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SELECTED ION MONITORING MASS SPECTROMETRIC METHOD FOR THE DETERMINATION OF HEXACHLOROCYCLOHEXANE ISOMERS IN SOILS

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SUMMARY

The methodology for the determination of isomeric 1,2,3,4,5,6-hexachlorocyclohexanes (HCHs) was investigated by gas chromatography–mass spectrometry–selected ion monitoring (GC–MS–SIM). In order to evaluate the extraction efficiencies, three types of field soils with various organic matter contents were selected, spiked with HCHs and fully deuterated analogues synthesized as internal standards, and ultrasonically extracted. The extracts were subjected to GC–MS–SIM without clean-up and monitored at both *m/z* 219 and 224. The method developed was applied successfully to soil samples from arable fields.

INTRODUCTION

The long-term persistence of organochlorine pesticides in soils is well known[1–7]. For example, Edwards[1] reported that the times required for 95% disappearance of insecticides applied at levels of 1–3 lb./acre as active ingredients were 1–6 years for aldrin, 4–10 years for DDT, 5–25 years for dieldrin, 3–5 years for heptachlor and 3–10 years for the γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane (HCH). Therefore, the soil environment is a major storage site for pesticides, and many pesticides and other organic chemicals applied have accumulated together in soils. These phenomena have led to many difficulties in determining these residues in soils, especially in arable soils.

In order to extract these pesticide residues from soils or sediments, high-speed mixing of the soil with a single solvent or a mixture in a blender[8], low-speed shaking with a reciprocal or a wrist-action shaker[9,10] or solvent extraction with a Soxhlet extractor[11] have commonly been employed. These methods have satisfactory results, but there were various problems. First, a complete explosion-free blender was necessary for the blending extraction and second, it took several hours for extraction with the shaking or the Soxhlet method. In contrast, the ultrasonic method with an ultrasonicator is simple and much faster[12]. When determining organochlorine pesticide residues in soil samples by gas chromatography with electron-capture detection

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(GC–ECD), it has been often observed that peaks derived from coextracted artifacts in arable soil samples overlapped with those from HCH isomers, heptachlor and/or aldrin[13–15]. Also, the peaks of HCH isomers were overlapped by those of aldrin, heptachlor and/or hexachlorobenzene[16]. The elimination of such problems by clean-up using column or thin-layer chromatography was tedious and time consuming. Therefore, a selective, sensitive and simple method for the determination of HCH isomers in soils is required in order to monitor environmental pollution by HCH congeners.

In a previous paper[17], a selective and sensitive analytical method for residual HCH isomers in the aquatic environment using deuterated analogues as internal standards and as the carrier was reported. In this work, gas chromatography–mass spectrometry–selected ion monitoring (GC–MS–SIM) methodology for the determiniation of HCH isomers in arable soils has been developed and applied successfully to soil samples taken from agricultural fields.

EXPERIMENTAL

Materials

Isomers of α-, β-, γ- and δ-HCH (99+%) were obtained commercially and used without further purification. Deuterated analogues of α- and β-HCH were synthesized and purified (99+%) as described previously[17]. These HCH isomers and deuterated HCH analogues were dissolved in pesticide-residue grade acetone to give a stock solution. The stock solution was diluted to appropriate concentrations when necessary. Other reagents used were commercial reagent-grade chemicals or pesticide-residue grade solvents. Chemical-grade acetonitrile was purified by the AOAC method[11]. Double distillation of *n*-hexane was carried out in an all-glass still equipped with a Norton–Otten fraction column. Anhydrous sodium sulphate and HCH-free water were prepared as described previously[17]. All glassware employed was rinsed twice with *n*-hexane before use.

Field soil sampling and pre-treatment

Agricultural soil samples were taken in September and December 1981 in arable fields in the Kitakyushu District. The soil textures varied from loamy to sandy. Soil A (sandy loam, organic matter content, OMC = 5.5%) was taken in a paddy field and soils B (Loam, OMC 10.5%) and C (sandy, OMC 2.1%) in vegetable fields. Pre-treatment was carried out as described previously[18]. For recovery experiments, three types of soils with various OMC values were selected, because the OMC is one of the major factors governing the extractability of organochlorine pesticides from arable soil[19].

