was found equivalent to 320 mg/m^2 of the field surface with a standard error of ±42 mg/m^2. This indicates that 2.4 kg/ha or 43% of the initial application was not accounted for, with a 95% confidence that the actual loss was between 29 and 57%.

Analysis of soil surrounding another field sprayed in the same way in 1968 (Caro, 1971) showed limited local deposition of drift, suggesting that almost all the lost material was injected into the atmosphere by evaporation of spray particles or from the treated surface before incorporation. It is thus clear that the amounts of soil-incorporated dieldrin and heptachlor volatilized to the air over the growing season were between 10 and 25% of those entering the atmosphere during the application.

ACKNOWLEDGMENT

The authors wish to acknowledge the contribution of E. R. Lemon, M. Johnson, and G. Drake of the Agricultural Research Service Microclimate Unit at Ithaca, N.Y., and L. H. Parmele of the Northeast Watershed Research Center, Agricultural Research Service, U.S. Department of Agriculture, University Park, Pa., for their role in measuring the micrometeorological parameters and calculating the flux densities.

Supplementary Material Available: A detailed map of the experimental field (Figure A) and tabulations of insecticide concentration as a function of height for each observation day (Tables A–E); 6 pages. Ordering information is given on any current masthead page.

LITERATURE CITED

Barrows, H. L., Caro, J. H., Armiger, W. H., Edwards, W. M., *Environ. Sci. Technol.* **3**, 261 (1969).
Caro, J. H., *J. Agric. Food Chem.* **19**, 78 (1971).
Caro, J. H., Taylor, A. W., *J. Agric. Food Chem.* **19**, 379 (1971).
Caro, J. H., Taylor, A. W., Lemon, E. R., Proceedings of the International Symposium on Measurement of Environmental Pollutants, National Research Council of Canada, Ottawa, 1971, pp 72–77.
Freeman, H. P., Taylor, A. W., Edwards, W. M., *J. Agric. Food Chem.* **23**, 1101 (1975).
Harris, C. R., Lichtenstein, E. P., *J. Econ. Entomol.* **54**, 1038 (1961).
Harrold, L. L., Dreibelbis, F. R., *U.S. Dep. Agric. Tech. Bull.* **No. 1367** (1967).
Hartley, G. S., *Adv. Chem. Ser.* **No. 86**, 115–133 (1969).
Jackson, R. D., Kimball, B. A., Reginato, R. G., Nakayama, F. S., *Soil Sci. Soc. Am. Proc.* **37**, 505 (1973).
Lemon, E. R., "Physiological Aspects of Crop Yield", Symposium of the American Society of Agronomists, Madison, Wis., 1969, pp 117–142.
Lichtenstein, E. P., Myrdal, G. R., Schultz, K. R., *J. Econ. Entomol.* **57**, 133 (1964).
Lichtenstein, E. P., Schultz, K. R., *J. Econ. Entomol.* **54**, 517 (1961).
Miller, G. A., Wells, C. E., *J. Assoc. Off. Anal. Chem.* **52**, 548 (1969).
Parmele, L. H., Lemon, E. R., Taylor, A. W., *Water, Soil Air Pollut.* **1**, 433 (1972).
Spencer, W. F., Cliath, M. M., *Environ. Sci. Technol.* **3**, 670 (1969).
Spencer, W. F., Cliath, M. M., *J. Environ. Qual.* **2**, 286 (1973).
Spencer, W. F., Cliath, M. M., Farmer, W. J., *Soil Sci. Soc. Am. Proc.* **33**, 509 (1969).
Spencer, W. F., Farmer, W. J., Cliath, M. M., *Residue Rev.* **49**, 1 (1973).
Taylor, A. W., Freeman, H. P., Edwards, W. M., *J. Agric. Food Chem.* **19**, 832 (1971).
Turner, B. C., unpublished data, Agricultural Chemicals, Management Laboratory, U.S. Department of Agriculture, Beltsville, Md.

Received for review November 11, 1975. Accepted February 6, 1976.

