Effect of Light on Chemical and Biological Properties of Parathion

JOHN P. FRAWLEY[1], J. WILLIAM COOK, JANE R. BLAKE, and O. G. FITZHUGH

Divisions of Pharmacology and Food, Food and Drug Administration, Department of Health, Education and Welfare, Washington 25, D. C.

The exposure of parathion to ultraviolet light gives rise to a mixture of compounds possessing greater in vitro anticholinesterase activity than parathion, but lower reactability to the Averell-Norris chemical method and lower toxicity to flies. As similar conversion might occur on crops sprayed with parathion, the ability of the method to detect all toxic residues was in question. Therefore, samples of parathion and ultraviolet light–exposed parathion were fed subacutely to rats, and the in vivo anticholinesterase activity was found to parallel closely the loss in response to the Averell-Norris chemical method.

ORGANIC PHOSPHATE INSECTICIDES have assumed an increasingly important role in agricultural practice. The problem of determining the safety of such compounds has been complicated by their lack of stability in the presence of light (*6*), and their metabolism by plant tissue (*2*). Thus, suspicion has been cast on the validity of toxicological studies conducted with the parent material, as human consumption of the fresh fruit and vegetables might include chemically and biologically different compounds.

Cook (*7*) reported that exposure of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) to light results in the formation of cholinesterase inhibitors chromatographically different from parathion. Frawley and associates (*8, 9*) have demonstrated cumulative plasma cholinesterase inhibition in the dog from feeding 1 p.p.m. of parathion (*8*) and cumulative erythrocyte cholinesterase inhibition in the rat and dog (*8, 9*) from feeding 5 p.p.m. of parathion. As these levels approach the tolerance level for this compound and as spray residue levels usually are determined by a chemical method developed for the unchanged parathion (*1*), the present study was undertaken to determine the ability of the chemical method to detect all of the toxic residues of parathion.

Methods

Chemical determinations of weighed samples of parathion, before and after exposure to ultraviolet light, were carried out following the procedure of Averell and Norris (*1*).

In vitro anticholinesterase determinations were performed by the method of Cook (*3*).

Fly bioassay, to determine toxicity of samples of parathion to flies, was conducted by the procedure of Laug (*11*).

The toxicity of the samples of parathion to mammals was carried out using young adult male and female rats of the Osborne-Mendell strain. Weighed samples of parathion were dissolved in chloroform and added to ground commercial laboratory feed. Each rat was housed individually and provided with water and appropriate diet *ad libitum*. All animals were maintained on control feed for a 4-week period prior to test diet to permit determination of the pretreatment whole blood cholinesterase activity.

Whole blood cholinesterase values were obtained by a modification of the electrometric method of Michel (*12*). Two or three drops of blood from the tail vein were collected in a heparinized spot plate and 0.1 ml. was transferred by micropipet to 0.9 ml. of distilled water. After laking, this was incubated *in toto* for 1 hour at 35° C. with 1 ml. of 0.004*M* sodium barbital, 0.0008*M* potassium dihydrogen phosphate, and 0.60*M* potassium chloride buffer, and 0.2 ml. of 0.17*M* aqueous acetylcholine chloride solution. Cholinesterase activity was measured by change in pH during the incubation period.

Paper chromatography was carried out using Whatman No. 1 paper sprayed with mineral oil as the immobile phase, and water as the mobile phase as described by Cook (*4*), and using the anticholinesterase spot test of Cook (*5*) to detect the presence of anticholinesterase agents.

Experimental Results

Technical parathion, 25-mg. portions, was weighed into a series of 3.5-inch Petri dishes. The same parathion, 175 mg., was put into a large flat borosilicate glass dish. This was calculated to have the same quantity of parathion per unit surface area as the Petri dishes. By the use of small portions of acetone, the parathion was distributed as uniformly as possible over the dish surfaces. These dishes were placed under a GE Uviark ultraviolet lamp—16 inches from the dishes—and were exposed directly to the light. At the end of 0, 2, 4, 6, 8, 12, and 16 hours, one of the Petri dishes was removed from underneath the lamp. Each was weighed to compute loss by evaporation and the residue was dissolved in chloroform. Aliquots were removed from each for Averell-Norris determination of parathion, for fly bioassay, for measurement of in vitro anticholinesterase activity, and for paper chromatography.

The residue in the large dish, exposed and showing 60% parathion by the Averell-Norris method, was dissolved in chloroform and was mixed into the animal feed, after correction for evaporation losses. A weighed sample of untreated parathion was dissolved in chloroform and mixed into animal feed as the unexposed control.

