of this group of pesticides in the Caucasian population is lacking. Following their review of many references as to the effects in human populations, some of which draw the association and some of which do not, the authors here conclude: "In most cases, the connection between the etiology of eye impairment and effects of anticholinesterases is purely speculative." (p. 8)

The authors describe a study of their own on workers who were employed in pesticide formulation and application for longer than 5 years. The study consisted of 63 workers, divided into three study groups. Group A (31 subjects, pesticide production and formulation), exposed to organophosphates and carbamates; group B (12 subjects, pesticide application, exposure described as irregular, there were good protective measures and good working conditions; group C (20 subjects, control, non-exposed subjects). The subjects received ophthalmologic exams which included assessment of changes in the retina and evaluations of corneal curvature, intraocular pressure, visual field, color vision and dark adaptation. Blood cholinesterase was also assayed. According to the authors, ophthalmoscopic examination of exposed patients revealed only mild degenerative changes of the retina, mostly in elderly patients. There was a slight but significant narrowing of the visual field. Dark adaptation was slower in exposed subjects, which may have been related to slower reaction of the pupillary sphincter. The authors concluded that their study did show visual impairment and eye abnormalities in exposed subjects, but appear to view these as of relatively minor importance in view of the magnitude of exposure. They believe heavy exposures of the type experienced by pesticide workers produce only transient lenticular changes, suggesting that serious effects from environmental exposure would not be anticipated.

While these investigators cited and discussed many of the Japanese papers, their review does not go far enough in conveying the magnitude of the Japanese effort, nor the rationale used by the latter authors in reaching their conclusion that an association

exists between organophosphate use and the increased incidence of citizens experiencing visual anomalies.

9. Mitsumi, T., et. al. (1975). Health survey of pest control operators exposed to mildly poisonous organophosphorus pesticides. J. Kumamoto Med. Soc. (Jap.), 49, 175-84 (TR-0800)

These authors conducted a health survey of personnel employed in the spraying of certain organophosphates, specifically fenitrothion and DDVP. The study cohort consisted of 12 pest-control personnel all of whom were males ranging in age between 23 and 40 years. As the control group the study employed 8 male office personnel in a similar age bracket who worked for the same agency, but who were not exposed to pesticides. Parameters evaluated included subjective symptoms, blood cholinesterase, p-nitrocresol in urine, hematology, urinalysis, liver function, ophthalmologic assessment (vision, visual field, intraocular pressure, retina, aqueous humor, etc.), EEG, EKG, blood pressure and physical examination.

The findings in the study cohort might be summarized as follows: significantly increased complaints of forgetfulness, heavy head feeling, headache, pressure feeling in chest, sweating in sleep, nausea, dry throat, twinkling in the eyes, eye trembling, easily tiring, etc. The workers had elevated p-nitrocresol in the urine. Plasma and erythrocyte cholinesterases were lowered, but not by more than 50%. EEGs were slightly abnormal in 33% of the study group before the spraying season and in 64% after the spraying season. According to the authors, in general, the incidence of similar abnormal brain waves is 9.6-21% in healthy persons. Hematology and urinalysis assays did not reveal any significant abnormality.

The basic finding in this study with respect to ocular effects was that in spite of clinical evidence of worker exposure to the organophosphates, the ophthalmological study did not disclose any significant effects.

## II. Animal Studies

There are several publications on animal testing. In general these studies were reasonably well conducted, but often, leave unanswered questions. For example, these studies would be more meaningful if individual ocular effects findings were reported in concert with individual cholinesterase data in various tissues. In any case, the studies do reveal adverse effects upon the visual system in response to organophosphates.

### Ethylthiometon

1. Suzuki, H. and Ishikawa, S. (1974) Ultrastructure of the ciliary muscle treated by organophosphate pesticide in beagle dogs. Brit. J. Ophthal. 59, 931-940.

This publication presents results of an assessment of ultrastructural changes in the ciliary muscle and development of myopia in the dog following chronic administration of ethylthiometon. Other findings from this study pertaining to the visual system are presented in additional publications to be cited.

Beagle dogs were administered ethylthiometon orally, via capsule, on a daily basis, 5 days/week, excepting holidays for two years, at the following dosage levels: 5 mg/dog/day (~0.5 mg/kg/day) (two dogs), 10 mg/dog/day (~1.0 mg/kg/day) (two dogs) and 15 mg/dog/day (~1.5 mg/kg/day) (1 dog). Five dogs used as controls received empty capsules. [Note: dosage selection was made on the basis of the lethal dose (LD<sub>50</sub>) which is variously reported as 6-12.5 mg/kg in the dog].

All treated dogs developed myopia within one year, a phenomenon which progressed until study termination. Fine structure (electron microscopy) of the ciliary muscle was normal in control dogs, however, the cytoplasm of the ciliary muscle in the low dose dogs exhibited "unique membranous structures (UMS)", resembling mesh-work or the layered coats of an onion. Such were present in about one-third of the cells examined. Fine structural changes were more remarkable in dogs from the higher dosed groups. The study authors consider myopia in this study to follow as a consequence of changes in the ciliary muscle resulting from exposure to ethylthiometon.

2. Araki, M., Otsuka, J. and Mizuhira, V. (1973) Histological study of electron microscope and elemental analytical study of the ciliary body of a dog chronically poisoned by organophosphates. Nippon Ganka Gakkai Zasshi, 77, 1923-1935.

These investigators also performed electron microscopic examinations of the ciliary muscle of a control beagle dog and one

that had been treated at the 1 mg/kg/day dosage level for 2 years. Apparently, these were animals from the Suzuki and Ishikawa (1974) study cited above.

