A Micro Derivatization Technique for the Confirmation of Trace Quantities of Kepone

Robert F. Moseman,* M. Keith Ward,[1] Howard L. Crist, and Robert D. Zehr

A rapid and simple procedure has been devised for the confirmation of nanogram quantities of Kepone that is sensitive to part per billion levels in environmental and biological samples. Electron-capture gas chromatography of the perchlorinated derivative enabled confirmation often not possible by other techniques such as gas chromatography combined with mass spectrometry. Conversion of Kepone to mirex was accomplished by a high-temperature closed-tube reaction. Mirex that might have been present in the original sample extract was separated from Kepone by a micro Florisil column cleanup step. The absence of mirex in cleaned-up sample extracts was verified during the electron-capture gas chromatographic quantitation for Kepone. The conversion of Kepone to mirex was quantitative, allowing for the estimation of Kepone by a separate technique. Thus, considerable confidence is added to analytical results. Details of the methodology and results obtained are discussed.

Confirmation of suspected pesticide residues in biological and environmental samples should be an integral part of an overall analytical scheme. The use of multiple gas chromatographic columns, element selective detectors, thin-layer chromatography, and sequential elution of sample extracts from clean-up columns add substantial confidence to residue identification. Unequivocal confirmation can be obtained by using combined gas chromatography–mass spectrometry, infrared spectroscopy, and/or nuclear magnetic resonance spectroscopy, providing that the instrumentation is available and sufficient compound is present in the cleaned-up extract.

When sample size and/or residue level is limited, chemical derivatization and subsequent determination of the reaction product(s) with the same instrumentation used for conventional pesticide residue analyses offers an attractive means of confirmation of part per billion levels of certain compounds (Shafik et al., 1971; Chau, 1972; Greenhalgh and Kovacicova, 1975; Crist et al., 1975).

In this paper we describe a rapid and simple technique for the confirmation of part per billion quantities of Kepone in biological samples. Kepone is also known as chlordecone. Since most readers are probably more familiar with the tradename Kepone, it will be used throughout this article. Following extraction, cleanup, and quantitative determination of Kepone by electron-capture gas chromatography, the sample extract was subjected to a perchlorination reaction which converted Kepone to mirex (Gilbert and Giolito, 1952). The reaction mixture was then cleaned up on a micro Florisil column. The resulting mirex was determined using the same gas chromatographic columns and conditions as were used for Kepone. Using this procedure, Kepone residues were confirmed in blood, shellfish, and fin fish extracts. This technique can undoubtedly be applied to confirmation of Kepone in many other types of environmental and biological samples.

EXPERIMENTAL SECTION

Apparatus. The gas chromatographic system used was a Tracor MT-222 equipped with a linearized ^{63}Ni electron-capture detector. The instrument was fitted with 1.8 m × 4 mm i.d. glass columns packed with the following materials: 1.5% OV-17/1.95% OV-210, or 4% SE-30/6% OV-210 coated on 80/100 mesh Gas-Chrom Q and operated at 200 to 210 °C with an argon/5% methane carrier gas flow rate of 60–80 mL/min.

A Kontes tube heater was used for maintaining the derivatization reaction temperature at 140–145 °C. Solvents were evaporated with the aid of an N-Evap (Organomation Associates).

Reagents and Solvents. Phosphorus pentachloride, aluminum chloride, and granular sodium sulfate were anhydrous reagent grade; Florisil, PR grade. Hexane, benzene, methanol, acetonitrile, and carbon tetrachloride were of pesticide quality or equivalent.

Glassware. Chromaflex columns, Kontes, size 22-7 were used for the micro Florisil cleanup. The derivatization reaction was carried out in 16 mm × 77 mm culture tubes fitted with Teflon-lined screw caps. Eluates from micro Florisil columns were collected in graduated centrifuge tubes.

Micro Florisil Column Preparation. A small loose plug of glass wool was placed in the tip of a size 22-7 Chromaflex column. Florisil (1.6 g) was added to the column in increments, followed by gentle tapping. The column was then topped with 1.6 g of granular sodium sulfate, washed with 30 mL of 1:1 benzene–methanol, allowed to air-dry, and activated in an oven at 130 °C for at least 16 h prior to use (Thompson, 1974).

