Isolation of the Chiral Isomers U82, MC5, MC7 and MC8 from Technical Chlordane by HPLC and Determination of ECD and NICI-MS Response Factors

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Abstract

The minor octachloro-isomers U82, MC5 and MC7 in the technical pesticide chlordane were isolated by a combination of reversed phase and normal phase high performance liquid chromatography (HPLC). The isolated compounds were identified and characterized by high resolution gas chromatography (HRGC) on two stationary phases of different polarity using the retention index by Dearth and Hites, a reference cod liver extract and negative ion chemical ionization (NICI) mass spectrometry. Concentrations of the isolated isomers were calculated assuming the same flame ionization detector (FID) response for all octachloro isomers. Response factors relative to cis-chlordane were determined for the electron capture detector and NICI mass-spectrometry.

Introduction

Several studies of the presence of the pesticide chlordane in the food web as well as in human samples have revealed high levels of the octachloro isomers MC5, MC7 and U82[1,2,3] which are minor constituents in the technical product compared to cis- and trans-chlordane. The concentrations of the latter are significantly lower than U82 in biota at the highest trophic level. The structure of MC5 and MC7 was assigned by Miyazaki et.al.[4]. Both are chiral. In general, little is known about the levels of these minor octachloro compounds in the environment as well as about their biochemistry and toxicology. U82 could be separated into enantiomers[5] but its chiral structure is still unknown. The high bioaccumulation of U82 compared to other octachloro isomers in human tissue[2] and in seals[3,6] makes a structure elucidation highly desirable.

Until now U82, MC5 and MC7 could not be determined quantitatively due to the lack of commercially available reference compounds. Semi-quantitative estimations were carried using the average response factor of cis- and trans-chlordane. The aim of this study was to find a method to isolate larger quantities of these octachloro isomers from technical chlordane in order to use them as reference standards and to develop a method which allows to collect a sufficient amount of U82 for structure elucidation. This work describes the isolation by different HPLC techniques and the gas chromatographic characterization of the single isomers including the determination of response factors for different detectors.

Experimental Methods

Standards and solvents: Technical chlordane and crystalline cis-chlordane of 99% purity were obtained from Ehrenstorfer (Germany). The n-hexane was of HPLC-quality (Machler, Switzerland). Acetonitrile of far UV quality from Romil (England) was used. Water-free sodium sulfate of trace analysis quality was obtained from Merck (Germany). Technical chlordane solutions of about 150 ng/µl were made in acetonitrile. Cis-chlordane was dissolved in hexane giving a concentration of

105.2 ng/µl. 100 to1000-fold dilutions were also prepared. The cod liver oil extract was prepared as described in[5].

Isolation by HPLC: All HPLC separations were carried out on a Hewlett-Packard 1050 HPLC-system with a multiple wavelength detector. Detection was performed at 240 nm. A pump flow of 4 ml/min was used for reversed phase separations and of 1 ml/min for normal phase separations. Volumes of 20-50 µl were injected. A C_{18} reversed phase column of 125 mm x 10 mm id was used filled with VP Nucleosil 100-7 and a silica column of 250 mm x 4.6 mm id containing the silica phase EC Nucleosil 100-5. Both columns were from Macherey-Nagel (Switzerland). The following mobile phase gradient was applied for reversed phase separations: acetonitrile/water 60:40 for 15 min, then within 20 minutes to 80:20 and then 15 min flushing with pure acetonitrile. The fraction indicated in Figure 1A was collected. Water was added before it was extracted three times with n-hexane and dried over sodium sulfate.

A first normal phase separation of the collected fraction was carried with n-hexane as mobile phase. To achieve stable retention properties n-hexane was stirred over sodium sulfate for minimum 24 h, and afterwards 1000 ppm acetonitrile was added as a modifier. Four fractions where collected as shown in Figure 1B. The fractions containing U82 (b1) and MC5 (b2) needed a further clean-up and were therefore separated once more on the normal phase column using dried n-hexane modified with 100 ppm acetonitrile. Fractions were collected according to Figure 1C-D.