Determination of Malathion, Malaoxon, and Mono- and Dicarboxylic Acids of Malathion in Fish, Oyster, and Shrimp Tissue

Gary H. Cook and James C. Moore*

A method is described for monitoring the presence of malathion and its metabolites in the aquatic environment. Malathion, malaoxon, malathion monoacid, and malathion diacid were determined in fish, oyster, and shrimp tissues by gas–liquid chromatography (GLC) using phenthoate and phenthoate acid as internal standards. GLC analyses were performed without cleanup, using a flame photometric detector operating in the phosphorus mode. Acid compounds were methylated with diazomethane. Pinfish exposed to 75 μg/l. of malathion in flowing seawater for 24 h contained no residues of malathion or malaoxon, although the concentration of the malathion monoacid in the gut was 31.4 μg/g. The data illustrate that pinfish rapidly convert malathion to the mono- and dicarboxylic acids of malathion.

Malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) has a broad spectrum of effectiveness against insects and is widely used along coastal areas for control of mosquitoes, flies, and other noxious pests. Although the chemistry and metabolism of malathion in various substrates have been studied extensively (Krueger and O'Brien, 1959; Corley and Beroza, 1968; Shafik and Enos, 1969; Shafik et al., 1971; El-Refai and Hopkins, 1972; Wolf et al., 1975) practically no residue data have been reported for malathion or its degradation products in aquatic species.

Binder (1969) studied the uptake of malathion in carp exposed to 5 mg/l. malathion for 4 days and found that residues in the flesh had an average half-life of 12 h, the liver concentrating the greatest amount. At our laboratory,

U.S. Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561.

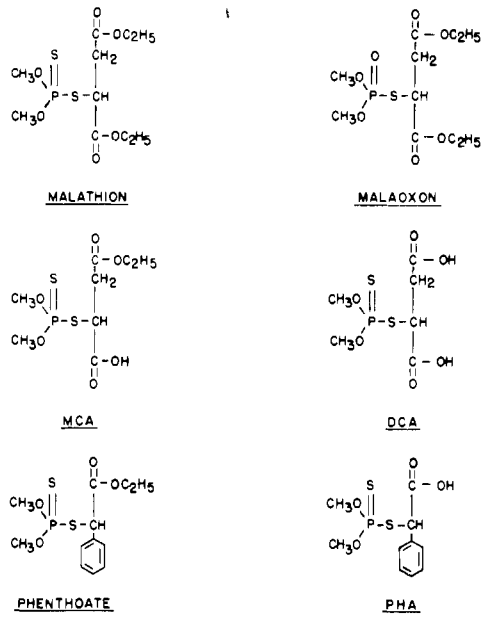

Figure 1. Malathion, malaoxon, monocarboxylic acid of malathion (MCA), dicarboxylic acid of malathion (DCA), phenthoate (PHE), and the carboxylic acid of phenthoate (PHA).

no residues of malathion have been found in the tissue of fish at exposures to 300 μg/l. in the water (Coppage et al., 1975; Tagatz et al., 1974).

Because of the different properties of malathion and its hydrolytic products, separate methods of analysis are usually performed for their extraction and cleanup. Shafik et al. (Shafik and Enos, 1969; Shafik et al., 1971) developed methods for monitoring human beings exposed to malathion by analyzing the urine for malathion, the monocarboxylic acid of malathion (MCA), the dicarboxylic acid of malathion (DCA), as well as the alkyl phosphate metabolites. Kadoum (1969) described a method of analysis for malathion and its hydrolytic products in stored grain.

In this investigation, a method was developed for analysis of malathion, malaoxon, MCA, and DCA in fish, oysters, and shrimp, using Phenthoate (PHE; *O,O*-dimethyl phosphorodithioate of ethyl mercaptophenylacetate) and its acid degradation product (PHA; *O,O*-dimethyl phosphorodithioate of mercaptophenyl acetic acid) as internal standards (Figure 1).

Each sample was spiked with PHE and PHA to permit evaluation of the integrity of the analysis. Recoveries of malathion were based on recoveries of PHE. MCA and DCA recoveries were based on recoveries of PHA. All residues were adjusted for recovery. The method is suitable for (1) monitoring the presence of malathion in the aquatic environment and species, (2) pointing out its path of degradation, and (3) explaining the lack of reported residues for malathion in fish.