Florisil Cleanup–Isolation of Kepone. A prepared column was removed from the oven and allowed to cool before beginning sample elution. The column was rinsed with 7 mL of solvent I (hexane containing 2% methanol and 4% benzene). When the solvent level was lowered to the top of the Na_2SO_4, an aliquot of sample extract not exceeding 2 g equiv in 0.2–0.3 mL was placed on the column. Quantitative transfer of the extract was accomplished with three 0.5-mL rinsings with solvent I. A first fraction of 7 mL total of the above solvent was collected and discarded. PCBs and most of the common organochlorine pesticides, particularly mirex, were eluted in this fraction. Kepone was collected by eluting with 30 mL of solvent II (hexane containing 1% methanol, 2% acetonitrile, and 4% benzene). The eluate was adjusted to an appropriate volume for GC injection. If concentration was required, methanol was added to the sample in an amount necessary to bring its concentration to 1% (Moseman et al., 1977).

Analytical Chemistry Branch, Environmental Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

[1]Present address: Virginia Institute of Marine Science, Gloucester Point, Va. 23062.

Derivatization. The cleaned-up extract (fraction II), or a portion thereof, was transferred to a 16 mm × 77 mm screw-cap culture tube. The solvent was evaporated just to dryness using an N-Evap with a gentle stream of nitrogen. Approximately 200 mg of phosphorus pentachloride, 50 mg of aluminum chloride, and 3.0 mL of carbon tetrachloride were added to the tube. The tube was closed with a Teflon-lined screw cap and placed in a 145 °C heating block for 3 h. The tube was removed from the heating block, allowed to cool to room temperature, and opened. Three milliliters of distilled water was added, and the tube was closed and shaken for 2 min. After phase separation, an aliquot of the lower layer (CCl_4) was transferred to a clean centrifuge tube and the solvent evaporated just to dryness. The residue was transferred to a micro Florisil column (preparation described previously) with three 0.5-mL portions of hexane. An additional 8.5 mL of hexane was used to elute the column. The collected eluate was then concentrated or diluted such that an injection into the chromatographic system produced a peak for mirex within the linear range of the detector. The same column and detector parameters used for Kepone determination were used for the confirmation. Reagent blanks and Kepone standards in approximately the same amount as expected in sample extracts were derivatized and analyzed along with the samples.

RESULTS AND DISCUSSION

During the initial stages of this work, several chlorinating agents were tested in attempts to quantitatively convert Kepone to mirex. The first encouraging results were obtained with phosphorus pentachloride. Derivatization was nearly complete most of the time but occasionally very low conversion efficiencies were noted. The presence of approximately 50 mg of aluminum chloride in the reaction mixture improved reproducibility.

The mode of action of the aluminum chloride in this system is not known. It may serve to complex with the carbonyl group (Georgoulis et al., 1968; Tronov et al., 1968), thereby facilitating chlorination by the dimer (Payne, 1961) of phosphorus pentachloride in a manner analogous to that proposed (Newman and Wood, 1959) for the mechanism of chlorination of ketones. Alternatively, the aluminum chloride may complex with the phosphorus pentachloride (Fialkov and Bur'yanov, 1953, 1955; Carlson, 1963; Petro and Shore, 1964), enhancing the chlorination of Kepone in this system (see, however, Newman et al., 1963).

A series of experiments was conducted with 200-ng quantities of Kepone in order to establish the optimum time and temperature required for maximum conversion to mirex. For a reaction time of 1 h, 19% of the original Kepone remained. After 2 h, 9% remained while 3, 4, and 5 h reaction times showed no detectable Kepone. Mirex subjected to the derivatization reaction showed no apparent decomposition.

Amounts of Kepone ranging from 10 to 1000 ng were derivatized in quadruplicate and produced a linear response for the resulting mirex (Figure 1). The overall average percent conversion of Kepone to mirex was 104% with a relative standard deviation of 8.2%.