Instrumentation: NICI mass-spectra were recorded with a HP5989B GC/MS-system. Methane with an ion source pressure of 0.45 torr was applied as chemical ionization gas, and the ion source was kept at 200°C. The transfer line temperature was 250 °C. NICI response factors of the isolated U82, MC5 and MC7 were determined using the (M+2) isotope at m/z 408 of the molecular ion cluster. Concentrations of the new standards were determined on a HP 5890 gas chromatograph equipped with a FID detector presuming the same response as for cis-chlordane which was added in known amounts as reference. Electron capture response factors were measured on a HP 6890 gas chromatograph using a ^{63}Ni electron capture detector (ECD) kept at 250 °C. Splitless injections of 1 µl with a splitless time of 2 min were carried out on all systems.

Gas chromatographic separation: A first identification and purity control as well as determination of concentrations and response factors was carried out on a capillary of 22 m length and 0.2 mm id coated with 0.11µm of Ultra 1 (polymethylsiloxane, Hewlett-Packard). Exactly the same separation conditions were applied as described by Dearth and Hites[7]. To confirm further the identity of U82, MC5, MC7 and MC8 and to carry out enantioselective separations, a tandem column was applied consisting of a 30 m x 0,25 mm id capillary in front coated with 0.1 µm 90% biscyanopropyl/10% phenyl cyanopropyl polysiloxane (RTx-2330, Restek Corp., Bellefonte, PA, USA) which was coupled to an enantioselective capillary of 23 m length and 0.25 mm id coated with 0.14 µm of heptakis(2,3,6-O-tert.-butyl-dimethylsilyl)-β-cyclodextrin (TBDMS-CD) in PS086 (1:10). Further details are given in[6]. The separation conditions were: Splitless injection of 1 µl at 90°C, splitless/isothermal period 2 min, 15°C/min to 180°C, isothermal 44 min, 180-230°C at 2°C/min, isothermal at 230°C for 2 min.

Results and Discussion

Originally the main target was to isolate U82. However, as can been seen below, the applied method allowed simultaneous collection of the also important octachloro isomers MC5 and MC7 as well as MC8.

Isolation by HPLC: To allow the collection of quantities in the high µg range, the column of the first step using reversed phase HPLC was overloaded. A fraction containing U82, MC5, MC7 and MC8 was isolated during a first run which removed the majority of the congeners of no interest including about 90% of cis- and trans-chlordane and most heptachloro isomers. A similar second separation was carried out which eliminated further interferences due to a reduced overload. In this run all compounds eluted within 35 min. In a second step normal phase HPLC on silica using n-hexane with 1000 ppm acetonitrile as mobile phase allowed to pre-separate all target compounds from each other. The compound amount separated during the last step was small enough so that column overload was not longer a problem. This together with a less polar mobile phase allowed a further separation of heptachlor isomers and cis-chlordane from U82. Furthermore, it was possible to remove U82 and some unknown compounds from MC5. Figure 2 shows the HPLC chromatograms of the isolation of some isomers.

Compound identification and purity control: Identification and purity control was carried out by NICI mass spectrometry using a well characterized cod liver oil and the retention index of Dearth and Hites[7]. In all cases the retention times on the polymethylsiloxane phase and the full scan mass spectra were identical with those earlier reported for U82, MC5, MC7 and MC8[5,7]. Identity and purity were further confirmed with the enantioselective tandem capillary. As can be seen from Figure 2, the NICI purity of MC7 and MC8 was more than 95%. U82 contained a few percent trans-chlordane and a dihydroheptachlor isomer. The MC5 fraction contained two unknown compounds with the same retention time as the U82 enantiomers. One co-eluted with the first enantiomer of the other compound. Some of the overload problems can be avoided by using larger columns. this is planned as the next step. All isolated isomers are chiral which could be confirmed by enantioselective separation except for MC7 which could not be separated on the applied stationary phase (see Figure 2). All four compounds gave a NCI fragmentation pattern typical for octachloro isomers with a pentachlorocyclopentene moiety. The molecular ion cluster (m/z 406) was most abundant followed by m/z 298 ($M-3Cl-3H^-$) and m/z 264 ($M-4Cl-2H^-$).

