US EPA ARCHIVE DOCUMENT

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D C 20460

MEMORANDUM March 30, 1990

SUBJECT: Malathion: Allergic and Ocular Effects

TO: Penelope Fenner-Crisp Ph.D.
Director
Health Effects Division (H7509C)

FROM: Robert P. Zendzian Ph.D. 3/30/90
Senior Pharmacologist
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THROUGH: Reto Engler Ph.D.
Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Action Requested

Review and comment on the following documents;

MALATHION REVIEW: ALLERGIC AND OCULAR EFFECTS
Dennis Shusterman MD, MPH
Chapter Outline
Undated

Draft Memo; re. Potential for Malathion Ocular toxicity,
From, Dennis Shusterman MD, MPH,
To, DHS Medfly Working Group
March 8, 1990

Conclusions

1. Allergic effects

The sensitizing agent in technical malathion has been identified as diethyl fumarate. Considering its removal from technical malathion and the lack of clinical reports of sensitization from direct dermal use of malathion, allergic effects from malathion are not considered a potential health problem. However, the possibility remains that a specific formulation or application form of malathion may contain sensitizing agents.

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2. Ocular effects

The human and animal data available to the Agency clearly establish the organophosphate cholinesterase inhibitors as having the potential for significant irreversable toxicity on the visual system. While the human data does not clearly implicate any one compound, the animal studies show ocular toxicity, similar to that observed in humans, from fenitrothion, ethylthiometon, fenthion and ethyl and methyl parathion. At this time we have no evidence from animal toxicology studies of ocular toxicity due to malathion. However, we do not have an acceptable chronic toxicity study with malathion and this is the only type of routine (guideline) study which has demonstrated this type of toxicity with organophosphate cholinesterase inhibitors.

The compounds demonstrating the toxic effects on the ocular system are all irreversable cholinesterase inhibitors and the evidence available strongly indicates that cholinesterase inhibition, at the target organ, is required for these effects. This information does not perclude the possibility that reversable inhibitors, carbamates, can produce functional abnormalities in the visual system. The toxicity testing available at this time is not capable of detecting functional abnormalities.

Note. The compounds which are implicated in the human toxicity and have shown toxicity in the animal tests do not produce organophosphate type delayed neurotoxicity. Their toxicity on the visual system is separate from that unique type of toxicity. However, none of the compounds which produce OP delayed neurotoxicity have been tested for toxicity on the visual system and it is expected that they can produce ocular toxicity.

Recommendations

It is recommended that;

1. Special testing for toxicity to the visual system be required for all irreversable cholinesterase inhibitory compounds. Such testing has been required for ethyl and methyl parthion and fenthion in the respective registration standards.

2. Special testing for toxicity to the visual system be required for malathion with particular attention to additions to the rat chronic toxicity study such as those performed in the second ethyl parathion study. The high dose must be sufficiently high so as to produce signs of cholinergic toxicity in order to assure a sufficiently severe challenge to the visual system. In vivo observations must include direct examination of the eyes and electroretinograms. Histopathology must include light and electron microscopy of the eye and the

optic nerve.

3. The Agency develop sensitive functional tests for toxicity to the visual system. Such tests must be validated with reversable and irreversable cholinesterase inhibitors including the toxic organophosphates. When validated, the tests must become part of the Agency guidelines for toxicity testing.

Background

1. Dr. Shusterman's Documents.

a. The outline (attachment I) is, as represented, an outline for a chapter on Malathion as follows;

"Evidence for allergic effects
IgE-mediated (e.g. anaphylasis, urticaria, angiodema, atopic dermatitis, allergic rhinitis/conjunctivitis, atopic asthma)
Cell-mediated (allergic contact dermatitis)

Evidence for ocular effects
Irritant/Allergic (e.g., conjunctivitis, keratitis)
Pharmacologic (miosis, accomodative disturbances)
Neuro-opthalmologic (oculomotor abnormalities)
Other (optic neuritis)"

Five references are cited for the allergic effects section. The references were not provided to the Agency. As noted below we do not consider it necessary to obtain and review them.

Nine references are cited for the ocular effects section. The references were not provided to the Agency. We have the four references in Japanese and the one in Slovic, in translation and have obtained the remaining references.

b. The Draft Memo is a one and one-half page document listing 24 references (attachment II). Included with the package are copies of several of the English language papers written by the Japanese investigators, three other papers in English on the subject and 'testimony' of two individuals on the effects of malathion on the visual system. We have most of these papers and, in translation, copies of additional background papers from the Japanese literature.

Dr. Shusterman briefly summarizes the literature and states that there are significant methodologic problems in the studies and concludes "At best, for public health policy-making purposes, the reports should be treated as clinical case series materials suggestive of the need for further study of the opthalmologic effects of chronic high-dose

organophosphate pesticide exposure." He states as "reasons for the non-applicability of these studies to the Medfly eradication program":

1. "The pesticides used in Japan includes parathion and other organophosphates with acute and subacute toxicities many times that of malathion. In the medfly program, the sole pesticide is malathion."

2. Different application rates. "In Japan, rates of more than 2.6 pounds per acre per application were used, whereas in California the application rate is 2.8 ounces per acre per application"

3. Form of spray, fine mist which could remain suspended in Japan, "predominately large particle" protein bait in California.

4. Contamination of drinking water in Japan.

He concludes;

"it is our conclusion that exposures from the Medfly eradication program will not produce eye effects of the sort discribed in the Japanese literature."

2. Agency background.

a. Allergic effects.

On May 9-11, 1988, Dr. Roy Sjoblad of this office attended the Workshop on "The Effects of Pesticides on Human Health" as a member of the "Immunotoxicology Working Group". Dr. Sjoblad has provided a galley proof of "Chapter 5, Immunologic Effects of Pesticides" which will be published as part of the Proceedings of the Workshop in Advances in Modern Toxicology. We believe that the working group's comments on malathion's immunological properties provide the most recent and authoritative evaluation.

b. Ocular effects

In 1979, as part of the EPN Rebuttable Presumption Against Reregistration, I evaluated a review article by Plestina & Liukovic-Plestina (1978) on the toxic effects of organphosphates on the eye. The authors discussed the cholinergic effects of organophosphates on the eye and the production of cataracts following topical administration for glaucoma. In addition they cited an extensive literature from Japan on the toxic effects of organophosphates on the visual system. We obtained the Japanese references and had them translated. We have also obtained additional, more recent, reports directly from Drs Satoshi Ishikawa and Kazui Mukuno. Bibliographies of these references are attached as Human Effects, 55 references,

attachment III and Animal Studies, 28 references, attachment IV.

We have also received registrant submitted reports of chronic toxicity studies in the rat with ethyl and methy parathion which show significant toxicity to the eye by routine eye examination and histology and, in the second ethyl parathion study, by additional special testing.

Based on these findings we are now requesting specific evaluations of the visual system for organophosphate cholinesterase inhibiting pesticides.

My evaluation of this material is presented in a briefing document, "Toxic Effects of Organophosphate Pesticides on the Eye", attachment V.

Discussion

1. Allergic effects

The following is quoted from the immunologic effects chapter galley proofs;

"The pesticides malathion, captan, benomyl, maneb, and naled are strong to extreme sensitizers by GPMT (Guinea Pig Maximization Test); however, human sensitization data do not always agree. For example, although human maximization test (HMT) data on technical grade malathion support its classification of a strong sensitizer (Kligman, 1966), such a rating was not confirmed by an International Contact Dermatitis Research Group survey of 455 individuals, in which one tested positive (Cronin, 1980). The lack of clinical reports of dermatitis from malathion use (including direct skin contact as a delousing agent) makes it apparent that the animal and human predictive tests (GPMT and HMT) that indicated strong to extreme sensitization potential overestimated the sensitization hazard. Furthermore, the offending chemical in technical malathion is diethyl fumarate (Fisher, 1988: Milby and Epstein, 1964); reduction of this constituent has further lowered the sensitization potential of this pesticide."

Considering the removal of the sensitizing agent diethyl fumarate from technical malathion and the lack of clinical reports of sensitization from direct dermal use of malathion, allergic effects from malathion are not considered a potential health problem.

However, this does not mean that the formulation, bait, used in California is not a sensitizer. In our experience formulations can contain sensitizing agents other than the active ingredient. For this reason the Agency requires sensitization testing for all formulations. We have no information as to whether the California bait formulation has been tested for sensitization potential.

b. Ocular effects

The pharmacologic effects of the organophosphate cholinesterae inhibitors (OPs) on the eye such as miosis and accomodative disturbances are well recognized, as is the production of cataracts following topical administration for glaucoma. The Japanese experience with extensive human poisoning by OPs showed a previously unreported syndrome of effects on vision ranging from myopia to congestion or atropy of the optic nerve.

The ocular syndrome was not typical of myopia, being generally more severe, accompanied by vertical astigmatism, concentric narrowing of the visual field, and abnormal eye movements. It was not correctable. Additional observations included lowered activity of serum cholinesterase, neurological abnormalities characteristic of anticholinesterase poisoning and relatively high levels of organophosphate insecticides in the blood of the patients compared with normal individuals from other areas.

Ishikawa and Miyata (1980) listed the organophosphates malathion, EPN, ethyl and methyl parathion, fenthion, dipterex, fenitrothion and diazinon as having extensive use in Japan. Earlier papers listed these orgaonphosphates and many more but are not clear as to the extent of their use.

