Translocation, Penetration, and Metabolism of *O*-(4-Bromo-2,5-dichlorophenyl)-*O*,*O*-dimethylphosphorothioate (Bromophos) in Tomato Plants

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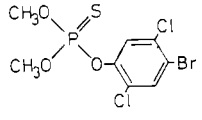
O-(4-Bromo-2,5-dichlorophenyl)-*O*,*O*-dimethylphosphorothioate (Bromophos) does not act systemically in tomato plants, but penetrates from the surface into the interior of the leaf and also from a nutrient solution into the root. In addition to unchanged Bromophos, dichlorobromophenol was found as a main metabolite, and small amounts of bromoxon, monodesmethylbromophos, dimethyl thionophosphate, and inorganic phosphate were detected.

The fate of *O*-(4-bromo-2,5-dichlorophenyl)-*O*,*O*-dimethylphosphorothioate (Bromophos) was recently investigated in rats (Stiasni *et al.*, 1967). Since this insecticide is also widely used in plant protection, its behavior in plants was considered of interest.



METHODS

Radioisotope-Labeled Bromophos and Formulations. Bromophos-^{32}P was synthesized by The Radiochemical Centre, Amersham, Bucks, England, and purified as described (Stiasni *et al.*, 1967). The specific activity of the individual lots was between 7 and 10 mc. per mmole. The radiochemical and chemical purity was more than 99%.

Tritium-labeled Bromophos (Bromophos-T) was synthesized from dimethyl thiophosphoryl chloride and 2,5-dichloro-4-bromophenol-3,6-T (Stiasni and Schweikert, 1966). The specific activity was 110 mc. per mmole and the radiochemical purity was more than 99%. The substance contained small amounts of tetramethylurea.

The emulsifiable formulations used are given in Table I. Emulsions 1 and 2 were applied to tomato leaves in the translocation, penetration, and metabolism studies with Bromophos-^{32}P. Similar experiments with Bromophos-T were carried out with emulsion 3, while emulsion 4 because of its higher Bromophos-T concentration was used for metabolism studies. The absorption of Bromophos-T through the roots was studied using emulsion 5. All formulations except the fairly stable emulsion 5 were freshly prepared before use.

Treatment of Plants. Tomato plants were cultivated in plastic flowerpots on standard soil for 6 weeks in a greenhouse. In the studies on absorption by roots the plants were cultivated on sand and approximately 50 ml. of a 0.1% (v./v.) Substral solution (Barnaengens Vademecum G.m.b.H., 502 Frechen, Germany) per plant were given weekly as nutrient. During the experiments the plants were kept in a growth chamber at 25° to 30° C. and a relative humidity of 60 to 80% and were irradiated for 12 hours per day by a daylight lamp (Philips HPLRH 400 W) which was kept approximately 30 to 50 cm. from the tops of the plants. The plants were watered when necessary.

In the volatility studies the treated leaves were enveloped in a polyethylene film. Because of difficulties in measuring the absorbed activity, in later experiments the polyethylene film was replaced by glass containers aired by a stream of 0.5 to 1 liter of air per hour.

Table I. Bromophos Emulsions Used

Emulsion	Number 1	2	3	4	5
Bromophos-^{32}P, mg.	25	32.5	—	—	—
Bromophos-T, mg.	—	—	27.5	27.5	10
Shellsol A, mg.	69	52.9	69	99	—
Epichlorohydrin, mg.	1	1.1	1	1	—
Emulsogen I 40,[a] mg.	5	8.7	5	5	—
Triton X 155,[b] mg.	—	—	—	—	10
Cyclohexanone, mg.	—	17.3	—	—	—
Acetone, ml.	—	—	—	—	2
Water redistilled, ml.	50	50	50	5	10.000[c]
Concentration, %	0.05	0.065	0.055	0.55	1[d]

[a] Farbwerke Hoechst, A. G., Frankfurt/Main, Germany.
[b] Rohm & Haas, Philadelphia, Pa.
[c] Addition of 10 ml. of Substral Liquid fertilizer (Barnaengens Vademecum G.m.b.H., 502 Frechen, Germany).
[d] P.p.m.

