POSTHARVEST INSECTICIDE RESIDUES

Extraction of Malathion Residues from Fruits

PEKKA KOIVISTOINEN,[1]
ANJA KARINPÄÄ,
MAILA KÖNÖNEN,
and LASSE VANHANEN

Department of Nutritional Chemistry, University of Helsinki, Helsinki, Finland

Stripping unmacerated crops with benzene as an extraction method for malathion postharvest residues on fruits was compared with three other methods: blending the crop in water with subsequent tumbling with benzene, blending in ethanol with subsequent tumbling with benzene, and drastic blending in chloroform followed by additional extractions with a methanol-acetone mixture. In all cases stripping the unmacerated crops gave the highest values of initial deposits and in most cases also the highest residue recoveries during the first few days after malathion application; later, slightly higher recoveries were obtained from the blended macerated materials.

I N THE method of Norris, Vail, and Averell (17), using colorimetric estimation, the total residues of malathion [O,O-dimethylS-(1,2-dicarbethoxyethyl)-phosphorodithioate] were extracted with carbon tetrachloride from crop material blended with water and the surface residues by stripping the unblended crop with the same solvent. Recoveries of added malathion were higher for the surface than for the total residues. This observation was confirmed by Conroy (2). The recoveries of total residues in his studies were increased when the initial extraction was made by blending in ethanol and the extract was diluted with water and re-extracted with carbon tetrachloride. Carbon tetrachloride is also used for extracting malathion from liquid (5) and freeze-dried milk (1). Malathion is removed from cottonseed by Soxhlet extraction with n-hexane (16) and from wheat with carbon tetrachloride (14). Koivistoinen and coworkers (8, 10) have used benzene alone for extracting unmacerated crops or benzene with ethanol as a blending cosolvent when the crops were macerated before extraction. Recoveries of added malathion were satisfactory with both methods.

The recoveries obtained with added malathion do not necessarily agree with the extraction efficiencies of the actual residues (6). In addition, Van Middelem, Waites, and Wilson (20) have shown with parathion that the extraction efficiency of pesticide residues from field-treated vegetables may greatly depend on the procedure applied.

In the present investigation the technical efficiencies of various extraction methods are evaluated by comparing the recoveries obtained by different procedures to remove postharvest residues of malathion from fruits.

[1] Present address, Massachusetts Institute of Technology, Cambridge, Mass.

Extraction Methods

Extraction A. A 500-gram sample of unmacerated crop was stripped with 500 ml. of benzene in a 2-liter glass container with a cellophane-lined lid by end-over-end tumbling for 1 hour at 44 r.p.m. After equilibration, the benzene was decanted and purified with a cleanup mixture of activated charcoal–Hyflo Super-Cel–anhydrous Na₂SO₄ for the colorimetric determination of malathion residues (8).

Extraction B. A 500-gram sample was blended with 500 ml. of water in a top drive macerator for 5 minutes. Malathion was extracted from the slurry by tumbling with 500 ml. of benzene for 1 hour. The benzene was then decanted, or, if emulsions had formed, an aliquot of benzene was obtained by centrifuging the extraction mixture. The benzene extract was purified and analyzed for malathion.

Extraction C. A 500-gram crop sample was blended with 250 ml. of 94% ethanol in a top-drive macerator for 0.5 minute. Malathion was extracted from the slurry by tumbling with 500 ml. of benzene for 1 hour. The benzene extract was washed twice with saturated NaCl solution, purified, and analyzed for malathion.

Extraction D. This method was used both on small crop samples (about 20 grams) containing P³²-labeled malathion residues and on larger samples (250 grams) having inactive residues. The method has many features similar to those used in studies on malathion metabolism in animal tissues (7, 18).