Determination of response factors: Since no reference compounds were commercially available for the isolated isomers, they were quantified in biota by using the average response factor of cis- and trans-chlordane[1,8]. The response factors for isomers with such a closely related structure are similar for the flame ionization detector (FID). This allows to determine the concentrations of the isolated isomers in solution within an uncertainty of about 10% using the response factors for cis- and trans-chlordane. However, for the electron capture detector (ECD) and in NICI-MS, the response of stereo isomers can be very different leading to systematic errors. Therefore, ECD and NICI-MS response factors relative to cis chlordane were determined for U82, MC5, MC7 and MC8. The first step was quantification by FID assuming the same response for all isomers as for cis-chlordane. The ECD response of U82, MC7 and MC8 was very similar to that of cis-chlordane (see Table 2).

Table 1. Response factors relative to cis-chlordane (rrf) of the isolated octachloro congeners for ECD and NICI-MS.

	rrf ECD	rrf NICI-MS
U82	1.06	0.95
trans-chlordane	0.98	1.61
cis-chlordane	= 1	= 1
MC5	0.66	2.08
MC7	1.07	2.61
MC8	0.98	1.99

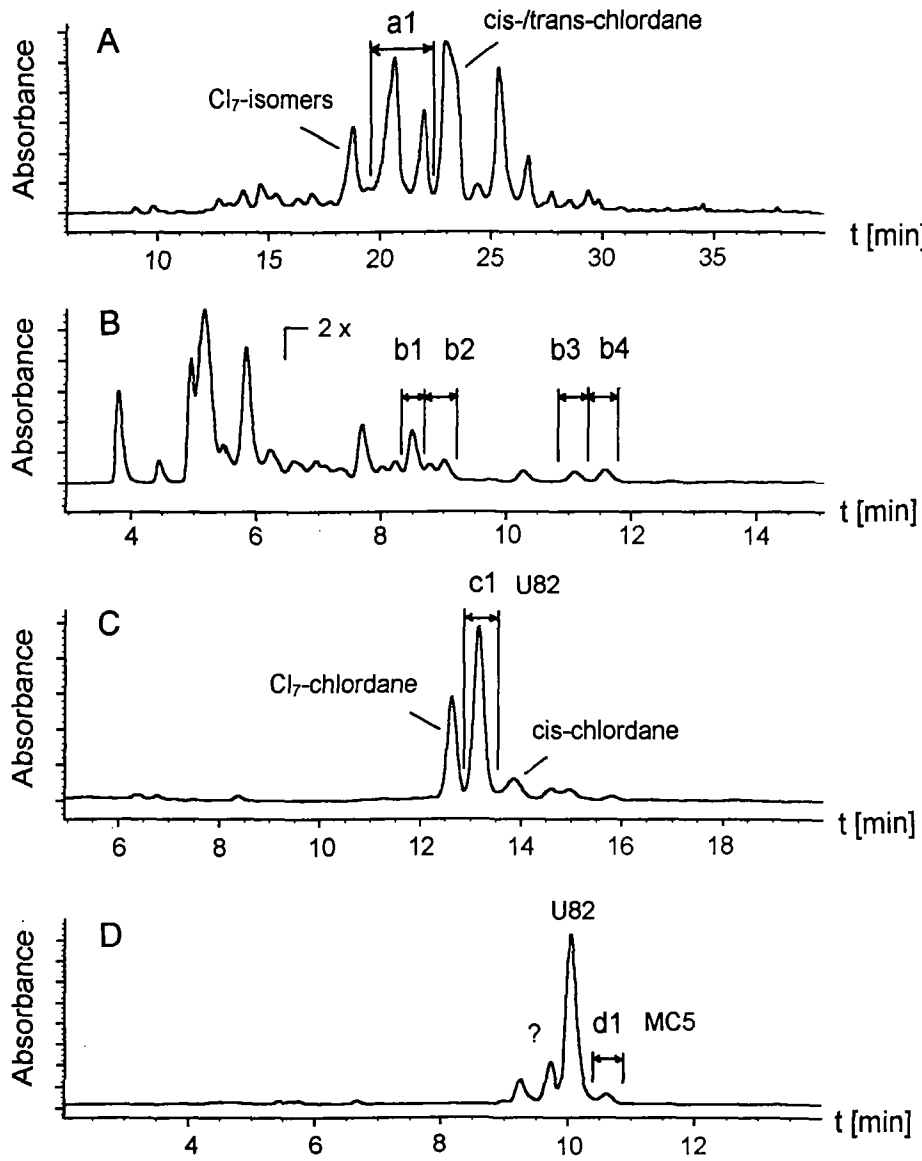

Figure 1: Isolation of U82, MC5, MC7 and MC8 by different HPLC techniques. For details, see experimental methods. A: Reversed phase HPLC with acetonitrile/water 60:40. Fraction a1 was collected containing all target isomers. B: Normal phase HPLC using n-hexane with 1000 ppm acetonitrile. Four fractions were selected, b1(U82), b2 (MC5), b3 (MC7) and b4 (MC8). C: Normal phase HPLC applying n-hexane with 100 ppm acetonitrile. Fraction c1 contained U82. D: Normal phase HPLC using n-hexane with 100 ppm acetonitrile. Fraction d3 was collected for MC5.