In contrast to the control, the treated dog exhibited degeneration of the ciliary muscle and marked hypertrophy of the basement membrane between muscle cells. In the normal dog the thickness of the basement membrane is 300-1000Å, while in the treated dog the thickness was 2000-5000Å. Some mitochondria were observed to have abnormal morphology and present evidence of various stages of degeneration. There was evidence of an enhanced level of sulfur in the ciliary muscle, presumably derived from ethylthiometon.

3. Tokoro, T., Suzuki, K., Nakano, H. and Otsuka, J. (1973) Experimental studies of organic phosphorus pesticide on beagle dog: long-term observations on the refraction and the intraocular pressure. Acta. Soc. Ophthal. Jap. 77, 1237-1245).

This study involved the assessment of such parameters as refraction, degree of astigmatism and intraocular pressure in dogs after 4, 12, and 24 months of treatment with ethylthiometon at the dosages indicated previously, i.e., 5, 10 and 15 mg/dog/day for the period of two years.

The following findings derive from all dosed animals combined vs. control animals. Dose dependent relationships are not provided except perhaps in so far as erythrocyte cholinesterase changes reflect dosage level.

Myopic changes were clearly evident in dosed groups after two years. The lower the erythrocyte cholinesterase level, the earlier the development of myopia.

Direct astigmatism attributable to corneal astigmatism was observed after four months. Astigmatism after one year is considered attributable to concerted effects upon the cornea and lens.

Refraction was observed to decrease and myopia to progress in a time dependent manner and were observed to correlate with the degree of lowering of erythrocyte cholinesterase.

From four months to one year, the optic axis length was inversely related to erythrocyte cholinesterase level.

Intraocular pressure relative to the control increased slightly after one year and decreased slightly after two years.

The authors advise that these responses in the dog were similar to those in humans of the Saku and Moriguchi areas. This study presents several good figures describing such findings as

changes in refraction angle and corneal refractive power in horizontal and vertical directions as a function of time, correlation between the refractive angle and blood cholinesterase values, and erythrocyte cholinesterase levels (evidently all dosed groups averaged) as a function of time. Differences by sex were not observed. The study did not identify any NOEL since dose-response relationships were not provided. The authors state that differences based upon the dose administered will be reported in a subsequent study.

4. Mukuno, K. and Imai, H. (1973) Histological research on dogs given long term administration of organophosphorus - especially the changes in the extraocular muscles. Nippon Ganka Gakkai Zasshi, 77, 1246-1253.

This paper presents considerable detailed histopathologic discussion of light microscopic and particularly electron microscopic examination of extraocular muscle from control and ethylthiometon treated dogs. The basic conclusions appear to be as follows: marked decrease in cholinesterase activity in extraocular muscle tissue [note: erythrocyte cholinesterase at the time of observation was markedly inhibited]. Normal light microscopic examinations using stains designed for the technique did not disclose abnormal findings. However, when samples were stained with toluidine blue for electron microscopic examination, such slides then by light microscopic examination revealed degeneration of intramuscular nerve fiber bundles in ethylthiometon treated dogs. By electron microscopy, pathological changes were clearly confirmed in extra-ocular muscle tissue, in the nerves, neuromuscular junction and muscle fibers - considered by the authors to constitute a state of neuromyopathy. In discussing the electron microscopic findings the authors emphasize the abnormal appearances of mitochondria (swollen mitochondria, huge mitochondria, degenerated huge mitochondria), diminution of mitochondria in the sole plate area, etc. The authors say that these findings with respect to mitochondria correspond to what has been called the mitochondrial degeneration picture and in this case is believed to be the consequence of ethylthiometon exposure. In consideration of all the electron microscopic findings, the authors state that it is difficult to believe that the mechanism of action of ethylthiometon is only that of cholinesterase inhibition. Of course, this alludes to another possible mechanism of action.

This study underscores how pathological effects can go undetected by ordinary light microscopic techniques, or stated differently, electron microscopic exams should be employed in detecting pathologic changes in their early stages.

The authors emphasize that the pathologic changes in question were never observed in the control dogs.

5. Uga, S., Ishikawa, S. and Mukuno, K. (1976) Microscopic

changes in the retina of dogs administered organophosphorus for prolonged periods. Jap. Rev. Clin. Ophthalm., 70, 282-283.

These investigators performed electron microscopic examinations of retinas in beagle dogs treated with ethylthiometon for two years at 0.5-1.5 mg/kg/day.

Degeneration of the retina was observed. It was most evident in the pigment epithelial cell layer in the vicinity of the papilla. Also, myelin-like structural material was evident in numerous cells of the retina. Mitochondria appear to constitute part of the morphologic picture of the degenerate condition, although it is not clear from the description whether this is indicative of an adverse effect of dosing upon mitochondria. The electron microscopic examinations are not presented as specific to any particular dosage level, and presumably were noted at all three dose levels.

6. Mukuno, K., Ishikawa, S. and Uga, S. (1973) An electron microscopic study of the extraocular muscles, optic nerve and sural nerve in experimental chronic organophosphate intoxication. J. Clin. Elec. Micro., 6, 3-4 (254-255).

According to the authors, electron microscopic examination of ocular tissue revealed that myelinated nerves of extraocular muscles occasionally showed demyelination. Functional folds in the motor end plate were become small and sparse and selective swelling of mitochondria was noted. In all treated dogs, nerve fibers decreased in number in the optic nerve, and septal tissue (mainly astrocytes and their process) increased in amount. Thus, ethylthiometon induced neuromyopathy in extraocular muscle, optic neuropathy and peripheral neuropathy in sciatic and sural nerves. No NOEL was observed for these phenomena.