In general the association between the toxic syndrome observed in Japan and exposure to organophosphate pesticides is well established but individual compounds could not be clearly connected with individual cases. Use data indicated possible exposure to more than one organophosphate. Urine analysis could only identify the presence of phosphate metabolites which are not indicative of a specific organophosphate or the presence of paranitrophenol which can be indicative of ethyl parathion, methyl parathion or EPN.

Animal experimentation in Japan showed that the OPs fenitrothion, ethylthiometon and fenthion could produce various aspects of the human syndrome. See the briefing paper and Ishikawa 1980 for details (Attachment V).

The Agency has received chronic toxicity studies on ethyl and methyl parathion which showed toxic effects on the eyes. These effects are similar to some aspects of the sydrome reported from Japan. Two studies with ethyl parathion showed effects at the high doses (50 and 32 ppm) consisting of retinal degeneration by direct observation and histopathology, decreased ERG activity, histopathology (EM) indicative of blindness, and a possible increase in cataracts in females. Lesions were observed in the males but there was no compound-related effect. The sex differencs may have been due to significant differences in compound intake. At the 32 ppm

dose the actual dose was 2.47 mg/kg/day females and 1.75 mg/kg/day males. The study with methyl parathion showed retinal degeneration by direct observation and histopathology at the high dose, 50 ppm, again only in the females.

The human and animal data available clearly establish the OPs as having the potential for significant toxicity on the ocular system. While the human data does not clearly implicate any one compound, the animal studies show ocular toxicity from fenitrothion, ethylthiometon, fenthion and ethyl and methyl parathion. The pattern of toxicity is such as to clearly implicate cholinesterase inhibition as a major factor, in this toxicity. Since malathion is an organophosphate cholinesterase inhibitor, we must consider whether our lack of data indicating toxicity to the eye is due to an intrinsic lack of this toxicity or to lack of the proper experiment to show such toxicity.

We have no data indicating a toxic effect of malathion on the eyes but this may well be due to the lack of an acceptable chronic toxicity study in the rat with malathion. The chronic rat study is the only routine toxicity study which has shown ocular toxicity, by ethyl and methyl parathion. These studies also show that the dose tested must be sufficiently high as to show signs of cholenergic toxicity to assure adequate testing to demonstrate the presence or absence of structural toxicity to the visual system.

Special testing for toxicity to the visual system is necessary for malathion with particular attention to additions to the rat chronic toxicity study such as those performed in the second ethyl parathion study. The high dose must be sufficiently high so as to produce signs of cholinergic toxicity in order to assure a sufficiently severe challange to the visual system. In vivo observations must include direct examination of the eyes and electroretinograms. Histopathology must include light and electron microscopy of the eye and the optic nerve.

However, one must note that the compounds, ethyl and methyl parathion, shown by chronic animal studies to have ocular toxicity are in the order of 200 times more toxic than malathion. If malathion demonstrates ocular toxicity in animal studies, it may occur at doses significantly higher than those of the prototype compounds.

The following conclusions can be made in relation to the organophosphate cholinesterase inhibitors;

1. Severe functional and structural damage has been demonstrated in human and experimental animals. Such damage can follow a single massive dose or repeated smaller doses.

In general the severe toxicity follows doses which demonstrate cholinergic toxicity.

2. Function and possibly structural damage can occur at doses which produce blood cholinesterase inhibition without signs of cholinergic toxicity.

3. Functional abnormalities can occur at doses which do not produce blood cholinesterase inhibition. These can occur in the highly sensitive cholinergic structures in the retina and in central areas of the brain. Such effects require sensitive and specific testing procedures which are not available for routine testing.

Attachments

I MALATHION REVIEW: ALLERGIC AND OCULAR EFFECTS
Dennis Shusterman, MD, MPH

II Draft Memo, re Potential for Malathion Ocular Toxicity, Dennis Shusterman, MD, MPH to DHS Medfly Working Program Mrr 8 ,1990

III Bibliography Toxic Effects of OPs on the Eye, Human Effects, Japan

IV Bibliography Toxic Effects of Organophosphate Insecticides on the Eye (Animal Experiments 1972-1977)

V Briefing Document Toxic Effects of Organophosphate Pesticides on the Eye, Robert P. Zendzian Ph.D.

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Attachment I

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MALATHION REVIEW: ALLERGIC AND OCULAR EFFECTS

Dennis Shusterman, MD, MPH

CHAPTER OUTLINE

Evidence for allergic effects
- IgE-mediated (e.g., anaphylaxis, urticaria, angioedema, atopic dermatitis, allergic rhinitis/conjunctivitis, atopic asthma)
- Cell-mediated (allergic contact dermatitis)

Evidence for ocular effects
- Irritant/Allergic (e.g., conjunctivitis, keratitis)
- Pharmacologic (miosis, accomodative disturbances)
- Neuro-opthalmologic (oculomotor abnormalities)
- Other (optic neuritis)

REFERENCES TO BE REVIEWED

Allergic/Dermatologic Effects

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Ocular Effects

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Gupta SK, Pandya MK, Jani JP et al. (1980) Health risks in ultra-low-volume (ULV) aerial spray of malathion for mosquito control. J Environ Sci Health [B] 15:287-94.

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Attachment II

DRAFT

DRAFT

DATE: March 8, 1990
TO: DHS Medfly Working Group
FROM: Dennis Shusterman, MD, MPH
RE: Potential for Malathion ocular toxicity

As you are probably aware, it has come to our attention that several articles originating in Japan have purported to implicate the high-level spraying of various organophosphate pesticides in the genesis of a variety of ocular disorders. While we are continuing to receive and review literature at this date, certain preliminary conclusions are possible.

To briefly summarize the issue: Around 1970, Japanese researchers became concerned with what they believed were increased numbers of cases of myopia among school children in a rural area of Japan. Closer examination of some of these children reportedly revealed other eye abnormalities as well (including disturbances of extraocular movement, visual fields, pupillary function, and ophthalmoscopic appearance of the optic nerve). An attempt was made to compare these children with "controls" from another area, but details of this comparison are obscure in the articles we have reviewed to date. A search for possible causal factors revealed that the increase in eye cases appeared to coincide in time with the institution of "massive and routine" aerial agricultural spraying of organophosphorus pesticides in an area of mixed residential and agricultural land use. Upon blood cholinesterase testing, many of these children had results indicative of absorption of large quantities of highly toxic organophosphate pesticides. This last point is critical, because the eye effects, if real, are apparently related to chronic systemic toxicity (hence, the *absorbed dose* and *degree of toxicity* of the pesticide), not to external (i.e., conjunctival) exposures.

Review of the scientific merits of these studies reveal a number of methodologic problems (including ambiguous study design, questionable comparability of controls, lack of specification of diagnostic criteria, *post hoc* interpretation of results, and questionable statistical procedures), leading to ambiguity in the interpretation of study results. At best, for public health policy-making purposes, the reports should be treated as clinical case series materials suggestive of the need for further study of the ophthalmologic effects of chronic high-dose organophosphate pesticide exposure. Reasons for the non-applicability of these studies to the Medfly eradication program include the following differences in exposure conditions:

1) The pesticides used in Japan included parathion and other organophosphates with acute and subacute toxicities many times that of malathion. In the Medfly program, the sole pesticide used is malathion.

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2) There are large differences in the application rates between the Japanese studies and the Medfly program. In Japan, rates of more than 2.6 *pounds* per acre per application were used, whereas in California the application rate is 2.8 *ounces* per acre per application (less than one-fifteenth of the Japanese rate).

3) The form of the spray in Japan was a fine mist, capable of remaining suspended in the air for extended periods of time, thus maximizing deep penetration into the respiratory tract of exposed individuals. By contrast, the Medfly eradication program employs protein bait mixed with pesticide, creating predominantly large particles which tend to settle out of the air quickly.

4) In most of the Japanese areas studied, contamination of drinking water with pesticides had also occurred, correspondingly increasing the dose to the population. By contrast, no such contamination is anticipated in Southern California.

Thus, when differences in the form of the spray, average potency of the product used, application rate, and alternate routes of exposure are considered, the difference in potential dose to people living in the Japanese areas and in the Medfly eradication areas is very large.

Given the fact that these eye effects, if real, appear to be related to long-term cholinesterase inhibition (not a topical effect), and given the concensus that such inhibition will not occur as a result of the aerial spraying of malathion bait, it is our conclusion that exposures from the Medfly eradication program will not produce eye effects of the sort described in the Japanese literature.

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Please send any additional references to:

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Public Health Effects Advisory Committee - 3/8/90

SUPPLEMENTAL REFERENCE LIST: OCULAR EFFECTS OF MALATHION

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Attachment III

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Attachment IV

Toxic Effects of Organophosphate Insecticides on the Eye
(Animal Experiments 1972-1977)

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Attachment V

TOXIC EFFECTS OF ORGANOPHOSPHATE PESTICIDES ON THE EYE

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Introduction

In Japan organophosphate pesticides have been observed to produce toxic effects on the eye. Extensive human poisoning produced a syndrome of effects on vision ranging in severity from myopia to congestion or atropy of the optic nerve. The Japanese researchers were able to duplicate the effects on refraction in an experimental study on dogs utilizing the organophosphates fenitrothion and ethylthiometon. Experimental studies with the organophosphate fenthion on rats by another Japanese scientist demonstrated a syndrome of toxic effects on the eye beginning with functional abnormalities in electrical activity and culminating in retinal degeneration following chronic dosing.

Background

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

Dr. Ishikawa had been involved in research on the treatment of glaucoma and recognized this pattern of toxicity as similar to that seen in glaucoma patients treated with organophosphates.

