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Note

Simultaneous XAD-2 resin extraction and high-resolution electron-capture gas chromatography of chlorine-containing herbicides in water samples

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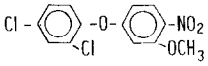
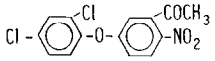
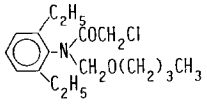
Environmental pollution by organochlorine pesticides is a serious problem with regard to public health. The agricultural use of organochlorine insecticides (OCIs) such as 1,2,3,4,5,6-hexachlorocyclohexane (HCH) and DDT, aldrin, dieldrin and endrin have been banned in Japan since 1971. However, residues have subsequently been found in soil[1] and water samples[2–4]. Also large amounts of chlorine-containing herbicides (CCHs) such as oxadiazon [5-*tert.*-butyl-3- (2,4-dichloro-5-isopropoxyphenyl) -1,3,4-oxadiazolin-2-one], CNP (*p*-nitrophenyl 2,4,6-trichlorophenyl ether), butachlor [2-chloro-2′,6′-diethyl-N-(butoxymethyl) acetanilide], chlormethoxynil (2,4-dichlorophenyl-3-methoxy-4-nitrophenyl ether) and bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate] have recently been used as pre- and post-emergence herbicides in paddy fields in Japan (Fig. 1)[5]. Monitoring of CCHs in river water and



CHLORMETHOXYNIL



BIFENOX



BUTACHLOR

Fig. 1. Structures of chlorine-containing herbicides.

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tap water samples is important for assessing environmental pollution by these CCHs, but as yet little information has been obtained on the simultaneous determination of CCHs.

When determining these CCHs at trace levels, conventional liquid–liquid extraction has required large volumes of samples and solvent. However, the adsorption of a variety of trace organics in water on XAD-2 resin has been one of the best choices for the preparation of extracts[6], because this method is simpler and needs less solvent than the liquid–liquid extraction. In previous studies, an XAD-2 resin column showed excellent retention and desorption for HCH, CNP and oxadiazon[2,7,8]. However, little information has been obtained on the extraction of butachlor, chlormethoxynil and bifenox with this resin. In this paper, the extraction of these herbicides with XAD-2 resin is described.

Gas chromatography with electron-capture detection (GC–ECD) and with a packed column has commonly been employed for the determination of residue levels of OCIs or CCHs. Because low-resolution GC with packed columns could not resolve each component of the CCHs from the co-extractives derived from environmetal samples, a clean-up procedure such as column chromatography has generally been utilized. However, the polarities of CCHs themselves are different, so that it is necessary to elute them from the column with various solvents whose polarities are also different. Therefore, the simultaneous determination of CCHs residues is extremely difficult. Up to now, each component of the OCIs or CCHs has been determined individually[2,7,8]. However, it is most desirable with regard to time and expense for a number of CCHs to be analysed simultaneously, with convenient data evaluation. Three chemically bonded fused-silica capillary columns (CB-FSCs), OV-17, OV-1701 and SE-52, were tested for the separability of 28 OCIs and related compounds, and excellent resolution was achieved, especially with OV-17 CB-FSC[1]. This paper also shows that the simultaneous determination of CCHs is possible using GC–ECD with CB-FSCs, which can resolve CCHs from the co-extractives without any clean-up.

EXPERIMENTAL

Materials

Hexane was redistilled as desribed previously[2]. Diethyl ether and methanol were commercial reagents of pesticide-residue grade and used without further purification. Liquid chromatographic grade isooctane was redistilled. Anhydrous sodium sulphate was heated at 625°C for 2 h to eliminate interferences. Amberlite XAD-2 resin was screened (20–60 mesh) and subsequently cleaned up with methanol, acetonitrile and diethyl ether in a Soxhlet extractor for 8 h for each solvent and immersed in methanol to maintain its activity until use. Pesticide-free water was prepared by distillation of tap water with an all-glass distiller and then passing the distillate through a column of XAD-2 resin (20 × 3 cm I.D.). All glassware employed was rinsed twice thoroughly with acetone and hexane before use.