The use of a micro Florisil column cleanup following the derivatization reaction removed a considerable amount of the early eluting components from the gas chromatograms. No significant interfering peaks from reagents or sample matrices were noted at the retention time of mirex on any of the gas chromatographic columns used. This clean-up step was found to be necessary when the total amount of Kepone present was less than 25 ng. In instances where more than 200 ng of Kepone was present, the derivatized

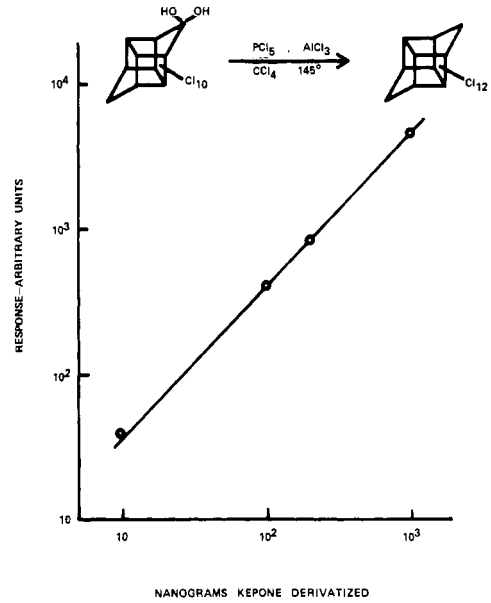


Figure 1. Standard curve for derivatization of Kepone to mirex.

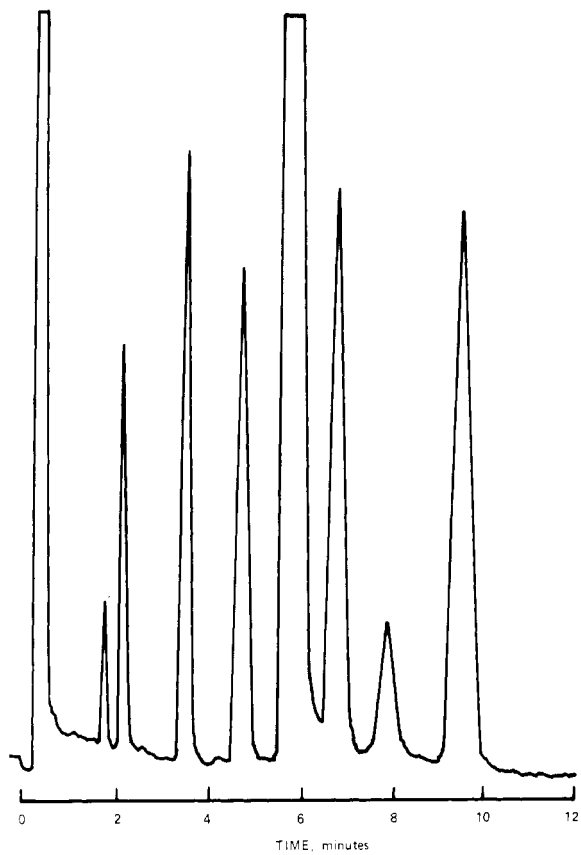


Figure 2. Electron-capture gas chromatogram of pesticide standards. Column: 4% SE-30/6% OV-210, 200 °C, argon/5% methane flow rate, 60 mL/min. Order of elution: HCB, β-HCH, aldrin, heptachlor epoxide, *p,p'*-DDE, dieldrin, *p,p'*-DDD, *p,p'*-DDT.

extract could be analyzed without Florisil cleanup. Special care was taken to evaporate all of the carbon tetrachloride prior to taking the residue up in hexane for electron-capture gas chromatography.

Since the resulting mirex is a pesticide often encountered in environmental and biological samples, close attention was required to insure that any mirex that may have been present in the original sample was separated from Kepone. It was demonstrated that the micro Florisil column