The small samples of plant material (about 20 grams) were placed in a 100-ml. homogenizing beaker with 10 ml. of distilled water and 60 ml. of chloroform, and then the mixture was blended for 30 minutes in an MSE Homogenizer (Measuring & Scientific Equipment, Ltd., London, England) at the top speed. The chloroform phase of this mixture was

then separated, and the water and solid layers were reblended with 40 ml. of fresh chloroform and 10 cc. of distilled water for 30 minutes. The chloroform layer obtained by centrifuging was separated, and the two chloroform fractions were combined. After the water fraction had been separated from the solid material, the latter was washed twice with 40 ml. of methanol-acetone mixture (1 to 1) in a Büchner funnel containing a Whatman No. 4 filter paper and 1 gram of Celite 535 (Johns-Manville, New York, N. Y.) to aid filtration. The methanol-acetone mixture was evaporated under reduced pressure and the residue was redissolved in a 1 to 1 chloroform-water mixture. After separation of the phases, each respective phase was combined with the previous fractions. The amounts of chloroform-soluble, water-soluble, and inextractable radioactivities were measured from the three fractions.

The solid material containing the inextractable activity was further processed by refluxing an aliquot with 40 ml. of methanol-acetone mixture (1 to 1) for 15 hours. The solvents were evaporated under reduced pressure and the residue was separated into chloroform-soluble, water-soluble, and solid fractions whose radioactivities were subsequently assayed.

The radioassays were performed by counting duplicate aliquots from the three fractions on planchets by a Geiger-Müller detector. The counting of each sample was continued until the standard deviation of the measurements reached a level of about 1%. All the glassware used was rinsed before extraction with a dilute solution of inactive malathion in order to prevent losses of radioactivity by adsorption to the glass surfaces.

The large samples (250 grams) were processed in the same way as the small ones, except that the entire procedure was scaled up. Only the combined chloroform fraction was analyzed. The chloroform extract was dried with anhydrous Na₂SO₄ and the solvent

Table I. Malathion Residues[a] (P.P.M.[b]) Extracted from Gooseberries and Tomatoes

Days after Application[c]	Extraction A[d]	Extraction B[e]	Extraction A/ Extraction B
		Gooseberries	
0	26.50 ± 0.67[e]	24.50 ± 0.67	1.082
1	17.55 ± 0.75	16.90 ± 0.60	1.038
2	11.75 ± 0.55	11.90 ± 0.40	0.987
4	7.00 ± 0.50	7.90 ± 0.40	0.886
7	4.68 ± 0.08	5.80 ± 0.56	0.807
		Tomatoes	
0	5.30 ± 0.60	4.07 ± 0.27	1.302
3	1.33 ± 0.11	0.99 ± 0.04	1.343

[a] Initial deposits are mean values of duplicate determinations on three 500-gram samples; subsequent values are from two samples. [b] ± Mean deviation. [c] Concentrations of dip emulsions: gooseberries 1140 p.p.m.; tomatoes 2850 p.p.m., actual malathion. [d] Extraction with benzene from unmacerated fruits. [e] Extraction with benzene from fruits blended with water.

Table II. Malathion Residues[a] (P.P.M.[b]) Extracted from Apples

Days after Application[c]	Extraction A[d]	Extraction C[e]	Extraction A/ Extraction C
0	13.28 ± 1.14[e]	10.90 ± 0.45	1.218
2	7.10 ± 0.40	8.10 ± 0.20	0.877
4	4.30 ± 0.00	5.65 ± 0.25	0.761
9	2.85 ± 0.05	3.25 ± 0.15	0.877
14	2.25 ± 0.15	2.35 ± 0.45	0.957

[a] Initial deposits are mean values of duplicate determinations on four 500-gram samples, subsequent values are from two samples. [b] ± Mean deviation. [c] Concentration of dip emulsion: 114 p.p.m. actual malathion. [d] Extraction with benzene from unmacerated apples. [e] Extraction with benzene from apples blended with ethanol.

