Toxicosis and Residues in Bromophos-Dipped Sheep

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Four lambs were dipped in 0.5% bromophos weekly for 9 weeks. No poisoning was observed. Omental biopsies were performed up to 3 weeks following the final dipping. The fat samples were analyzed for bromophos residues by microcoulometric gas chromatography. Average recovery of bromophos added to control fat was 79% for the range of 0.05 to 15 p.p.m. Residues of bromophos found in the omental fat of treated sheep averaged 9.75, 2.5, and 0.33 p.p.m. at 1, 8, and 22 days, respectively, after the final dipping.

BROMOPHOS (*O,O*-dimethyl-*O*-(2,5-dichloro-4-bromophenyl) phosphorothioate, Cela S1942, Landwirtschaftliche Chemikalien G.m.b.H., Ingelheim/Rhein, Germany) has shown promise as a broad spectrum insecticide of low mammalian toxicity (*2*). As a part of the studies of the chronic toxicity of bromophos, lambs were dipped weekly for 9 weeks at the probable maximum dosage. Residues of the pesticide in omental fat were determined to broaden the knowledge of the toxicity of bromophos and to establish levels that might be available for human consumption.

Materials Used

Chloroform, redistilled.
Petroleum ether, redistilled.
Acetonitrile, redistilled.
Celite, analytical filter aid.
Bromophos, technical, minimum 92% active material.

Methods

Four shorn lambs 6 to 9 months old of mixed sex (average weight 70.5 pounds) were dipped in a 15-gallon vat containing 0.5% bromophos prepared from a 25% emulsifiable concentrate in xylene–Triton 100. The animals were dipped once a week for 9 weeks and were observed daily for signs of toxicosis. Blood samples were drawn at appropriate intervals before and after each treatment. On the days that the animals were dipped, blood samples were collected just prior to treatment. Cholinesterase activities were determined by a modification of the Michel electrometric method (*3*).

Omental fat samples for residue analysis were taken by the biopsy technique of Radeleff (*4*). Samples were collected from each of the four test animals and the one untreated control on the first, eighth, and 22nd day following the final dipping and stored in a freezer until the time of analysis.

Residue Analysis. Bromophos residues were extracted from 10-gram omental fat samples by homogenization with 1 gram of Celite and two 50-ml. portions of hot chloroform. The undissolved material was filtered out and the extracts were combined. The chloroform was removed by evaporation. The remaining solid material was dissolved in petroleum ether and partitioned into acetonitrile saturated with petroleum ether. The acetonitrile was evaporated off and the residue was transferred to a 10-ml. volumetric flask with petroleum ether and diluted to the mark. Aliquots of this solution were injected into the gas chromatograph for quantitation.

Gas Chromatography. The gas chromatograph is a Micro-Tek 2500R fitted with a Dohrmann microcoulometric detection system and a Honeywell 1-mv. recorder. The column effluent passes from the column outlet through a heated transfer line into the quartz combustion tube, where the organic compounds in the column effluent are oxidized in the presence of oxygen. The resulting hydrogen halides are subsequently titrated in the halogen titration cell.

The analytical column was $^1/_8$-inch i.d. by 5 feet, stainless steel, filled with 15% Dow 710 on acid-washed Chromport XXX (60- to 80-mesh). The operating temperature of the inlet block, column, outlet block, transfer line, and combustion tube inlet was 240° C. The combustion furnace temperature was 835° C. Prepurified grade nitrogen (The Matheson Co., LaPorte, Tex.) was the carrier gas (30 cc. per minute, 39 p.s.i.).

The microcoulometer sensitivity was 450 ohms. Retention time for bromophos was 2.1 minutes with no interfering peaks (Figure 1). Under these conditions, as little as 2 nanograms of bromophos could be measured quantitatively.

Standard Curve. A series of standards of bromophos was prepared by dissolving the technical material in petroleum ether, then making the appropriate dilutions. Samples ranging from 2 to 35 nanograms were injected and a standard curve was prepared by plotting peak heights against nanograms of bromophos injected. This gave a straight-line concentration curve with a slope of 2.65. Peak heights were directly

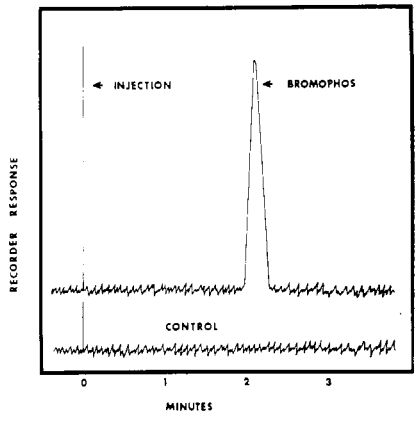

Figure 1. Gas chromatograms of bromophos extracted from omental fat of dipped and nontreated sheep

Table I. Bromophos Residues in Omental Fat of Lambs of Mixed Sex Dipped Repeatedly in 0.5% Bromophos

	Residues, P.P.M.		
Sheep	1[a]	8	22
Control	0	0	0
1163	5	1.3	0.07
1167	14	2.5	0.24
1168	10	2.5	0.25
1170	10	1.0	0.43

[a] Day of omentectomy following final treatment.