Recovery experiments

The extraction methodology with XAD-2 resin was developed for butachlor, chlormethoxynil and bifenox. The extraction system has been described previously[2]. For recovery experiments, standard solutions containing butachlor, chlormethoxynil

and bifenox in acetone were prepared. Acetone standard solutions (0.50 ml) were spiked into 2 l of pesticide-free water to give concentrations of 25, 50, 250 and 500 ng/l for chlormethoxynil and 50, 100, 500 and 1000 ng/l for butachlor and bifenox. Then the sample was passed through the XAD-2 column (10 cm × 1 cm I.D.) at a flow-rate of *ca.* 50 ml/min under gentle suction. The herbicide standards adsorbed on the resin were desorbed with 60 ml of diethyl ether after standing for 15 min to equilibrate. Then the extractant and an additional 40 ml of diethyl ether eluent were drained into a separating funnel, to which 50 ml of hexane were added to remove the aqueous layer. Following the removal of the aqueous layer, the organic layer was dried by passing it through a column of anhydrous sodium sulphate and concentrated with a Kuderna–Danish evaporative concentrator. The final concentration was accomplished by passage of a stream of nitrogen and the residue was dissolved into 1 ml of isooctane and subjected to GC–ECD on a CB-FSC.

Gas chromatography

A Shimadzu GC-9APFE gas chromatograph equipped with an electron-capture detector and an OV-17 CB-FSC (25 m × 0.32 mm I.D., 0.25 μm film) was employed. A Shimadzu C-R2AX integrator performed data processing. Aliquots of standards and sample extracts, normally 1 μl, were automatically introduced through a Shimadzu AOC-9 autosampler by splitless injection. The temperature programme was as follows: 90°C for 3 min, then increased to 170°C at 20°C/min and further to 275°C at 3°C/min, the final temperature being maintained for 8 min. The injector temperature was 290°C throughout the experiment. The flow-rate of make-up gas was 70 ml/min. Identification of CCHs was performed by the internal standard method, comparing relative retention times (RRTs) with respect to heptachlor epoxide. Other CB-FSCs, OV-1701 (25 m × 0.25 mm I.D., 0.25 μm film) and SE-52 (25 m × 0.25 mm I.D., 0.30 μm film) were used to confirm identification. Calibration graphs for each CCH were prepared, using the internal standard method. Quantification was performed by peak-height ratio measurements of each CCH relative to the internal standard.

Field experiments

Water samples were taken at the sampling sites shown Fig. 2. Sampling site 1 was by the Onga River, which is one of the longest rivers in Kyushu Island and its total catchment is 1032 km^2. Sampling sites 2, 3, 5 and 6 were located in argricultural areas, the streams being used for agricultural drainage. Sampling site 4 was by the Murasaki River, which is located in an urban area of Kitakyushu City. Sampling site 7 was laboratory tap water which is supplied after conventional treatment of Onga River water. The water samples were taken once a month, provided that no rain had fallen for three days before sampling. Grab water was taken in a 3-l glass vessel and rapidly transferred to the laboratory for analysis. The samples were filtered through a G4 Büchner filter (150–200 mesh) to remove sediments. The filtered water sample (2 l) was drained into another glass vessel and stirred for 15 min to achieve thorough mixing after addition of an acetone solution of heptachlor epoxide (0.015 μg) as an internal standard. The subsequent procedures were the same as in the recovery experiments.