The results of the chemical analysis and the fly bioassay are shown in Figure 1. As parathion is exposed to light under these conditions, its ability to react under the standard conditions of the Averell-Norris chemical determination decreases. Likewise the toxicity of the material to flies appears to decrease at about the same rate as the Averell-Norris chemical determinations. However, the in vitro anticholinesterase activity steadily increased at the same time that the other measurements were decreasing. Paper chromatography revealed that there was an increase in anticholinesterase compounds that were more hydrophylic than parathion itself. The paper chromatographic work revealed that the qualitative effect of light on parathion—spread as a film on glass—was essentially the same as the effect of light on parathion—spread as a

[1] Present address, Hercules Powder Co., Wilmington, Del.

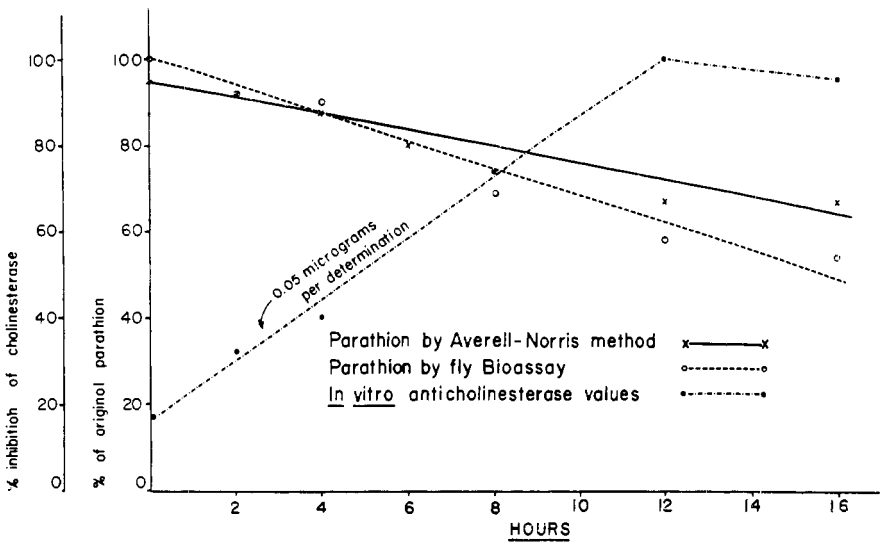

Figure 1. Parathion as determined by Averell-Norris and fly bioassay methods and in vitro anticholinesterase values following exposure of parathion to ultraviolet light

spot on paper—as reported by Cook (*4*) and studied quantitatively by Cook and Pugh (*7*).

To determine whether the mammalian toxicity of the light-exposed sample of parathion increased in toxicity in accordance with the in vitro anticholinesterase activity, or decreased in toxicity in accordance with the Averell-Norris chemical analysis and fly toxicity, the two samples were fed at various levels to two groups of rats.

Toxicity was measured by determining, at weekly intervals, the whole-blood cholinesterase activity of each animal and by comparing this activity with the mean of 5 pretreatment cholinesterase levels obtained during a 4-week control period prior to the start of the experiment. Initially, control cholinesterase levels were obtained on 20 male and 20 female rats. Five males and five females selected at random from this group then received 2 p.p.m. of the light-exposed parathion, and the same number received 2 p.p.m. of the unexposed parathion. Five males and five females also received 5 p.p.m. of either of these two samples. After 4 weeks, no significant effect was observed with either of the 2 p.p.m. diets, and the animals were placed on a diet containing 10 p.p.m. The animals fed 5 p.p.m. also showed no significant effect after 6 weeks, and they were then placed on 20 p.p.m. of the respective samples. Figures 2 and 3 show average blood cholinesterase activities of the groups of the five male and five female animals, respectively, and clearly show that the toxicity of the exposed parathion is less than that of unexposed material. The loss in toxicity is approximately 50%, which closely parallels the Averell-Norris chemical method.

Discussion

Undoubtedly, the material resulting from the ultraviolet light treatment used in these experiments is a mixture of parathion, paraoxon, and other oxidation and degradation products. The increased anticholinesterase activity is at least partially explained by the conversion of parathion to paraoxon (*7*). The mixture is less subject to diazotization and coupling; this suggests destruction of the *p*-nitrophenol moiety. That the toxicity parallels the chemical method, supports the belief that the increased in vitro anticholinesterase activity is due to the formation of the oxygen analog, or some other equivalent normal metabolite, as the mammalian liver is capable of making this same conversion (*10*). As long as the oxidative capacity of the liver is not exceeded, the same net toxicity would be expected whether the oxidation takes place in vitro or in vivo.