Table III. Extraction D[a] of P[32] Malathion Residues from Gooseberries[b]

Days after Application[c]	Radioactivity[d]							
	Chloroform-Soluble		Water-Soluble		Inextractable		Total	
	C.p.m.[e]	%[e]	C.p.m.[e]	%[e]	C.p.m.[e]	%[e]	C.p.m.[e]	%[e]
0	30,800 ±1226	93.6 ±3.7	1,176 ±722	3.6 ±2.2	911 ±549	2.8 ±1.8	32,887 ±690	100.0 ±2.1
3	13,037 ±150	54.7 ±0.6	9,753 ±560	40.9 ±1.6	1,053 ±560	4.4 ±2.4	23,843 ±228	72.5 ±0.7
7	11,575 ±1395	48.5 ±5.9	10,459 ±1489	43.9 ±6.2	1,818 ±327	7.6 ±1.4	23,852 ±421	72.5 ±1 3

[a] Extraction with chloroform and methanol–acetone mixture from macerated berries.
[b] Three replicate samples.
[c] Concentration of dip emulsion: 570 p.p.m. actual malathion.
[d] Values corrected for decay of P[32] to same point of time.
[e] ± standard deviation.

evaporated under reduced pressure. The residue was taken up in benzene, which was purified and analyzed for malathion by the colorimetric method (8).

Experiments and Results

Extraction A *vs.* **B.** Extraction Method A (stripping the unmacerated crop) and Method B (blending the crop in water) were compared in trials on gooseberries (var. Houghton) and tomatoes (var. Grower's Pride). A batch of fruit was treated after harvest by dipping in a water emulsion of malathion and stored at about 20° C. At the beginning of storage and later at regular intervals random samples of gooseberries were analyzed for malathion using these two extraction methods.

The results, presented in Table I, show that Method A gave higher values of the initial deposits and of the residues during the first days of storage, but that later Method B showed higher recov-

eries. The differences, however, were small.

Extraction A *vs.* **C.** Extraction Method A (stripping the unmacerated crop) and Method C (blending the crop in ethanol) were compared in a trial on apples (var. Winesap). The fruit was treated after harvest and stored at about 20° C. Method A gave a higher initial deposit value than Method C (Table II). In the later determinations Method C removed slightly more malathion than Method A.

Efficiency of Extraction D. To study the efficiency of Extraction Method D, P[32]-labeled malathion was applied to gooseberries after harvest by dipping in a malathion emulsion and the fruit was then stored at about 20° C. The P[32]-malathion was synthesized according to Krueger and O'Brien (12) and purified by reversed-phase column chromatography (11). The chloroform-soluble, water-soluble, and inextractable radio-

The results of this trial (Table III) show that most of the total radioactivity was in the chloroform and water fractions. Only small amounts (2.8 to 7.6%) were retained in the solid material. When this material was later re-extracted with methanol-acetone, both chloroform- and water-soluble activity were detected, but the relative amount of the chloroform-soluble fraction had definitely decreased in a week, while that of the water-soluble and inextractable fractions had correspondingly increased (Table IV).

In Table III it can be seen that during the first 3 days of storage the proportion of water-soluble radioactivity increased very rapidly, but only slightly during the subsequent 4 days. The total radioactivity decreased during the first 3 days by 27.2% Perhaps this was due primarily to evaporation and partly to mechanical abrasion during handling; the last 4 days, however, showed no additional loss of total activity. On the basis of these results it can be presumed that with Extraction D, practically all the malathion is recovered from the plant material.

Extraction A *vs.* **D.** To compare Extraction A, the standard method in this investigation, to Extraction D, which was found to remove malathion completely from plant tissues, postharvest residues of different ages were extracted from gooseberries by both methods. The results (Table V) showed that Extraction A gave slightly higher residue values than Extraction D. These differences were of the same magnitude as those between Extractions A and B (Table I).