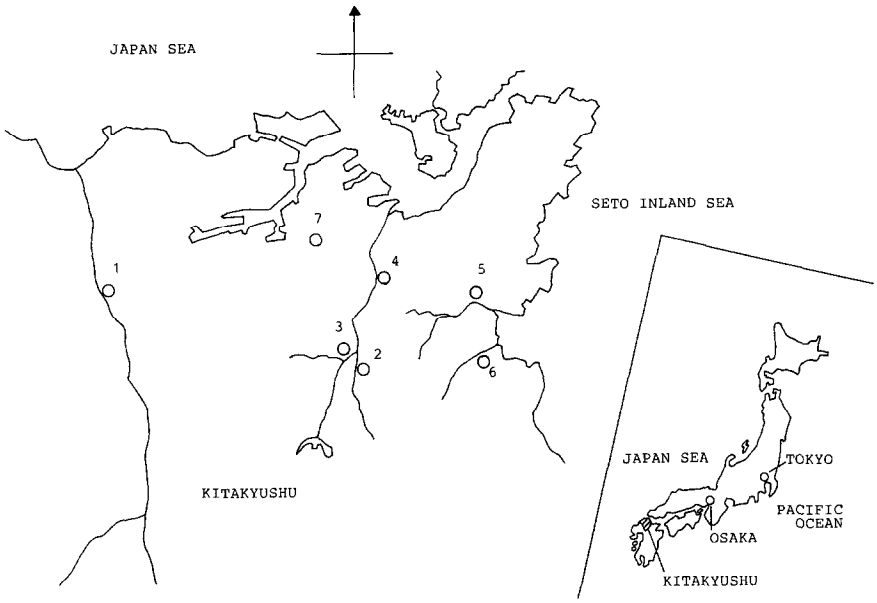

Fig. 2. Sampling sites: 1, Onga River; 2, Higashitani River; 3, Nishitani River; 4, Murasaki River; 5, Chikuma River; 6, Nuki River; 7, tap water.

RESULTS AND DISCUSSION

Recovery experiments

It is known that polar compounds are poorly adsorbed on XAD-2, resulting in low recoveries[6,9]. As butachlor, chlormethoxynil and bifenox have some polarity, their recovery using the method was examined. Recoveries from water samples at four fortification levels are given in Tabel I. All recoveries exceeded 90% and the

TABLE I

RECOVERIES OF BUTACHLOR, CHLORMETHOXYNIL AND BIFENOX FROM FORTIFIED SAMPLES USING THE XAD-2 EXTRACTION METHOD

Compound	*Fortification level (μg/l)*	*Recovery ± C.V.* (%)*
Butachlor	0.05	96.7 ± 3.1
	0.10	96.3 ± 4.0
	0.50	95.5 ± 8.2
	1.00	94.3 ± 6.2
Chlormethoxynil	0.025	102.0 ± 5.3
	0.05	103.7 ± 9.0
	0.25	100.3 ± 8.4
	0.50	102.8 ± 0.4
Bifenox	0.05	93.7 ±4.9
	0.10	103.0 ± 9.2
	0.50	93.7 ± 3.2
	1.00	102.9 ± 2.5

* C.V. = coefficient of variation (n = 3).

standard deviations were less than 10%. Only the mean recoveries of chlormethoxynil were over 100% at all levels spiked. Although some fairly large deviations of the recoveries can be seen in Table I, they are within the ranges of deviations for GC determinations. Therefore, almost quantitative adsorption–desorption kinetics of these pesticides from XAD-2 resin at all the fortified levels was demonstrated.

These results were considered to be satisfactory for determining the three CCHs in a water matrix. Also, these CCHs could be simultaneously and rapidly extracted from water samples together with HCHs, CNP and oxadiazon, as in previous studies[2,7,8]. XAD-2 resin adsorbed less co-extractives such as organic materials with high molecular mass which cause more serious problems with GC–ECD than liquid–liquid extraction[9]. In many instances the extracts could be directly applied to GC–ECD without any clean-up. High blank levels derived from impurities in the resin have been one of the disadvantages of the method, but few interfering peaks were seen in the chromatogram of the extracts from pesticide-free water using the GC–ECD method (Fig. 3a). Further, the resin could be used repeatedly after washing with an appropriate amount of methanol.