EXPERIMENTAL SECTION

Apparatus. We employed a Tracor MT-220 gas–liquid chromatograph equipped with a flame photometric detector operating in the phosphorus mode and a ^{63}Ni electron-capture detector. The 180 cm × 3 mm i.d. glass column was packed with 2% OV-101 on Gas-Chrom Q 100/120 mesh. Operating conditions for the flame photometric detector were: column, 175 °C; inlet, 225 °C; detector, 165 °C; nitrogen carrier flow, 65 ml/min; hydrogen, 200 ml/min; air, 50 ml/min; oxygen, 15 ml/min. Operating conditions for the electron capture detector were: column, 175 °C; inlet, 225 °C; detector, 250 °C; nitrogen carrier flow, 65 ml/min.

New columns were conditioned by placing a small piece of glass wool in the inlet end of the column and adding approximately 10 cm of 10% Carbowax (Chemical Research Services, Inc.,) on Chromosorb W, acid-washed 80/100 mesh. The column was heated overnight at 230 °C with a nitrogen flow of 20 ml/min. (During the conditioning period, the column was not connected to the detector.) The Carbowax and the glass wool separator plug were replaced with 2% OV-101, the carrier flow was adjusted to 50 ml/min, and the column was conditioned an additional hour at 230 °C, when sensitivity and efficiency of the column became unacceptable because of peak tailing, lack of peak separations, or the lack of reproducibility. Replacement of the glass wool and approximately 5 cm of the OV-101 at the injector end usually returned the column to its original efficiency.

Reagents. All solvents were Nanograde, distilled in glass (Mallinckrodt Inc., or equivalent).

Standards. Malathion, malaoxon, and phenthoate were obtained from the Pesticide Reference Standard Section, Environmental Protection Agency, Washington, D.C., MCA and DCA were obtained from the American Cyanamid Co., Princeton, N.J., and PHE was obtained from Thomson-Hayward Chemical Co., Kansas City, Kan.

Primary standard solutions of malathion, malaoxon, and PHE were prepared by diluting 100 mg to 100 ml with benzene. DCA, MCA, and PHA primary standard solutions were prepared by diluting 100 mg to 100 ml with benzene–acetone (3:1). Working standards and spike solutions were prepared by diluting the primary standard solutions with petroleum ether to the desired concentrations. Primary standards were kept refrigerated at 3 °C in amber bottles closed with Teflon-lined screw caps.

Acidified Sodium Sulfate. Anhydrous sodium sulfate (Baker Chemical Co.) was stirred into a smooth slurry with 9 N sulfuric acid and the excess acid removed by vacuum filtration. The sodium sulfate was washed twice with methyl alcohol, then with ethyl acetate, the mixture being stirred each time into a smooth slurry before vacuum filtration. The sodium sulfate was allowed to air-dry for 2 h and then heated at 130 °C overnight. All lumps were removed by blending in a Waring blender.

Procedure. A 0.5 to 5.0 g (wet weight) sample of tissue was placed in a 25 × 150 mm culture tube and fortified with 20 μg of PHE and PHA. Ten milliliters of acetonitrile, acidified by adding 2% (v/v) 2 N HCl, was added to the tube and the tissue extracted at 20 000 rpm for 30 s with a Willems Polytron. The culture tube was centrifuged and the acetonitrile extract decanted into a 150-ml beaker containing 100 ml of 2% aqueous sodium sulfate. The tissue was extracted a second time with 5 ml of acidified acetonitrile and centrifuged and the extract decanted into the 150-ml beaker. The aqueous solution was adjusted to pH 8.5 with 5% aqueous sodium carbonate and transferred to a 250-ml separatory funnel.