7. Hikita, H., Miyata, M. and Ishikawa, S. (1973) Experimental study of chronic organophosphate (OP) intoxications in the beagle dogs activities of cholinesterases and residue of OP. Nihon Ganka Gakkai Zasshi, 77, 1254-65.

This study affirms the presence of both AChE and pseudo ChE in extraocular muscles and various tissues of the eye itself. Ethylthiometon inhibited cholinesterases of the extraocular muscles in a dose-related manner. At the highest dose level, 1.5 mg/kg/day, for example, inhibition of cholinesterase was 60-70% in the lateral rectus muscle. Cholinesterases in other ocular tissues were also inhibited in a dose-related manner. In the retina, the enzyme was inhibited to the extent of approximately 30%, 50% and 70% at the low, mid and high dose levels, respectively. Dosage levels employed did not include a NOEL. Serum pseudo-ChE was not inhibited by ethylthiometon, but erythrocyte AChE did respond to this organophosphate.

### Summary Conclusions from the Ethylthiometon study

The concerted findings as reported by the many aforementioned investigations of various ocular parameters following ethylthiometon administration in the dog at dosage levels of 0.5-1.5 mg/kg/day for two years is summarized as follows. These studies involved assessments of refraction, myopia, astigmatism, cholinesterase measurements in certain tissues, and electron microscopic examinations of extraocular muscle, ciliary muscle, retina and optic nerve. All treated dogs developed myopia within one year, a condition which was progressive throughout the study period. Astigmatism was observed at four months. Degeneration of ciliary muscle occurred in a dose related manner across all doses. This degeneration was marked by the appearance of abnormalities of mitochondria. There was a dose dependent lowering of erythrocyte AChE, but not of plasma pseudo-ChE. The study authors attribute myopia to degeneration of the ciliary muscle and note the relationship with the lowering of erythrocyte AChE. Degeneration of extra ocular muscle was also observed. Since the effect involved both nerve and muscle changes, the authors employ the descriptive term neuromyopathy in reference to this degenerative phenomenon. Cholinesterase inhibition in extraocular muscle was considerable (60-70%) at the high dose level. Retinal degeneration was observed particularly so in the pigmented epithelium in the vicinity of the papilla. Retinal cholinesterase was inhibited in a dose-related manner across all doses. The study disclosed decreases in the number of nerve fibers in the optic nerve, accompanied by increases in septal tissue. Peripheral neuropathy of the sciatic nerve was also observed. The authors suggest that in view of degenerative changes which often involve changes in the appearance of mitochondria, that the mechanism of action of this organophosphate may not be limited to that of cholinesterase inhibition, though no further elaboration of possible mechanism of action is offered. The authors also note that many of the responses seen in the dog were similar to those observed in humans in the Saku area.

This study as a whole provides convincing evidence of an effect of ethylthiometon on ocular tissues. Cholinesterase inhibition, erythrocytes and that of extraocular muscle, ciliary muscle and retina, was observed. The study did not identify a NOEL for these findings. The dosage range selected for the study, 0.5-1.5 mg/kg/day for two years was a relative high dose in relation to the LD<sub>50</sub> for ethylthiometon in the dog. Though the study confirms a manifold effect of this organophosphate upon the eye, it does not enable one to conclude at what dosage level the effects would be first noted, nor does it enable one to say whether or not the eye is uniquely sensitive or exquisitely sensitive to the organophosphates, since cholinesterase was inhibited at all levels, many systems in addition to the eye could be affected.

## Fenthion

1. a - Imai, H. (1974) Toxicity of organophosphorus pesticide (fenthion) on the retina. Electroretinographic and biochemical study. Acta Soc. Ophthal. Jap. 78, 163-172.

b - Miyata, M., Imai, H. and Ishikawa, S. (1973) Electroretinographic study of the rat after fenthion intoxication. Jap. J. Ophthal., 17, 335-343.

These investigators evaluated the effects of fenthion on the retina of the Wistar rat. This involved the assessment of electroretinographic (ERG) changes and cholinesterase inhibition in the retina of subject animals. Fenthion was administered in single doses subcutaneously (s.c.) to 6 animals in each of eight study groups. Dosage levels employed were 0.005, 0.05, 0.5, 5.0, 25, 50, 100 and 500 mg/kg. ERG readings were made on day 4 post-dosing. Upon sacrifice, AChE activity was measured in the retina and brain.

All rats in the 500 mg/kg group died before ERG measurements could be performed. At 50 and 100 mg/kg, subnormal ERGs were observed. At 25 mg/kg, the ERG was virtually normal and at doses below 25 mg/kg supernormal ERGs were observed. The authors consider the 25 mg/kg dose to correspond to a transitional band from supernormal to subnormal ERG responses. There was a minimal change in ERG even at the lowest dose, 0.005 mg/kg, or in the author's estimation, 1/60,000 of the LD<sub>50</sub>. Hence, the NOEL for ERG responses was not identified in the study. AChE activities in the retina and cerebellum were inhibited in a dose-related manner over the 0.5-100 mg/kg dose range. Neither was measurably inhibited at 0.005 or 0.05 mg/kg. Suppressions in both tissues at 0.5, 5 and 100 mg/kg were approximately 23%, 50% and 100%, respectively. Rats receiving less than 0.5 mg/kg showed no evidence of intoxication in terms of behavior. However, constriction of pupils (miosis) was noticeable in all animals. At doses exceeding 5 mg/kg, the rats exhibited general muscular tremor, exophthalmus and conjunctival discharge, phenomena which were dose dependent.