Recovery experiments

A soil sample (20 g), which had been dried at ambient atmosphere, screened through a 20-mesh sieve and well mixed, was placed in a 200-ml conical beaker and 1 μg of both deuterated analogues as internal standards and 0, 0.1, 0.5 or 2.5 μg of HCH isomers were added. The concentrations of HCH isomers in the spiked soil samples were therefore 0, 0.005, 0.025 and 0.125 ppm (μg/g soil), and those of deuterated analogues were 0.050 ppm. The concentrations of HCH isomers were in al-

most the same ranges as in the survey data for the Kitakyushu District, which were obtained from 1969 to 1974[20]. The spiked sample was allowed to stand overnight to equilibrate, then 15 ml of 0.2 *M* ammonium chloride solution were added to deactivate organic chemicals at active sites in the soil and the mixture was allowed to stand for a further 15 min to deactivate. A 50-ml volume of acetonitrile was then poured into the beaker and sonication was carried out for 5 min with a Branson-32 ultrasonicator (Branson Cleaning Equipment Co.). The resulting supernatant was decanted into a glass Büchner funnel (150–200 mesh) and filtered. the residual soil cake was extracted three times with 30-ml volumes of acetonitrile. Each time, the supernatant was decanted into the funnel and filtered. The combined extracts and several rinsings were drained into a 1-l separating funnel that contained 400 ml of HCH-free water, 100 ml of *n*-hexane and 20 ml of saturated sodium sulphate solution and the funnel was shaken vertically for 5 min to effect partitioning. The aqueous layer was removed into a second separating funnel and shaken with 100 ml of *n*-hexane. Following the second shaking, both organic layers were combined, then washed twice with 100-ml volumes of HCH-free water and dried by passing through an anhydrous sodium sulphate column (2 × 5 cm). The organic layer was concentrated to less than 1 ml with a Kuderna–Danish evaporating concentrator with a three-ball Snyder column. An appropriate aliquot, normally 7–8 μl, was subjected to GC–MS–SIM analysis after diluting the concentrate to 1 ml.

Field soil experiments

To pre-treated agricultural soil (20 g), 1 μg of both α-HCH-d_6 and β-HCH-d_6 as internal standards were added, the sample was allowed to stand overnight and the extraction was carried out as described above.

GC–MS–SIM analyses

GC–MS–SIM measurements were made with a Nihon Denshi JEOL JGC 20KP-JMS 01SG-2 GC–MS system. The operating conditions were almost the same as described previously[17]. Ions at *m/z* 219 $[M - HCl_2 + 2]^+$ for HCH isomers and at *m/z* 224 $[M - {}^2HCl_2 + 2]^+$ for deuterated analogues were monitored, being recorded at a 500 msec dwell time. Mass spectra of both the HCH isomers and the deuterated analogues were also obtained. Calibration graphs were obtained daily by injecting appropriate standard solutions. The mass spectra of the HCH isomers and the deuterated analogues were measured with the above-mentioned GC–MS system.

RESULTS AND DISCUSSION

Recovery experiments

Mass spectra of the HCH isomers and the deuterated analogues are shown in Figs. 1 and 2, respectively, and typical SIM traces of both standard HCH isomers and deuterated analogues are shown in Fig. 3a and b, respectively. The retention times of the deuterated analogues were almost identical with those of the HCH isomers.

Linear calibration graphs for HCH isomers *versus* deuterated analogues as internal standards are shown in Fig. 4. The graphs for the HCH isomers were linear over the range 0–40 ng for α-HCH and 0–60 ng for the other isomers. The detection

