Disappearance of Malathion Residues from Gooseberries at Different Residue Levels

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Postharvest applications of P^{32}-labeled malathion at different initial deposit levels were made on gooseberries which were then subsequently stored at 22° to 25° C. The berries were analyzed after 3 and 7 days of storage, and the distribution patterns of malathion-P^{32} into chloroform-soluble, water-soluble, and inextractable fractions as well as the loss from the berries were determined. In the 7-day pattern, which was, except the lowest residue level, essentially the same as the 3-day pattern, the proportion of the P^{32}-activity in the chloroform fraction was 31% at the initial deposit level of 2.2 p.p.m., 44% at the 130-p.p.m. level, 85% at the 330-p.p.m. level, and 95% at the 1802-p.p.m. level. The amounts of malathion-P^{32} found in the water fractions varied from 59% at the lowest to 3% at the highest residue level. The actual figures in malathion equivalents for the four application levels were 1.1, 51, 38, and 38 p.p.m., indicating the presence of a malathion-degrading enzyme system in gooseberries with a limited capacity. The amounts of radioactivity not extractable from the berry solids to either the chloroform or water fractions varied from 10% at the lowest to 2% at the highest level. Of the initial loads of radioactivity, 14 to 23% or 0.3 to 324 p.p.m. in malathion equivalents was not recovered. This study indicates that the disappearance behavior of residues of malathion may depend on the amount of the initial deposit.

The disappearance of malathion residues from crops after both field and postharvest treatments has generally been found to proceed very rapidly (*5*, *7*, *13*). Little is known, however, about the different disappearance routes and the effect of the amount of initial malathion deposits on the disappearance rate. Certain studies (*1*, *12*) indicate that evaporation may play an important role in the disappearance of this pesticide and that malathion is effectively metabolized in the rice plant (*14*) and in fresh plant homogenates (*5*).

This paper describes studies which were made to estimate the role of different disappearance routes on malathion residues from gooseberries treated after harvest with malathion and to determine whether the amount of initial malathion deposits may have an effect on the disappearance.

Materials and Methods

Preripe gooseberries (var. Houghton), whose perianth remnants were removed, were treated with malathion in the laboratory by immersing them for 1 minute in P^{32}-labeled malathion emulsions containing 0.01, 0.10, 0.25, and 1.00% actual malathion. The inactive ingredients of the formulation were Triton X-100 (Rohm & Haas, Philadelphia, Pa.) and xylene. Each group contained 140 berries which had been selected from the same initial berry batch after drying for 2 hours; two 20-berry random samples with the same total weight were analyzed. The rest of the berry samples were stored in open cartons for 1 week at 22° to 25° C. and at a relative humidity of 50 to 60%. Further analyses were performed after 3 and 7 days.

P^{32}-labeled malathion was prepared by the method described by Krueger and O'Brien (*11*), starting with an initial radioactivity of 10 mc. of $H_3P^{32}O_4$ and using benzene as the reaction solution instead of the higher boiling toluene. The crude material synthesized was extracted with chloroform–10% sodium carbonate solution and the chloroform-soluble fraction was finally purified by reversed-phase chromatography on a silicon-coated 35 × 250 mm. Celite column (10% Silicon AP150, Wacker-Chemie, Germany, on Celite 535, Johns-Manville, New York, N. Y.). Two volumes of an acetone-ethanol-water mixture was used as the eluting solvent; first 400 ml. in a ratio 1:1:4 and then 500 ml. in a ratio 1:1:2. The malathion peak appeared from the column after a total run of about 650 ml. of effluent. Its behavior on the paper chromatograms and its infrared spectrum gave no indication of impurities; therefore, this purification method proved to be superior to the other methods used for this purpose. In the various cases, 4.2 to 15.7% of the initial radioactivity was recovered in the purified malathion with specific activities ranging from 102 to 370 dps. per μg. This product was used directly for the 0.01 and 0.10% water emulsions, while for the other two concentrations it was first diluted with 95% technical-grade malathion (American Cyanamid Co., Princeton, N. J.).

The gooseberry samples were extracted by the following method (*8*). The samples were placed in a 100-ml. homogenizing beaker with 10 ml. of distilled water and 60 ml. of chloroform, and the mixture was blended for 30 minutes in a MSE Homogeniser (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 together were reblended with 10 ml. of distilled water and 40 ml. of fresh chloroform for an additional 30 minutes. The chloroform layer obtained by centrifuging was separated, and the two chloroform fractions were combined. After separating the water fraction from the solid material, the latter was washed twice with 40 ml. of methanol-acetone mixture (1:1) 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 evaporation residue taken up into a chloroform and a water fraction (1:1) which was then combined with the corresponding previous fractions. The amounts of chloroform-soluble, water-soluble, and inextractable radioactivities were measured from the three fractions. The radioactivity losses through evaporation and mechanical