Acknowledgment

The authors wish to thank John F. Tighe for his technical assistance in conducting the fly bioassay.

Literature Cited

(1) Averell, P. R., Norris, M. V., *Anal. Chem.* **20,** 753 (1948).
(2) Casida, J. E., J. Agr. Food Chem. **4,** 772 (1956).

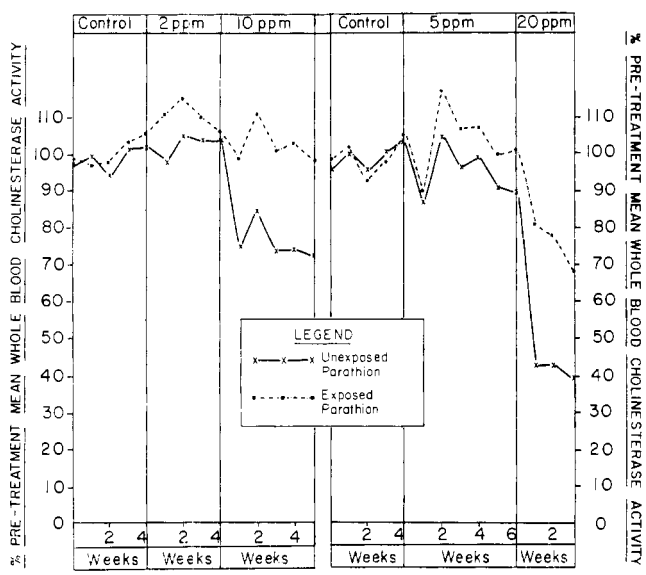

Figure 2. Effect of light on toxicity of parathion to male rats

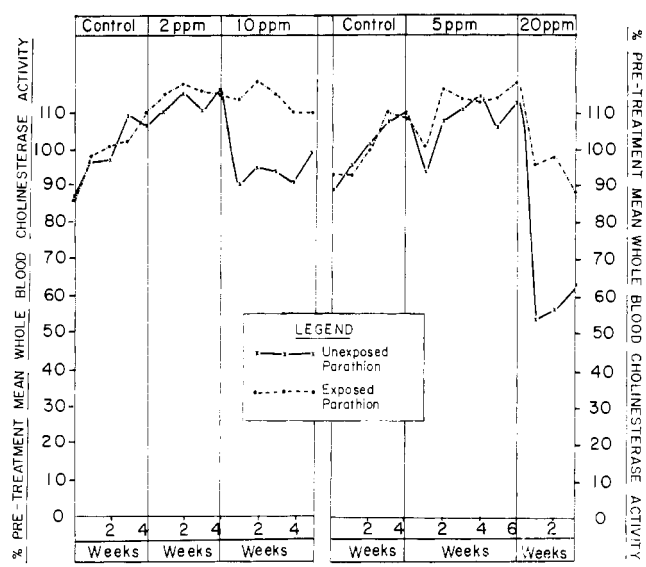

Figure 3. Effect of light on toxicity of parathion to female rats

(3) Cook, J. W., *J. Assoc. Offic. Agr. Chemists* **37,** 561 (1954).
(4) *Ibid.*, p. 987.
(5) Cook, J. W., *Ibid.*, **38,** 150 (1955).
(6) *Ibid.*, p. 826.
(7) Cook, J. W., Pugh, N. D., *Ibid.*, **40,** 277 (1957).
(8) Frawley, J. P., Fuyat, H. N., J. AGR. FOOD CHEM. **5,** 346 (1957).
(9) Frawley, J. P., Hagan, E. C., Fitzhugh, O. G., *J. Pharmacol. Exptl. Therap.* **105,** 156 (1952).
(10) Gage, J. C., *Biochem. J.* **54,** 426 (1953).
(11) Laug, E. P., *J. Pharmacol. Exptl. Therap.* **84,** 324 (1946).
(12) Michel, H. O., *J. Lab. Clin. Med.* **34,** 1564 (1949).

Received for review March 23, 1957. Accepted July 25, 1957.

INSECTICIDE ASSAY

Analysis of Mixtures of Isomers of Demeton

KERMIT GROVES

Department of Agricultural Chemistry, State College of Washington, Pullman, Wash.