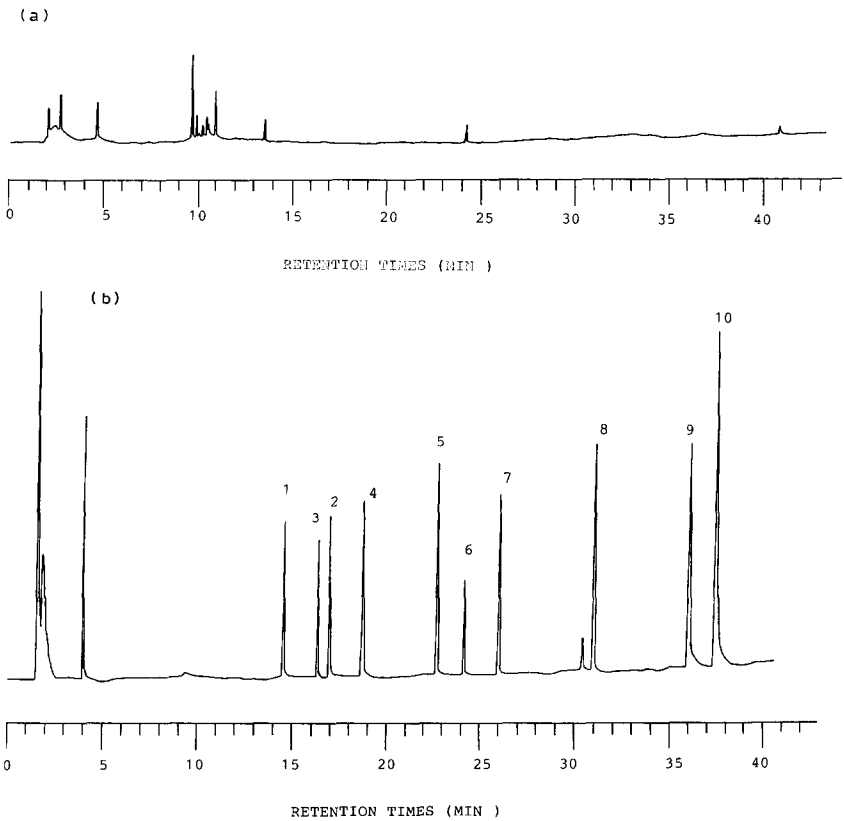

Fig. 3. (a) GC–ECD trace of blank run on an OV-17 CB-FSC. A = Diethyl ether extract was obtained from the XAD-2 resin column after passing pesticide-free water. (b) GC–ECD trace of standards of chlorine-containing pesticides on an OV-17 CB-FSC column. Peaks: 1, α-HCH; 2, β-HCH; 3, γ-HCH; 4, δ-HCH; 5, heptachlor epoxide (internal standard); 6, butachlor; 7, oxadiazon; 8, CNP; 9, chlormethoxynil; 10. bifenox.

High-resolution GC–ECD

A chromatogram of standards of HCH isomers, butachlor, oxadiazon, CNP, chlormethoxynil and bifenox on the OV-17 CB-FSC is shown in Fig. 3b. Under the GC conditions applied, the peaks of each component were clearly resolved from each other. The internal standard method was employed to avoid misunderstandings or false identification due to fluctuations in retention times and to minimize the deviation of the recovery during the procedure. The peaks derived from pesticide components could be identified by comparison of the RRTs with respect to the internal standard, heptachlor epoxide. The retention times fluctuated periodically owing to changes in room temperature, whereas the RRTs were almost constant (the coeffi-