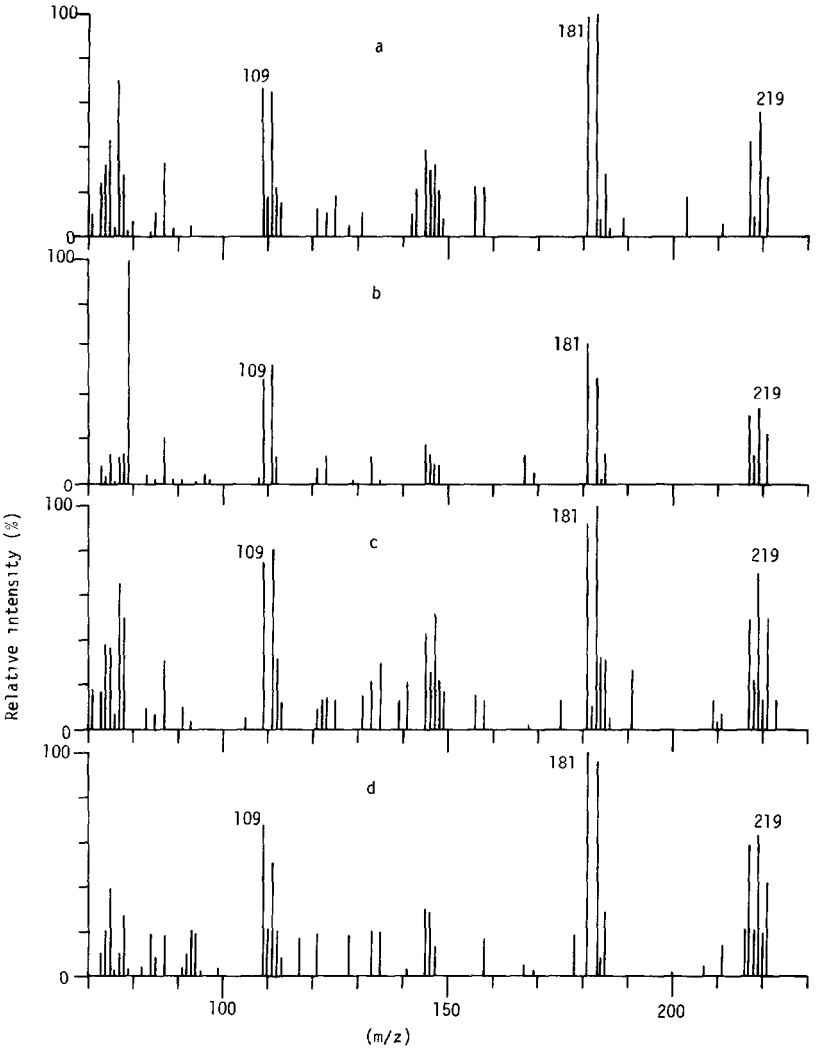

Fig. 1. GC–MS traces of HCH isomers: (a) α-HCH; (b) β-HCH; (c) γ-HCH; (d) δ-HCH. Operating conditions: 2% OV-17 on 60–80-mesh Uniport HP, 2 m × 2 mm I.D. glass column; column temperature, 200°C; injector temperature, 240°C; enricher temperature, 250°C; resolution, 800.

limits of HCH isomers in a 20-g soil sample were 0.00035 ppm for α-HCH and 0.0007 ppm for the other isomers at signal-to-noise ratio (S/N) $\geqslant$ 2, respectively, and it could be suggested that these levels were sensitive enough for evaluating environmental pollution by HCH isomers.

Recoveries of fortified HCH isomers are shown in Table I for soil A, Table II for soil B and Table III for soil C. These recoveries were corrected from the levels for none-spiked samples. With soil A (sandy loam), better recoveries and smaller standard deviations were obtained than those with soils B and C of the three fortification levels, the best recoveries were obtained at 0.125 ppm, because this level was