The isomers of demeton [O,O-diethyl O(and S)-ethyl-2-mercaptoethyl phosphorothioate] are determined in their mixtures by titration with bromine before and after alkaline hydrolysis. The reliability of the method is shown by the results of titrations of known mixtures of the isomers and related compounds. Duplicate analyses can be made in less than 40 minutes.

DEMETON (Systox, Chemagro Corp.), a commercial insecticide, consists of two isomeric forms, *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (thiono isomer), and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiol isomer) (*3, 7*). Both isomers have insecticidal properties but differ in their chemical and physiological behavior, and the determination of the isomer content of experimental mixtures is of considerable importance for the evaluation of such mixtures as insecticides.

Gardner and Heath (*3*) separated the isomers by column chromatography and determined the phosphorus content of the eluted fractions. Henglein, Schrader, and Mühlmann (*5*) and Hensel and Kessler (*6*) have described determinations of the isomers based on infrared absorption by the P → S and P → O bonds. Hensel and Kessler gave methods based on the different rates of alkaline hydrolysis of the thiol and thiono isomers, and on determinations of the sulfate formed from the thiono sulfur when the isomer mixtures were oxidized with nitric acid.

All of these methods are subject to interferences by impurities. The presence of foreign esters interferes in the hydrolysis method. Other phosphates may interfere with the infrared (*4*) and chromatographic methods. Nitric acid oxidation gave unreliable results for the analysis of demeton isomer mixtures (*6*).

The procedure reported herein determines the isomers in a mixture by titration with bromine before and after alkaline hydrolysis. The method is rapid and requires no special equipment.

The thiono isomer reacts with 10 bromine equivalents:

$$(C_2H_5O)_2P(S)OC_2H_4SC_2H_5 + 5Br_2 + 6H_2O \rightarrow (C_2H_5O)_2P(O)OC_2H_4S(O)C_2H_5 + H_2SO_4 + 10HBr$$

After hydrolysis,

$$(C_2H_5O)_2P(S)OC_2H_4SC_2H_5 + H_2O \rightarrow (C_2H_5O)_2P(S)OH + HOC_2H_4SC_2H_5$$

the same amount of bromine is required for the titration,

$$(C_2H_5O)_2P(S)OH + 4Br_2 + 5H_2O \rightarrow (C_2H_5O)_2P(O)OH + H_2SO_4 + 8HBr$$
$$HOC_2H_4SC_2H_5 + Br_2 + H_2O \rightarrow HOC_2H_4S(O)C_2H_5 + 2HBr$$

The thiol isomer requires two bromine equivalents before hydrolysis,

$$(C_2H_5O)_2P(O)SC_2H_4SC_2H_5 + Br_2 + H_2O \rightarrow (C_2H_5O)_2P(O)SC_2H_4S(O)C_2H_5 + 2HBr$$

and eight bromine equivalents after hydrolysis,

$$(C_2H_5O)_2P(O)SC_2H_4SC_2H_5 + H_2O \rightarrow (C_2H_5O)_2P(O)OH + HSC_2H_4SC_2H_5$$
$$HSC_2H_4SC_2H_5 + 4Br_2 + 3H_2O \rightarrow C_2H_5S(O)C_2H_4SO_2Br + 7HBr$$

The diethyl phosphoric acid does not react with bromine.

Siggia and Edsberg (*8*) reported a method for determining alkyl sulfides and thiols by titrating these compounds with 0.1*N* potassium bromate–potassium bromide in acetic acid solution. Their titration procedures have been used in the method to be described.

Reagents

Bromate-bromide, 0.1000*N*. Dissolve 2.784 grams of high-grade potassium bromate and 15 grams of potassium bromide in water and make to 1 liter.

Sodium hydroxide, 2*N*, in methanol-water. Dissolve 8 grams of sodium hydroxide in 50 ml. of water and add 50 ml. of methanol.

Glacial acetic acid and hydrochloric acid. Use high-purity reagents.

Procedure

First Titration. Weigh accurately a sample of 0.1 gram into a 300-ml. Erlenmeyer flask. Add 40 ml. of glacial acetic acid, 25 ml. of concentrated hydrochloric acid, and 10 ml. of water. Titrate the mixture with the bromate-bromide solution to a faint permanent yellow color, which persists about 1 minute. Make a reagent blank titration in the same manner, adding water to make the total volume about the same as that of a titrated sample.

Second Titration. Weigh accurately a sample of 0.1 gram into a 25-ml. glass-stoppered volumetric flask, and add 7 ml.