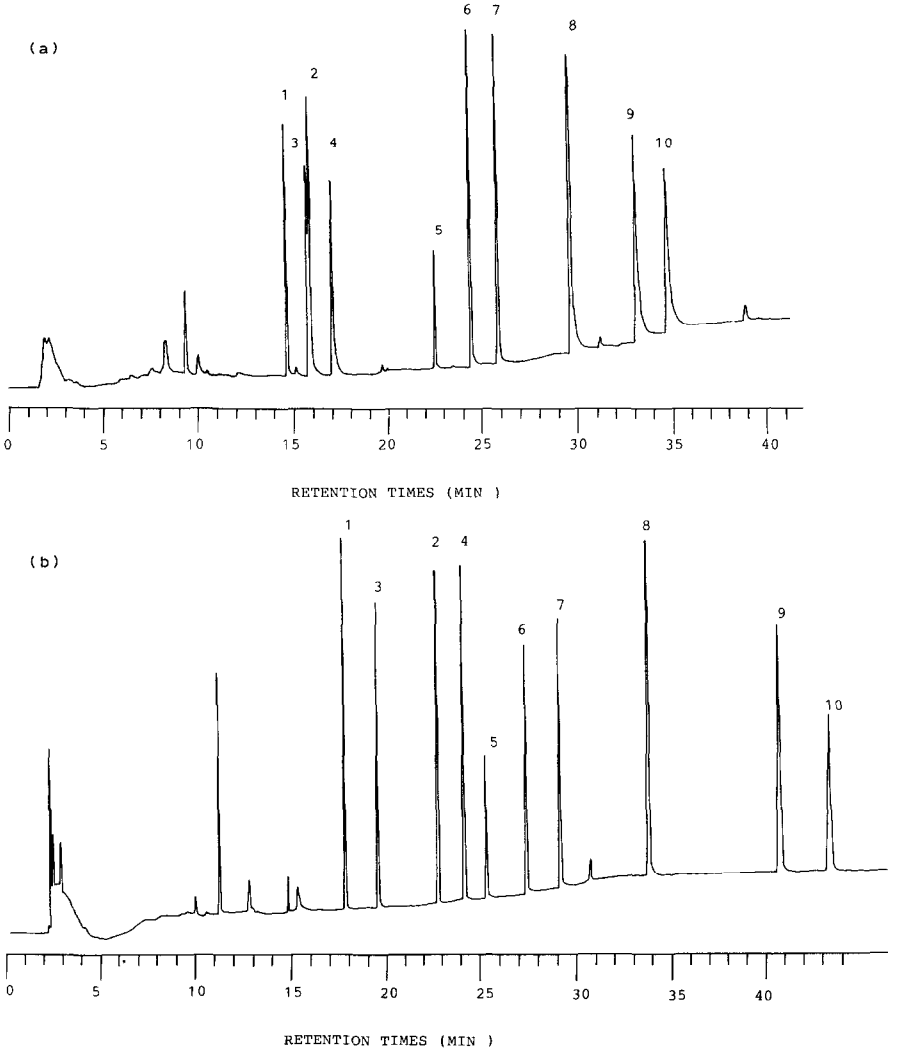

Fig. 4. GC–ECD traces of standards of chlorine-containing pesticides on (a) SE-52 and (b) OV-1701 CB-FSC. Peaks as in Fig. 3b.

cients of variation of the RRTs with ten replicate injections of CCHs were less than 0.1%). Therefore, comparison of RRTs was adequate for the identification of CCHs in river water samples.

For further identification, it is desirable to use a column of different polarity, *i.e.*, a non-polar column for confirmation of the identification, but chlormethoxynil and bifenox, which are slightly polar, could not be resolved satisfactorily, and they were absorbed on the column readily. In such instances an SE-52 or OV-1701 CB-FSC could be used (Fig. 4). With the SE-52 CB-FSC, β-HCH and γ-HCH could not be resolved, which was a disadvantage as HCH isomers have to be monitored simultaneously and they have often been detected in water samples. The OV-1701 CB-FSC was more suitable for confirmation of the identification.

Quantification was performed by peak-height ratio measurements relative to the internal standard. Calibration graphs were obtained with an amount of internal standard of 0.015 ng and amounts of chlormethoxynil, bifenox, CNP and oxadiazon in the range 0.01–0.15 ng and of butachlor in the range 0.04–0.60 ng. Linear graphs were obtained between 0 and 0.25 ng for chlormethoxynil, bifenox, oxadiazon and CNP and between 0 and 1 ng for butachlor. The minimum detectable amounts were 0.01 ng for chlormethoxynil and bifenox and 0.02 ng for butachlor, which corresponded to 0.005 and 0.01 μg/l respectively, with concentration of the water samples under the experimental conditions.

On extended use, late-eluting CCHs, *i.e.*, chlormethoxynil and bifenox, tended to be adsorbed on the column wall, resulting in diminution of the peak heights. This might be due to the discrimination[10]. Some measurements were made by determination of the peak-height ratio of the CCHs relative to the internal standard. The discrimination was improved by injection of a silylating reagent or by cutting a part of the column of the injector side.

Field experiments

CCH residues in river surface water could be identified and determined using the present methodology. Fig. 5a showed a typical gas chromatogram of CCH resi-

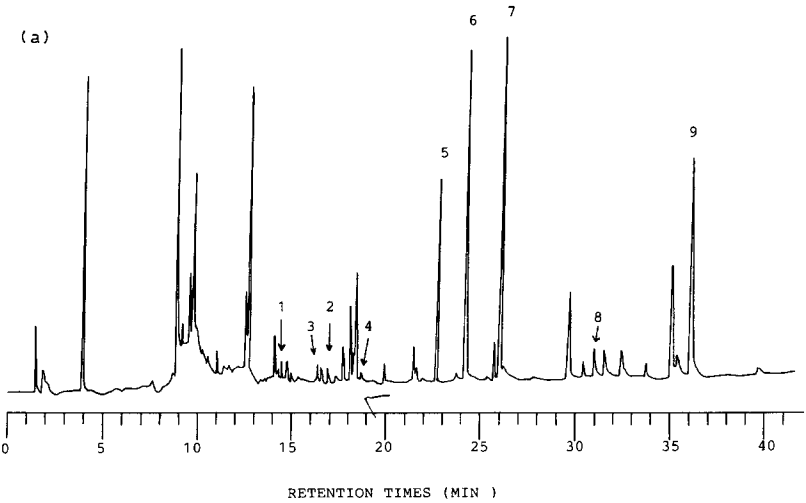

Fig. 5.

