Effect of Bromine and Ultraviolet Light on Eight Pesticides Detected with Liver Esterases of Five Species

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Bromine vapor was more effective than UV irradiation in converting carbaryl, ethion, oxydemeton-methyl, demeton, demeton sulfone thiol isomer, and dimethoate to more active inhibitors of esterases. Increased inhibition was not observed with dichlorvos and dimethoxon, after exposure to bromine or UV. The pesticides were exposed to bromine or UV before or after resolution and were detected by a TLC (thin-layer chromatographic)–enzyme inhibition technique using liver esterases of beef, sheep, pig, monkey, and chicken. The sheep and chicken esterases were slightly inhibited by the demeton compounds. The products produced by exposure of dimethoate to bromine were markedly different from those produced by UV; none of the major products correspond to dimethoxon. Unlike bromine, UV had only a minor effect on ethion conversion to a potent enzyme inhibitor. After UV and bromine exposure, differences in ability to inhibit esterases and in migration rates on TLC plates found among the pesticides and their various products may be used in the pesticide identification procedure.

Chemical compounds such as organophosphorus and carbamate pesticides were converted to more potent enzyme inhibitors after exposure to bromine vapor (McKinley and Read, 1961; McKinley and Johal, 1963; Bunyan, 1964; Irudayasamy and Natarajan, 1965; Mendoza *et al.*, 1968*a* and 1968*b;* Wales *et al.*, 1968; Winterlin *et al.*, 1968; Mendoza *et al.*, in press), bromine water (Fallscheer and Cook, 1956; Ackermann, 1968) or UV (ultraviolet light) (McKinley and Johal, 1963; Ackermann, 1968). O'Brien (1960) summarized the effect of UV on pesticides. McKinley and Johal (1963) reported that dimethoate (Rogor) on paper chromatograms was not converted to an active inhibitor as efficiently with 20-minute UV exposure as with bromine. However, Ackermann (1968) observed increased enzyme inhibition after 20-minute UV exposure of this compound on TLC (thin-layer chromatographic) plates. The discrepancy might have been due to the difference in UV intensity or supporting media. The increased enzyme inhibiting properties after UV irradiation of the carbamate pesticides might be attributed to several antiesterase breakdown products (Wales *et al.*, 1968).

This study was carried out to determine the effect of bromine or UV on certain pesticides and to evaluate this effect in pesticide detection, identification, and confirmation using liver esterases from beef, sheep, pig, monkey, and chicken. The products obtained after bromination and UV irradiation were compared with the parent compounds as to their ability to inhibit the esterases.

MATERIALS AND METHODS

Pesticide Standards. Purity is expressed in percentage.

Carbaryl = 1-naphthyl methylcarbamate 99%
Dichlorvos = 2,2-dichlorovinyl dimethyl phosphate 99%
Ethion = *O,O,O',O'*-tetraethyl *S,S'*-methylene bisphosphorodithioate 95.5%
Oxydemetonmethyl = *S*-2-(ethylsulfinyl)ethyl *O,O*-dimethyl phosphorothioate
Demeton = a mixture of *O,O*-diethyl *S* (and *O*)-2 (ethylthio) ethyl phosphorothioates 96%
Demeton sulfone thiol isomer = *O,O*-diethyl *S*-2 (ethyl sulfonyl) ethyl phosphorothioate
Dimethioate = *O,O*-dimethyl *S*-(methylcarbamoylmethyl) phosphorodithioate 98.6%
Dimethoxon = *O,O*-dimethyl *S*-(methylcarbamoylmethyl) phosphorothioate

Each pesticide was dissolved in methanol. Under the conditions used, oxydemetonmethyl, demeton sulfone thiol isomer, and dimethoxon standards gave single spots and were, therefore, considered 100% pure.

Extraction of Esterases. In a VirTis homogenizer, homogenize 50 grams of ground fresh livers for 2 minutes with 180 ml. of cold distilled water or Tris-buffer solution containing nicotinamide, each at 0.01*M*. Centrifuge the homogenate at 2000 G at approximately 4°C. for 5 minutes; freeze the supernatant in 13 × 100-mm. test tubes. The frozen extracts can be used even after several months.

Fresh livers from beef (steer), sheep (wether), pig (barrow), male monkeys, and mixed livers of male and female chicken were used.

Enzyme Spray Solution. Dilute one part of the defrosted enzyme extract with 8 parts of 0.05*M* Tris-buffer solution just before use. Dilution may be varied depending on intensity of the azo dye produced on the TLC plate, indicating the activity of the enzyme preparation. For the preparation used, one tube diluted 8 times was enough for 6 plates.

Chromogenic Spray Solution. Dissolve 15 mg. of 5-bromoindoxyl acetate in 5 ml. of absolute ethanol. Add 2 ml. of freshly prepared solution of potassium ferro- and ferricyanide, each at 0.05*M*, to 13 ml. of 0.05*M* Tris-buffer, pH 8.32. Thoroughly mix the resulting solution with 5-bromoindoxyl acetate in ethanol before spraying. This spray solution is stable and can be kept for at least a day for subsequent use.