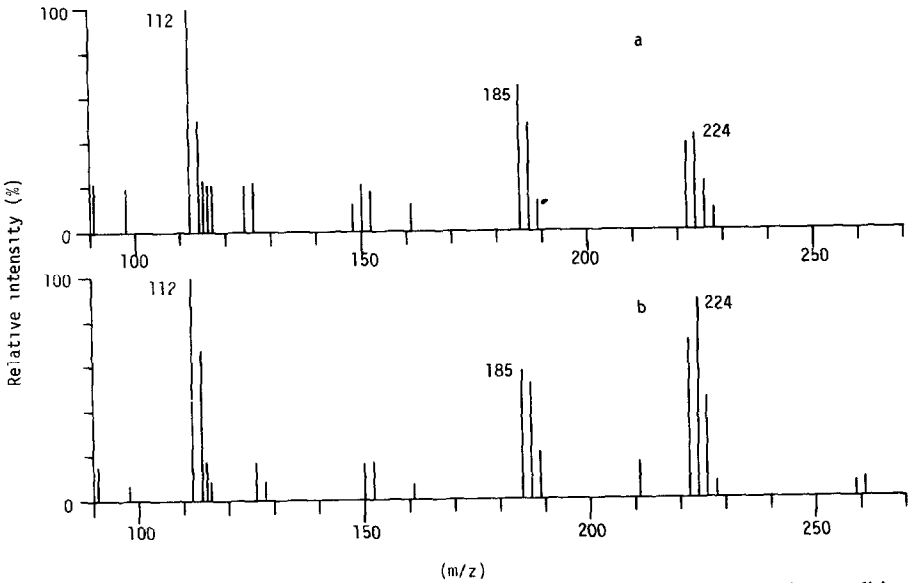

Fig. 2. GC–MS traces of deuterated analogues: (a) α-HCH-d_6; (b) β-HCH-d_6. Operating conditions as in Fig. 1.

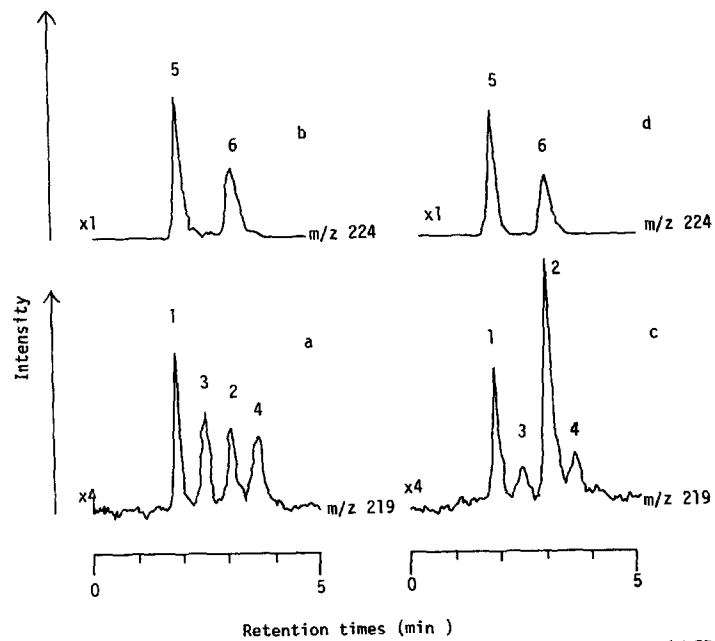

Fig. 3. GC–MS–SIM traces of HCH isomers and deuterated analogues: (a) HCH standards; (b) deuterated analogue standards; (c) HCH isomers from a field soil sample; (d) deuterated analogues spiked in a field soil sample. Peaks: 1 = α-HCH; 2 = β-HCH; 3 = γ-HCH; 4 = δ-HCH; 5 = α-HCH-d_6; 6 = β-HCH-d_6. Operating conditions as in Fig. 1.

