Simplified Method of Determining Chlordanes in the Soil Using Partial Dechlorination

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(Received September 5, 1989)

A gas chromatographic procedure has been developed for determination of chlordanes in environmental samples such as soil. The basis of the method is the conversion of many of these chlorinated C₁₀ hydrocarbons into a single partial dechlorinated product, which is determined by gas chromatography with an electron capture detector. Soil is extracted with hexane. The extract is cleaned up on a Florisil cartridge and dechlorinated with Zn/HCl. The dechlorinated compound is injected into a gas chromatograph and chlordanes determined as one partially dechlorinated compound. The detection limit in this method is 0.002 $\mu\text{g/g}$. The coefficient of variation was 3.2% for soil samples while the average recovery was 94.3%. Although capable of determining only 4,7-methanoindanes, which comprise more than 80% of the chlordanes, the significance of the method is recognized as the first means of screening for chlordanes. To date application has been made only to soil samples, but it is believed that with selection of the proper solvent for extraction, the method can be more broadly applied to samples such as food and biological samples.

Keywords — gas chromatography ; chlordane ; determination ; environment ; soil ; dechlorination

Introduction

Chlordanes had been used as soil pesticides or insecticides for termites and other pests until 1986, when they were prohibited in Japan. However, since these organochlorine insecticides are chemically stable, they have been persistent in the environment and are still detected in various samples of sludge,¹⁾ soil,²⁾ canine and feline fat,³⁾ fish and shellfish,^{4,5)} meat,⁶⁾ blood,⁷⁾ mother's milk⁸⁾ and Black-tailed Gull (*Larus crassirostris*).⁹⁾

Technical chlordane is a mixture of various chlordanes, and consists of more than 50 components. Chlordane is decomposed in the environment, making the composition of the mixture more complex. For practical determination of chlordanes in the environment, five

major types were isolated and determined by GC-ECD and GC-MS, and their combined total is shown as the total chlordane.¹⁰⁾ Determination of the presence and quantity is therefore extremely complicated, and it is difficult to precisely assess the environmental pollution by chlordanes. We report here a method to determine chlordanes by dechlorinating 4,7-methanoindanes, which comprise more than 80% of chlordanes, into one partially dechlorinated compound under mild conditions. This method effectively removes interference from samples and enables precise and simple determination of total chlordane.

Materials and Methods

Chemicals — Heptachlor (HC) and technical chlordane (tec-CLD) were obtained from Gasu-

kuro Kogyo, Inc. (Tokyo, Japan), and *trans*-chlordanes (t-CLD) and *cis*-chlordanes (c-CLD) were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). *Trans*-nonachlor (t-NC), and *cis*-nonachlor (c-NC) were kindly supplied by Velsicol Chemical Co. (Tokyo, Japan). Florisil was obtained from Floridin Co. and activated at 130°C for 5 h. Sep-Pak Florisil Cartridge was obtained from Japan Millipore, Ltd. (Tokyo, Japan).

Gas Chromatography — A Shimadzu GC-4CM equipped with an electron capture detector (^{63}Ni) was used and a 0.3 cm i.d. $\times$ 2 m glass column packed with 2% OV-1 on chromosorb W AW DMCS. Column and injection temperatures were 165°C and 220°C, respectively. Flow rate of carrier gas (nitrogen) was 50 ml/min.

Gas Chromatograph-Mass Spectrometer — A Shimadzu QP-1000 was used, together with a 0.3 cm $\times$ 2 m glass column packed with 2% OV-1 on chromosorb W AW DMCS. Column and injection temperatures were 165°C and 190°C, respectively. Flow rate of carrier gas (helium) was 30 ml/min. The ion source was operated at EI (70 eV), CI (CH_4).

Nuclear Magnetic Resonance — A JEOL GX-400 NMR Spectrometer (400 MHz) was used. Chemical shifts were reported in ppm *vs.* Me_4Si as internal standard.

Infrared Spectrometer — A Hitachi 260-30 infrared spectrometer was used and spectra were determined in KBr pellets.

Dechlorination of Heptachlor — Two hundred mg of heptachlor was dissolved in 10 ml of *n*-hexane, and the resultant solution was added to powdered zinc. After hexane was removed under reduced pressure, the powder was placed in a 2 l separatory funnel with 1 l of 1 N HCl and shaken gently for 10 minutes for dechlorination. The dechlorinated product was extracted three times with 200 ml of *n*-hexane. After washing with water, the hexane layer was dehydrated with anhydrous sodium sulfate and concentrated to 2 ml under reduced pressure. The resultant solution was placed on a Florisil column (1.5 $\times$ 12 cm) eluted with hexane. The first 60 ml eluate was

discarded and the subsequent 60–130 ml fraction of the eluate was concentrated under reduced pressure to obtain 53 mg of white crystals (mp 103–104°C). Yield was 41.7%.