UV Source. Use four 15-watt germicidal lamps (products of General Electric Co., Ltd.) arranged 7.5 cm. between tubes. Expose the TLC plates 10 cm. from the center of the tube.

TLC Procedure. Coat the TLC plates with a 450-micron thick layer of Kieselgel G-HR following the method reported previously (Mendoza *et al.*, 1968*a*). Apply 10 μl. of each pesticide solution on the plates 2.5 cm. from the bottom edge; then, expose the plates to bromine vapor (Mendoza *et al.*, 1968*a*) or to UV for 10 to 60 minutes either before or

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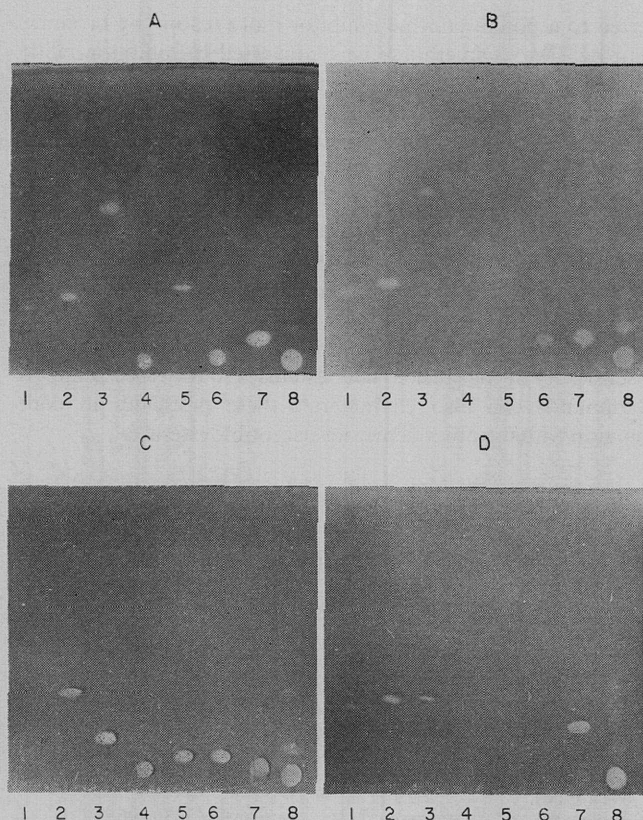

Figure 1. Typical TLC plates exposed to (*A*) bromine after, (*B*) UV after, (*C*) bromine before, and (*D*) UV before resolution of pesticides with 20% acetone in hexane

(1) Carbaryl, (2) dichlorvos, (3) ethion, (4) oxydemetonmethyl, (5) demeton, (6) demeton sulfone thiol isomer, (7) dimethoate, (8) dimethoxon. (A and D were sprayed with the Tris-extracted monkey liver esterases and B and C with the water-extracted monkey liver esterases)

after resolutions. Resolve the pesticides in 20% acetone in hexane (20 ml. of acetone plus hexane to make 100 ml.) until the solvent front reaches 15 cm. from the origin. When the solvent dissipates, spray the enzyme solution evenly until the plate is just thoroughly wet. Allow the plate to dry before spraying the chromogenic solution. In a few minutes the areas of inhibition will appear as white spots on intense blue background. Whenever the azo dye produced tends to obliterate the spots, stop the reaction by bromination. To record, trace on clear plastic sheets or photograph the developed plates.

The sprayer used for enzyme spray solutions had a spray tube of about 3-mm. i.d. that tapered to about 1 mm. at the nozzle. Clogging of the tube experienced with the use of capillary tube sprayers was eliminated.

RESULTS AND DISCUSSION

Figure 1 shows the marked differences in detection when the pesticides were exposed to bromine or UV before and after TLC resolution. There was a marked difference between the products of bromination or UV irradiation. There was evidence that certain products, particularly those of dimethoate, were not oxygen analogs. The number of inhibitors detected by a TLC-enzyme inhibition technique are summarized in Table I.

CARBARYL. After bromination carbaryl inhibited beef, pig, sheep, chicken, and monkey esterases with the exception of the water-extracted monkey esterases. Carbaryl exposed to UV inhibited the pig, sheep, and monkey esterases but it was not detected with beef and chicken esterases. The discrepancy between the detection of the UV-irradiated and brominated carbaryl with water-extracted monkey liver esterases could indicate that the products were not similar. The pig liver esterases were most susceptible to carbaryl inhibition.

DICHLORVOS. Dichlorvos exposed to bromine or UV was neither activated nor changed to compounds with different rates of migration. This pesticide was detected with the liver esterases of beef, sheep, pig, monkey, and chicken.