~~The~~ ~~effect~~ ~~of~~ ~~fenthion~~ ~~upon~~ ~~the~~ ~~ERG~~ ~~reported~~ ~~in~~ ~~this~~ ~~study~~ ~~at~~ ~~very~~ ~~low~~ ~~doses,~~ ~~below~~ ~~those~~ ~~that~~ ~~inhibited~~ ~~brain~~ ~~and~~ ~~retinal~~ ~~cholinesterases,~~ ~~is~~ ~~an~~ ~~important~~ ~~finding~~ ~~that~~ ~~solicits~~ ~~confirmation.~~

2. - Miyata, M., Imai, H. and Ishikawa, S. (1974) Rat retina cholinesterase and the effects of organophosphates. Nippon Ganka Kiyo, 25, 89-93. (TR-81-0016).

In studying retinal cholinesterases, these investigators performed both enzymologic and histochemical procedures. The investigation confirmed the presence of cholinesterases in the retina, including the optic nerve ganglia cell layer. When

fenthion was administered at 100 mg/kg, cholinesterase activity was completely non-detectable in the retina. There was a slight reduction in retinal cholinesterase activity when fenthion was administered at 5.0 mg/kg. The authors suggest that changes in ERG at doses as low as 0.005 mg/kg are conceivably due to cholinesterase inhibition. If so, this would suggest an exquisite sensitivity to the organophosphate, one which is not detectable by usual means of assaying cholinesterase activity.

3. - Imai, H. (1975). Research on the ocular toxicity of organophosphorus agents. Report 2. Residue properties in 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 activity). Hcta. Soc. Ophthalm. Jap. 79, 1067-1076 (TR-81-0235).

The authors investigated the duration of effects on ERG and cholinesterase activity following single dose administrations (s.c.) of fenthion. Wistar rats (6 rats/group) received dosages of 0, 5, 25 and 50 mg/kg. Dosage selection was designed to include ones which in the former studies yielded supernormal ERGs (5 mg/kg), corresponded to a transitional dose (25 mg/kg) and yielded a subnormal ERG response (50 mg/kg). In addition to ERG measurements, the study assessed serum pseudo ChE and retinal AChE. Cholinesterase measurements were performed only on the high-dose group.

At 5 mg/kg, supernormal ERGs were obtained which increased until about day 10 post-administration. This response decreased thereafter, returning to normal after about 2 months. At the mid dose, a transitional effect on ERG was confirmed wherein up to 4 days the ERG was increased, but later on reduced and apparently required 2 months for recovery. At 50 mg/kg, a subnormal ERG was observed which did not recover even after 66 days.

Serum pseudo ChE as measured at the high dose only declined to about 15% of normal at day 4. Substantially reduced activity was observed for 14 days. Recovery occurred at 30 days. Retinal AChE was decreased to about 7.5% of the pretreatment level on day 4. There was slight recovery by day 8 to about 45% of pretreatment level. The retinal AChE activity was decreased over a long time interval with recovery occurring by day 49. The author notes that ERG changes in the high dose group corresponded with the recovery curve for retinal AChE.

4. a - Imai, H. (1977) Experimental retinal degeneration due to organophosphorus agents. Acta. Soc. Ophthalm. Jap. 81, 925-932 (TR-81-0237).

b - Miyata, M. Imai, H. and Ishikawa, S. (1979) Experimental retinal pigmentary degeneration by organophosphorus pesticides in

rats. Excerpta Medica. Int. Cong. Ser. 450 vol. 1, ophthalmology, 901-902.

c - Uga, S., Imai, H. Miyata, M. and Ishikawa, S. (1979). Retinal degeneration in Long-Evans rats induced by fenthion intoxication: an electron microscopic study. Excerpta Medica Int. Cong. Ser. 450, vol. 1, Ophthalmology, 915-918.

The authors performed a multi-dosing experiment with fenthion designed primarily to assess the effects of chronic dosing upon retinal histopathology. Fenthion was administered (s.c.) once every 4 days at the dosage level of 50 mg/kg to a group of 20 Long-Evans black rats for 1 year. Ten rats served as controls. This high dose was selected in order to clarify whether the agent can cause retinal degeneration. Parameters studied included body weight change, ERG measurements, fundus morphology and retinal histopathology.

Dosed animals exhibited signs of toxicity, including exophthalmus, shivering, diarrhea, etc. Two animals died on both the third and fourth days of dosing. Deaths also occurred at one month (2 rats), at four months (1 rat) and after eleven months (2 rats).

ERG values in dosed animals were subnormal at three months post-dosing (measurements apparently were not performed prior to this time point). After 9 months, ERG a waves disappear leaving only b waves, and after 1 year no ERGs were recordable, i.e., extinguished.

Fundus examination disclosed paleness of the papilla where veins and arteries were constricted and pigmentary spots were found in the posterior pole. These effects were described as strong. Histologic examination confirmed the above. In addition the retinal pigmentary epithelial layer disappeared and the outer nodes, inner nodes and outer granular layer also disappeared. Migration of melanin was seen. Election microscopy showed that the photoreceptor cells had completely disappeared. Other peculiarities of the pathological changes are discussed in these papers. The authors emphasize that degenerative changes were principal findings, and these changes were never seen in the controls.

5. - Imai, H. Miyata, M., Uga, S. and Ishikawa, S. (1983) Retinal degeneration in rats exposed to an organophosphate pesticide (fenthion). Env. Res. 30, 453 - 465.