Dechlorination of Chlordanes — To 1 ml each of hexane solution containing 20 ppm of each chlordanes was added 10 g of powdered zinc. Hexane was distilled off at room temperature. The residue was transferred into a 200 ml separatory funnel with 100 ml of 1 N HCl and was shaken gently for 5 min. The dechlorinated product was extracted twice with 50 ml of hexane, washed with water, dehydrated and concentrated to 1 ml under reduced pressure.

Analytical Method — Five g of soil sample was taken in a separatory funnel, 50 ml of hexane was added and it was shaken for 5 minutes for extraction. The hexane layer was taken in another separatory funnel and an additional 50 ml of hexane was added. This was shaken for 5 minutes for extraction and the hexane layer was combined with the previous one. The hexane layer was washed with 50 ml of water and 10 g of anhydrous sodium sulfate was added for dehydration; the layer was then condensed to 2 ml under reduced pressure. The condensed solution was applied on a Sep-Pak Florisil cartridge preliminarily washed with 20 ml of hexane, and was eluted with 30 ml of hexane at a flow rate of 3 ml/min. The eluate was collected and condensed to 1 ml under reduced pressure. The resultant hexane solution was added with 1 g of powdered zinc, and hexane was distilled off at room temperature. Subsequently, 5 ml of 0.5 N HCl warmed to 40°C was added, the solution was stirred occasionally and left standing in a 40°C water bath for 5 minutes for dechlorination. The mixture was immediately cooled in a water bath to room temperature, added with 4 ml of hexane and shaken vigorously for 2 min for extraction, following which the hexane layer was transferred to a separate tube. The residual water layer was added with 4 ml of hexane, shaken for 2 min, and centrifuged at 3000 rpm for 1 minute. The hexane layer was then added to the previous one. After washing this layer with 5 ml of water, it was

dehydrated with anhydrous sodium sulfate and made to a definite volume with hexane. The resultant solution was determined by GC-ECD.

The calibration curve was prepared by treating the known amount (0.01–0.4 μg) of tec-CLD in 2 ml of hexane with powdered zinc and hydrochloric acid, and determination by the same method as described above.

Results and Discussion

1. Dechlorination of Chlordane Isomers

There are many reports dealing with the dechlorination and reduction of chlordanes: Chau *et al.*¹¹⁾ treated heptachlor with chromous chloride in order to identify hepta-

chlor metabolites. Cochrane^{12,13)} treated heptachlor with reducing reagents to establish its structure. Dennis and Cooper¹⁴⁾ reduced chlordane with sodium borohydride and discussed its possible application for waste treatment techniques. Chlordane reduction has thus mainly been done to identify or decompose its compounds. On the other hand, Cochrane and Chau¹⁵⁾ reported that heptachlor can be determined by replacement of the allyl chlorine of heptachlor to a hydroxyl group and then converting it into its corresponding silyl derivative. However, there has been no reported method by which various chlordanes are dechlorinated and determined as a single compound. We studied a means to do this using a relatively mild reduction reagent, *i.e.* Zn/HCl, to obtain the same compound on a gas chromatogram, and thus to determine total chlordane.

Figure 1 shows gas chromatograms of tec-CLD and its dechlorinated product. Dechlorinated products of c-CLD, t-CLD, c-NC, t-NC and HC were revealed to have the same retention time as that of tec-CLD. Mass (EI, CI) spectra of various dechlorinated chlordanes were identical with that of dechlorinated HC. These results suggest that t-CLD, c-CLD, t-NC, c-NC and tec-CLD generate the same product as HC by partial dechlorination.

2. Structure of the Dechlorinated Product

To elucidate the structure of the dechlorinated product (II), NMR and mass spectra

Fig. 1. Gas Chromatogram of tec-CLD, 0.2 ng (a) and Dechlorinated tec-CLD, 0.03 ng (b)

heptachlor (I)

dechlorinated heptachlor (II)

Fig. 2. Chemical Structure of Heptachlor (I) and Dechlorinated Heptachlor (II)

(MS) were determined for II and the precursor HC (I). From the ^1H - and ^{13}C -NMR (including the 2D-NMR) spectra, the structure of II was suggested as depicted in Fig. 2. The results are given in Table I and Figs. 3–7. This assignment of proton spectrum was also supported by H-C COSY spectra (Fig. 6).

Although configuration of the proton of bridge-headed carbon in II remains undetermined, Dennis and Cooper¹⁴⁾ reported that the proton on the methylene bridge of the catalytic dechlorination product of HC was *anti* to the dichloro olefin moiety; therefore, the bridge-headed methylene proton of compound II is also considered to be *anti* to the olefin moiety.