Table II. Recovery of Bromophos Added to Omental Fat Samples

Bromophos Added		Bromophos Recovered	
μg.	P.p.m.	μg.	%
0.5	0.05	0.34	68.0
2.5	0.25	2.0	80.0
5.0	0.5	4.0	80.0
10.0	1.0	8.5	85.0
25.0	2.5	21.0	84.0
50.0	5.0	38.5	77.0
50.0	5.0	36.3	72.5
50.0	5.0	36.3	72.5
100.0	10.0	80.0	80.0
150.0	15.0	133.5	89.0

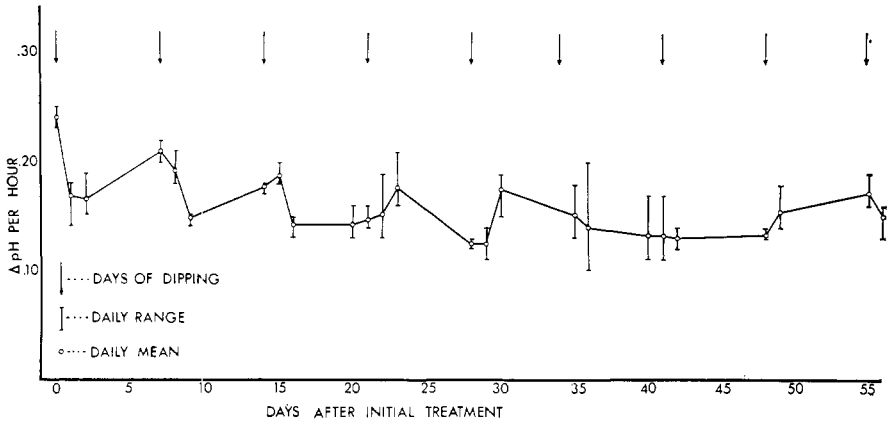

Figure 2. Effect of 9 weekly dippings in 0.5% bromophos emulsion on whole blood cholinesterase (means and range) of four lambs

Cholinesterase expressed in Δ pH per hour per ml. of blood and time in days after initial treatment

proportional to the nanograms of bromophos injected and were found to be more reliable than area under the peak as determined by the recorder disk integrator.

Results

No clinical signs of toxicosis were observed in any of the test animals. The only apparent effect was temporary reddening of the skin immediately after dipping. The effect of dipping on the daily cholinesterase activities is shown in Figure 2.

Bromophos residues found in the omental fat samples are given in Table I. The figures are the residues actually found, not corrected for extraction loss. The recoveries of bromophos which was added to omental fat from nontreated animals are shown in Table II. The oxygen analog of bromophos was not analyzed for specifically; however, under the chromatographic conditions used, there was only one peak corresponding to a halogenated compound.

Chlorinated hydrocarbon and some of the organophosphorus insecticides, when applied dermally, may be absorbed through the skin and stored in the fatty tissues of the animals. The residues found in other tissues can usually be attributed to the fat content of the tissues (*1,5*).

In view of the low toxicity, as demonstrated by the lack of clinical symptoms and low cholinesterase inhibition, and the fairly rapid depletion of residues, bromophos appears to offer advantages that many other halogenated and organophosphorus insecticides do not.

Literature Cited

(1) Claborn, H. V., Bushland, R. C., Mann, H. D., Ivey, M. C., Radeleff, R. D., J. AGR. FOOD CHEM. **8,** 439 (1960).
(2) Immel, R., Geisthardt, G., *Overdruk Medelingen Lanbouwhogeschool Opzoekingstations Staat Gent* **29** (3), 1242 (1964).
(3) Michel, H. O., *J. Lab. Clin. Med.* **34,** 1564 (1949).
(4) Radeleff, R. D., *Vet. Med.* **45,** 125 (1950).
(5) Radeleff, R. D., Claborn, H. V., Wells, R. W., Nickerson, W. J., *Ibid.*, **47,** 94 (1952).

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RESIDUE ESTIMATION

Determination of Bromophos Residues

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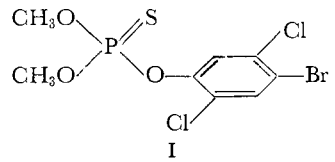
Procedures for the estimation of bromophos residues on mud, the commonest surface in the West African village, are described. 4-Aminoantipyrine coupling was used in the colorimetric procedure. The possible determination of its metabolite, 2,5-dichloro-4-bromophenol, in urine as a means of determining the degree of exposure to the insecticide is discussed. It was possible to separate bromophos from its hydrolysis products and to determine both quantitatively by using a spot area-to-weight relationship in thin-layer chromatography.

IN A SEARCH for insecticides to replace DDT and dieldrin (to which resistance is developing) in antimalarial campaigns, a collaborative scheme for insecticide testing was launched by the World Health Organization. The Insecticide Testing Unit in Lagos has evaluated a few compounds suitable for mosquito control (*1, 6, 7*). Bromophos [*O,O*-dimethyl *O*-(2,5-dichloro-4-bromophenyl) phosphorothioate (I), Cela G.m.b.H., Ingelheim, West Germany] has been selected as a candidate compound for evaluation of its mosquito control properties. This paper deals with the chemical analysis of bromophos and the estimation of its residues on mud, the commonest surface encountered in the West African village. The authors' main concern was the accurate determination of the initial deposit of bromophos, and the possible estimation of its residues at intervals thereafter. Entomological aspects of this work will be covered separately. A method is also proposed for the estimation of residues absorbed by people exposed to this insecticide.



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