Malathion and PHE were removed by extracting the aqueous solution with two 25-ml portions of petroleum ether. The petroleum ether extracts were dried by eluting through a 15-g plug of anhydrous sodium sulfate in a 30-mm powder funnel. The sodium sulfate was rinsed with 15 ml of petroleum ether. The combined extracts (collected in a 70 × 50 mm crystallizing dish) were placed on a 50 °C slide warmer in a hood and evaporated to about 5 ml by pulling a gentle stream of air over the dish. The extracts were then diluted to a standard volume of 10 ml for determining the percentage recovery of PHE. The sample volume was then further adjusted as required for

Table I. Recoveries of Malathion, Phenthoate, Malaoxon, MCA, DCA, and PHA from Fortified Fish, Oyster, and Shrimp Tissue

Compound[a]	Added, μg	Percentage recovery ($\bar{X}$ ± SD)		
		Fish	Oyster	Shrimp
Malathion	20	93.0 ± 3.8	91.7 ± 3.2	91.4 ± 5.0
Phenthoate (PHE)	40	92.0 ± 4.6	94.1 ± 3.5	91.6 ± 4.8
Malaoxon	20	68.9 ± 5.8	71.0 ± 11.8	80.6 ± 3.8
MCA	20	81.0 ± 3.6	86.5 ± 9.4	88.2 ± 3.7
DCA	20	74.5 ± 5.2	91.8 ± 9.6	84.4 ± 4.3
PHA	40	73.6 ± 5.6	81.7 ± 13.4	84.8 ± 3.8

[a] Tissue samples were 1–5 g (percentage recovery based on total micrograms recovered). Fish tissue are averages of 10 samples; shrimp tissue, 5 samples; oyster tissue, 8 samples.

determination of malathion.

Malaoxon was removed by extracting the aqueous solution twice with 25-ml portions of methylene chloride. The extracts were dried by eluting through sodium sulfate and then evaporated to about 1 ml on a slide warmer as described for malathion. The volume was adjusted with acetone as necessary for GLC analysis.

To extract MCA, DCA, and PHA, the aqueous solution was transferred to a 150-ml beaker and 5% (w/v) solid sodium chloride was added. The pH was adjusted to pH 2 with 6 N HCl. The solution was returned to the 250-ml separatory funnel and extracted twice with 50-ml portions of ethyl acetate. The ethyl acetate extracts were dried by eluting through a plug of acidified sodium sulfate, collected in a crystallizing dish, and esterified with diazomethane.

Esterification of the acidic compounds was carried out in a diazomethane generating apparatus described by Schlink and Gellerman (1960). Diazomethane was bubbled into each sample until a slight yellow color of excess diazomethane persisted. Excess diazomethane was removed during concentration on the slide warmer. The samples were diluted to 10 ml for the analysis of PHA and then adjusted as required, for the analysis of MCA and DCA. (**Caution:** Diazomethane is explosive, carcinogenic, and extremely toxic.)

Samples to be analyzed by electron-capture gas chromatography were cleaned up by the procedure of Hansen et al. (1974) before analysis. Malathion, PHE, and the methyl esters of MCA, DCA, and PHA were eluted from the Florisil column with 20 ml of 50% ethyl ether in hexane. Malaoxon was eluted with 20 ml of acetone. The eluate was concentrated to 3–5 ml under a gentle stream of N_2 in a Kontes concentrator apparatus at 45 °C and diluted to the desired volume with petroleum ether. Acetone was removed from the malaoxon fraction by evaporation to 1–2 ml, adding 10 ml of hexane, and reevaporating before adjusting to the desired volume.

RESULTS AND DISCUSSION

The chromatograms in Figure 2 were from the extract of a 2-g sample of fish tissue which had been fortified with 20 μg of malathion, malaoxon, MCA, and DCA and 40 μg of PHE and PHA and extracted by the procedure described above. The extract was concentrated to 10 ml and analyzed on the flame photometric detector without cleanup. Table I shows the average percentage recovery of malathion, malaoxon, MCA, DCA, and the internal standards, PHE and PHA, from fortified tissue samples. The petroleum ether extract contained 10–15% of the malaoxon present. This malaoxon was added to the malaoxon recorded in the methylene chloride extract to obtain total malaoxon recovery.

