PESTICIDE ANALYSES BY GAS CHROMATOGRAPHY AT THE LAKE MICHIGAN BASIN OFFICE*

by

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ABSTRACT.

Records of organic pesticide usage by farmers show that, in 1966, 37 percent were herbicides, 29 percent insecticides, 4 percent fungicides, and 8 percent miscellaneous pesticides. More than 150 million pounds of pesticides are purchased by urban dwellers; 44 to 109 pounds of DDT per acre was used to control Dutch Elm disease in Wisconsin urban communities. This high urban usage of pesticides contributes a large pollution load to lakes and streams. The evolutionary growth of gas chromatography is meeting the Federal Water Pollution Control Administration (FWPCA) laboratories' need to measure low level and changing pesticide characteristics.

The FWPCA's Lake Michigan Basin Office (LMBO) has the responsibility for conducting pesticide surveillance programs in the Great Lakes and the Central Mississippi River Basins waters. Assistance and consultation is also provided to federal, state, and local agencies for pesticide analysis by gas chromatography.

This paper elaborates on analytical methods employed for chlorinated and thiophosphated pesticides, types of samples analyzed, typical concentrations, and the significance of findings.

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INTRODUCTION

The Lake Michigan Basin Office (LMBO) is responsible for conducting pesticide surveillance programs in the Great Lakes and the Central Mississippi River Basins waters. Assistance and consultation is also provided to federal, state, and local agencies for pesticide analysis by gas chromatography. Pesticide pollution of these regions presents a potential hazard for aquatic organisms, fish, birds, wildlife, and man. The chlorinated pesticides are the most persistent, while the thiophosphates are more highly toxic.

Records of organic pesticide usage by farmers show that, in 1966, 37 percent were herbicides, 29 percent insecticides, 4 percent fungicides, and 8 percent miscellaneous pesticides. More than 150 million pounds of pesticides were purchased by urban dwellers; 44 to 109 pounds of DDT per acre was used to control Dutch Elm disease in Wisconsin urban communities. This high urban usage of pesticides contributes a large pollution load to lakes and streams. Total U.S. production of pest control chemicals in 1967 was approximately 1.25 billion pounds having a market value of about 800 million dollars. The total U.S. consumption was over one billion pounds of active pesticide chemicals (1). Of all the insecticides used today, 75% is applied to less than 2% of the land, and of the 457 million acres of farmland in the United States, it is estimated that only 15% of total crop acreage receive pesticides (2).

Thousands of pounds of chlorinated, thiophosphated, and other pesticides run off into lakes and streams yearly. The application of pesticides has been so extensive that DDT is found in antarctic penguins and arctic life forms, such as lichens. Chlorinated pesticides are so persistent that toxaphene was found ten years after application in Wisconsin lakes (3).

The concentrations of pesticides are determined by several factors such as kind of pesticide, sorption ability and climate. As a general statement, the concentration of pesticides in waters is based upon the amount directly received, that portion from runoff, and the stability of molecules towards physicochemical effects. For example, aldrin decomposes to form its epoxide, dieldrin; DDT generally decomposes to its isomers, pp'-DDT, op-DDT, DDE, etc.; and heptachlor is converted to heptachlor epoxide.

The initial higher concentrations of chlorinated and thiophosphated pesticides after spraying, dusting or runoff have the most detrimental effect on fish and wildlife. Dosages necessary to produce immediate kills vary from species to species but generally 0.1 mg/l will seriously effect or kill most game fish and benthic fauna. Numerous fish and fowl kills have been reported in and around Lake Michigan (4).

This paper presents some highlights on how pesticides are routinely analyzed at the Lake Michigan Basin Office by gas chromatography with electron capture and microcoulometric titration detection, thin layer chromatography, and positive identification by infrared spectroscopy (IR).

FWPCA's Lake Michigan Basin Office routinely analyzes for the following chlorinated pesticides: lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, op-DDT and pp' DDT. Analyses have been performed according to the U.S. Public Health Service revised methods (5).

The Lake Michigan Enforcement Conference Pesticides Committee recommends the following compounds be determined in the water, fish and clams of Lake Michigan: DDT, dieldrin, DDD, DDE, methoxychlor, chlordane, and endrin (6).