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Table II. R_f Values (× 100) of Bromophos and Its Derivatives in Different Solvent Systems by TLC

Compound	Solvent System[a] I	II	III	IV	V	VI
Bromophos	54	70	72	87	81	—
Bromoxon	20	64	29	84	79	—
Dichlorobromophenol	35	52	31	81	84	7
Monodesmethylbromophos	0	0	0	62	58	28
Monodesmethylbromoxon	0	0	0	62	57	41
Dimethyl thionophosphate	0	0	0	29	30	—

[a] Solvent systems used on silica gel G (Merck, Darmstadt, Germany) + fluorescent dye ZS Super

I. Hexane-acetone — 4 to 1
II. Benzene-methanol — 95 to 5
III. Gasoline-benzene-ethanol — 30:10:3
IV. Butanol–acetic acid–water — 4:1:5 (upper phase)
V. Butanol–formic acid–water — 50:5:25 (upper phase)
Cellulose powder 300 G (Macherey & Nagel, Düren, Germany) + fluorescent dye ZS Super
VI. Saturated $(NH_4)_2SO_4$ solution-water–1*M* Na-acetate solution–2-propanol — 52:28:18:2

Determination of Radioactivity. To avoid postexperimental diffusion of Bromophos, immediately after the experiment the plants were cut into several pieces and analyzed separately.

For the autoradiographic detection of ^{32}P-activity the plant parts were dried under slight pressure between sheets of blotting paper. The dried samples were fixed with tape on a PVC film (Lohmann-Oclufolbinde) mounted on a glass plate. An x-ray film was placed between the film and the glass plate. The plant sections were covered by filter paper, foam rubber sheets, and a second glass plate. The whole packet was pressed together by means of rubber rings. After the required exposure, the films were developed and fixed. To avoid any misinterpretations due to artefacts, several untreated plants were handled in the same manner.

In experiments in which the wax layer of the treated leaf was investigated separately, the layer was removed by slight rubbing with benzene or chloroform. The remaining part of the leaf, as well as the other parts of the plant, was homogenized with methanol or acetone in an Ultra-Turrax homogenizer (Janke & Kunkel, Staufen, Germany) under ice cooling and centrifuged.

In the metabolism studies of Bromophos-^{32}P the plants were homogenized and first extracted with hexane-acetone (9 to 1) and then with water.

The radioactivity was measured by liquid scintillation counting in a Packard TriCarb 3000. The quench effect correction was done by the internal standard technique, using the applied solution as standard. The correction for the half life of ^{32}P was carried out simultaneously. Extracts containing tritium were burned by the flash-combustion technique of Kalberer and Rutschmann (1961) and the activity was measured by the external standard technique.

Table III. Translocation of ^{32}P-Activity from One Leaf into Residual Plant Estimated by Autoradiography

Time, Hours	Radioactivity Applied d.p.m.[a]	Translocated, %
8	2.3×10^6	<0.5
24	2.3×10^6	<1
48	1.3×10^6	<5

[a] Disintegrations per minute.

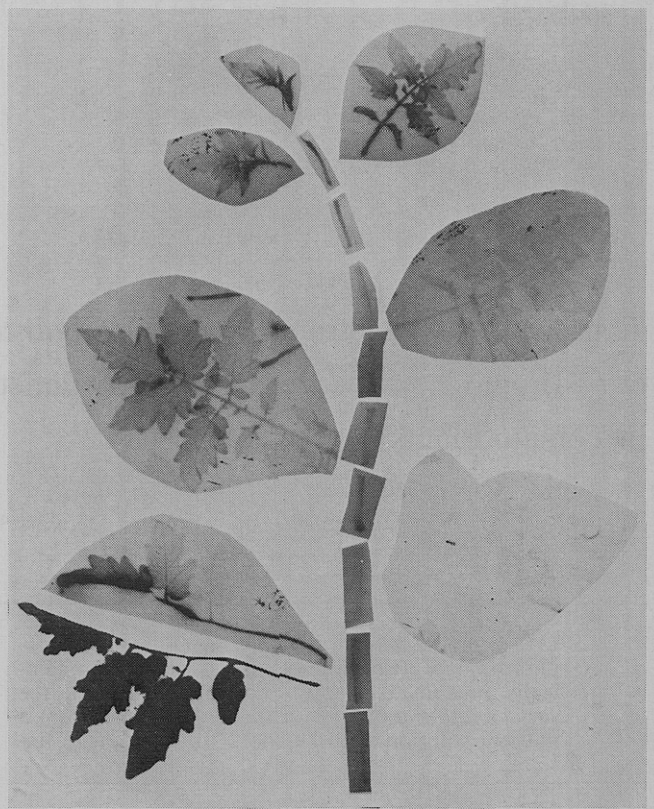

Figure 1. Photographic montage of autoradiogram of tomato plant 48 hours after application of Bromophos-^{32}P

Half leaf on left lower corner treated

For the detection of tritium-labeled compounds on thin-layer chromatograms a thin-layer scanner (manufactured by Prof. Berthold, Wildbad, Germany) was used.

Osray x-ray film (Gevaert, Antwerp, Belgium) was used in autoradiographic studies (Stiasni *et al.*, 1967). The amount of translocated ^{32}P-activity was estimated having regard to the exposure time, by comparing the blackening of treated and untreated material.