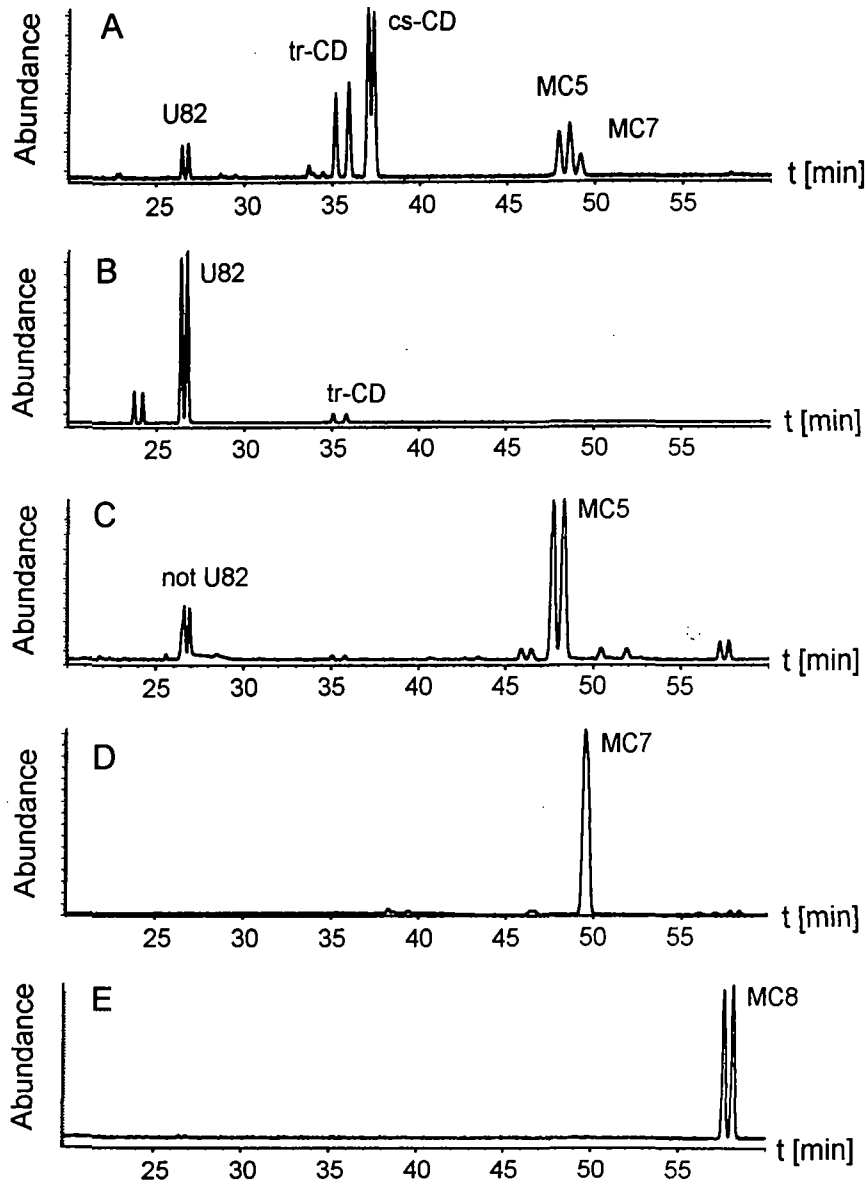

Figure 2: NICI mass chromatograms of m/z 408 (full scan mode except A)of isolated octachloro isomers using the enantioselective tandem column (see experimental methods). A: Cod liver oil extract (selected ion monitoring) for comparison[5]. B: Fraction c1 with U82. C: Fraction dl containing MC5. D: Fraction with MC7. E: Fraction with MC8. tr-CD, cs-CD: cis/trans-chlordane.

As expected, differences of nearly a factor of 3 were observed for the NICI-MS response factors making the use of single reference compounds mandatory for quantification. MC5 had a significantly lower response factor than all other congeners which cannot be explained yet. Further work is in progress to exclude a possible interference.

Conclusions

- Reversed phase HPLC followed by normal phase HPLC is a suitable tool for the isolation of U82, MC5, MC7 and MC8 from technical chlordane.
- A better separation of trans-chlordane from U82 and MC5 is needed before they can be used in a reference standard containing all important octachloro isomers. This should be possible by up-scaling the separation which avoids stationary phase overload.