Ishikawa and Miyata (1980) list the organophosphates malathion, EPN, ethyl and methyl parathion, fenthion, dipterex,

*Appendix I contains a copy of this paper.

fenitrothion and diazinon as having extensive use in Japan. Earlier papers list these and many more but are not clear as to the extent of their use.

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

Ishikawa and his coworkers were able to reproduce the human eye toxicity, myopia with vertical astigmatism, in a two-year study of ethylthiometon and fenitrothion in dogs. This effect was detected by measuring the shape of the refractive surface of the eye. The results of the study are summarized briefly in Ishikawa and Miyata (1980). Ethylthiometon was administered daily for two years at doses of 5 to 15 mg/dog/day. A reduction in erythrocyte acetylcholinesterase activity was the only additional sign of toxicity. Histopathology revealed a doserelated destruction of the ciliary muscle. Fenitrothion was administered for one year at doses of 5 to 50 mg/kg twice weekly and the dogs were observed for an additional year. The eye effect was produced and persisted for the one year observation period. Both plasma and RBC cholinesterase activity were depressed. Histopathology was similar to that observed in the ethylthiometon treated dogs.

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

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

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

at 5 mg/kg showed recovery from the compound effect at 30-49 days after dosing.

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

Additional data available

1. Japanese reports on human effects.

Fifty-five papers from the Japanese literature on the toxic eye effects of organophasphates are available in translation in Toxicology Branch. Although there is a great deal of duplicative reporting in these papers, they contain the primary data source on this type of poisoning in Japan. The papers present detailed descriptions of the toxic syndrome and its treatment, detailed individual case reports, exposure and incidence surveys and critical comments on the issue. In general the connection between the toxic syndrome and organophosphate pesticides is well established but individual compounds could not be clearly connected with individual cases. Use data indicated possible exposure to more than one organophosphate. Urine analysis could only identify the presence of phosphate metabolites which are not indicative of a specific organophosphate or the presence of paranitrophenol which can be indicative of ethyl parathion, methyl parathion or EPN.

2. Japanese reports of studies in experimental animals

Twenty-eight papers from the Japanese literature on the toxic eye effects of organophosphates in animal experiments are available in translation in Toxicology Branch. These studies were able to reproduce various aspects of the toxic eye effects seen in the human cases with ethylthiometon and fenitrothion in dogs and fenthion in rats. These studies also provide information on dose-response relationships. Their results have been presented briefly above in the Background section. These studies also provide experimental methodology and design suitable for testing other organphosphates for the toxic eye effects.

3. Studies submitted to the Agency

a) Ethyl Parathion.

A two-year chronic study of ethyl parathion was performed in rats. The compound was supplied in the feed at concentrations

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

Light microscophy detected 2.5 times as many lesions in the controls and 3 times as many in the high dose females as well as detecting lesions in the low and intermediate dose females. Percent incidence was 17% controls, 8% 0.5ppm, 10% 5ppm and 43% 50ppm. Lesions were observed in the males but there was no compound-related effect.

In a second chronic rat study animals were dosed at 0, 2, 8 & 32 ppm and special eye examinations, electro-retinograms (ERGs) and special microscopic examinations (including electronmicroscopy) were performed at termination. Terminal observations on the eyes indicated decreased ERG (LEL 8 ppm, NOEL 2 ppm), gross retinal abnormalities, histopathology indicative of blindness and possible increase in cataracts (LEL 32 ppm, NOEL 8 ppm) in the females. Effects on the eyes of the males at 32 ppm were possible but not clearcut. The sex differences were most probably due to the higher doses actually ingested by the females (2.47 versis 1.75 mg/kg/day for the high dose).

The critical factor in the special eye studies was that the animals examination by electronmicroscopy were randomly selected from the clinically healthy and opthalmologically normal rats intended for the ERG examination. The EM examination showed histological abnormalities of retina and optic nerve indicating that most, if not all, of the high dose animals were blind as a result of treatment.

A testing problem identified in this study was the lack of corrilation between the results of the various optic tests. The animal selected for special histopath were grossly normal by direct ophthmoscopic examination but histological abnormalities of the retina and optic nerve were severe. Animals with severe histological abnormalities showed ERGs which could not be distinguished from histologically normal controls. The study clearly showed histopathology as the most sensitive indicator of optic system abnormality.

b) Methyl Parathion

A two-year chronic study of methyl parathion, identical to the first study with ethyl parathion, was performed in rats. The compound was supplied in the feed at concentrations of 0.5, 5.0 and 50 ppm. At the 24 month observation, retinal

degeneration was observed by the veterinary opthalmologist in 15 of the 35 living females at the high dose. No additional high dose females showed the effect at 28 months so that 15 was the total number detected for an incidence of 43% (15/35). No retinal effect was observed in any other group of females and no retinal effect was observed in the males.

Histopathology detected retinal degeneration in the controls, low and intermediate dose females which were not detected by the opthalmologist and an additional five high dose females. Percent incidence was 5% controls, 5% 0.5ppm, 5% 5ppm and 36% (20/55) 50ppm. Lesions were not observed in the males.

Discussion

The Japanese reports available, on both human and animal, are a very mixed lot. Many are duplicative, incomplete or in abstract. Yet sufficient hard evidence exists to clearly show that organophosphate cholinesterase-inhibiting pesticides produce a spectrum of toxic effects on the eye both functionally and structurally. The mechanism of these effects is reasonably delineated in the animal studies. However, one cannot attribuite a specific compound as causal in the human cases.

In order to make a case based on the studies available it will be necessary to prepare a detailed, written evaluation of the reports and write a document presenting the evidence. There are 82 reports in house, most in translation from the Japanese and a few written in English. In addition, some 20-30 other reports cited therein will have to be obtained, translated and evaluated. To do the task will take 2.5-3 man months of a senior reviewer's time and, if the additional studies are ordered early, can take 3-3.5 calender months for completion.

What will we have when this is completed?

1) In the human cases we will have a clearer picture of the toxic syndrome but probably still not be able to indict any single compound or determine a dose-relationship.

2) In the case of the animal studies of ethyl and methyl parathion, we will know that chronic administration causes retinal atropy and as the Registrant(s) completes the studies requested, we will have information on acute and chronic effects on nerve function. The Registrant(s) has indicated interest in performing the studies. These, the most sensitive studies will provide a dose-response relationship and NOELs.

3) In the case of ethylthiometon and fenitrothion we will have studies in a limited number of dogs showing effects on the geometry and optics of the eye and indications of their cause through effects on the retinal, ciliary and

oculomotor muscles. The doses used and the dose regimen are not clear and we will not have a NOEL(s).

4) In the case of fenthion in the rat we will have studies of the acute dose response on the electrical activity, cholinesterase inhibition in discrete parts of the eye and indications of the duration of the effect. The chronic study provides information on the effects on electrical activity and histology showing degenerative effects. Unfortunately all of this is the result of subcutaneous abministration, a route that is not normal for pesticide administration. Even so the electrical effects reported occurred at incredibly low doses, 0.005mg/mg/kg the lowest dose tested. Rats in the study survived a single dose of 100mg/kg and all died at 500mg/kg.

5) There is some indication in the Japanese literature of an effect on color vision. At this time we have no experimental test system for determining effects on color vision.

Recommendations

It is recommended that a formal written evaluation of the Japanese reports be performed. The task may be performed either immediately with a completion in three months or to be completed in 6-9 months. The latter course is recommended as this will allow time for the receipt of preliminary information on the eye toxicity studies on parathion.

Upon completion of the evaluation the following possible actions can be available.

1) Require acute eye studies, electroretinograms, in rats on the organophosphate pesticides identified as being used in Japan during the toxic eye epidemic and on the compounds tested in animals in the Japanese studies. The acute studies will determine which of the suspect compounds causes an eye effect by monitoring electrical activity and will determine a dose-response relationship with NOEL for the active compounds.

2. Evaluate all organophosphate pesticides with acute eye studies.

3. Present the problem to SAP and request guidance.

4. Something unexpected may come of the acute parathion eye studies and an unpredictable course may be necessary.

Of the four possibilities number one appears most likely.

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Parathion, study for chronic toxicity and cancerogenicity in Wister rats (administration in diet for twenty-six months). R. Eiben; Histopathology report, Pappritz; Report on ERG examination, Rubin; Report on EM examination, Liebich; Bayer AG, Toxicology Division, Study No. T 1016770, Report No. 16305, Dec 16, 1987, MRID 406447-04 (2 vols)

The paper attached is a good short general review paper on the Japanese experience with the effects of organophosphate pesticides on the eye. It presents the human experience and the animal studies which were used to show that organophosphate pesticides can cause the effects seen in humans. It is of necessity incomplete and should not be used as a primary data source. Translations of the Japanese papers cited as well as numerous other Japanese papers on this subject are on hand in Toxicology Branch. These papers present the origional reports of the human experiences and the animal studies which should be used in developing an understanding of the toxic eye effects of these compounds

Ishikawa, I. & M. Miyata, Development of Myopia Following Chronic Organophosphate Pesticide Intoxication: An Epidemiological and Experimental Study,. in Neurotoxicity of the Visual sustem. Ed by W.H. Mergin and B. Wise, Raven Press, New York © 1980.

