Rapid Microdetection of Organochlorine Pesticides in Submilligram Fish Tissue Samples[1,2]

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The picogram sensitivity to organochlorine pesticides which the electron capture detector offers the analyst has generally been used inefficiently. Samples to be analyzed usually range from a gram to several kg and after extraction and clean-up, the pesticide residues are taken up in several mls of solvent from which only a few microliters are taken for gas-chromatographic determination. However, if the accumulation of pesticides is to be studied on the cellular level, then the high efficiency of the electron capture detector should be exploited maximally.

Levins and Ikeda (1) described the advantages of loading volatile samples in a section of open capillary tubing which was then placed in the column inlet and the septum replaced. The main disadvantage of this procedure for pesticide residue analysis is that the sample is slowly swept from the tubing,

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resulting in unacceptably broad and unresolved peaks, even with temperature programming. Freund (2) noted the desirability of having an exchangeable injection port cartridge to retain nonvolatile material from syringe injected biological samples. However, after each use the inlet must be disassembled and the cartridge removed for cleaning to prevent the buildup of interfering substances. Nye, Coler, and Wall (3) measured the pesticide residue in a single marine oligochaete by evaporating 5 ml of extractant to 0.01 ml in a stream of nitrogen in a serological pipet with one end flame sealed. They reported only slight loss of pesticide by vaporization. After concentration the sample was transferred to a syringe for injection into a gas chromatograph.

This report describes a method of determining sub-ppm concentrations of organochlorine pesticides in submilligram amounts of fish tissue. This method incorporates the advantages of internal loading, exchangeable cartridges, and micro concentration, but has none of the drawbacks mentioned above. It is both rapid and accurate and requires only slight modification of commercially available instrumentation.

Principle

A small amount (0.1-500 mg) of fish tissue is hydrolyzed in alcoholic KOH and then extracted with hexane in a vial sealed with silicone rubber. The extract is then taken directly into a capillary where it may be concentrated if desired. The

charged capillary is sealed and placed in a solid sampler for introduction into the gas chromatograph. The sealed capillary is broken and its contents flushed onto the column where the separation of the pesticide occurs. Interfering residues remain on the wall of the capillary, which is removed and discarded.

Experimental Detail

Sample Preparation. The tissue sample is placed in a serum vial (W. H. Curtin & Co., Inc., Houston, Texas, No. 2507), sealed, and frozen until saponification. The serum bottle stopper used (Curtin No. 2512) fits firmly over the outside of the vial lip. A 9 mm silicone rubber septum of the type used for gas chromatographic inlets (Analabs, Inc., College,Park, Pa., No. JU18) is inserted into the hollow center of the stopper. The septum must be free of volatile organic contaminants. The serum stopper holds the septum firmly against the lip of the vial so that the sample is exposed only to glass and silicone rubber.

A fresh saponification solution is prepared by dissolving 15 g of KOH in 9 ml of water to which 51 ml of ethanol are added with stirring.

At least 10 volumes of this reagent are added to the vial by syringe needle through the silicone rubber septum, thus avoiding opening the vial. Heating for one hour at 40°C in a water bath generally completes the saponification. The

vial is cooled to room temperature and an equal volume of hexane is added by syringe after withdrawing an equal volume of air.

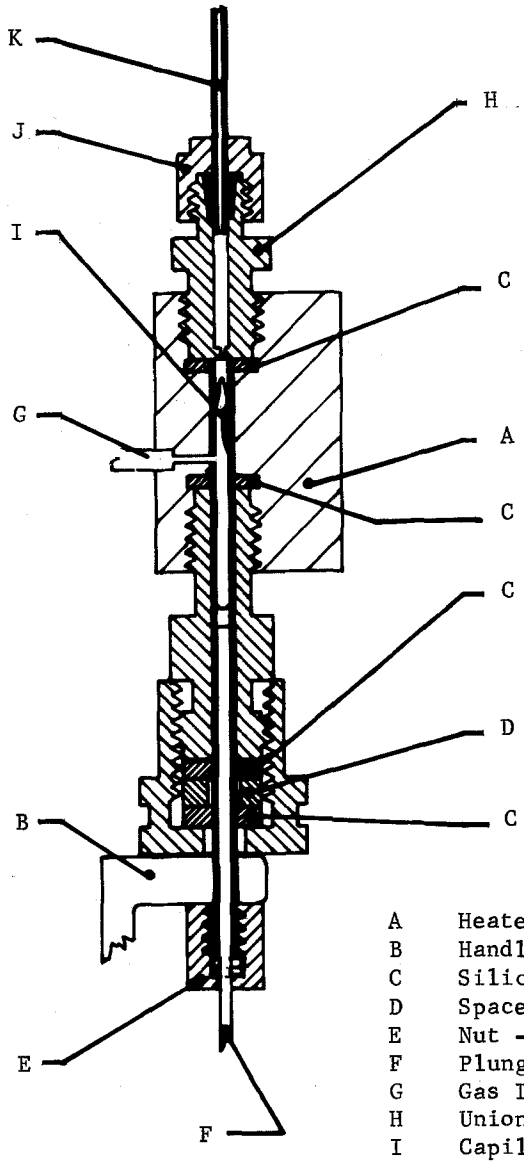
Extraction is aided by shaking, or for very small amounts of reagent, by placing the vial in water in an ultrasonic cleaner. Phase separation occurs rapidly and the alkaline soap layer is removed by syringe with a fine needle. At this point the procedure may be interrupted at least several hours.