An additional assessment of the potential for fenthion to cause retinal degeneration as assessed in Long-Evans and Wistar rats. Fenthion at the 50 mg/kg dosage level was administered to rats, s.c., twice a week for one year. In the Long-Evans group there were 40 rats in the dosed group and 20 controls. Among the

Wistar group there were 20 dosed rats and 15 controls.

ERG examinations were performed every 3 months. Retinas from one rat of each strain from the experimental groups were examined every 3 months. On the day following the initial injections of fenthion, rats exhibited typical clinical cholinergic signs which generally subsided after 2 or 3 days, though some signs persisted. ERGs were remarkably subnormal as early as the 3rd month and extinguished at the 12th month. Wistar rats exhibited a more rapid onset of ERG extinction, where ERG responses had disappeared in 7 out of 15 rats at the 6th month time point. All treated Long-Evans rats exhibited retinal degeneration after one year. Among treated Wistar rats, 8 out of 12 survivors developed retinal degeneration at the end of one year. The authors note that pigmented animals (Long-Evans) were more sensitive than albino animals (Wistar).

In the case of the Long-Evans rats, there were ophthalmoscopically visible changes in the optic nerve, but no "frank" optic nerve atrophy. For Wistar rats, no degenerative changes of the optic nerve were observed.

The authors advise that rbc AChE was too low in dosed animals to be reliably assayed. This statement implies dosed groups as having essentially totally inhibited AChE, but this was not clearly stated. Plasma pseudo ChE was significantly reduced in treated animals at the 3rd and 12th months.

This study affirms the adverse effects of fenthion on the retina of the rat. The study authors note that these effects have been seen in patients chronically exposed to organophosphates.

#### Comments

- 1 - ERG changes occurred progressively across the dosage range 0.005 - 50 mg/kg following single dose administration. The 0.005 mg/kg level corresponds to 1/60,000 of the LD<sub>50</sub>. AChE activity in the retina and cerebellum were inhibited in a dose-related manner over the 0.5-100 mg/kg range, but neither was measurably inhibited at 0.005 or 0.05 mg/kg. This indicates an exquisite sensitivity of retinal physiology to this agent, which extends down to dosage levels well below those that would be detected by cholinesterase assays in the tissue of concern.
- 2 - Effects on ERGs following single doses in the 5-50 mg/kg range extend for 30 to 66 days. At the 50 mg/kg dose (one dose), recovery of serum pseudo ChE inhibition occurred by day 30 and retinal AChE by day 49.
- 3 - A chronic (1-year) study in the rat at 50 mg/kg (biweekly) yielded histopathologic evidence of retinal degeneration.

It appears as though retinal effects of fenthion in the dosage range 0.005 - 100 mg/kg may be progressive ones, from early measurable changes in ERG, to the extinction of the same, accompanied by progressive, ultimately severe, retinal degeneration at the high doses. We cannot say at this point what the effects upon vision might be at the low doses where ERG changes first occur, but it appears reasonable that at high doses, where retinal degeneration is severe and the ERG extinguished, that the animal in question would probably be blind. One would anticipate a wide gradation in visual competence at doses in between. An NOEL was not identified in this overall investigation. It does not appear that cholinesterase assays could be used to provide assurances that adverse physiological effects are not occurring at the lower doses.

## Fenitrothion

Ishikawa, S. and Miyata, M. (1980). Development of myopia following chronic organophosphate pesticide intoxication: an epidemiological and experimental study. In, Neurotoxicity of the Visual System; Merigan, W. and Weiss, B., eds., Raven Press, N.Y., pp. 233-254.

This publication includes a presentation of a study which evaluated the effects of fenitrothion upon the ciliary muscle and attendant changes in refraction (myopia), thickness of the lens and ocular axial length.

Experimental dogs (beagle) were administered fenitrothion orally (capsule), twice a week, at dosage levels of 10 mg/dog/week (2 dogs), 20 mg/dog/week (1 dog), and 100 mg/dog/week (1 dog) for a period of one year. Apparently 4 dogs served as controls and were administered empty capsules. The dogs were sacrificed one year after cessation of dosing.

Myopia was statistically significantly increased in dosed dogs (combined) vs controls at the 9th month after exposure was initiated, and persisted at 24 months, or for one year beyond cessation of dosing. Thickness of the lens was significantly increased in treated dogs. Intraocular pressure was also increased.

Histopathology of the ciliary muscle after one year of dosing revealed swollen muscle fibers even at the lowest dose level. Destruction of the ciliary muscle is described by the authors as obvious. This particular pathologic finding in the ciliary muscle was reportedly almost identical with that seen following ethylthiometon treatment. AChE was significantly reduced in the ciliary body, but not in the lens or erythrocytes at this terminal point in the study.

It should be noted that both erythrocyte AChE and serum pseudo ChE "tended to fall" during administration of fenitrothion, but these enzyme activities returned to normal level three months after cessation of treatment.

We should note that these findings indicate that cholinesterase inhibition in the ciliary body may be a more sensitive indicator of exposure to an organophosphate, such as fenitrothion, than would be erythrocyte AChE.

## Mevinphos and Malathion

Carricaburu, P., Lacroix, R. and Lacroix, J. (1981) Electroretinographic study of the white mouse intoxicated by organo-phosphorus: mevinphos and malathion. Tox. Eup. Res. III, 87-91.

In this study, Swiss white mice of both sexes weighing approximately 30g were employed in the assessment of acute effects of mevinphos and malathion upon ERG responses. Animals were administered either 300 mg of malathion or 0.015 mg of mevinphos via single intraperitoneal injection. These are high doses and the animals generally died a few hours after the beginning of the experiment. The dose of malathion was considerably higher than that of mevinphos, but in contrast to mevinphos, malathion requires activation (oxidation) before significantly inhibiting cholinesterase.