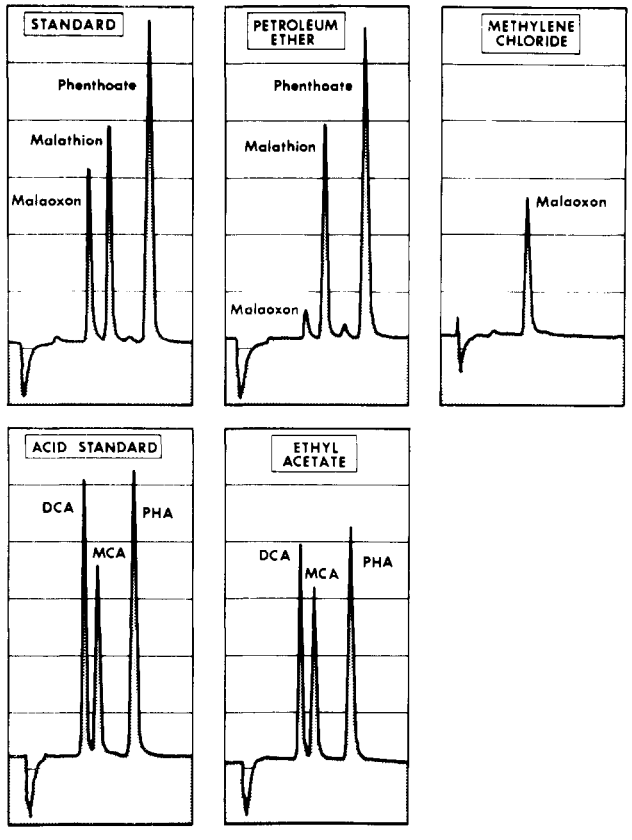

Figure 2. Flame photometric chromatograms of the extracts of fish tissues fortified at 10 μg/g of tissue with malathion, MCA, and DCA and 20 μg/g of tissue with PHE and PHA.

A single methylene chloride extraction which will remove malathion, malaoxon, and PHE may be used instead of separate petroleum ether and methylene chloride extractions. This has the advantage of eliminating one injection; however, methylene chloride also removes approximately 80% of the acetonitrile from the aqueous phase. Because of the higher boiling point of acetonitrile and the volatility of malathion and PHE, evaporation was slow and low recoveries of malathion and PHE were obtained. This accounts for the lower recovery obtained for malaoxon. If the analysis for malaoxon is eliminated, the methylene chloride extraction should be made at pH 8 and discarded; otherwise, the acetonitrile will be removed in the ethyl acetate extract and will interfere with methylation of the acids.

The aqueous phase must be kept to a minimum due to the solubility of the acid compounds in water. The distribution coefficients of the acids are made favorable for extraction of the compounds with ethyl acetate by adjusting to pH 2 and adding enough NaCl to reach 5% w/v.

To check the applicability of the method to real samples, 5 to 7 cm long pinfish (*Lagodon rhomboides*) were exposed for 24 h to 75 μg/l. malathion in flowing seawater as described by Coppage et al. (1975). The pinfish were then rinsed with distilled water and whole-body analysis was performed. Table II shows the concentrations of malathion, and its degradation products and the percentage recovery of the internal standards. Concentrations were adjusted for percentage recovery of the internal standard. The FPD chromatogram of each extract is shown in Figure 3. The internal standards peaks, phenthoate and PHA, represent 20 μg/g of tissue diluted to 10 ml for analysis.

For routine analysis, the sensitivity of the FPD is 0.1 ppm for a 1-g sample compared to 0.01 ppm for the

Table II. Whole-Body Residues of Malathion and Its Metabolites in Pinfish Exposed to 75 μg/l. Malathion in Flowing Seawater for 24 h

Sample no.	Phenthoate (PHE), % recovery	Malathion, μg/g	Malaoxon, μg/g	MCA, μg/g	DCA, μg/g	PHA, % recovery
1	88	ND[a]	ND[a]	3.8	0.28	76
2	87	ND	ND	3.0	0.22	78
3	93	ND	ND	6.0	0.43	81
4	93	ND	ND	4.1	0.25	73
5	100	ND	ND	4.3	0.34	79
$\bar{X}$ ± SD	92 ± 5	ND	ND	4.2 ± 1	0.30 ± 0.08	77 ± 3

[a] ND = not detected; <0.10 μg/g.