At the appropriate time, the stopper is removed and the hexane transferred to micro-hematocrit tubes (Curtin No. 3-532) one end of which is sealed, and the open end drawn to a fine point. To transfer the hexane extract, the sealed end of the capillary is gently flamed and the open end immediately immersed in the hexane. As the capillary cools it aspirates the hexane from the extraction vessel. A gentle tap of the sealed end of the capillary on the bench top transfers the hexane to the base of the sealed capillary. The volume of hexane extract taken may be varied at will between one and 20 microliters and replicate samples may be taken if desired. The hexane rapidly evaporates when the capillary is placed on its side and the fine tip removed, leaving a residue of pesticide. The capillary containing the pesticide residue and residual hexane is partially evacuated and sealed in a microflame. Subsequent breakage of the capillary in the solid sampler is facilitated if the seal incorporates a small bend in the glass.

Sample Analysis. A solid sampler (Hewlet-Packard, Inc., Avondale, Pa., No. SI-4) was adapted to the flash vaporization inlet of a Beckman GC-5 gas chromatograph equipped with an electron capture detector. The solid sampler comprises a hollow stainless steel tube in which the sealed capillary is inserted. A stainless steel rod crushes the capillary against a tungsten carbide block. Vapors pass through a slot in the heated tube into the injection chamber whence they are swept onto the column. Silicone rubber O-ring seals on the rod and tube prevent gas leakage while allowing movement of the plunger.

The solid sampler was modified as follows (Figure 1). The tungsten carbide block was removed and an O-ring seal placed around the proximal end of the tube. The distal end of the tube was sealed by abutting the tube end against the injection-port side of the Swagelok column union fitting. When the union is tightened, the O-ring is sealed, which forces the carrier gas to flow into and through the tube directly to the column. Thus the vapors escaping from a broken capillary are swept immediately and completely onto the column. Since the injection-port side of the union is flat-faced, with only a 0.25 mm opening for gas passage, the capillary is easily broken against it. Although standard for the inlet used, a similar Swagelok fitting could be fabricated for any gas chromatograph.

After a capillary is inserted, the column pressure generally returns to an equilibrium value within two minutes. The



A	Heated Injection Port
B	Handle of Solid Sampler
C	Silicon O Ring
D	Spacer
E	Nut - Seal Retainer
F	Plunger
G	Gas Inlet
H	Union
I	Capillary
J	Swagelok Fitting
K	Column

FIGURE 1.

MODIFIED SOLID SAMPLER

capillary seal is then broken and the sample placed on the column as in the on-column injection procedure. When the analysis is completed the broken capillary is removed by rapidly withdrawing the stainless steel plunger. The elevated pressure existing at the column head expels the capillary fragments. Any remaining glass fragments in the tube can be quickly removed by rotating the tube 180° causing the gas entrance slot to open downward into the surrounding injection port chamber. Pushing the plunger in will shove any fragments remaining in the tube into the chamber. The fragments which accumulate do not affect performance and may be removed at the operator's convenience. The entire removal-cleaning-loading cycle is accomplished in 15 sec.

Results

Samples of gill, brain, blood, liver, gall bladder, intestine, and muscle of the sailfin molly, Poecilia latipinna and of the tarpon Megalops atlanticus, have been routinely analyzed. Several common pesticide columns, SE-30, DEGS, AN600, SE-30 + QF-1, and QF-1 have given equally good performance. Recovery of added pesticide from tissues is virtually 100%, with precision increasing from ±20% for 0.1 mg samples to ±5% for 25 mg or greater samples. Recovery was erratic, and often only 10% until the solid sampler was modified as described. As little as 0.01 ppm of kelthane, heptachlor epoxide, chlordane, DDD, DDE, DDT, and dieldrin in 1 mg of tissue have been measured.

Because of the picogram sensitivity of the electron capture detector, the chromatograms obtained indicate that the limit of the method would be about 0.01 ppm in 0.1 mg of tissue. However, this limit has not been achieved in practice because of inaccuracy introduced in measuring and handling the extremely small amounts of material involved.

Discussion

The use of saponification makes this method applicable to most organochlorine pesticides (4,5). It is unnecessary to remove the soaps when analyzing even the most fatty samples (6). Direct tissue injection with the solid sampler, or direct extraction as reported for human tissue (7) introduced many interfering compounds that irreversibly contaminated the column.

It has been noted (8) that the most time-consuming portion of pesticide analysis is the gas chromatographic determination. This is particularly true in time-dependent pesticide studies where large numbers of animals are sacrificed at the termination of an exposure period. Since the samples can be stored indefinitely in the sealed capillaries in our procedure, scheduling of the gas chromatograph can be made independent of sample load and preparation.

The chief advantage of this method is its ability to measure the entire pesticide burden of a discrete, sub-milligram tissue sample, rather than an aliquot from an homogenate of a heterogeneous larger sample.

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