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edited by W. H. Merigan and B. Weiss
Raven Press, New York © 1980

Development of Myopia Following Chronic Organophosphate Pesticide Intoxication: An Epidemiological and Experimental Study

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A tremendous increase of cases of myopia in children that began several years after World War II is one of the most serious public health problems in Japan. Until recently, its etiology had never been analyzed. The correlation between this myopia and organophosphate pesticides was first suggested by the outbreak of cases in the Saku district, where massive amounts of parathion and malathion had been sprayed (6). Thereafter, detailed epidemiological, clinical, and experimental investigations were conducted in the district by a government task force that had recommended specific diagnostic criteria (7-10). The patients with myopia differed from ordinary myopes in that they had mild clinical complications of the central, peripheral, and autonomic nervous systems. In addition, all patients showed minor abnormalities in the biochemical profile of their peripheral blood (7).

Organophosphate compounds produce an intense cholinomimetic response in the nervous system. They can result in delayed neurotoxicity, both in humans and in animals, involving the cerebrum, brainstem, spinal cord, peripheral nerve, and muscle as well as the eyes (2,3). These neurotoxic effects are best documented in a monograph from the Stockholm International Peace Research Institute (1).

Japan appears to use more pesticides, especially organophosphate compounds, per unit of cultivated area than almost any other country in the world (16). Japan also has a high incidence of visual disturbances in school children in several districts (12) throughout the country. In this chapter, organophosphate pesticides are examined from an epidemiological viewpoint as a cause of myopia in school children of Tokushima and the Saku districts. In addition, the development of myopia is reported in young Beagle dogs following chronic, low dosage, oral administration of one potent and one less potent organophosphate pesticide.

The patients described below from the Saku district were chosen by the following diagnostic criteria (10).

Major signs:

A. Ocular signs
   1. Reduced vision
   2. Narrowing of the peripheral visual field and/or central scotoma
   3. Abnormal refraction or myopic tendency with or without vertical corneal astigmatism

B. General signs
   1. Pyramidal signs
   2. Reduction of the proprioceptive sense and difficulty standing on one foot
   3. Autonomic signs, dizziness, headache, nausea, vomiting, diarrhea, constipation, perspiration, numbness, polydipsia, impotence, vesicular and rectal signs, etc.

C. Laboratory-detection of organophsphate compounds in the blood or urine over 0.01 ppm

D. Therapeutic-recovery from the signs with the administration of 2-PAM or atropine

Additional signs:

A. Ocular Signs
   1. Congestion or atrophy of the optic nerve
   2. Difficulty with ocular smooth pursuit movement
   3. Abnormal ERG

B. General signs
   1. Reduction of cholinesterase activity (primarily of erythrocytes)
   2. Mild abnormalities of liver function (mainly LDH, CPK, alkaliphosphatase, etc.)
   3. Sensory disturbance of the glove-stocking type
   4. Foot drop
   5. EEG abnormalities

METHODS

Use of Organophosphorous Pesticides in Japan

Volume of organophosphorous pesticide use was calculated from the annual book issued by the Japanese Agricultural Department. A comparison between the use of pesticides in Japan and other countries was also calculated from the Production Year Book.

Epidemiological Study of Myopia

The epidemiological study was done by the committee members, Drs. O. Tamura and Y. Mitsui, of the Tokushima Prefecture (14). During the period from 1957 to 1973, eye tests were carried out in about 40,000 school children

of the primary and junior high schools in the prefecture. The ocular refraction was determined by school nurses and orthoptists with trial lenses and visual acuity charts. The results from each school were accumulated by the statistics section of the prefectural office and the incidence of myopia was calculated separately for boys and for girls in primary and junior high schools. In 1970, when the incidence of myopia in school children was investigated on a nationwide scale, the sampling method differed from that of the present survey, and therefore, 1970 data were omitted from the present statistics. Three kinds of pesticides, i.e., organophosphates, carbamates, and organochlorines have been used in the prefecture and the total amount sold was calculated for each pesticide.

Experimental Studies

Prior to the experiment, the optimal dose of each pesticide required to produce myopia was determined by using 20 young and old mongrel dogs. The subsequent experiment started with 19 purebred Beagle dogs (6 months of age). All dogs were accustomed to the procedure before the start of the experiment. Five were treated with oral administration of ethylthiometon and four with fenitrothion. Ten others, given empty capsules, served as controls. Ethylthiometon dosage was 5 mg/dog/day for two dogs, 10 mg/dog/day for two dogs, and 15 mg/dog/day for one dog. The capsules were given 5 days a week for 2 years from November 1, 1970, to November 1, 1972. Total amount of ethylthiometon ranged from 2,300 mg to 6,900 mg at the termination of administration. Fenitrothion was given in the following manner. Administration by the oral route was made twice a week —10 mg/dog/week for two dogs, 20 mg/dog/week for one dog, and 100 mg/dog/week for one dog. Total amount of fenitrothion ranged from 520 mg to 5,200 mg, respectively, for 1 year. The administration started June 1, 1973 and terminated June 1, 1974. The dogs weighed 5 to 9.4 kg at the start and 7.1 to 10.6 kg at the end of administration. Refraction in dipoters was measured before exposure began and then once a month using the coincidence optometer (Hartinger-Jena) 30 min after the topical administration of 3 drops of 1% cyclopentolate hydrochloride over a 5-min period (11). The dog was placed in a comfortable box and the vernier adjustment performed by the same examiner at a point on the retina approximately two disc diameters temporal to the disc. Horizontal and vertical meridians of both eyes were measured. In addition, corneal curvature (ophthalmometer), ocular axial length (ultrasonographic method), depth of the anterior chamber, thickness of the lens, and intraocular pressure (Mackay-Marg tonometer) were determined. All ophthalmological measures were averaged and compared with those of the controls. Other general measures included hemogram and various chemical analyses as well as a measurement of pseudocholinesterase in the blood (5).

All dogs were killed within 10 days after November 1, 1972 for the ethyl thiometon group and July 1, 1975 for the fenitrothion group. Intravenous pentobarbital anesthesia (20 mg/kg) was used before sacrifice. All dogs were ex-

using conventional chemical histopathologic and electron microscopic techniques. All data were compared with those of the controls.

RESULTS

Clinical Study

Use of Organophosphate Pesticides in Japan

Organophosphate pesticides began to be used around 1953 in Japan. However, precise records were not kept until 1957. After this time, the use of the pesticides increased at a tremendous rate throughout the country. The usage of seven major pesticides in tons or kiloliters (kl) is plotted against time (1958 to 1971) in Fig. 1A.

Until 1971, malathion, EPN, and ethyl and methylparathion were the major compounds, but these were replaced by fenthion (MPP), dipterex (DEP), fenitrothion (MEP), and diazinon since these were assumed to be less toxic to human beings (chemical structures are shown in Table 1). Over 30 other organophos-

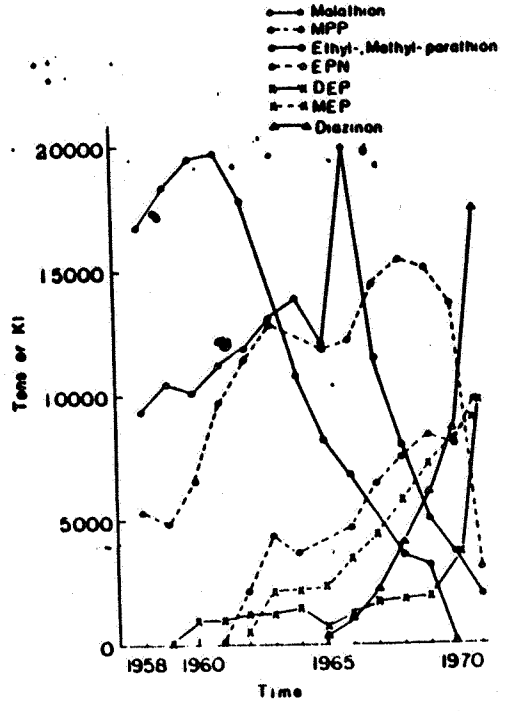


FIG. 1. A: Major organophosphate pesticide usage in Japan as a function of time from 1958 until 1971

phate pesticides are still used, but for brevity these are omitted in the figure. The use of pesticides per hectare in Japan was greater than that in other countries in 1963 (16). This is shown in Fig. 1B. Actual usage of organophosphate pesticide in Japan and other countries per 1,000 hectares from 1963 to 1967 is given in Table 2. The use of pesticides per unit area in Japan was definitely greater than that in other countries.

Epidemiological Study of Myopia and Organophosphate Pesticides

The incidence of myopia, together with the annual amount of pesticides sold, is plotted in Fig. 2. In all groups, the incidence of myopia has tended to increase since 1957. Of particular interest is the fact that the increase in incidence was not gradual, but showed three distinct peaks: in 1962–1965, in 1969, and in 1973. The presence of such peaks was particularly striking in junior high school girls. The amount of OP increased during the same period in parallel fashion. The amount of carbamate pesticides, which were not used before 1963, increased sharply during the 4-year period, 1964–1968, and then gradually until 1973. Organochlorine compounds were withdrawn completely in 1971.

The three peaks in the incidence of myopia coincided with the three peaks in the amount of organophosphate pesticides, although a 1-year time lag was found in myopia incidence in 1969 and 1973. This was attributable to the fact that eye examinations in schools were scheduled every year before the season for pesticide application.

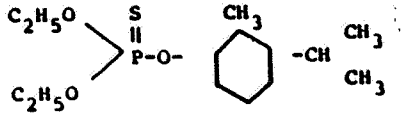
These results indicate that the amount of organophosphates used during 1 year is significantly correlated with the incidence of myopia in the following year. No significant correlation was found between the amount of carbamate or organochlorine pesticides and the incidence of myopia.