Discussion

The trials on the extraction of postharvest residues of malathion from fruits indicated that simple stripping of the unmacerated crops gave the highest values for the initial deposits and usually for the residues 2 to 3 days of age; later, the other methods tested, which included maceration of the plant tissues, proved to give somewhat higher recoveries. Why the recoveries of the initial and young residues were better by Extraction A than by other procedures is not known, but one could reason that the simpler the procedure the higher the recoveries if the extractability of malathion from plant tissue is not an important limiting factor. The differences between the extraction methods, however, seem to be of small practical significance in routine work. Therefore, it is proposed that, at least for this kind of plant material, stripping the unmacerated crop alone is a practical and relatively quantitative extraction method. It is possible, of course, that the efficiency of this method could be

Table IV. Methanol-Ethanol Re-extraction of Radioactivity from Solids[a] Unremovable by Extraction D

Age of Residues, Days	Radioactivity, %[b]		
	Chloroform-soluble	Water-soluble	Inextractable
0	43.9 ± 11.5	32.5 ± 7.7	23.6 ± 7.2
7	5.7 ± 1.4	54.5 ± 27.5	39.8 ± 26.2

[a] 2 to 3 replicate samples. [b] ± mean deviation.

Table V. Malathion Residues[a] (P.P.M.[b]) Extracted from Gooseberries

Days after Application[c]	Extraction A[d]	Extraction D[e]	Extraction A/ Extraction D
0	132.0 ± 6.3[e]	125.0 ± 6.0	1.056
3	68.0 ± 1.8	63.0 ± 4.7	1.079
7	41.7 ± 1.5	39.5 ± 3.4	1.056

[a] Each value is mean of duplicate determinations on four samples.
[b] ± standard deviation.
[c] Concentration of dip emulsion: 570 p.p.m. actual malathion.
[d] Extraction with benzene from unmacerated berries.
[e] Extraction with chloroform and methanol–acetone mixture from macerated berries.

different for very aged residues and for other kinds of plant material—e.g., citrus fruits.

The decrease in efficiency of the stripping method after the passage of time is thought to be due primarily to the penetration of malathion into the plant tissue, from which it is difficult to remove without prior maceration with a solvent. The fixation of malathion to the plant constituents in a firmly chemically bound and therefore inextractable form is evidently of minor importance (Tables III and IV).

It is assumed that after malathion is applied to the crop surface, it starts to penetrate through the cuticle into the outermost cell layers. Here the chemical is at first rapidly degraded into water-soluble metabolites (Table III), and therefore no malathion can be accumulated in the plant tissue during this initial period. At this time, stripping the crops

without maceration removes malathion quantitatively. Later, the breakdown of malathion into water-soluble products (4, 8, 18, 19) decreased (Table III), probably because of inhibition of the malathion-degrading enzymes (3, 7, 8, 9, 18) by malathion itself or its anti-esterase derivatives (7, 12, 13, 15, 18). When the rate of degradation of malathion in the plant tissue is thus reduced, its lifetime and amount are gradually increased and it may penetrate further into the tissue. At this time, maceration of the crop somewhat increases the efficiency of the extraction methods.

Acknowledgment

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FUMIGANT RESIDUES

Bromide Residues in Chicken Tissues and Eggs from Ingestion of Methyl Bromide–Fumigated Feed

M. E. GETZENDANER

Bioproducts Department, The Dow Chemical Co., Midland, Mich.

Earlier reports have dealt with bromide residues in milk from cows fed on diets containing bromide residues. Young et al. (4) fed peanut vines grown in soil which had been fumigated with ethylene dibromide and Lynn et al. (2) fed rations to which so-

dium bromide had been added or which had been fumigated with methyl bromide. Another consideration, when crops are fumigated with organic bromide fumigants or are produced from fumigated land, is that chickens eating such feed could produce eggs and meat

containing inorganic bromide residues. This study was carried out to determine what residues would occur in eggs and chicken tissues as the result of feeding rations which contain bromide residues resulting from fumigation with methyl bromide.