Gas Chromatographic Analysis of Malathion in Water and in Fish[1]

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The successful use of malathion in the field of pest control and its possible translocation in the environment has necessitated the development of a simple and sensitive method for its determination in water and in fish. The present paper describes a reliable sequence of techniques for such determinations.

Recovery of Malathion from Fortified Water Samples

Add 5 ml of nanograde hexane to fortified filtered water samples (100 ml) in a series of 100 ml volumetric flasks with long neck above the graduation mark or with bulb reservoir in neck. Shake vigorously for 2 minutes during which time loosen the stopper to release pressure. Add about 5 g sodium chloride and shake vigorously for an additional three minutes. Add more NaCl or hexane if necessary to effect sharp separation. Remove the hexane layer by means of a disposable Pasteur pipet

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and dry it by passing through anhydrous sodium sulfate in another pipet with a small plug of clean glass wool (washed with water, baked in an oven, and then rinsed with hexane) in the bottom. Repeat extraction four more times. Evaporate hexane, redissolve malathion residue in a suitable volume of hexane and assay it by electron capture gas chromatography using the following operating conditions:

Gas chromatograph, Research Specialties Model 660 fitted With Sr 90 electron capture detector; Column, glass, U-shaped, 5'x¼" o.d., packed with 5% DC 11 on 60/80 mesh acid washed Chromosorb W and conditioned at 220°C for 4 days; Oven temperature, 180°C; Nitrogen flow rate, 48 ml/min.; Detector temperature, 260°C; Cell voltage, 1.47 V.D.C.; Recorder, Sargent, 1 mv, 1 sec. full scale.

Recovery of Malathion from Fortified Fish Tissues

Trap the fish alive between two blocks of sponge covered with plastic sheet and installed on a strong stand (in case of carp, the sponge blocks are operated by compressed air). Take blood by heart puncture and quickly remove brain and then desired tissues.

(a) Blood - Immediately centrifuge at high speed in a refrigerated centrifuge. Extract malathion from fortified serum by shaking vigorously one part serum with three parts of hexane three times in a centrifuge tube. Wait for phase separation or centrifuge. Dry an

aliquot of the hexane layer with sodium sulfate and assay malathion by GLC as described for water samples.

(b) Brain, Liver, and Small Gills - Macerate fortified samples with hexane in a tissue grinder and proceed as in (a).

(c) Skin, Flesh, and Large Gills - Blend fortified chopped samples with 3 to 5 parts of hexane in a Lourds stainless steel homogenizer for 5 min. at high speed. Shake mechanically in a wide-mouth Erlenmeyer flask for one hour. Centrifuge in a centrifuge bottle or tube for 15 min. at 2000 rpm. Decant hexane layer and reextract pellet. Combine extracts, dry an aliquot with sodium sulfate and analyse by gas chromatography.

Cleanup of Malathion Extracts from Fish

If the extracts from fish contain excessive co-extractives cleanup is necessary. An aliquot may be subjected to: (I) the conventional liquid-liquid partitioning procedure (hexane - dimethylformamide) and/or (II) a florisil (activated at 130°C for 6 hours) column using petroleum ether and acetone for elution. (2). Since in many cases only a small volume of extract is required for analysis by GLC, cleanup using (III) a modified "wedge-layer" (1) chromatographic technique was found to be efficient and less time consuming. TL plates 2"x8" are coated with a slurry of Al_2O_3 : water (1 : 1.2,w/v) with a TL applicator (Research Specialties

Model 200) adjusted to 125 μ at one end and 1000 μ at the other end. The thickness of the layer thus varies uniformly from 125 μ to 1000 μ along the length of the plate. The dried plate is washed in water using a filter paper wick from the thick to the thin end of the wedge, air-dried, and then activated at 80°C for 40 min. About 1" of the adsorbent at the top of the plate is scraped off or isolated by scoring a thick horizontal line. On the remaining rectangle of adsorbent a triangle is drawn with base about 1 3/4" and height about 6¼" (Fig. 1). The adsorbent outside the triangle is then removed. An aliquot of the concentrated extract is applied as a streak about ½" above the base of the triangle. On developing in acetonitrile : water (8 : 2, v/v) malathion migrates to the vertex of the triangle while most of the impurities remain near the origin. The malathion area is scraped off, extracted with hexane and assayed in the gas chromatograph. Several triangles may be constructed on a 8"x8" plate. If the original extract is extremely oily, prior use of the liquid-liquid partitioning technique (I) is required.

Concentrated extracts of water and carp flesh fortified at 1 ppm were gas chromatographed (flame ionization detector fitted with splitter). Peak effluents were collected in U-shaped glass tubes of 1/8" o.d. immersed in dry ice and malathion was

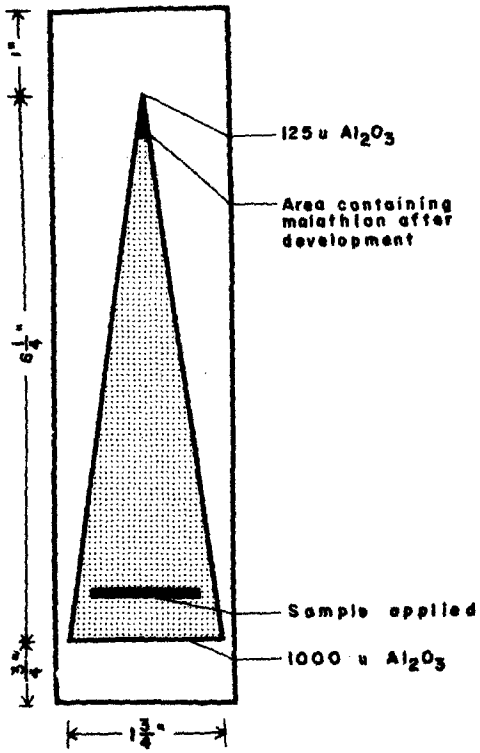

Fig. 1. Modified wedge-layer for chromatographic cleanup of fish extracts containing malathion.