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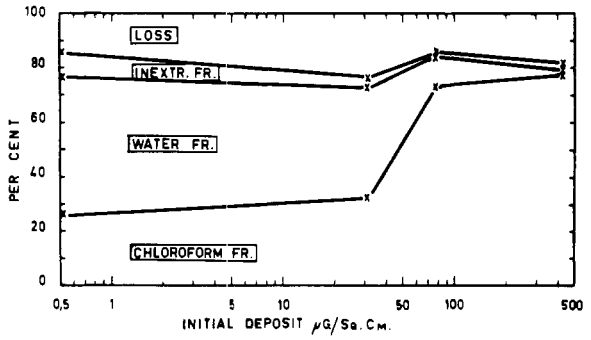

Figure 1. Relative amounts of radioactivity of chloroform-soluble, water-soluble, and inextractable fractions derived from P^{32}-labeled malathion applied 7 days previously to gooseberries at different initial deposits

Loss. Difference of total radioactivities of 0-day and 7-day residues

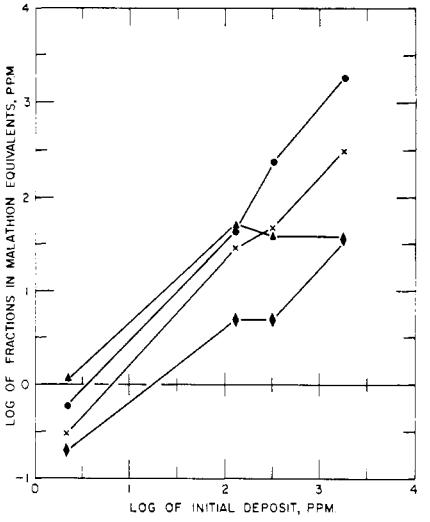

Figure 2. Logarithmic regression between initial deposits and subsequent extract fractions for 7-day residue

● Chloroform fraction
▲ Water fraction
● Inextractable
X Lost

abrasion were calculated as the difference between the total activity at the start of the experiment and the activity at the time of analysis.

The radioassays were performed by counting duplicate aliquots from the three different 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.

Results and Discussion

The distribution pattern of malathion-P^{32} into different fractions is shown in Table I. The first analysis (0-day residue), in which the extraction was made about 2 hours after the application of malathion, showed that at the lowest residue level 0.2 p.p.m. or 9% of the initial application was converted into a water-soluble form. At the three higher levels, approximately the same amounts or 9, 8, and 7 p.p.m. representing 7, 2, and 0.4% of the respective total residues were found. In the three subsequent days (3-day residue), the water-soluble fraction both in absolute and relative quantities greatly increased at all residue levels. The highest absolute amount, 56 p.p.m. in malathion equivalents, of water-soluble activity was found at the 130-p.p.m. residue level, while there was somewhat less at the two higher levels. The last four days of the experiment did not markedly change the fraction pattern, except for the increase in the water-soluble fraction at the lowest residue level.

The composition of these chloroform and water fractions was not studied. Malathion and its oxidized form malaoxon are undoubtedly recovered in the chloroform fraction (*10*). In plants, several water-soluble compounds are formed from malathion (*5*, *10*, *14*) and it could be possible that some of those would be extracted in chloroform at the low pH values—i.e., pH 3 to 4—for the gooseberry homogenates. In this case, however, it is believed that these types of compounds did not contribute substantially to the radioactivity of the chloroform fractions. This assumption is sup-

Table I. Distribution of Radioactivity into Fractions from P^{32}-Labeled Malathion Residues of Different Ages and Different Initial Deposits on Gooseberries

	Initial Deposit, P.P.M. (μg. per Sq. Cm.[a])							
	2.2 (0.5)		130 (31)		330 (78)		1802 (424)	
	P.p.m.[b]	%[c]	P.p.m.[b]	%[c]	P.p.m.[d]	%[e]	P.p.m.[b]	%[c]
0-day residue								
$CHCl_3$-soluble	1.7 ± 0.0	78 ± 0.3	113 ± 1.8	87 ± 1.4	315 ± 0.7	96 ± 0.2	1757 ± 28.8	98.0 ± 1.6
H_2O-soluble	0.2 ± 0.0	9 ± 0.3	9 ± 3.0	7 ± 2.3	8 ± 1.3	2 ± 0.4	7 ± 7.2	0.4 ± 0.4
Inextractable	0.3 ± 0.0	13 ± 0.7	9 ± 4.9	7 ± 3.8	7 ± 1.7	2 ± 0.5	38 ± 19.8	2.0 ± 1.1
Lost	0	...	0	...	0	...	0	...
3-day residue								
$CHCl_3$-soluble	1.0 ± 0.1	54 ± 5.3	37 ± 3.5	37 ± 3.5	233 ± 11.1	82 ± 3.9	1388 ± 20.7	94.0 ± 1.4
H_2O-soluble	0.8 ± 0.1	44 ± 5.7	56 ± 1.3	56 ± 1.3	36 ± 7.7	13 ± 2.7	43 ± 4.4	3.0 ± 0.3
Inextractable	<0.1 ± 0.0	2 ± 0.4	7 ± 4.8	7 ± 4.8	15 ± 3.1	5 ± 1.1	47 ± 25.1	3.0 ± 1.7
Lost[d]	0.3	...	30	...	46	...	324	...
7-day residue								
$CHCl_3$-soluble	0.6 ± 0.1	31 ± 2.7	44 ± 0.2	44 ± 0.2	241 ± 2.0	85 ± 0.7	1404 ± 11.8	95.0 ± 0.8
H_2O-soluble	1.1 ± 0.1	59 ± 4.0	51 ± 0.5	51 ± 0.5	38 ± 3.1	13 ± 1.1	38 ± 2.8	3.0 ± 0.2
Inextractable	0.2 ± 0.0	10 ± 1.3	5 ± 0.7	5 ± 0.7	5 ± 1.1	2 ± 0.4	35 ± 8.4	2.0 ± 0.6
Lost[e]	0.3	...	30	...	46	...	324	...