The study authors advise that both pesticides had identical influence upon electrical ocular responses as reflected in the ERG tracing. The a-waves were slightly decreased in amplitude, accompanied by substantial increases in time lag over a 40 minute observation period. The b-wave amplitude behaved similarly except that the decreased amplitude was more remarkable. The authors consider these findings to indicate that these agents, as reflected in the a-wave responses, exert direct action on photoreceptor cells via disruption of cholinergic neurotransmission. Similarly, alterations in b-wave responses could be attributable to disruption of cholinergic neurotransmission in bipolar neurons or to direct damage to bipolar or ganglion cell neurons, according to the study authors.

DEF

Letter of John S. Thornton, Mobay Corporation, Kansas City, MO. to Mr. Robert Taylor, Product Manager 25, OPP, dated April 30, 1990. Subject: DEF Technical, EPA Reg. No. 3125-96 - Interim Results from A Chronic Study Reportable Under FIFRA 6(a)(2).

This 6(a)(2) letter derives from an interim (12-month) micropathologic examination of rats in an on-going chronic study of DEF. The dosage levels being employed in this study are 0, 4, 40 and 320 ppm. This interim study evaluated 20 rats/sex of the control and high dose groups and 10 rats/sex at the low and mid doses.

The report advises under the 6(a)(2) reporting requirement that retinal degeneration was apparent in all rats at the high dose. The visual layer of the retinas in these rats apparently no longer existed. Retinal degeneration was not observed at the lower dosage levels at this point in time.

The registrant acknowledges that some organophosphates are known to cause this effect, but this is the first time it has been found true of DEF. A final report is anticipated in about one year.

## PARATHION

- a) Zendzian, R. (1984). Ethyl parathion, review of chronic/oncogenic rat study: A memorandum (review) by R. P. Zendzian dated 8/29/84.
- b) Zendzian, R. (1986). Electroretinographic evaluation, G.A. Edwards, Monsanto, R.D. No. 693, July 23, 1986, Accession #263986. A data evaluation report dated 8/86.
- c) Zendzian, R. (1987). Report of results of findings of electron microscopy of retina, nervus opticus --- chronic toxicology long-term study in rats. A data evaluation reported dated 7/1/87.
- d) Zendzian, R. (1989). Parathion, chronic/oncogenicity study in rats. A memorandum (review) by R. P. Zendzian dated 3/15/89.

Two studies are under discussion here. In the first of these, a Biodynamics Inc. study reviewed by Zendzian (1984), parathion was administered via the feed to rats in groups of 60 each at dosage levels of 0, 0.5, 5.0 and 50 ppm. Findings relevant to effects on the eye were: inhibition of plasma pseudo ChE in both sexes at the mid- and high-dose levels, but not at the low dose, brain cholinesterase was inhibited only at the high dose; cholinergic signs, more pronounced in females than in males, occurred at the high dose only; evidence of retinal atrophy was observed in females at the high dose level.

The second study was performed by the Institut fur Tieranatomie der Universitat Munchen. As reviewed by Zendzian (1987) parathion was tested in the rat at 0, 2, 8 and 32 ppm. Plasma pseudo ChE and erythrocyte AChE, LEL = 8 ppm, NOEL = 2 ppm; brain LEL = 32 ppm, NOEL = 8 ppm. Decreased ERGs occurred at the mid- and high-dose levels only. Gross retinal abnormalities were evident in females at the high dose. Of the 50 animals per group, 20 (40%) received ophthalmoscopic examination, 10 (20%) received ERG examinations and one-half, 5 (10%), of the latter group selected at random received retinal histopathologic examinations.

The ophthalmoscopic exam yielded evidence of choroidal pallor in males at all doses, and evidence of cataract in males at the high dose and in females possibly at all doses.

The ERG assessment indicated statistically significant reduced b-wave amplitudes among females at the two high doses accompanied by a numerical increase at the low dose. In males, there was a marginal (20%) reduction in b-wave amplitude in all dosed groups, which was not statistically significant.

Electron microscopy yielded a compound related toxicity in the retina and optic nerve of males and females at the high dose level.

Zendzian (1989) expressed the view based upon the concerted ERG, ophthalmoscopic and histopathologic effects that most if not all rats in the high dose group are blind.

Dr. Zendzian notes that ophthalmoscopic exams failed to reveal abnormalities which were clearly evident by electron microscopy. Also, ERGs were not as effective as electron microscopy in identifying (characterizing) the problem.