Optico-autonomic-peripheral Neuropathy (Saku Disease)

The effect of organophosphate pesticides on the visual system was carefully examined in Japan in 1969 by Ishikawa (7). A specific ocular and systemic syndrome has been isolated in agricultural regions of that district, where parathion and malathion are used extensively. In 1969, 71 children, ages 4 to 16 years, who were seen at Asama Hospital, were examined. These patients presented similar signs of reduced visual acuity, a narrowing of the visual fields, and optic neuritis. All of the children were from the Saku area, an agricultural area in central Japan where rice and a variety of fruits are grown. The signs presented by these children were first noted in the residents of the area in 1965, shortly after insecticides became used on a massive scale. The organophosphates of malathion and vamidothion were routinely applied by helicopters at a rate of 30 g per 100 square meters from twice to a maximum of six times each year for 5 years prior to the time of the study. A series of tests was performed on these children. The results were compared with those of a control

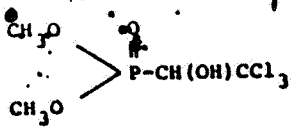
TABLE 1. *Structures of the compounds described*

Diazinon *0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate



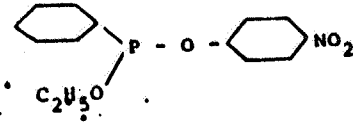
L.D. 50 = 135*

Dipterex *0,0*-dimethyl 1-hydroxy-2,2,2-trichloroethyl phosphonate (DEP)



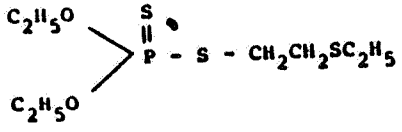
L.D. 50 = 610

EPN *0*-ethyl *0*-*p*-nitrophenyl benzenephosphonothioate



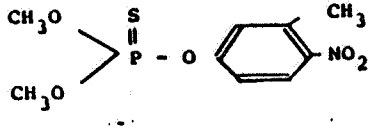
L.D. 50 = 24

Ethylthiometon diethyl-*S*(2 ethylthioethyl) phosphorothionate



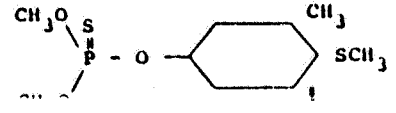
L.D. 50 = 14

Fenitrothion (sumithion) MEP *0,0*-dimethyl-*0*-(4-nitro-*m*-tolyl) phosphorothioate



L.D. 50 = 788

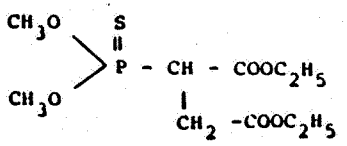
Fenthion (MPP) *0,0*-dimethyl *0*-[(4-methylmercapto-3-methyl) phenyl] phosphorothioate



L.D. 50 = 88

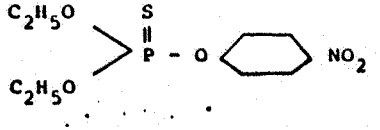
TABLE 1 *Continued*

Malathion *0,0*-dimethyl *S*-(1,2 dicarbethoxy)ethyl phosphorodithionate



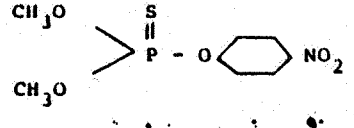
L.D. 50 = 369

Parathion (ethyl) *0,0* diethyl *0*-*p* nitrophenyl phosphothioate



L.D. 50 = 3.5

Parathion (methyl) *0,0* dimethyl *0*-*p* nitrophenyl phosphorothioate



L.D. 50 = 5.2

*Lethal dose 50 (mg/kg) in mouse in acute experiments with oral administration.

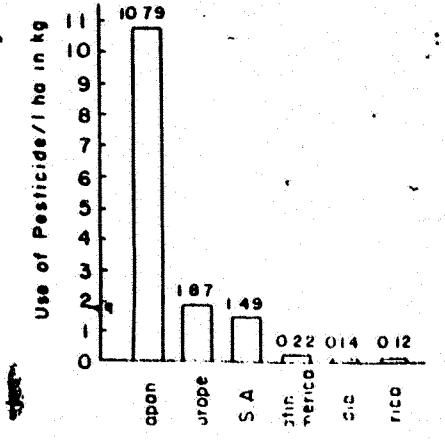


FIG. 1. B: Comparison of pesticide usage in kilograms per hectare against countries. Japan had the highest rate of usage

TABLE 2. *Use of organophosphate pesticides per 1,000 hectares in various countries*

Countries	1963	1964	1965	1966	1967
Japan	3.55	3.82	3.55	3.95	3.96
Italy	1.93	2.26	2.26	2.56	2.82
Germany	0.35	0.41	0.31	0.44	0.56
U.S.A.	1.81	1.98	2.33	3.03	2.81
Canada	0.02	0.02	0.02	0.91	0.19
Indonesia	0.006	0.04	0.06	0.09	0.13

group which consisted of 100 subjects at Tokyo University Hospital. The control subjects were similar in age to those in the study group but had no known exposure to organophosphate pesticides.

Vision

Ninety-eight percent of those in the study group were found to have reduced visual acuities, most of which were in the range of 20/100 to 20/200. Fifty percent of these could not be corrected with lenses to 20/20 because of high vertical astigmatism.

Refraction

Refractive anomalies were present in 88% of the children. 1.00 to 3.00 diopters of myopia with 2.00 to 3.00 diopters of "with the rule astigmatism" was most common. Heredity was ruled out as a cause of the high astigmatism, since examination of 40 of their parents revealed mostly 1.50 to 2.00 diopters of simple spherical myopia.

Visual Field

A concentric narrowing of the visual fields in both eyes was revealed in 95% of the study group. There were no central scotomas; however, the fields were 10 to 20 degrees smaller than those obtained from members of the control group having 0.50 to 3.00 diopters of simple myopia. Red and green fields were also constricted.

Eye Movements

Of the 53% of the study group who had abnormal eye movements, most had some disturbance of smooth pursuit, especially vertical movements. Many also showed an increased latency for saccadic eye movements. A biopsy of the lateral rectus on several of these patients revealed a total inhibition of cholinesterase activity and a slight increase in phosphorylase activity with some

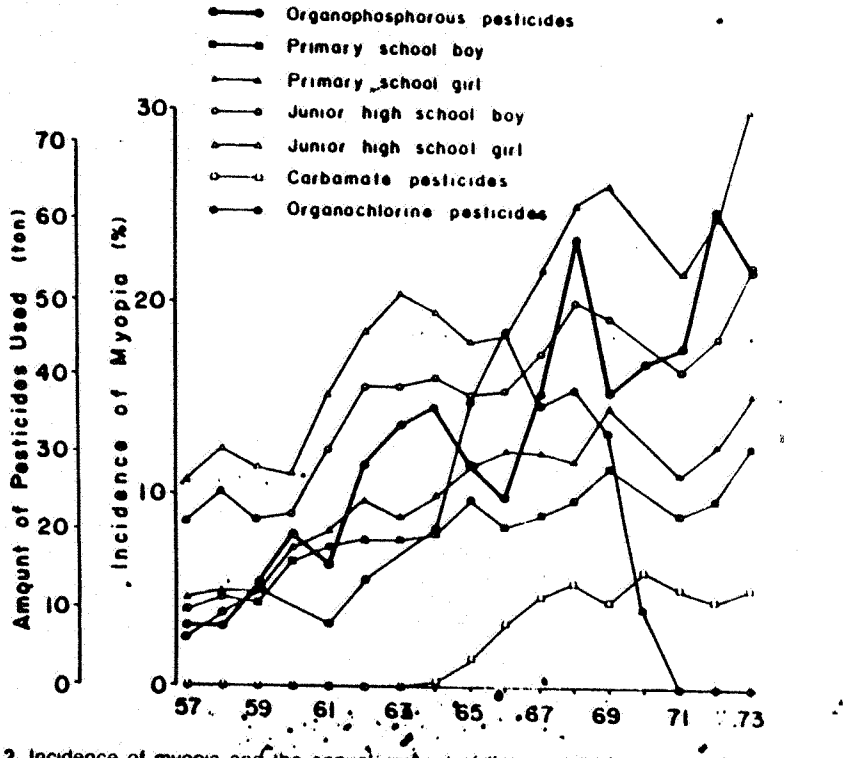


FIG. 2. Incidence of myopia and the annual amount of three pesticides used in Tokushima Prefecture. The increase in the use of organophosphate pesticides and the incidence of myopia coincided with a 1-year time lag.

Pupil

Abnormal pupils and pupillary responses were found in 52% of the children. Most had pupils that were slightly dilated under dark conditions compared with those in the control group. Further dilation of the pupil by tropicamide or atropine was extremely difficult. Five cases of pinpoint pupils were also observed and seemed to be associated with acute intoxication and strong ciliary spasm. Under dark conditions, pupil constriction with the presentation of a strong light (approximately 100 lux) was less in the study group than in the control group. Those exposed to the pesticides also had a longer mean latency for pupillary responses.

Fundus

Forty-nine percent of the study group were found to have an abnormal optic nerve head including optic neuritis, temporal pallor of the disc, and optic atrophy. Where observed, these findings were all bilateral.