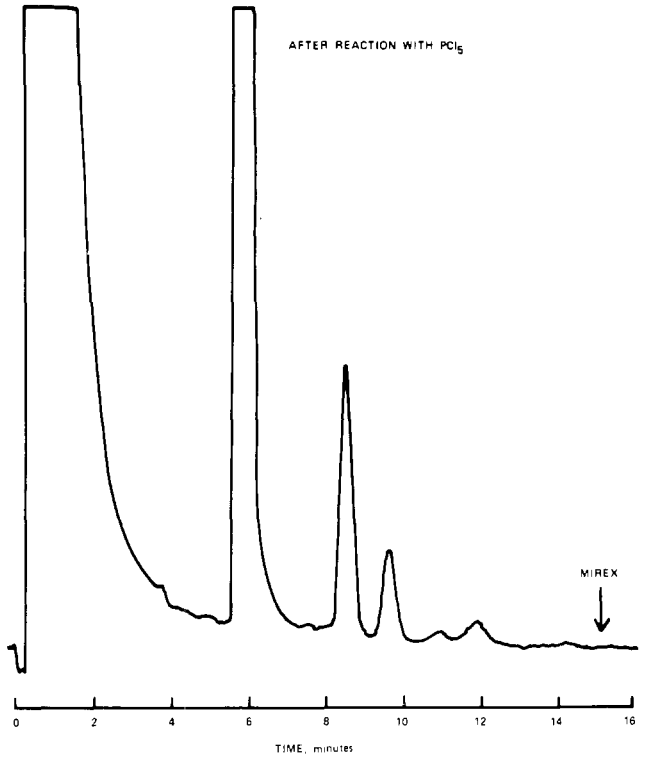


Figure 3. Electron-capture gas chromatogram of pesticide standards after perchlorination. Column and parameters same as in Figure 2. Order of elution: *p,p'*-DDE, derivative of dieldrin, *p,p'*-DDT.

clean-up procedure used for Kepone analysis eluted mirex in the first (discarded) fraction. The absence of mirex in the second (collected) fraction was verified by allowing sufficient time for the elution of mirex in each gas chromatographic run used for the quantitative determination of Kepone.

Figure 2 shows a chromatogram of several common chlorinated hydrocarbon pesticides. These pesticides were then subjected to the derivatization reaction. Figure 3 shows that no interfering gas chromatographic peaks resulting from products of these pesticides were observed at the retention time of mirex. The peak which appears just before *p,p'*-DDT resulted from dieldrin. Although the structure of this compound is unknown at present, this procedure may have a potential application to the confirmation of dieldrin as well as Kepone.

Figure 4 illustrates representative gas chromatograms of an oyster extract before and after derivatization. The Kepone residue in this particular sample averaged 0.07 ppm (upper chromatogram). A portion representing 500 mg of original sample was subjected to perchlorination and yielded the lower chromatogram.

Figure 5 demonstrates the sensitivity of the procedure. Replicate analyses of this blood sample indicated a Kepone level of 11 ppb. The initial sample size was 2 g and only two-thirds of the total was carried through the Florisil column cleanup after derivatization. A 5-μL aliquot was injected from a final volume of 1 mL to yield an easily discernible peak for mirex. In order to confirm the presence of Kepone is this particular sample using gas chromatography combined with chemical ionization mass spectrometry, where the detectable limit for Kepone in standard solutions was determined to be 5 to 10 ng, approximately one-fourth to one-half of the total sample extract would have been required for injection into the system. The few attempts that were made resulted in considerable instrument down time because of the presence

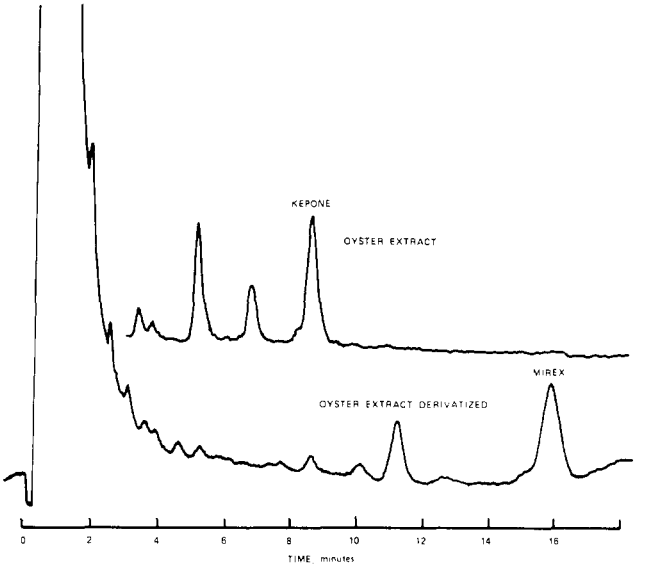


Figure 4. Electron-capture gas chromatograms of an oyster extract before and after derivatization, 0.66 mg injected. Column and parameters same as in Figure 2.