ETHION. Exposure to bromine converted ethion to a more potent enzyme inhibitor that migrated much slower than the parent compound. Marked inhibition of the esterases was observed. As we reported previously (Mendoza *et al.*, in press), the unknown component in the standard disappeared after bromination. UV irradiation before resolution slightly converted ethion to a stronger enzyme inhibitor; however, irradiation after resolution converted ethion more effectively to an enzyme inhibitor. UV also caused minor degradation of the unknown component in the standard.

OXYDEMETONMETHYL AND DEMETON SULFONE THIOL ISOMER. After bromination, oxydemetonmethyl and the thiol isomer of demeton sulfone were detected with the liver esterases except that of sheep, which was not inhibited by the thiol

Table I. Summary of the Number of Inhibitors Detected with Liver Esterases of Five Animal Species after Bromination or UV Irradiation

Chemicals[a]	Treatment	Beef		Sheep		Pig		Monkey		Chicken	
		Water	Tris	Water	Tris	Water	Tris	Water	Tris	Water	Tris
1) Carbaryl, 1	Br_2	1	(1)[b]	(1)	(1)	1	1	0	1	1	1
	UV	0	0	1	1	1	1	1	1	0	0
2) Dichlorvos, 8	Br_2	1	1	1	1	1	1	1	1	1	1
	UV	1	1	1	1	1	1	1	1	1	1
3) Ethion, 10	Br_2	1	1	1	1	1	1	1	1	1	1
	UV	1	1	1	1	2	2	1	2	(1)	1
4) Oxydemetonmethyl, 50	Br_2	1	1	1	1	2	2	2	2	(1)	(1)
	UV	0	0	0	0	0	0	0	0	0	0
5) Demeton, 50	Br_2	2	1	1	1	2	2	2	2	(1)	(1)
	UV	(1)	0	0	0	1	1	(1)	(1)	0	0
6) Demeton sulfone thiol isomer, 50	Br_2	2	1	0	0	2	2	2	2	(1)	(1)
	UV	0	0	0	0	0	0	0	0	0	0
7) Dimethoate, 10,000	Br_2	6	6	2	2	5	5	3	2	2	2
	UV	2	2	2	2	5	5	2	2	2	2
8) Dimethoxon, 10,000	Br_2	3	3	1	1	3	3	3	3	2	2
	UV	7	7	1	1	7	7	3	4	2	2

[a] Numbers after the chemical names indicate nanograms per application.
[b] Numbers in parentheses indicate very weak inhibitors.

isomer. Another but faint inhibition spot was observed with the brominated thiol isomer; it travelled just ahead of the thiol isomer standard. Very weak inhibition of chicken esterases was observed when these pesticides were brominated before but not after resolution. The diffusion of the parent compounds may be an explanation why they were not detected when brominated after resolution. UV-irradiated oxydemetonmethyl and the thiol isomer of demeton sulfone were not detected with any of the extracts.

DEMETON. Demeton exposed to bromine inhibited the liver esterases of the five species studied. Demeton exposed to UV did not inhibit the sheep, chicken, and the Tris-extracted beef esterases. Weak inhibition of the monkey and the water-extracted beef esterases was obtained with the UV-irradiated demeton. Likewise, very weak inhibition of the chicken esterases was observed with demeton exposed to bromine. Bromine converted demeton to a compound that migrated as far as the thiol isomer of demeton sulfone. Brominated demeton had an additional but a weak enzyme inhibitor similar to that in the thiol isomer. However, UV-irradiated demeton travelled as far as the nonirradiated demeton, suggesting that there was no, or negligible, conversion of demeton to its analogs.

DIMETHOATE AND DIMETHOXON. Dimethoate and dimethoxon after bromination or UV irradiation were detected with all five liver esterases (dimethoxon, being an oxygen analog, was expected to be a strong enzyme inhibitor even without exposure to bromine or UV). Both bromination and irradiation converted dimethoate to as many as five enzyme inhibitors that travelled faster than dimethoxon. Lucier and Menzer (1968) found as many as 18 metabolites of dimethoate in treated bean plants. The major spot produced after irradiation travelled much faster than that produced after bromination. The UV-irradiated dimethoxon had as many as six unidentified components while the brominated had two. Under the conditions used, dimethoate exposed to UV for 60 minutes gave stronger enzyme inhibition than that exposed for 30 minutes.

CONCLUSION

Bromine was more efficient than UV in converting the pesticides studied to more potent inhibitors. Ethion was converted to a potent enzyme inhibitor more readily by bromine than by UV. Dimethoate was converted to inhibitors with different migration rates; the products of UV irradiation migrate much faster than those of bromination. Therefore, identification of ethion and dimethoate may be based on these effects.

The difference in inhibition of liver esterases may also be used in the identification procedure using TLC-enzyme inhibition technique. As compared with inhibition of liver esterases of beef, pig, monkey, or chicken, the inability of the thiol isomer of demeton sulfone to inhibit the sheep esterases may be a useful criteria to characterize this isomer. The insensitivity of the chicken liver esterases to the three demeton compounds can also characterize these pesticides in conjunction with the other demeton susceptible esterases.

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