Liver Function

Numerous tests were done to evaluate liver function. Most of the study group showed some slight involvement of liver function. Thirty-three percent had lowered serum cholinesterase.

Neurological Findings

Positive neurological findings were seen in 71% of the study group. These included dysmetria, an excitation (mild case), and a diminution (severe case) of deep and superficial reflexes and a reduced proprioceptive sense. Sixty-six subjects showed a sensorial neuropathy of glove-stocking type. EEGs were done on 55 subjects and 16 were found to be abnormal, whereas 15 were borderline. Only 44% were considered to be normal.

Autonomic Nervous System

A variety of symptoms ascribed to autonomic dysfunction was seen in most patients. Among these symptoms were vertigo, headache, nausea, orthostatic anemia, diarrhea, constipation, numbness, hyperperspiration, thirst, impotence, and cystic and rectal disturbances. About 7% of the control group showed such symptoms.

Results of Inquiries

After the above examinations, inquiries were carried out on the selected patients mentioned above and selected control subjects. They were 59 patients with a mean age of 9.0 years and 49 controls with a mean age of 9.8 years. About 50 questions were asked, but only those questions whose answers differed significantly from those of the controls are listed in Table 3. These results suggested that the syndrome seen in the Saku district was closely related to the presence of pesticides (4).

Blood Level of Organophosphate Pesticides from Patients

Organophosphate compounds have been detected in high levels in the blood of inhabitants of the Saku district, although not in the blood of inhabitants of other districts. The above 59 patients who had myopia with vertical astigmatism and peripheral neuropathy were examined. The examination was made in the spring and the blood measurement was made in the winter of the same year. Incidence and blood levels of pesticides detected are shown in Table 4. Pesticides that were detected from the retention time of the chromatographic chart were: salithion, formothion, ethyl and methyl parathion, DDVP, S-Seven, and Diazinon. In one patient, several pesticides were detected.

TABLE 5. *Concentrations of organophosphate pesticides (OP) from specified drinking area. Relationship between residue of OP, rate of prevalence of myopia, and mean vertical refraction of the right eye in children with Saku disease*

Saku district	No. of patients	Water concentration of OP (ppm)		Prevalence rate (per 1,000 in each area)	Mean refraction (diopters)
		Maximum	Minimum		
Koumi	30	4.29	0	3.9	−3.25
Saku	169	2.14	0.05	3.4	−2.80
Asashina	16	0.[illegible]	0	2.5	−2.97
Mochizuki	25	0.54	0	2.1	−3.50
Karuizawa	25	1.82	0	1.9	−2.11
Myota	10	2.30	1.00	1.1	−3.56
Nagato	4	0	0	0.7	−2.11
Omaki	4	0.28	0	0.7	−1.76
Yachio	3	0.83	0	0.6	−1.11
Komoro	21	0.83	0	0.5	−1.25
Tateshina	1	0	0	0.1	−0.75
Shiota	2	0	0	0.1	−0.95
Control area in Tokyo	49 Subjects	< 0.001			−0.15

[a] < 0.001 ppm

TABLE 3. *Results of inquiries in Saku patients (N = 59)[a] and control subjects (N = 49) in Tokyo*

Inquiries directed to parents	χ^2
Nausea and/or vomiting	6.21
Stomach pain	7.42
Numbness of the lower legs	8.33
Narrowing of the eye lids	7.21
Photophobia	5.93
Involuntary eye movement	5.59
Reading with near distance	9.86
Myopia in sister and brother	4.26
Poor school record of exercise	4.78
Playing in the rice field or fishing in the field	5.94
Swimming in the brook	4.09
Use of well water	4.68
Pesticides use at their own house	3.94
History of definite contact with the sprayed pesticides	6.95

χ^2 over 1.38 shows significant difference from controls. Mean age: 9.0 years in patients and 9.8 years in the controls.

[a] All patients had optico-autonomic-peripheral neuropathy.

Concentration of Pesticides in Drinking Water

Concentration of organophosphate pesticides in drinking water was measured at about the same time as the blood measurements. The results are given in Table 5. The prevalence of myopia was higher in the districts where higher pesticide concentrations were found. The degree of myopia, measured at the vertical meridian after cycloplegics, was also correlated with organophosphate concentration. No such relationship was found in the control population from Tokyo. The higher the organophosphorus level in the blood, the more intense were the clinical signs. We have previously demonstrated that the symptoms of poisoning were mild at levels of less than 0.01 ppm (9).

The Japanese Ministry of Health and Welfare later organized a project team to investigate chronic poisoning brought about by organophosphorus compounds and to make a full-fledged study of the disease. This study confirmed that the

TABLE 4. *Blood level of organophosphate pesticides detected from the patients[a]*

No. of patients who had peak	Detected pesticides	Mean value (ppm)
59	Salithion	0.265
14	Formothion	0.040
4	Ethyl and methyl parathion	0.425
2	DDVP	0.070
2	S-Seven	0.035
1	Diazinon	0.006

[a] All patients had optico autonomic peripheral neuropathy

visual disorders occurring with high incidence in the Saku district arose from chronic poisoning by those compounds. The project team of the Japanese Ministry of Health and Welfare published the criteria for diagnosing chronic organophosphorus poisoning in 1973 as described previously (12). According to their survey, the disease is not confined to the Saku district, but is widespread in various districts of Japan, and is found not only in children but in adults as well (8). Sporadic cases of the disease in urban districts as well as in the factory where the organophosphate compounds are produced have been observed (9).

In summary, most of the Saku disease patients revealed myopia with vertical astigmatism but differed only from ordinary myopia in that they had neurological complications. The experiment described below was carried out to follow these findings.

Experimental Study

Ethylthiometon

All treated dogs developed myopia with vertical astigmatism during the 2 years of observation (13).

Averaged data for refraction in diopters, corneal curvature in diopters, and acetylcholinesterase activity in erythrocytes (percent of initial) against time in months are given in Figs. 3A, B, and C. For brevity, only six measurements of refraction, five of corneal curvature, and 14 of acetylcholinesterase are shown. Vertical bars denote standard error both for refraction and corneal curvature and standard deviation for acetylcholinesterase. As compared with controls (dotted line with triangle), the treated group (solid line with closed circle) developed myopia with significant negative refraction 13 months after the start of exposure ($p < 0.05$) and this continued until the termination of the experiment ($p < 0.01$). The corneal curvature of the control group changed from 39.5 to 37.8 within 2 years in both the horizontal and the vertical meridians. An equivalent change took place in the treated group for the horizontal meridians of both eyes (solid line with x). However, the vertical meridians of the right eye (closed circle) and left eye (open circle) remained at 39 to 40 diopters until 13 to 18 months after the start of exposure ($p < 0.05$) and then approached the control values. Therefore, a significant development of "with the rule astigmatism" between the 13th and 18th months was found. Acetylcholinesterase activity was significantly reduced by the fourth month of treatment (closed circle with solid line) ($p < 0.01$) and remained depressed until the termination of treatment. The reduction of acetylcholinesterase activity coincided with the development of myopia. No difference was seen in the serum cholinesterase of the two groups. These results indicate that the development of myopia as well as "with the rule astigmatism" and reduction of erythrocyte cholinesterase are the major findings in treated animals. No other general symptoms were seen in the treated animals. The most obvious pathological finding was observed in the ciliary muscle (co-

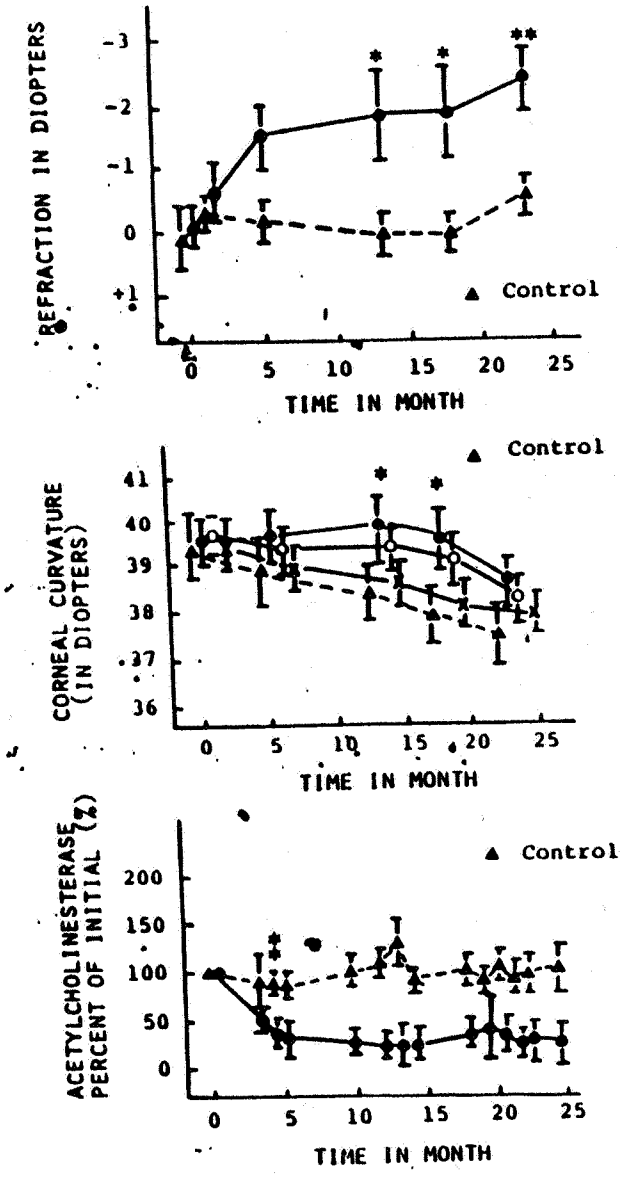


FIG. 3. Experimental study of oral administration of ethylthiometon in Beagle dogs. Averaged values of refraction in diopters, corneal curvature in diopters, and acetylcholinesterase (percent of initial) against time in months. *Dotted line* indicates controls. **A:** Significant myopia developed 13 months after the start of treatment. *Asterisks* denote the difference between the treated and the control groups * $p < 0.05$, ** $p < 0.01$ **B:** The transient development of "with the rule astigmatism" is noted in the treated group. *Closed circle*, vertical meridian of the right eye; *open circle*, left eye; ×, Averaged values of the horizontal meridian of the right and the left eyes. Mean ± SEM is given from refraction and corneal curvature. **C:** Acetylcholinesterase