### Cholinesterase activity values in various organs- (dogs) I

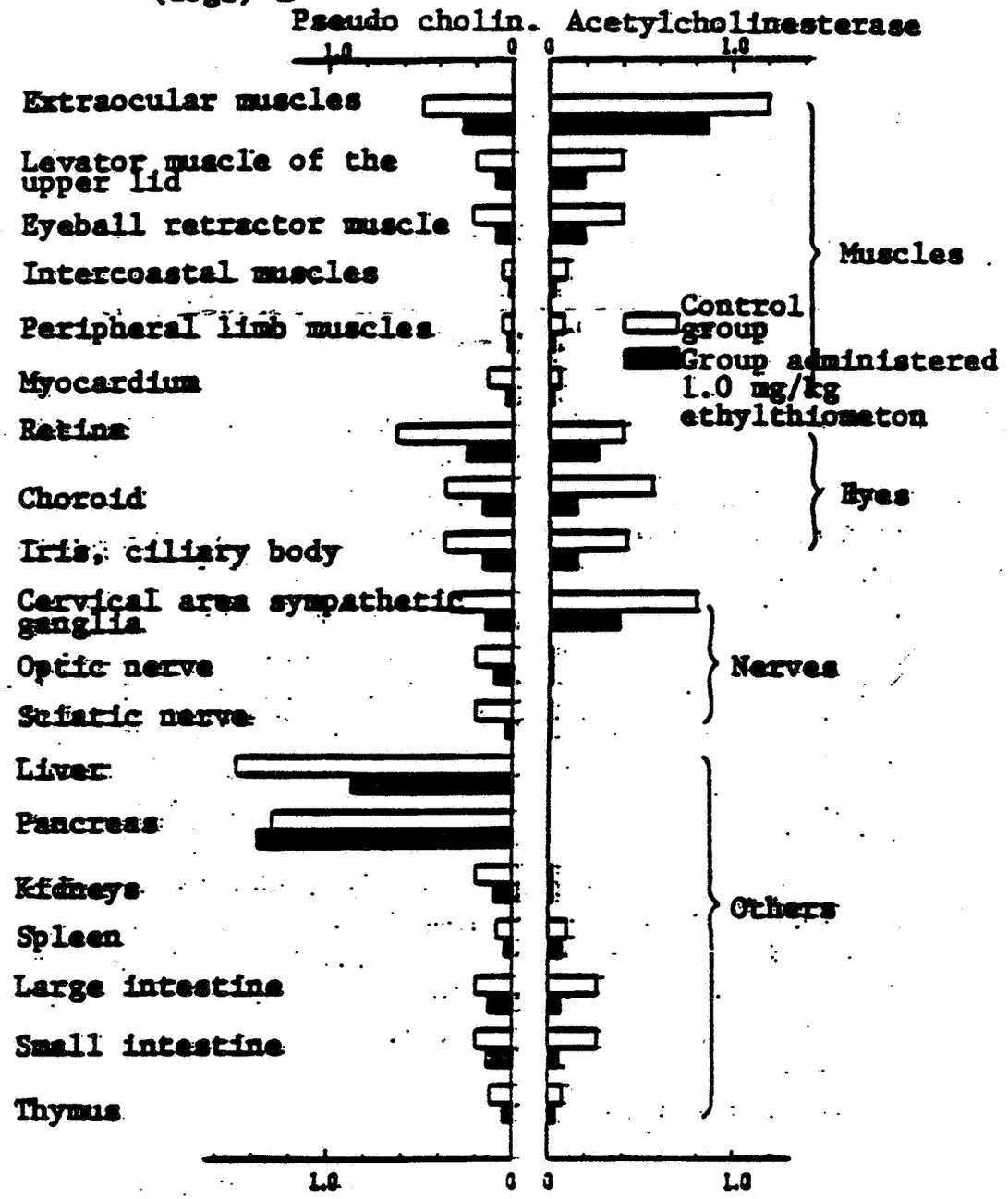


Fig. 3: Results of measuring acetyl and pseudo cholinesterase activity values in various organs. □ control group. ■ experimental group. Acetyl cholinesterase values were in the order of extraocular muscles, cervical area sympathetic ganglia, choroid, retina, iris ciliary body, levator muscle of the eyelid. Pseudo cholinesterase values are highest in the liver and pancreas, followed by the retina and extraocular muscles.

### III. Cholinesterases in Ocular Tissues

1. Hikita, H., Miyata, M. and Ishikawa, S. (1973). Experimental study of chronic organophosphate (OP) intoxications in the beagle dogs activities of cholinesterases and residue of OP. Nihon Ganka Gakkai Zasshi, 77, 1254-1265. (TR-81-0242).

Cholinesterases are widely found in ocular tissues. These investigators studied the distribution of cholinesterase activity at various anatomic sites as measured in the dog. The findings are reflected in the appended copy of Figure 3 (p.9) from the study. Cholinesterases, both acetyl- and pseudo- are shown to be present in extraocular muscles, eyeball retractor muscle, retina, choroid, and iris (ciliary body). Pseudocholinestrerase activity was present in the optic nerve. Notable findings included the fact that the highest level of AChE activity observed in any tissue in the study was found in extraocular muscles. The extraocular muscles exhibited 10-20 times higher cholinesterase activity than peripheral limb muscle. The lateral rectus muscle contained the highest total cholinesterase activity of all the extraocular muscles. High levels of cholinesterase activity were also identified in the retina, choroid and ciliary body.

2. Atterwill, C., Mahoney, A. and Neal, M. (1975). The uptake and subcellular distribution of <sup>3</sup>H-choline by the retina Br. J. Pharmacol., 53, 447.

These investigators studied choline uptake and its conversion to acetylcholine in the retina (rat and rabbit). Rates for both of these processes were considered to be very remarkable. This information in concert with other published works suggests a cholinergic activity in the retina.

3. Laties, A.M. (1969). Localization in cornea and lens of topically-applied irreversible cholinesterase inhibitors. Am. J. Ophthalm., 68, 848-857.

This investigator reported the presence of AChE in the corneal epithelium of the rabbit and human. He found not only cholinesterase activity, but choline acetyltransferase activity and acetylcholine as well. According to Plestina and Piukovic-Plestina (1978), the corneal epithelium contains the highest concentration of acetylcholine of any animal tissue. The presence of the two enzymes and acetylcholine in the cornea is indicative of the presence of cholinergic physiology, which, though not understood, has been speculated to involve pain perception. Laties indicates that organophosphorus cholinesterase inhibitors are bound in high concentration by the corneal epithelium. Such agents pass across the anterior chamber to enter the lens and the iris.