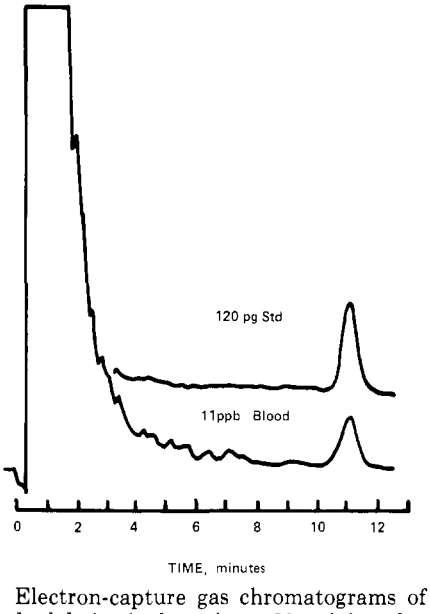


Figure 5. Electron-capture gas chromatograms of 50 ng of Kepone standard derivatized to mirex, 120 pg injected, and a blood extract after derivatization, 6.7 mg injected. Column: 1.5% OV-17/1.95% OV-210, 200 °C, argon/5% methane flow rate, 60 mL/min.

of large amounts of coextracted interfering materials.

In conclusion, the procedure described for the confirmation of trace quantities of Kepone offers a rapid and simple alternative approach for verification of analytical results. Determination of the derivative was accomplished with the same electron-capture gas chromatographic system and parameters used for routine Kepone analyses. This technique should be especially useful in laboratories where expensive and sophisticated instrumentation is not available. Although not tested in this work, the procedure would probably be applicable to the mono- and dihydrokepone photodecomposition products (Wilson and Zehr, 1977; Alley et al., 1974), which may be found in environmental and biological samples along with Kepone (Borsetti and Roach, 1977; Harless et al., 1977).

LITERATURE CITED

Alley, E. G., Layton, B. R., Minyard, J. P., Jr., *J. Agric. Food Chem.* **22**, 442 (1974).

Borsetti, A. P., Roach, A. G., presented at the 91st AOAC Meeting, Washington, D.C., Oct 1977.
Carlson, G. L., *Spectrochim. Acta* **19**, 1291 (1963).
Chau, A. S. Y., *J. Assoc. Off. Anal. Chem.* **55**, 519 (1972).
Crist, H. L., Moseman, R. F., Noneman, J. W., *Bull. Environ. Contam. Toxicol.* **14**, 273 (1975).
Fialkov, Y. A., Bur'yanov, Y. B., *Dokl. Akad. Nauk SSSR* **92**, 585 (1953); *Chem. Abstr.* **48**, 5708c (1954).
Fialkov, Y. A., Bur'yanov, Y. B., *Zh. Obshch. Khim.* **25**, 2391 (1955); *Chem. Abstr.* **50**, 9197h (1956).
Gilbert, E. E., Giolito, S. L., U.S. Patents 2616825 and 2616928 to Allied Chemical & Dye Corp. (Nov 1952); *Chem. Abstr.* **47**, 2424e (1953).
Georgoulis, C., Gross, B., Ziegler, J. C., Prevost, C., *C. R. Hebd. Seances Acad. Sci., Ser. C* **266**, 1465 (1968).
Greenhalgh, R., Kovacicova, J., *J. Agric. Food Chem.* **23**, 325 (1975).
Harless, R. L., Harris, D. E., Sovocool, G. W., Zehr, R. D., Wilson, N. K., Oswald, E. O., *Biomed. Mass Spec.*, **5**, 232 (1978).
Moseman, R. F., Crist. H. L., Edgerton, T. R., Ward, M. K., *Arch. Environ. Contam. Toxicol.* **6**, 221 (1977).
Newman, M. S., Wood, L. L., *J. Am. Chem. Soc.* **81**, 4300 (1959).
Newman, M. S., Fraenkel, G., Kirn, W. N., *J. Org. Chem.* **28**, 1851 (1963).
Payne, D. S., *Q. Rev., Chem. Soc.* **15**, 1973 (1961).
Petro, V. P., Shore, S. G., *J. Chem. Soc.*, 336 (1964).
Shafik, M. T., Bradway, D. E., Enos, H. F., *Bull. Environ. Contam. Toxicol.* **6**, 55 (1971).
Thompson, J. F., Ed., "Analysis of Pesticide Residues in Human and Environmental Samples", U.S. Environmental Protection Agency, Research Triangle Park, N.C., 1974.
Tronov, B. V., Romanchukova, L. A., Tronov, A. B., *Zh. Obshch. Khim.* **38**, 2171 (1968); *Chem. Abstr.* **70**, 52648g (1969).
Wilson, N. K., Zehr, R. D., presented at the 29th Southeast Regional ACS Meeting, Tampa, Fla., Nov 1977.