An electron micrograph of the ciliary muscle of a control animal showing a longitudinal section of approximately ten muscle fibers is presented in Fig. 4A. The cell organelles are almost identical in shape, size, and distribution. An inset indicates smooth endoplasmic reticulum in the normal muscle. In the low dosage group (5 mg/dog/day) of ethylthiometon, the cytoplasm of the muscle cell was occupied by a unique membraneous structure (ums) resembling a finger print. This was seen in about one-third of the muscle cells examined. In the high dosage group (15 mg/dog/day), which is illustrated in Fig. 4B, widespread destruction of the ciliary muscle was noted. Two affected cells (A) are almost amorphous in their cytoplasm with slight preservation of the vacuolar system (V) and the ums. Myofilaments and so-called dense bodies are almost extinguished, and, if preserved, are in disarray. There are two normal-looking muscle cells (N) among the affected cells with a concentric lamellar structure of the endoplasmic reticulum (arrow; bar = 1 μm). Amorphous swollen changes in the cytoplasm indicate intracellular accumulation of fluid. These changes were attributed to ethylthiometon since they were not seen in the controls. Therefore, the binocular development of myopia in the treated group was considered to be secondary to changes within the ciliary muscle. Severe histopathologi-

FIG. 4. A: An electron micrograph of the control ciliary muscle. M, Mitochondria; E, endoplasmic reticulum; D, dense body. **Inset:** Smooth endoplasmic reticulum in normal muscle cell. Bars 5 μm and 1 μm, respectively.

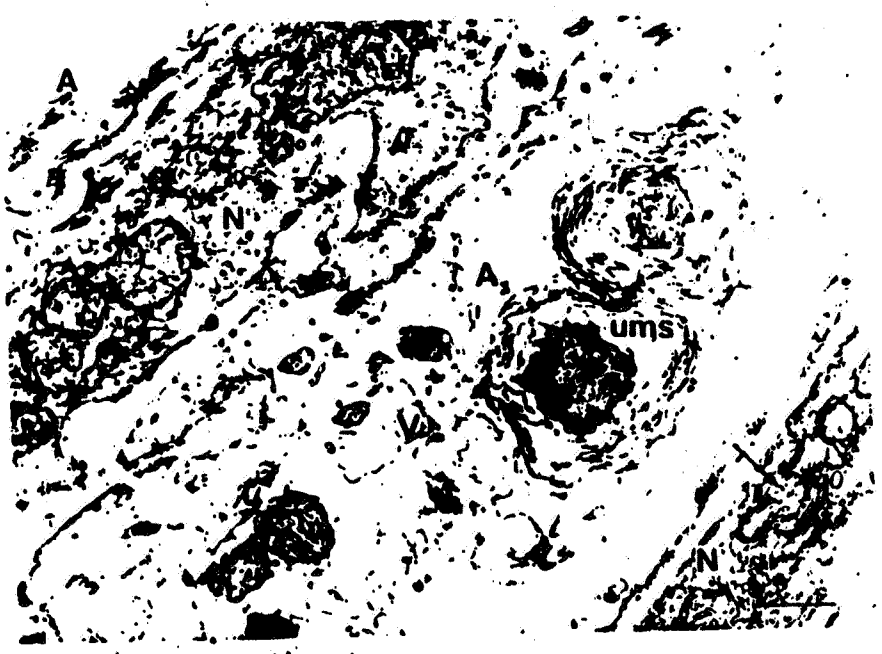


FIG. 4. B: Ciliary muscle after administration of ethylthiometon, 15 mg/dog/day for 5 days a week for 2 years. Highly affected muscle cells among normal cells (N). Two affected cells (A) are almost amorphous in their cytoplasm with slight preservation of the vacuolar system (V) and a unique membrane structure (ums) similar to a finger print is seen. The *arrow* indicates a ums within normal-looking fibers.

cal findings were also noted at optic nerve, spinal ganglia, sural nerve, and upper brainstem (15). Cerebellum and cerebral cortex were almost intact.

Fenitrothion

In the fenitrothion-treated group, all dogs also developed myopia. Refraction in diopters, thickness of the lens (mm) and ocular axial length (mm) are shown against time in months in Figs. 5A, B, and C. Myopia was significantly larger than in controls at 9 months after exposure started ($p < 0.05$) and persisted until 18 months ($p < 0.01$) when compared with the controls. Thickness of the lens increased significantly in the left eye ($p < 0.01$) and at 9 months in the right eye ($p < 0.05$). There were no significant differences in axial length between treated and control dogs. The myopia persisted at 24 months, which was 1 year after the cessation of the fenitrothion.

Both erythrocyte and serum cholinesterases tended to fall during administration, but these measures returned to their original level about 3 months after the cessation of treatment. Other chemical analyses of the blood proved normal

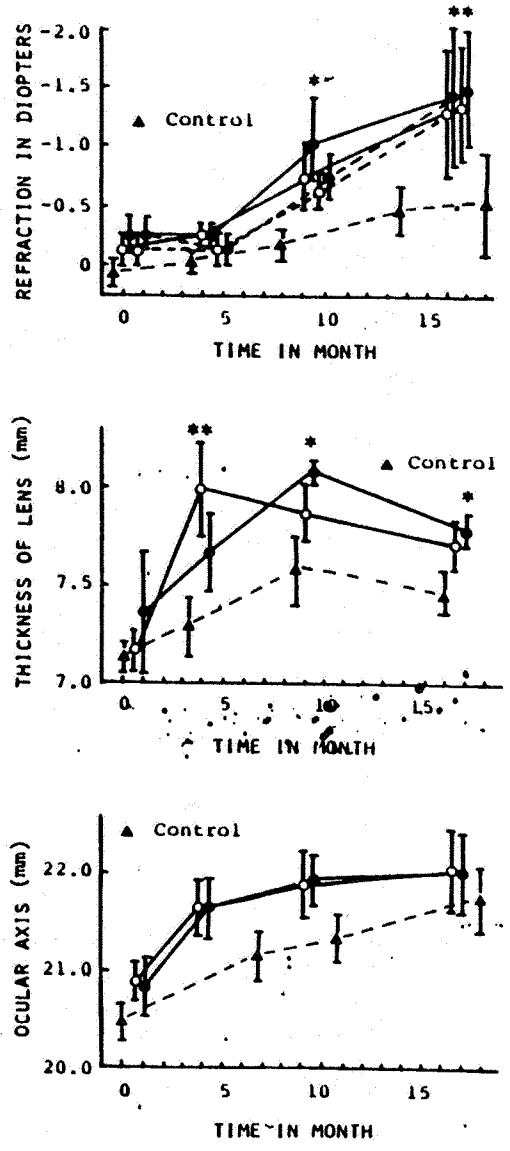


FIG. 5. Experimental study in Beagle dogs with oral administration of fenitrothion. A: Refraction in diopters. B: Thickness of the lens in mm. C: Ocular axial length in mm against time in months are shown. Myopia developed 9 months after the start of treatment ($p < 0.05$) and continued until 18 months ($p < 0.01$). *Closed* and *open circles* with *solid line* denote vertical meridians of right and left eyes. The same symbols with dotted line denote horizontal meridians. *Solid triangle:* controls. In B thickness of the lens increased 4 months after the treatment in the left eye ($p < 0.01$) and 9 months in the right eye ($p < 0.05$). In C ocular axial length tended to increase with time, not significantly. *Closed* and *open circles*: right and left eyes

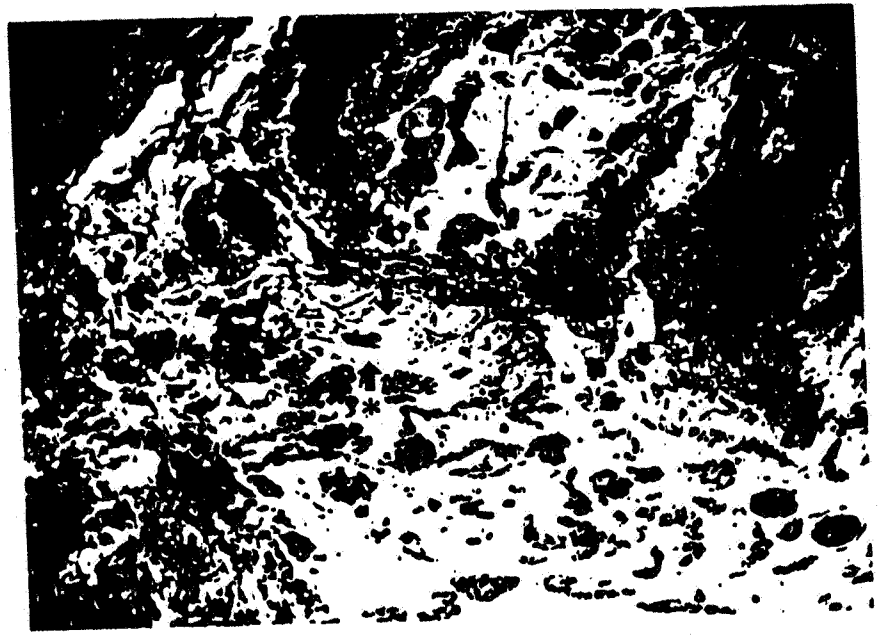


FIG. 6. Ciliary muscle after 10 mg/week for 1 year of fenitrothion. Swollen muscle fibers *(downward arrows)* adjacent to the nerve *(upward arrow with asterisk)* are seen in the radial bundle intermingling with the intact fibers.