The thin-layer chromatography (TLC) was carried out by the method of Stahl (1962), using the solvent systems in Table II.

To detect Bromophos, Bromoxon [*O*,*O*-dimethyl-*O*-(4-bromo-2,5-dichlorophenyl)phosphate], and 4-bromo-2,5-dichlorophenol by the reverse dilution analysis (Broda, 1967) the plant extracts were divided into three parts and to each part approximately 300 to 500 mg. of nonlabeled material of the compound to be determined were added. The cleanup was done by column chromatography (Kieselgel, 0.05 to 0.2 mm., E. Merck, AG, Germany). Bromophos was eluted with carbon tetrachloride, dichlorobromophenol with benzene, and Bromoxon with benzene-methanol (1 to 1). After evaporation of the eluates the substances were recrystallized until constant specific activity was reached.

RESULTS AND DISCUSSION

Translocation of Bromophos. To one half of a leaf 0.2 ml. of emulsion 1 (100 μg. of Bromophos-^{32}P) were applied and two plants each time were analyzed by autoradiography 8, 24, and 48 hours after application. The results are shown in Table III. Figure 1 is a photographic montage of an autoradiogram, showing the location of the individual leaves and stems.

Though the values in Table III are only estimated, it becomes clear that Bromophos and its ^{32}P-containing metabolites

Table IV. Translocation of Tritium-Activity from Treated Leaf into Other Parts of Plant

Plant Parts	Fraction of Part	Per Cent of Total Dose Applied 8 hours	24 hours	48 hours	7 days
Treated part of handled leaf	Wax layer	21.6	9.4	12.7	14.2
	Soluble	19.4	14.3	13.6	11.3
	Residue	0.8	0.7	0.9	2.4
	Sum	41.8	24.4	27.2	27.9
Untreated part of handled leaf	Wax layer	0.2	1.4	0.3	0.7
	Soluble	0.5	2.4	0.6	1.6
	Residue	0.0	0.1	0.1	0.9
	Sum	0.7	3.9	1.0	3.2
Top leaves (apical meristem)	Soluble	0.1	0.2	0.1	0.2
	Residue	0.0	0.0	0.0	0.0
	Sum	0.1	0.2	0.1	0.2
Residual plant	Soluble	0.5	0.9	0.6	1.2
	Residue	0.0	0.1	0.1	0.1
	Sum	0.5	1.0	0.7	1.3
Root	Soluble	0.0	1.8	0.0	0.2
	Residue	0.0	0.2	0.0	0.0
	Sum	0.0	2.0	0.0	0.2
Soil		—	0.1	0.0	0.2
Total activity found		43.1	31.5	29.0	32.8

Table V. Penetration of ^{32}P-Activity from Leaf Surface into Interior

	8 Hours	24 Hours	48 Hours
Leaf surface			
% of applied	18	8	5
Bromophos, %	95	85	83
Metabolites, %	5	15	17
Leaf interior			
% of applied	21	17	9
Bromophos, %	86	73	42
Metabolites, %	14	27	58
Sum, % of applied	39	25	14
Not extracted, % of applied	0.5	1.8	3
Total recovery, %	39.5	26.8	17

are scarcely translocated. The translocation of the phenolic part of Bromophos was examined by the application of 0.2 ml. of emulsion 3 (110 μg. of Bromophos-T to one half of a leaf). The determination of the total radioactivity in the individual parts of the plant by liquid scintillation counting shows (Table IV) that only very small amounts of tritium activity were translocated. The considerable loss in the recovered activity was doubtless due to the evaporation during the experiment as it also was found with other substances—e.g., Parathion (El-Rafai and Hopkins, 1966), Dursban (Smith *et al.*, 1967), and carbamates (Abdel-Wahab *et al.*, 1966).

The rate of evaporation was determined by keeping treated leaves in polyethylene bags or glass containers, and reached after 50 hours more than 60% of the total activity applied. The evaporation rate decreased during the experiment and had nearly ceased after 24 hours (Table IV). This may be due to penetration of Bromophos into the wax layer of the leaf or to decomposition to nonvolatile compounds.

According to the definition of Unterstenhöfer and Frehse (1963), a systemic insecticide should not only be absorbed and distributed in the plant, but also insecticidal effective amounts should be stored during a limited period. In this sense Bromophos cannot be defined as a systemic insecticide. The slight activity found in the untreated parts of the plant may be attributed partly to contamination by evaporated Bromophos. On the other hand, the obviously, greater activity found in the top leaves (Table IV, Figure 1) indicates that transport to apical meristem regions in the plant has taken place.