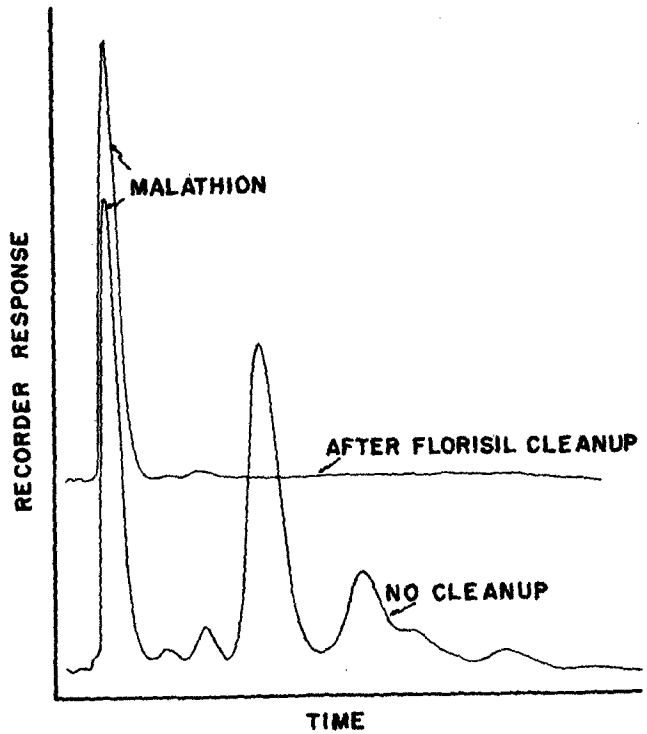

Fig. 2. Typical gas chromatogram of extract from carp flesh fortified at 0.5 ppm malathion (R_t = 7.2 min.)

identified by its R_f values on TL plates (4) and IR spectrum.

Results and Discussion

Table 1 shows recoveries of malathion from fortified water samples. The procedure is much simpler than the use of activated carbon (3). It also eliminates the use of large numbers of separating funnels and requires smaller volumes of solvent for extraction. No cleanup of these water samples was necessary but the cleanup techniques described for fish could be used if required.

Table 2 shows recoveries of malathion from fortified tissues of five species of fish. Figure 2 shows the efficiency of the florisil column in removing interfering peaks from malathion extract of carp flesh. No major interfering peaks were produced before the malathion peak. Extracts from frozen tissues contained less co-extractives and were less colored than those from fresh materials. The retention time of malathion under the conditions described in this study was 7.2 min. and that of lindane used as internal standard was 3.1 min.

The use of modified wedge-layer chromatography for cleanup was very satisfactory and it is believed that this technique may have wider application in pesticide residue analysis. It combines the capacity and resolution of wedge-layer chromatography (1) with the concentrating

TABLE 1

GLC determination of malathion in waters fortified at different levels

Water source *	% Recovery			
	1 ppb	5 ppb	10 ppb	50 ppb
Distilled	96.0	97.5	96.5	98.8
	101.0	100.3	100.0	97.3
	100.1	98.0	99.6	102.9
Av.	99.0	98.6	98.7	99.6
Log Cabin, New Brunswick, N. J.	94.7	102.1	95.3	103.1
	96.1	98.0	91.0	98.0
	92.9	99.3	93.1	94.2
Av.	94.5	99.8	93.1	98.4
Johnson Park, Highland Park, N. J.	86.0	95.7	93.6	103.1
	105.3	98.0	100.0	92.1
	93.1	100.2	96.0	97.9
Av.	94.8	97.9	96.5	97.7
Shiners' Well, Ohio	83.9	101.2	91.1	92.1
	93.0	88.9	96.0	101.3
	87.1	87.3	89.5	89.1
Av.	88.0	92.4	92.2	94.1

* pH = 6.6 - 6.9

TABLE 2

GLC analysis of malathion in fish organs and parts fortified at 10 ppb.

	% Recovery					
Fish	Skin	Flesh	Gills	Liver	Brain	Blood
Carp, *Cyprinus carpio*	98.3	99.8	98.0	79.1	87.0	93.8
	95.5	100.1	96.1	81.3	85.9	90.0
	91.8	95.7	93.9	84.7	84.6	94.1
Av.	95.2	98.5	96.0	81.7	85.8	92.6
White sucker, *Catostomus commersoni*	97.7	99.3	97.9	83.1	83.6	89.1
	93.9	97.1	92.1	-	-	-
Av.	95.8	98.2	95.0	83.1	83.6	89.1
Yellow perch, *Perca flavescens*	83.1	99.7	94.0	85.8	85.1	87.7
	89.0	94.9	91.3	-	-	-
Av.	86.0	97.3	92.6	85.8	85.1	87.7
Chain pickerel, *Esox niger*	90.2	98.8	93.6	-	81.8	83.3
	95.5	93.3	97.1	-	-	-
Av.	92.8	96.0	95.3	-	81.8	83.3
Golden shiner,* *Notemigonus crysoleucas*		91.0				
		90.6				
		88.0				
Av.		89.8				

*The whole fish was used.

effect of the triangle-shaped coating. The pesticide residue is thus cleaned up and concentrated in the vertex of the triangle. By varying the size of the plate and the dimensions of the triangles a number of samples may be cleaned up on the same plate.

Unwashed glass wool and various plastic films produce interfering peaks in the electron capture gas chromatograph and therefore, teflon liners should be used for caps of vials used for storing malathion extracts or solutions.

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