Histopathological findings in the ciliary muscle were almost identical with that of the ethylthiometon group after 1 year of administration of fenitrothion. Figure 6 shows a photomicrograph (toluidine blue) of the ciliary muscle following a dose of 10 mg/dog/twice a week. Swollen muscle fibers are clearly seen (arrows)

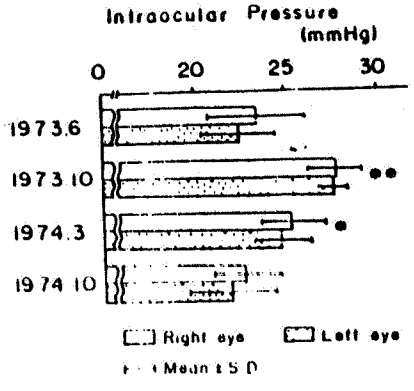


FIG. 7. Averaged intraocular pressure in mm Hg as a function of time when measured in fenitrothion treated group. Mean ± SD. A transient binocular elevation of intraocular pressure was noted 4 to 9 months after the start of treatment in all treated animals. *Asterisks* indicate the difference from the controls. $^{*}p < 0.05$; $^{**}p < 0.01$.

intermingling with normal fibers adjacent to the nerve (upward arrow). This was seen in the radial fibers.

The intraocular pressure (mean ± SD) of the treated group rose significantly at 4 months after the start of treatment in both eyes of all dogs. This continued for 9 months, then gradually approached the normal level at 14 months (Fig 7). The same result was found in the ethylthiometon group.

Since the destruction of the ciliary muscle was so obvious, chemical analyses of several enzymes of the ciliary body were made and compared with controls. There was a significant reduction of acetylcholinesterase, hexokinase, pyruvatekinase, and creatinekinase. Other enzymes, e.g., glutamic oxaloacetic and pyruvate transaminases, were within normal limits. There was no abnormal increase or decrease of 20 enzymes, including the above, in the lens or the erythrocyte. These data indicate that the etiology of the myopia is revealed either by histopathology or by enzyme measurement of the ciliary muscle. It is impossible to perform these tests with humans.

DISCUSSION

Clinical Study

A high correlation existed between myopia with astigmatism and blood level of organophosphate compounds in the blood of the inhabitants in the Saku district, but not in the blood of inhabitants of other districts. The serum cholinesterase was decreased in the blood of most of those in the former group and was inversely proportional to the levels of organophosphate (9).

Organophosphate levels in blood during November, after agrochemical treatment of crops had ended, was about 10 times as high as that prevailing during the period of application, reaching as high as 3 ppm in some inhabitants. The higher the organophosphate level in the blood, the more intense were the clinical signs. We have previously demonstrated that the symptoms of poisoning were mild at less than 0.01 ppm (9).

Malathion, parathion, fenitrothion (sumithion), diazinon, fenthion, ethylthiometon, demeton, and salithion are the major agrochemicals available at present. However, the final metabolic pathways are known only for parathion and possibly fenitrothion. Therefore, exact determination of given compound in the blood is sometimes difficult.

According to the retention life of various organophosphate agrochemicals as published in Residue Review (5), the half-life of some of the chemicals in fruits at ordinary temperature can be as long as 100 days or longer. It is, therefore, apparent that the chronic accumulation and toxicity of organophosphate compounds are comparable with—or even worse than—those of organochlorine compounds such as DDT. The synergistic toxicity of a mixture of two or more organophosphorous compounds or an organophosphate compound

ith an organochlorine compound such as DDT, BHC, etc., may be even more evere. Study of these interactions is in progress in our laboratory.

Unfortunately, organophosphate compounds have not only been used as insecicides but have also been spread in large quantities over vegetables before shipment in order to retain their freshness. Due to this indiscriminate use, not nly suburban but also urban districts have been exposed to agrochemicals as esidue in the foods and water.

Opportunities for exposure to agrochemicals in the Saku district are varied. The primary route is application of agrochemicals by farmers. Secondarily, there s the application of agrochemicals in backyard gardens for the extermination of harmful insects on shade trees and on miniature trees such as rosebushes nd pine trees. Undesirable effects are produced in those living or working in areas where agrochemicals are applied, such as upland farms, paddy fields, nd large gardens. Those people who go to their working places and schools by passing through such areas, and children who play there, are among the individuals affected. Possible exposures at home include the handling of antimoth agents, the eating of fresh vegetables and fruits without thorough washing, and the drinking of the residues in fresh juices. Contaminated drinking water, fishing in contaminated rivers, and playing in such rivers can, of course, comprise additional exposures.

Although organophosphate compounds are widely distributed as agrochemicals, in ophthalmology they serve as therapeutic agents, being applied by instillation in the treatment of myasthenia gravis, glaucoma, squint, and amblyopia. It has long been known that prolonged application of these drugs by instillation causes symptoms such as myopia, astigmatism, cataract, optic neuritis, diarrhea, perspiration (8), and a sensation of paralysis in the distal region of the extremities. These were the clues to the discovery of Saku disease, since the symptoms observed in the patients in that district were basically consistent with the symptoms observed in patients under long-term treatment with these drugs.

Experimental Myopia

Although an extensive experimental study has been carried out by our group, only the results obtained from young beagle dogs are described here. The development of myopia was clearly demonstrated in young beagle dogs during long-term, low-dosage oral treatment with two organophosphate pesticides; ethylthiometon [strongly toxic, as shown by the lethal dose (LD50) of mouse] and fenitrothion (less toxic). An epidemiological study also demonstrated a marked increase of myopia in young children in Tokushima Prefecture that coincided with an increase in the usage of the above pesticides. According to previous reports (6,7), there was definite myopia with or without "with the rule astigmatism" in patients with chronic organophosphate intoxication. The epidemiological survey was important, since these cases would have escaped detection if only an ordinary ophthalmological examination had been made (14).

Myopia possibly develops as the result of a change in corneal curvature and edema of the ciliary muscle (13). A transient rise of intraocular pressure may be related to the edema of the ciliary body as well as to the increased thickness of the lens. The reduced size of the ring diameter of the ciliary muscle with increased thickness of the lens could be the cause of the myopia. Ocular axial length showed slight elongation both in patients and the treated dogs (11). However, experimental myopia was not seen in dogs over 3 years of age treated by fenitrothion (9). This may be related not only to the elastic nature of the eyeball during development but also to the activity or sensitivity of cholinesterase. In general, cholinesterase activity is higher in the newborn and during middle age and is lower before puberty and in advanced age (2,3,5). Since a reduction of acetyl and/or butyryl cholinesterase activity was noted in patients, pesticide-induced myopia may develop only during certain age periods. Furthermore, genetic determinants of this enzyme are known to exist both in humans and in animals and the activity differs among ethnic groups (2-4). It is highest in Caucasians and Negroes, lower in Orientals, and the lowest in Eskimos. A great increase of myopia among young children after World War II has been reported (17). This may be related to the use of pesticides in modern agriculture after that period.

It is also important to note that both highly toxic and less toxic pesticides produce myopia. Conventional toxicity criteria are not correlated with the capacity to cause ocular complications, especially in chronic exposures. Since the eye is a highly cholinergically innervated organ, especially in the iris-sphincter and ciliary muscle, a supersensitivity to organophosphate pesticides must exist. We need more detailed evaluation of these hazards in the future; otherwise, the number of undiagnosed victims will definitely increase throughout the world.

According to an estimate from the Japanese Ministry of Health and Welfare, this disease occurred at a rate of 2 per 1,000 people in the Saku district, and about 17 in 10,000 people throughout Japan in 1972 (12). The prognosis of this disease is favorable if it is discovered and treated by the antidotes atropine and pralidoxime methiodide during its early stage. If, however, the discovery of this disease is delayed, the prognosis is very poor in advanced cases; i.e., retinal degeneration and optic atrophy followed by blindness will occur (9).

Despite the seriousness of the potential damage, organophosphate agrochemicals are still in use. Moreover, general practitioners are not yet sufficiently aware of organophosphate poisoning, and uncomplicated analytical techniques for the detection of organophosphate compounds, which is one key to the diagnosis of this disease, lag behind the available technology. It is strongly urged that rigid controls be applied to the use of organophosphate compounds, and that techniques that permit accurate and quick analysis of these compounds be established as early as possible.

ACKNOWLEDGMENTS

This work was in part supported by grants from The Japanese Ministry of Health and Welfare and from the Ministry of Education.

We wish to thank Drs. J. Otsuka, T. Tokoro, Y. Mitsui, O. Tamura, and K. Ohto for their kind help during this work.

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