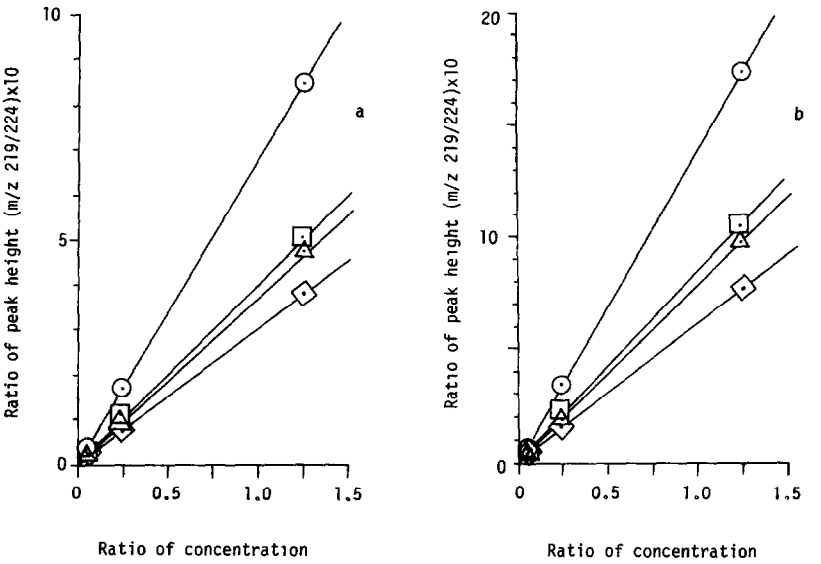

Fig. 4. Calibration graphs for HCH isomers *versus* deuterated analogues: (a) HCH isomers *vs.* α-HCH-d_6; (b) HCH isomers *vs.* β-HCH-d_6. ○, α-HCH; △, β-HCH; □, γ-HCH; ◇, δ-HCH.

TABLE I

RECOVERIES OF HCH ISOMERS SPIKED IN SOIL A WITH AN ORGANIC MATTER CONTENT OF 5.5%

Compound spiked	*Level (ppm)*	*Recovery ± standard deviation (%) (n = 4)*			
		α-HCH	*β-HCH*	*γ-HCH*	*δ-HCH*
α-HCH-d_6	0.005	100 ± 3	74 ± 9	73 ± 9	75 ± 7
	0.025	95 ± 5	80 ± 5	79 ± 4	96 ± 4
	0.125	98 ± 2	86 ± 4	95 ± 3	103 ± 4
β-HCH-d_6	0.005	134 ± 5	89 ± 9	94 ± 10	104 ± 7
	0.025	109 ± 6	90 ± 5	91 ± 5	111 ± 2
	0.125	106 ± 3	92 ± 3	103 ± 4	108 ± 7

the least influenced by the intrinsic HCH levels in the soil samples. These results were different from those obtained previously[17]. However, there was no clear correlation between the recoveries in the three soil samples and the OMC. Therefore, it could be said that the OMC has no effects on the recoveries of HCH isomers from soil samples.

Experiments with α-HCH-d_6 gave better recoveries than with β-HCH-d_6. This is of interest because β-HCH is the most persistent HCH isomer in soils[18], but no explanation can be given. α-HCH-d_6 was chosen as an internal standard, but β-HCH-d_6 was also used as an alternative to prevent unpredictable analytical problems.

TABLE II

RECOVERIES OF HCH ISOMERS SPIKED IN SOIL B WITH AN ORGANIC MATTER CONTENT OF 10.5%

Compound spiked	*Level (ppm)*	*Recovery ± standard deviation (%) (n = 4)*			
		α-HCH	*β-HCH*	*γ-HCH*	*δ-HCH*
α-HCH-d_6	0.005	117 ± 6	114 ± 17	124 ± 10	95 ± 10
	0.025	94 ± 2	75 ± 5	100 ± 3	80 ± 7
	0.125	93 ± 3	86 ± 1	91 ± 3	83 ± 1
β-HCH-d_6	0.005	122 ± 10	102 ± 25	132 ± 10	98 ± 17
	0.025	100 ± 4	74 ± 4	109 ± 4	88 ± 7
	0.125	102 ± 5	85 ± 2	99 ± 3	92 ± 4

TABLE III

RECOVERIES OF HCH ISOMERS SPIKED IN SOIL C WITH AN ORGANIC MATTER CONTENT OF 2.1%

Compound spiked	*Level (ppm)*	*Recovery ± standard deviation (%) (n = 4)*			
		α-HCH	*β-HCH*	*γ-HCH*	*δ-HCH*
α-HCH-d_6	0.005	105 ± 10	120 ± 15	86 ± 11	101 ± 14
	0.025	96 ± 7	95 ± 7	102 ± 6	98 ± 6
	0.125	96 ± 2	92 ± 1	96 ± 2	98 ± 2
β-HCH-d_6	0.005	98 ± 12	123 ± 15	81 ± 14	96 ± 12
	0.025	100 ± 7	104 ± 3	106 ± 4	101 ± 7
	0.125	100 ± 2	99 ± 3	101 ± 5	101 ± 2