This finding was also observed after absorption through the roots when Bromophos emulsion 5 was used. One day after application the concentration of activity in the roots was approximately equal to the initial concentration in the nutrient solution (1 p.p.m. of Bromophos-T), and after 7 days had increased to five times the amount of the total activity in the nutrient solution. The concentration in the apical meristem region also increased from 0.16 p.p.m. after 1 day to 0.44 p.p.m. after 7 days. The activity in the residual plant also increased during the experiment, but its concentration was significantly lower: 0.06 p.p.m. after 1 day, 0.26 p.p.m. after 7 days. Because of the low concentration it was not possible to differentiate between radioactivity due to Bromophos and that due to the metabolites.

In general, the transport of plant physiological active compounds is directed to areas of relatively intense metabolic activity (Mitchell *et al.*, 1960). Therefore the accumulation of radioactivity in the apical meristem region does not represent extraordinary behavior of Bromophos.

The penetration of Bromophos into the inner parts of the leaf is shown in Tables IV and V. The fact that Bromophos penetrates into the inner parts of the leaf of a tomato plant without acting systemically explains its activity against leaf-mining insects.

Metabolism of Bromophos. The rate of metabolism of Bromophos after application of 0.2 ml. of emulsion 4 (1.1 mg. of Bromophos-T) to a leaf is shown in Table IV. Most of the active ingredient was found in the interior of the treated leaf, but very small amounts of unchanged Bromophos could also be detected in other parts of the plant.

Dichlorobromophenol was found to be the main metabolite, reaching 13% of the total dose applied (70% of the activity found) after 7 days. Whether dichlorobromophenol is changed to a glycoside, or to an ester, forming a water-soluble compound as described by Towers (1964), could not be demonstrated. In addition to unchanged Bromophos and dichlorobromophenol, the following metabolic products could be detected: *O*-(4-bromo-2,5-dichlorophenyl)-*O*,*O*-dimethyl phosphate (Bromoxon), monodesmethylbromophos, dimethyl thionophosphate, and inorganic-phosphate. Desmethylbromoxon was not found.

This metabolic pattern in plants is identical with that found in rats (Stiasni *et al.*, 1967), except that Bromoxon could not be detected in animals by the usual technique.

The actual amount of Bromoxon found in tomato plants, however, was very small, reaching the highest level 48 hours after application with 0.2% of the total dose applied and decreasing after 7 days to 0.1% (Table VI). The formation of small amounts of Bromoxon in the metabolic pathway was also reported by Rowlands (1966) in his work on stored wheat grains treated with Bromophos. These findings were unexpected, in view of the opinion of Heath (1961) that plant enzymes in general favor the breakdown of thionophosphorus pesticides by oxidation rather than by hydrolytic cleavage. To investigate this quantitative difference a high disappearance

Table VI. Distribution of Bromophos and Its Metabolites in Tomato Plant[a]

Time, Days	Bromophos and Metabolites	Treated Leaf: Wax Layer, % of extract	Treated Leaf: Wax Layer, %[b]	Treated Leaf: Leaf Interior, % of extract	Treated Leaf: Leaf Interior, %[b]	Residual Plant (without root), % of extract	Residual Plant (without root), %[b]	Total Plant, %[b]
1	Bromophos	96	6.0	80	33.8	66	0.2	40
	Bromoxon	0.1	0.0	0.4	0.1	1	0.0	0.1
	DCP[c]	5	0.3	10	4.2	8	0.0	4.6
	Others[d]	—	—	9.6	4.0	25	0.1	4.0
	Sum	101.1	6.3	100	42.1	100	0.3	48.7
2	Bromophos	74	1.2	63	11.5	46	0.2	12.9
	Bromoxon	0.4	0.1	0.5	0.1	7	0.0	0.2
	DCP	15	0.2	26	4.8	13	0.1	5.1
	Others	10.6	0.2	10.5	1.8	34	0.1	2.1
	Sum	100	1.7	100	18.2	100	0.4	20.3
7	Bromophos	9	0.6	7	0.8	11	0.1	1.5
	Bromoxon	0.4	0.0	0.4	0.1	1	0.0	0.1
	DCP	94	6.4	58	6.6	16	0.1	13.1
	Others	—	—	34.6	3.6	72	0.4	4.0
	Sum	103.4	7	100	11.1	100	0.6	18.7

[a] After application of 1.1 mg. Bromophos-T on leaf of one plant, each.
[b] % of total dose applied.
[c] Dichlorobromophenol.
[d] Make up to 100 %.

rate of Bromoxon was considered. Therefore the stability of Bromoxon in buffered juice of tomato plants at pH 5 by the reverse dilution analysis was tested. The compound was found to be fairly stable under these conditions. Accordingly, the low content of Bromoxon is due to the low rate of transfer from Bromophos to Bromoxon and not to the high rate of disappearance of Bromoxon.

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