[a] In calculation, gooseberries are assumed to be spherical, with a density of 1.0 g./cc.
[b] ± mean deviation.
[c] Per cent composition (± mean deviation) of radioactivity found in samples; not per cent of initial deposits.
[d] In this series, calculations based on evidence (*8*) that loss of radioactivity in 3 days is same as for 7 days.
[e] Difference of malathion equivalents recovered in p.p.m. between 0-day and 7-day residues.

ported by studies with other fruits having the same range of acidity. When reversed phase paper chromatography was used, there was no indication of more hydrophilic compounds than malaoxon (*10*) in the corresponding chloroform fractions.

The amounts of inextractable malathion-P^{32} varied from 0.1 to 47 p.p.m. of malathion equivalents or 2 to 13% of the total radioactivity of the samples. The chemical form of the inextractable radioactivity, presumably firmly bound to the plant solids, is poorly known; it has been shown (*8*) that a certain part of such material can be extracted by a more drastic procedure, and that the radioactivity obtained is partly water soluble and partly chloroform soluble.

The amount of malathion-P^{32} not recovered after 7 days of storage was equivalent to 0.3 to 324 p.p.m. of malathion or 14 to 23% of the initial residue, depending on the initial level of application. It has been previously shown (*8*) that beyond 3 days no additional losses of P^{32} occurred under similar conditions; this is why in this experiment the losses of malathion-P^{32} in the first 3 days are assumed to be the same as the carefully estimated losses after the 7-day storage. What is responsible for the losses is unknown, but it is assumed that the evaporation of malathion from the fruit surface may account for the major part (*1*, *12*). This may primarily take place just after the application of malathion and before the residue is completely dissipated into the plant cuticle, as is indicated when fruits are washed after treatment with malathion emulsions (*9*).

Figure 1 illustrates how the relative amounts of malathion-P^{32} in the different fractions were affected by the rate of the malathion initial load. At the two lowest residue levels the conversion of malathion into water-soluble derivatives was the most important disappearance route of the residues, but between the residue loads of 130 and 330 p.p.m. (31 to 78 μg. per sq. cm.) its importance was greatly decreased. The absolute amounts of the water-soluble fractions at the three highest levels indicate that the gooseberries were able to degrade only a limited amount of malathion, after which practically no further degradation into the water-solubles took place. The upper limit of the water-soluble fraction was somewhat higher at the 130-p.p.m. residue level than at the two highest levels. If this observation is not an artifact, one could propose the presence of an optimum residue load range where the maximum absolute values for the water-soluble fractions are obtained.

The reason for the change in the fraction pattern with gooseberries in this experiment is most likely the fact that the enzymatic mechanism (*5*, *6*, *14*) which converts malathion into its water-soluble derivatives has a limited capacity which is exceeded at least for the two highest residue levels. When this mechanism is inhibited at a certain residue level, there is reason to assume that the remaining residues are very stable and the subsequent disappearance pattern of the residues would be drastically changed. This type of phenomenon could also be involved in those generally known cases in which the rapidly degrading residue is changed into a more persistent residue; this could happen, however, only under those conditions when the amount of the initial residue exceeds the degradation capacity of the enzyme system.

In Figure 2 the regression between the logarithm of the initial residue (in parts per million) at 7 days and that of the resultant malathion equivalents in each extract fraction is presented. This figure indicates that the overloading of the enzymatic degradation mechanism in the initial residue range, 130 p.p.m. (log. 2.11) to 330 p.p.m. (log. 2.52), more or less causes a shifting of all of the regression curves and thus apparently interferes with the probable linear regression.

It is generally believed that the rates of disappearance of pesticide residues are independent of the amount of initial deposits (*2–4*). The present study, however, indicates that at least the postharvest behavior of malathion may be an exception.

On the basis of this study it can be assumed that either a heavy, uniform application of malathion or a spotty application of concentrated formulations with sites of very high local concentrations of malathion on plant surfaces may greatly depress the degradation rate of malathion and thus extend the life of the residues. This might be of considerable interest both for the practical application of the pesticide and for the regulatory authorities on pesticide residues.

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