- The errors introduced by quantifying U82, MC5 and MC7 with the average response factor of cis- and trans-chlordane are substantial for NICI-MS but less than 10% for U82 and MC7 when employing the ECD.
- So far, MC8 has not been found in fish analyzed by our group. In case of absence due to quick metabolisation and/or insignificant bioaccumulation, this isomer could be a suitable internal standard for chlordane quantification.

Acknowledgment

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Literature cited

(1) Dearth, M.A.; Hites, R.A. *Environ. Sci. Technol.* **1991**, *25*, 1279-1285.
(2) Lindström, G.; van Bavel, B.; Broman, K. *Organohalogen Compd.* **1996**, *27*, 253-258.
(3) Wiberg, K.; Oehme, M.; Haglund, P; Karlsson H,; Olsson, M.; Rappe, Ch., *Mar. Pollut. Bull.*, submitted.
(4) Miyazaki, T.; Yamagishi, T.; Matsumoto, M. *Arch. Environ. Contam. Toxicol.* **1985**, *14*, 475-483.
(5) M. Oehme, M.; Kallenborn, R.; Wiberg, K.; Rappe, C. *J. High Resol. Chromatogr.* **1994**, *17*, 583-588.
(6) Oehme, M.; Karlsson, H.; Kallenborn, R. *J. Toxicol. Environ. Chem.* **1997**, in press.
(7) Dearth, M.A.; Hites, R.A. *Environ. Sci. Technol.* **1991**, *25*, 245-254.
(8) Dearth, M.A.; Hites, R.A. *Environ. Sci. Technol.* **1991**, *25*, 1125-1128.