SAMPLING PROCEDURE

The type of samples analyzed at the Lake Michigan Basin Office are: water, bottom sediments, algae or aquatic plants, soils, and fish.

The water samples are generally grab samples collected in a modified Kemmerer Sampler. The bottom samples are taken with an Ekman or Petersen dredge or by core sampling. Algae or small aquatic plants are collected through the use of a plankton net. Soil samples are taken from top soil in areas having been treated with pesticides.

The containers are 4 to 10 liter glass bottles and quart jars cleaned with dichromate cleaning solution and rinsed with distilled water, alcohol, acetone, ether, then chloroform. The caps are lined with aluminum foil (3).

Carbon adsorption filters may be used as a means of collecting or concentrating pesticides from water. This method involves the filtering of 300 to 5,000 gallons of water through a cartridge (3 x 18 inches in size) packed with granular carbon at the rate of 0.03-0.5 gallons per minute. The cartridge is filled with 4.5 inches of 4 x 10 mesh carbon, followed by nine inches of 30 mesh carbon and finally 4.5 inches of 4 x 10 mesh carbon.

A rapidly analyzed grab sample of water, bottom sediments, algae, aquatic plants and fish may be more reliable since it permits less degradation of pesticides. Grab sampling also offers a savings in manpower and equipment.

Composite sampling of the above may give a more representative picture of the pesticide content over a period of 2 or 3 days. However, some pesticides, such as thiophosphated and carbamates, may be degraded before the analysis can be performed.

All types of samples, except water, may be preserved for a few days or months by freezing depending upon type of pesticide. However, when collecting water samples in the winter months, it is necessary to add sodium chloride or ethyl alcohol to the water sample to prevent freezing and breakage of the sample containers while in the field. Water samples are stored at 5°C until analyzed.

ANALYTICAL PROCEDURES

Sample and Reagent Preparation

All water samples analyzed for pesticides by the Lake Michigan Basin Office are subjected to liquid-liquid extraction, using redistilled chloroform as the solvent. One gallon of sample is placed in a 4-liter separatory funnel and 25 ml of saturated sodium sulfate solution and 5 ml of 1:1 hydrochloric acid are added for each liter of sample. The sample is extracted three times using 100 ml of redistilled chloroform for each extraction. The extract is dried by pouring over a 2-inch column of anhydrous sodium sulfate. The extract is cleaned-up by passing through a column of Florisil topped with 1-inch of anhydrous sodium sulfate. The extract is then evaporated to a volume of 0.5 ml. Further clean-up may be accomplished by thin-layer chromatography separation or trapping of specific peaks by gas chromatography.

Bottom sediments are spread in aluminum lined pans approximately 12 x 24 x 2 inches, covered with gauze, then air dried by forcing air currents across the surface of the sediment until dry enough to grind to approximately 30 mesh. This requires from one to four days. Pesticides sorbed on particulate matter may be desorbed by a 24 hour continuous Soxhlet extraction of a 25-200 gram sample with chloroform. The bottom sediments extracts are cleaned-up in the same manner described in the paragraph above.

Algae, aquatic plants and soil samples are dried, ground, extracted and the extracts cleaned-up in the same manner as bottom sediment samples.

Fish and other aquatic organisms are ground in a blender and an aliquot removed for analysis. The aliquot is dried with anhydrous sodium sulfate, extracted with chloroform and the extract cleaned-up in the same manner as described above.

Carbon filter samples are dried at 40°C, then extracted with redistilled chloroform in a Soxhlet apparatus for 35 hours. The chloroform extract is then concentrated and cleaned-up in preparation for further analyses (7).

All solvents are redistilled or commercially available redistilled solvents are employed. Reliability of solvent purity is checked by running a blank.

Analysis of Samples

Water analyses are generally performed according to procedures in the Federal Water Pollution Control Administration's Interim Official Methods for Chlorinated Hydrocarbon Pesticides in Water and Wastewater by Gas Chromatography, as recommended by the Pesticide Committee of the Lake Michigan Enforcement Conference (6), (8).

Bottom sediment samples are analyzed in accordance with the procedures described in the FWPCA Great Lakes Region's Chemistry Laboratory Manual - Bottom Sediments (9).

The analyses of fish samples are performed according to procedures described by the Food and Drug Administration (10).