Field experiments

Table IV gives the concentrations of HCH isomers in 19 agricultural soil samples as determined by GC–MS–SIM measurements. Typical GC–MS–SIM traces of HCH isomers and deuterated analogues from a soil sample are also shown in Fig. 3c and d, respectively. The peak of β-HCH was the highest. No interfering peaks due to co-extractives from the soil or the insecticides aldrin, heptachlor and/or hexachlorobenzene were found in any of the SIM traces, because these compounds have no fragmentations near *m/z* 219 or 224. Therefore, this method is selective and simple. Also, none of herbicide CNP (2,4,6-trichlorophenyl 4'-nitrophenyl ether) peaks, which were detected in the earlier experiments[17], could be found. This might be due to the reduction of CNP to amino-CNP in soils and adsorption of the amino-CNP to soil colloids and/or organics such as fumic acids or fulvic acids[21]. Levels of overall HCH (ΣHCH) isomers in the present survey ranged from 0.169 to 0 ppm. In the previous survey[20], the ranges and means of the ΣHCH residues were 1.573–0.006 ppm and 0.196 ppm in 1973, and 1.533–0.003 ppm and 0.177 ppm in 1974, respectively. The maximum residue level in the present study was almost one tenth of that

TABLE IV

RESIDUE CONCENTRATIONS OF HCH ISOMERS IN FIELD SOILS DETERMINED BY GC–MS–SIM

Soil No.	*Concentration (ppm)*				
	α-HCH	*β-HCH*	*γ-HCH*	*δ-HCH*	*ΣHCH*
1	0.004	0.013	0.002	0.003	0.022
2	0.007	0.024	0.004	0.005	0.040
3	0.003	0.007	0.001	0.002	0.013
4	0.003	0.029	0.002	Tr*	0.034
5	Tr*	N.d.**	N.d.**	N.d.**	Tr*
6	0.003	0.019	0.001	0.003	0.026
7	0.003	0.025	0.001	0.004	0.033
8	0.002	0.008	0.001	0.001	0.012
9	0.005	0.019	0.002	0.004	0.030
10	0.003	0.030	0.001	0.003	0.037
11	0.002	0.029	0.001	0.002	0.034
12	0.018	0.063	0.007	0.013	0.101
13	0.016	0.123	0.011	0.019	0.169
14	0.001	0.015	Tr*	N.d.**	0.016
15	0.007	0.147	0.004	Tr*	0.158
16	0.004	0.025	0.003	0.003	0.035
17	0.001	0.004	Tr*	N.d.**	0.005
18	0.001	Tr*	Tr*	N.d.**	0 001
19	N.d.**	N.d.**	N.d.**	N.d.**	N.d.**
Mean ± S.D.	0.004 ± 0.004	0.031 ± 0.039	0.002 ± 0.003	0.003 ± 0.005	0.040 ± 0.048

* Tr = Trace (0.001 ppm).
** N.d. = None detected.

in the surveys conducted in 1973 and 1974. Also, the mean obtained was about one fifth to one quarter of those obtained in the previous survey. These results suggest that degradation of HCHs was rapid at high concentrations, but the decomposition of HCHs was slow when its concentration reached at a certain level in the soil. More than 75% of the ΣHCH was attributed to β-HCH, so the β-isomer was the most persistent of among HCH isomers, as shown previously[18].

Insecticidally active γ-HCH seemed to be less persistent than the other HCH isomers. The decomposition of γ-HCH is well known. Yule *et al.*[22] showed that γ-HCH was gradually degraded to γ-pentachlorocyclohexene (γ-PCCH) in soil, and its degradation was accelerated by the soil water content. Further, γ-PCCH was decomposed to 1,2,4-trichlorobenzene, 1,2,3,5-tetrachlorobenzene (TCB), 1,2,4,5-TCB, 1,2,3,4-TCB and tetrachlorocyclohexene[23,24]. However, with the GC–MS–SIM system I was unable to detect such metabolites, unless there were prominent ions around the ions monitored. In combination with other analytical methods such as GC or high-performance liquid chromatography, GC–MS–SIM will provide more useful results for monitoring organic pollutants in the natural environment.

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