Cholinesterase is also present in the lens, but Laties indicates it is pseudo ChE. Michon and Kinoshita (1967) also claim the lens contains cholinesterase, however, in contrast to Laties, claim that it is true cholinesterase, i.e., AChE.

4. a) Guyton, A.C. (1991). The Eye: II. Receptor and neural function of the retina. In Textbook of Medical Physiology, eighth edition, W.B. Saunders Company, Phila., PA. (pp. 546-559).

b) Hutchins, J.B. (1987). Review: Acetylcholine as a neurotransmitter in the vertebrate retina. Exp. Eye Res., 45, 1-38.

The retina is an extremely complex neural tissue which deserves additional comment here. The retina is properly viewed as an extracranial extension or aspect of the central nervous system. The complexity of the retina is on an order of magnitude equivalent to that of the brain. According to Guyton, there are essentially six types of neurons within the retina. These include photoreceptor cells (rods and cones), horizontal cells, interplexiform cells, bipolar cells, amacrine cells and ganglion cells. The cell bodies of ganglion cells are located in the retina, but the axons from these cells make up the optic nerve upon emergence from the retina as such. All synapses between these various neurons are effected by chemical mediation and, hence, employ neurotransmitter substances. The latest information as summarized in Guyton would indicate that the neurotransmitter profile within the retina is not fully established. At least five neurotransmitter substances have been identified: gamma-aminobutyric acid (GABA), glycine, dopamine, indolamine and acetylcholine.

Hutchins, in the abstract of his review article, indicates that there is evidence for the existence of a cholinergic system in the retina of every species studied to date, and that acetylcholine as a neurotransmitter is both essential and ubiquitous at this level of the visual system. This author sites several published works in support of his claim. The evidence for acetylcholine as a retinal neurotransmitter derives from studies of acetylcholine uptake, choline acetyltransferase, acetylcholine release, acetylcholine receptors and effects of cholinergic drugs on ERGs and acetylcholinesterase.

It is noteworthy that according to the author, in the mammalian retina (e.g. rabbit, cat) the ERG responds to acetylcholine and its agonists with an initial increase followed by a decrease in b wave amplitude, and anticholinesterase agents have similar effects.

Cholinergic drugs affect horizontal cells, ganglion cells, amacrine cells, etc. "In light of the studies discussed here, it seems likely that the retina is at least one locus for the action

of systemic anticholinesterases on vision." (p. 23) "The cholinergic system in the vertebrate retina is arguably the most completely understood and thoroughly studied neurotransmitter system in the central nervous system." (p. 25)

The evidence presented in this section attests to the wide spread distribution of cholinesterases and cholinergic innervations in the visual system which in principal should render the system vulnerable to a host of visual anomalies as a consequence of cholinesterase inhibition.

#### IV - Case Report

- a) Letter (June 12, 1990) of Alfredo Sadun, M.D., Dept. of Ophthalmology and Surgery (Neurological), University of Southern California School of Medicine, to Kenneth Cohn, M.D., South Gate, CA.
- b) Letter (August 1, 1990) of Alfredo Sadun, M.D., Dept. of Ophthalmology and Surgery (Neurological), University of Southern California School of Medicine, to Brian Dementi, Ph.D., USEPA, Washington, DC.

Dr. Sadun, ophthalmologist, advises of his determination that a patient, Mr. Juan Macias, experienced a remarkable loss of vision to the point of legal blindness following his exposure to malathion during helicopter application of this pesticide in the Los Angeles, CA area. He diagnosed the case as that of Saku disease, after the characteristics of that disease as set forth by Dr. S. Ishikawa.

The particular findings available in this case are summarized as follows. Juan Macias was exposed to malathion at his home when he looked upward at a helicopter as it flew over spraying malathion. He felt wetness on his face and shortly thereafter experienced a burning sensation in his eyes accompanied by redness. He subsequently experienced blurred vision and difficulty in reading the blackboard at school. He received medical attention by the school nurse and perhaps four physicians, none of whom could diagnose his condition, before being referred to Dr. Sadun. Additional visual symptoms that he has experienced, according to Dr. Sadun, include yellow spots in the center of his visual field, a remarkable deterioration in vision from a normal of 20/20 prior to the exposure episode, a reliance upon his peripheral fields in order to see, severe compromises in color vision and brightness perception, compromised pupillary responses, abnormalities (saccadic intrusion) in visual pursuit, narrowing of visual fields, bilateral optic atrophy with severe drop-out of the nerve fiber layers, abnormal appearance of the retina, etc. An ERG performed while under Dr. Sadun's care was normal.

In diagnosing the patient as having Saku disease, Dr. Sadun feels that he has satisfactorily ruled out all other possible explanations for the illness. By more recent communication, Dr. Sadun has advised that the patient's vision remains impaired to the extent of legal blindness and he offers little hope for a dramatic improvement.

The case report of Dr. Sadun was subsequently submitted to an OPP consultant in this field, Sheldon Wagner, M.D., for an assessment. In his August 24, 1990 letter to Mr. Frank Davido, OPP Pesticide Incident Response Officer, Dr. Wagner expressed his belief that Juan Macias' condition was probably that of Saku disease secondary to malathion exposure. In addition, he expressed his concern that although the condition would probably be rare, it could go unrecognized by most physicians.

We should note that the opinions of Drs. Sadun and Wagner do not establish a cause and effect relationship between malathion spraying and Juan Macias' condition.