Table III. Concentration of Malathion, Malaoxon, MCA, and DCA in Various Organs of Pinfish Exposed to 75 μg/l. of Malathion in Flowing Seawater for 24 h

Organ	Malathion, μg/g	Malaoxon, μg/g	MCA, μg/g	DCA, μg/g
Brain	ND[a]	ND[a]	1.7	0.22
Liver	ND	ND	6.0	0.25
Gills	ND	ND	2.5	0.36
Flesh	ND	ND	3.9	0.34
Gut	ND	ND	1.4	0.7

[a] ND = not detected; <0.10 μg/g.

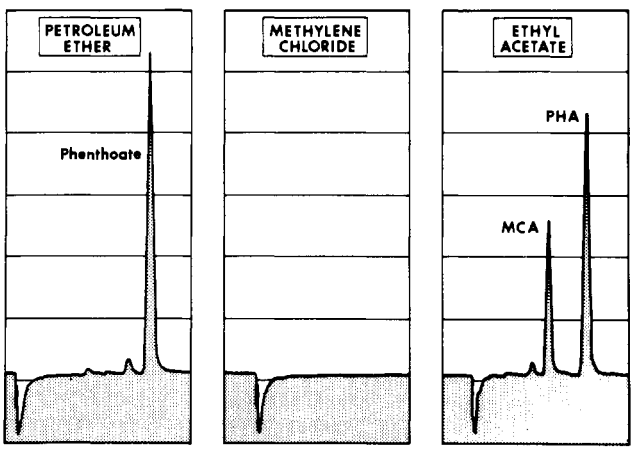

Figure 3. Flame photometric chromatograms from whole-body residue extracts of pinfish exposed to 75 μg/l. malathion for 24 h in flowing sea water.

electron-capture detector. However, the flame photometric detector offers advantages of specificity for phosphorus and a larger linear range of detection (three or more orders of magnitude). Also, the latter detector is not flooded with solvents, such as ethyl acetate, acetone, and small amounts of acetonitrile and methylene chlorine, and the extracts may be analyzed without cleanup.

Knowledge of the location of the pesticide residues in various tissues is important for understanding the route of detoxification and degradation, as well as for attaining increased analytical sensitivity by analyzing the organ of highest concentration. Table III shows the concentrations of malathion and its metabolites found in the various organs of pinfish exposed to 75 ppb of malathion for 24 h. Pinfish very rapidly convert malathion to mono- and diacids, whose greatest concentrations were found in the gut. Malathion itself was not found in any organ.

ACKNOWLEDGMENT

We thank P. R. Parrish and Edward Matthews for assistance in collecting and exposing the test animals used in this study and Steven S. Foss for preparation of tables and illustrations.

LITERATURE CITED

Binder, M. E., *Prog. Fish Cult.* **31**, 155–159 (1969).
Coppage, D. L., Matthews, E., Cook, G. H., Knight, J., *Pestic. Biochem. Physiol.* **5**(6), 536–542 (1975).
Corley, C., Beroza, M., *J. Agric. Food Chem.* **16**, 361 (1968).
El-Refai, Hopkins, T. L., *J. Assoc. Off. Anal. Chem.* **55**(3), 526–531 (1972).
Hansen, D. L., Parrish, P. R., Forester, J., *Environ. Res.* **7**, 363–373 (1974).
Kadoum, A. M., *J. Agric. Food Chem.* **17**(6), 1178–1180 (1969).
Krueger, H. R., O'Brien, R. D., *J. Econ. Entomol.* **52**, 1063–1067 (1959).
Schlink, H., Gellerman, J. L., *Anal. Chem.* **32**(11), 1412–1414 (1960).
Shafik, M. T., Bradway, D., Enos, H. F., *J. Agric. Food Chem.* **19**(5), 885–889 (1971).
Shafik, M. T., Enos, H. F., *J. Agric. Food Chem.* **17**, 1186 (1969).
Tagatz, M. E., Borthwick, P. W., Cook, G. H., Coppage, D. L., *Mosq. News* **34**(3), 390–315 (1974).
Wolf, N. L., Zepp, R. G., Baughman, G. L., Gordon, J. A., *Bull. Environ. Contam. Toxicol.* **13**(6), 707–713 (1975).

Received for review November 3, 1975. Accepted February 17, 1976. Contribution No. 273 from the Gulf Breeze Environmental Research Laboratory. Mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.