Determination of Dursban Insecticide in Water, Mud, Vegetation, Fish, Ducks, Insects, and Crustacea

Investigations to determine the effect of Dursban insecticide (Dow Chemical Co.) on wildlife when used as a mosquito larvacide required information on residues of the insecticide in both wildlife and the habitat. Analytical procedures were developed for the substrates of interest and used to make more than 1000 analyses. Extraction and cleanup procedures varied between substrates, but all analyses were made with a gas chromatograph equipped with either thermionic or a flame photometric detector. Both detectors are quite specific for phosphorus and the choice was a matter of personal preference and/or availability.

Dursban insecticide (Dow Chemical Co.) [phosphorothioic acid, *O*-*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) ester] is an effective mosquito larvacide currently under investigation to determine its effects on aquatic wildlife resulting from use in aquatic habitats. It was necessary, for analyzing the various substrates for residues of the insecticide, to modify published procedures, or develop new ones, for extraction and cleanup of samples prior to analysis by gas chromatography. Procedures for grass, published by Winterlin, Moilanen, and Burgoyne (1968) and by Bowman and Beroza (1967), could not be used because of strongly interfering peaks of unknown origin. The first-named authors also reported methods for water, mud, fish, and invertebrates that, with the exception of the one for water, were not usable in our laboratories. The gas chromatographic procedures reported by the previously mentioned authors should be satisfactory, although we preferred a 3% Carbowax 20M column with Gas-Chrom Q, 60/80 mesh because it gave excellent separation of Dursban from other organophosphorus pesticides that might be used for mosquito control, and from minor peaks resulting from unknown compounds in the injected samples.

EXPERIMENTAL

Water. A 2-liter sample of water, slightly acidified with 2 ml. of concentrated sulfuric acid, was placed in a 1-gallon glass jug with 500 ml. of hexane. The jug was then placed horizontally on a reciprocating shaking machine and agitated vigorously as possible without forming emulsions for 20 minutes. The phases were separated in a separatory funnel and the hexane extract was stored over a few grams of anhydrous sodium sulfate under refrigeration. No cleanup was required for water samples. Benzene may be used for extraction [Winterlin, Moilanen, and Burgoyne (1968)] but hexane is less prone to form emulsions and less toxic to personnel.

Mud. The samples of mud were frozen awaiting analysis, then thawed and extracted with 2 ml. of acetone per gram of mud by end-over-end tumbling at 58 r.p.m. for 1 hour in glass jars. After settling, the supernatant suspension was filtered through Sharkskin filter paper and the turbid filtrate was stored under refrigeration.

Prior to analysis the whole sample was measured for final calculation and the acetone largely removed in a Kuderna-Danish apparatus. The aqueous residue (about 20 ml.) was then transferred to a separatory funnel and extracted three times with 20-ml. portions of hexane, the three extracts were combined, filtered through about 3 cm. of anhydrous sodium sulfate in a 35-mm. filter tube, and concentrated to the desired volume in a Kuderna-Danish apparatus followed by a gentle current of air at room temperature. No further cleanup of the mud extracts was required.

Vegetation. The method for extraction and cleanup of aquatic grass samples was that used by Rice and Dishburger (1968) for oysters, with minor modifications. It includes blending with acetone, transfer to hexane, partitioning into acetonitrile, then back into hexane, and column chromatography using a silicic acid column eluted with 20% methylene chloride in hexane. Efforts to devise a shorter procedure for these samples were fruitless.

Fish. A 50-g. sample was macerated with 100 ml. of acetonitrile, the macerate filtered with suction, and container and filter cake were washed three times with 25-ml. portions of acetonitrile. The combined acetonitrile extracts were concentrated in a rotary vacuum evaporatory to an aqueous residue of about 25 ml. to which was added an equal volume of acetone. Coagulation solution (1.25 g. of ammonium chloride and 2.5 ml. of phosphoric acid in 1 l. of water) was then added, using a volume about equal to that of the acetone–water solution, and mixed by swirling. This mixture was placed in the refrigerator for at least 30 minutes, filtered with suction through a 5-mm. bed of Hyflo-Supercel, and the flask and filter were rinsed twice with 20-ml. portions of cold coagulation solution. The combined filtrates were extracted three times with 70-ml. portions of chloroform, the combined chloroform extracts were taken to dryness in a rotary vacuum evaporator, and the residue was dissolved in acetone. For very small fish samples (1–10 g.) the volumes were reduced and a 50-ml. Omni-mixer container was used.

Duck. Brain, fat, heart, kidney, liver, and muscle tissues were analyzed individually. Each sample of tissue was macerated with 100 ml. of acetonitrile in an Omni-mixer. The macerate was filtered with light vacuum, filter and cake were washed with three 10-ml. portions of acetonitrile, and the filtrate and washings concentrated to an aqueous residue in a rotary vacuum evaporator. This residue was diluted with an equal volume of water, transferred to a separatory funnel, and extracted three times with portions of hexane approximately equal in volume to that of the aqueous solution. The combined hexane extracts were concentrated to about 5 ml. in a rotary vacuum evaporator.

A 10-mm. chromatographic column was packed with 7.5 cm. of activated Florisil, 60/100 mesh (for fat samples, 10 cm. of Florisil was used). After wetting the column with hexane, the sample was introduced and the flask rinsed twice with 5-ml. portions of hexane which were also added to the column. As soon as all the solution had entered the column, the insecticide was eluted with 50 ml. of acetone. The eluate containing the insecticide was taken to dryness in a rotary vacuum

evaporator and the residue was dissolved in hexane. For fat, both the hexane and the acetone eluates were collected in one receiver.

Insects and Crustacea. A 0.3 to 6-g. sample was macerated with 20 ml. of acetonitrile (for the extraction of crustacea an additional 5 ml. of water was added) in a 50-ml. Omni-mixer container. The macerate was then treated as described for duck tissues other than fat.

DISCUSSION

The recoveries of Dursban from control samples of the various substrates fortified by adding the insecticide in acetone solution are shown in Table I. The data resulting from the analysis of samples from treated habitat are being published by Hurlburt *et al.* (1969).

Under the operating conditions employed for gas chromatography, 0.5 ng. was the minimum amount that could be reliably measured. In a 10-g. sample, the minimum detectable level was 0.01 p.p.m., where 5 μl. was injected from a final volume of 1 ml. Two detectors, the flame photometric and the thermionic (CsBr pellet), were used and found to be equally satisfactory. The thermionic unit was used for the routine analyses because of its better stability and ease of operation; hexane and acetone were used as the final solvent for injection, and they performed equally well with both detectors.

The oxygen analog of Dursban, if present, probably could be detected by the procedure, but no attempt was made to do so.

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Table I. Recovery of Dursban from Various Substrates

Amount Added, p.p.m.	Amount Recovered, %					
	Water	Grass	Mud	Fish	Insects	Crustacea
0	No correction necessary for untreated controls					
0.1	92	80	110	75	100	85
0.5	...	90	...	75	93	90
1.0	...	89	...	85	98	103
5.0	...	...	...	...	103	98
10.0	...	...	...	92	...	...
	Duck Tissues					
	Liver	Heart	Brain	Kidney	Muscle	Fat
0	No correction necessary for untreated controls					
0.1	90	90	90	80	105	100
0.5	...	...	...	...	...	100
1.0	80	105	...	...	93	...

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