On the other hand, Chau *et al.*¹¹⁾ dechlorinated chlordanes using chromous chloride-ethylenediamine complex, and reported on

Fig. 3. ^1H -NMR Spectrum of Heptachlor (I)

Fig. 4. ^1H -NMR Spectrum of Dechlorinated Heptachlor (II)

^1H -NMR chemical shift data of the dechlorinated products. These data are shown in parentheses in Table I and well agree with the data we obtained. The dechlorinated heptachlor was, therefore, assumed to be the compound shown in Fig. 2.

The mass spectra (EI) of dechlorinated HC are shown in Fig. 7. The molecular ion was 302. It appeared to undergo a retro Diels Alder reaction since the fragments observed were $(\text{C}_5\text{Cl}_5\text{H})^+$: 236 and $(\text{C}_5\text{H}_6)^+$: 66. In the IR spectra (Fig. 8) were observed an intense absorption at 1600 cm^{-1} by $\text{ClC}=\text{CCl}$ and an

Fig. 5. COSY Spectrum of Dechlorinated Heptachlor (II)

Fig. 6. C-H COSY Spectrum of Dechlorinated Heptachlor (II)

Fig. 7. Mass Spectrum of Dechlorinated Heptachlor (II)

Fig. 8. IR Spectrum of Dechlorinated Heptachlor (II)

absorption at 1440 cm^{-1} by CH_2 in the five-membered ring. Based on these measurement data, the dechlorinated product was identified to be 4,5,6,7,8-pentachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene.

3. Discussion of Dechlorination Conditions

The effects of the amount of zinc, reaction time, reaction temperature and concentration of hydrochloric acid on dechlorination of HC by zinc and hydrochloric acid were studied. The yield increased with the amount of zinc up to 0.5 g and thereafter remained constant, demonstrating that 0.5 g was sufficient for the reaction (Fig. 9). As to the reaction time, the yield increased up to 5 min, and tended to decrease with time thereafter (Fig. 10); five minutes was the proper time for the reaction. In respect to temperature, the yield increased with temperature up to 40°C where it reached a plateau (Fig. 11); this result made it clear that 40°C was sufficient for the reac-

TABLE I. Proton NMR Chemical Shifts^{a)} of Heptachlor and Dechlorinated Heptachlor

Compd.	H ₁	H ₂	H ₃	H _{3a}	H _{7a}	H ₈
I	4.82	5.96	5.91	4.09	3.66	—
II	2.46 (2.48) ^{b)}	5.84 (5.83)	5.66 (5.65)	3.67 (3.66)	3.19 (3.18)	4.36 (4.36)

a) pm down field from internal TMS (CDCl_3)

b) Reference No. 11.

Fig. 9. Effect of Amount of Zinc on Dechlorination of Heptachlor

Fig. 10. Effect of Reaction Time on Dechlorination of Heptachlor

tion. A concentration of hydrochloric acid, 0.5–1 N, was regarded as the most appropriate for dechlorination, because the yield of dechlorinated products decreased at over 1 N (Fig.

Fig. 11. Effect of Temperature on Dechlorination of Heptachlor

Fig. 12. Effect of Concentration of Hydrochloric Acid on dechlorination of Heptachlor

12).

After dechlorination, the dechlorinated product was extracted at 100% by shaking for 2 min in 4 ml of *n*-hexane. The calibration curve was linear at 0.01–0.4 μg of tec-CLD.

4. Recovery of Chlordanes from Soil Samples

The recovery test was performed according to the determination procedure using 5 g of a soil sample added with 0.1 μg of tec-CLD.

Fig. 13. Gas Chromatogram Obtained by the Proposed Method for the Soil Around a Termite-Proof House

Average recovery was 94.3% and CV 3.2% ($n=5$). The determination limit was 0.002 $\mu\text{g/g}$.

Figure 13 shows an example of a GC-ECD chromatogram obtained applying the present determination method to a sample taken from the soil around a termite-proof house. The chromatogram obtained from the soil without application of the method has many peaks of chlordanes and matrix extracts, making it very complicated to determine the total amount. But the cleanup procedure on a Sep-Pak Florisil cartridge and reduction with zinc and hydrochloric acid eliminated interfering peaks and enabled determination of CLD as a single component which is shown by an arrow.

Acknowledgement We express our great appreciation for the assistance rendered by Dr. M. Zushi, Meiji Seika Co. in obtaining NMR spectra and for the useful discussion with Dr. T. Miyazaki. Tokyo Metropolitan Institute of Public Health.

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