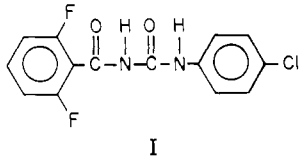
Received for review January 26, 1978. Accepted April 19, 1978. Presented at the 173rd National Meeting of the American Chemical Society, Division of Pesticide Chemistry, New Orleans, La., March 1977. The use of tradenames is for identification purposes only and does not constitute an endorsement by the Environmental Protection Agency.

Analysis of Diflubenzuron Residues in Environmental Samples by High-Pressure Liquid Chromatography

Susan J. DiPrima,* Richard D. Cannizzaro, Jean-Claude Roger, and C. Duane Ferrell

Residue methods for the routine analysis of diflubenzuron in a wide range of samples from both agricultural and nonagricultural ecosystems were developed. The procedures involve Celite liquid–liquid partition, and Florisil–alumina–silica gel colum chromatography, followed by detection using high-pressure liquid chromatography (HPLC), with a μ-Bondapak C-18 (reverse phase) or μ-Porasil (normal phase) column. The methods are reproducible and sensitive to 0.01 ppm in water and 0.05 ppm in soil, sediment, aquatic and forest foliage, fish and shellfish, agricultural crops, milk, eggs, and animal tissues.

Diflubenzuron, (I) TH-6040, *N*-[[4-chlorophenyl)-amino](carbonyl)]-2,6-difluorobenzamide, is an insect growth regulator developed under the trade name of Dimilin. Due to its inhibition of chitin biosynthesis,



I

excellent control of a variety of insects has been observed. Some of the more important insects include various forest insects, soybean and cotton insects, mosquitoes, and citrus pests.

Several residue procedures have been developed previously for the analysis of diflubenzuron. Corley et al. (1974) reported the determination of residues of diflubenzuron in milk by extraction with ethyl acetate, a partition between *n*-hexane and acetonitrile, followed by detection with high-pressure liquid chromatography. DeWilde et al. (1975) described a method in crops, soil, mud, and water by extraction with dichloromethane, cleanup of the extract on a Florisil column, and detection by HPLC. Schaefer and Dupras (1976) reported the stability and persistence of diflubenzuron in water utilizing HPLC methodology. The determination of diflubenzuron by HPLC in manure has been described by Oehler and Holman (1975).

Based on ^{14}C-radiotracer studies, residue methods for water, soil, sediment, fish, shellfish, agricultural crops, aquatic vegetation, forest litter and foliage, cow and poultry tissues, milk, and eggs were developed in order to assess the fate of diflubenzuron in both agricultural and nonagricultural ecosystems. The procedures reported here describe extraction, cleanup, and detection techniques routinely utilized for the determination of diflubenzuron residues at 0.01 ppm in water and 0.05 ppm in all other samples.

EXPERIMENTAL SECTION

Reagents. All reagents used were doubly distilled in glass from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. All aqueous solutions were prepared with water which had been deionized and distilled in glass. Mobile phases used for normal phase HPLC chromatography were isooctane–isopropyl alcohol (93:7, v/v) and dichloromethane–methanol (500:1, v/v). Mobile phases used for reverse phase were acetonitrile–water (60:40, v/v) and methanol–water (75:25, v/v). HPLC mobile phases were degassed by evacuation coupled with ultrasonic vibration.

The Florisil was Floridin's 60–100 mesh, pesticide grade. The silica gel and neutral alumina were Woelm's Activity

Thompson-Hayward Chemical Company, Kansas City, Kansas 66110 (S.J.D., R.D.C., C.D.F.) and Cannon Laboratories, Inc., Reading, Pennsylvania 19603 (J.C.R.).

0021-8561/78/1426-0968$01.00/0