Samples of water, bottom sediments, algae, aquatic plants and fish are analyzed by thin layer chromatography and gas chromatography with electron capture and microcoulometric titration. The Lake Michigan Basin Office utilizes gas chromatographs with an electron capture Nickel-63 detector, flame ionization and microcoulometric halogen and sulfur titration cells. The operating parameters of the gas chromatographs and microcoulometric titration have been as follows:

1. Electron Capture
   a. Volume injected: 0.5-4.0 µl
   b. Inlet temperature: 225°C
   c. Column temperature: 175°C
   d. Detector temperature: 195°C
   e. Attenuator setting: 32×10^2
2. Microcoulometric Titration
   a. Volume injected: 5.0 to 50.0 µl
   b. Column temperature: 195°C
   c. Inlet temperature: 250°C
   d. Furnace temperature: 850°C
   e. Sensitivity setting: 500 ohms

The following columns are used in gas chromatography with electron capture detector.

Material	Length	OD	Support	Coating*
1) Aluminum	5 ft.	1/4"	100/120 Mesh Gas Chrom Q	7% OV-17
				9% QF-1
2) Aluminum	5 ft.	1/4"	80/100 Mesh Gas Chrom Q	10% DC-200
				15% QF-1

For microcoulometric detection, the gas chromatography column used is as follows:

Stainless Steel	5 ft	1/4"	70-80 Mesh D-Dusted Gas Pack	5% DC-200

*OV-17 = Methyl Phenyl Silicone
QF-1 = Fluorosilicone
DC-200 = Dow Corning Silicone Oil 200 (12,500 centistokes)

Thin layer chromatography involves spotting microliter portions of an extract from a pesticide sample on a 200 x 200 mm glass plate coated with a 0.25 mm silica gel or alumina layer impregnated with silver nitrate. This procedure is used to semi-quantitate, separate, or clean-up a sample. The prepared plate is developed in a closed developing tank containing a 1 cm depth of carbon tetrachloride. The plate is permitted to develop to a 10 cm finish line. The plate is removed and placed under strong ultraviolet (U.V.) light to develop the chlorinated pesticides which, if present, react with the silver nitrate to precipitate silver as black spots. If TLC is employed for clean-up, plain silica gel or alumina plates are developed with rhodamine B solution and the spots are identified by the R_f value (ratio of spot travel to the travel of the solvent front). Samples are semi-quantitated by comparing visually with standards.

Instrumentation

Infrared spectrophotometry is used for identification when pesticides are present in relatively high concentrations.

Gas chromatography results may be coupled with mass spectroscopy (MS), IR, UV, Nuclear Magnetic Resonance (NMR) or Differential Thermal Analysis(DTA). The resulting data can be fed into a computer for extended studies and data processing. This multiple instrumentation is available commercially for use when analysis or studies require further confirmation.

DISCUSSION

Clean-up of samples is a major problem in pesticide analysis. Water samples relatively free of oil may be cleaned-up by passing through a 4-6 inch Florisil-1-inch-anhydrous sodium sulfate column after extraction. If the sample is oily, acetonitrile partitioning is used to separate the pesticides from the oil. Bottom sediment, algae, aquatic plants, and fish extracts are cleaned-up in the same manner as oily water samples. If further clean-up is necessary, the extracts are passed through a Florisil-anhydrous sodium sulfate column. After concentrating the cleaned-up samples, containing a drop of 0.001% paraffin oil (a pesticide holding agent), the samples are ready for analysis by gas chromatography, thin layer chromatography or infrared spectroscopy (3).

Gas chromatography with electron capture is used as a rapid quantitative method for screening pesticides at the Lake Michigan Basin Office. By employing shorter columns (approximately 2-5 feet), the retention time has been shortened; however, if better resolution is desired, longer columns are employed. Two or more columns of different polarities are used to change the retention time of the standard and unknown, thereby obtaining a means for tentative identification (11).

There are numerous problems associated with the gas chromatograph. The electron capture detector may give improper response due to low standing current. This condition may be corrected by cleaning the detector, unless this effect is due to a slow bleeding column; this latter condition may be corrected by replacing the old column with one that has an immobile phase with a higher boiling point. Carrier gas filter, poor voltage, defective detector, and gas leaks in the system cause low standing voltage.

Poor resolution can be corrected by employing the proper column substrate, temperature, replacing defective columns, correcting gas flow and improving or correcting injection techniques.

Thin layer chromatography is a useful tool that LMBO employs to isolate, clean-up, and semi-quantitatively or qualitatively analyze for pesticides. This is one of the most rapid methods of analysis and clean-up procedures. However, the sensitivity of this procedure is much less than by gas chromatography and less quantitative.

Since TLC is approximately 1,000 times less sensitive than gas chromatography (GC), TLC is useful only when pesticide concentrations are present in microgram quantities. Visual estimation of concentrations makes this method only semi-quantitative. However, what seems to be a problem becomes an asset if the purified spots are employed in GC or IR for confirmatory or positive identification.

Infrared spectroscopic identification is employed to give positive identification of questionable pesticides. This method relies upon obtaining purified samples so that there are a minimum number of overlapping absorption peaks in the fingerprint. Approximately 50 to 100 µg of sample are required for infrared identification. A purified sample is obtained from a column-chromatography fraction, thin layer chromatography spot or gas chromatography trapping. The large sample requirement prevents the maximum use of ordinary infrared spectroscopy, since many water samples have less than 50 µg of material after preparation and purification.

Infrared spectroscopy is one of the most powerful scientific tools for giving positive identification, but presents the problem of obtaining 10,000 times the amount of residue required for gas chromatography, even when employing a beam condenser and scale expansion. For good fingerprints the sample extract must be thoroughly cleaned-up and separated into relatively pure compounds prior to analysis.

Microcoulometric titration after gas chromatography is a specific method for determining halogenated or thiophosphated pesticides. The sensitivity of the method is approximately 1/3 to 1/20 of that obtained in gas chromatography with electron capture.

Microcoulometric gas chromatography combines the principles of gas chromatography, combustion, and coulometry into one operation. The problem of separation of extract into pure fractions is handled through the use of an appropriately packed column of the correct length and gas flow to give good resolution. The problem of incomplete combustion is minimized by adjusting furnace temperature, oxygen or hydrogen and nitrogen flow rate. Also, resistance is adjusted so that one nanogram of sulfur or chloride produces at least 5 percent of a full recorder scale deflection during microcoulometric titration.

Maintaining a uniform amount of electrically generated silver ion in the coulometer cell is insured by maintaining the correct amount of silver plate on the electrodes, flushing electrodes with electrolyte, and quickly cleaning with dilute nitric acid and rinsing with distilled water. When sulfur dioxide is being titrated with the triiodide ion, the platinum electrodes may become coated, reducing the sensitivity. This difficulty can be corrected by cleaning the electrodes with dilute hydrochloric acid followed by a rinsing with distilled water, and/or changing the electrolyte.

SUMMARY

The Lake Michigan Basin Office has the responsibility for conducting pesticide surveillance programs in the Great Lakes and the Central Mississippi River Basins waters.

The detection of pesticides at low levels in water is made possible through the use of gas chromatography and other sophisticated analytical instrumentation.

Grab sampling and analysis of water, bottom sediment, algae, aquatic plants, soils, and fish are used for the rapid assessment of pesticide levels in streams and lakes.

Composite sampling over a period of 2 or 3 days, however, is more representative of pesticide loadings.

After sample preparation, extraction, clean-up and concentration, samples are analyzed by the following methods:

Gas chromatography with a Ni-63 electron capture detector and micro-coulometric titration is employed with special columns to separate and analyze pesticide samples.

Thin layer chromatography is a practical way to semi-quantitatively screen pesticide samples; this method may also be employed to clean-up or purify samples for infrared spectroscopy and gas chromatography.

Infrared spectroscopy is employed as a more absolute method for identifying unknown pesticides.

CONCLUSIONS

1. Pesticide concentrations in lakes and streams may be kept under surveillance through the use of gas chromatography with electron capture and microcoulometric detection, with infrared and thin layer chromatography as associated or back-up methods.
2. The analysis of water, bottom sediments, algae, aquatic plants, soils, and fish gives information required to adequately assess pesticide levels of lakes and streams.

Disclaimer:

Mention of products and manufacturers is for identification only and does not imply endorsement by the Federal Water Pollution Control Administration or the U. S. Dept. of the Interior.

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