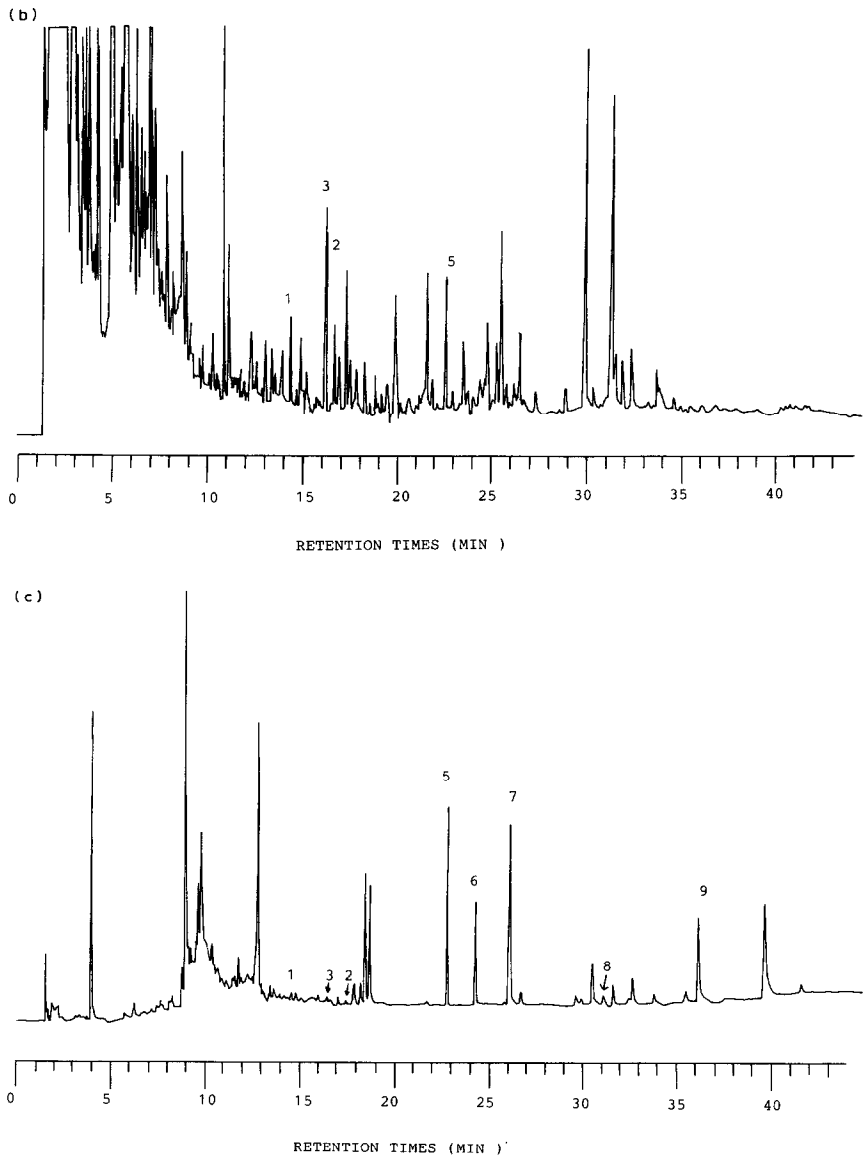

Fig. 5. Typical GC–ECD traces on an OV-17 CB-FSC column of samples taken from the Onga River in (a) June 1987 and (b) April 1987, and (c) tap water sample taken in June, 1987. The sample (2 l) was concentrated to 10 ml.

dues in a sample taken from the Onga River in June 1987, when high concentrations of herbicide residues were to be expected. The extract was concentrated to 10 ml. Many peaks derived from co-extractives were recorded. Overlapping of peaks with similar retention times commonly occurred when GC–ECD with a packed column without clean-up of the sample extracts was performed, but excellent separations of CCHs and other co-extractives were obtained with the OV-17 CB-FSC. In the surface

TABLE II

RESIDUE CONCENTRATIONS (μg/ml) OF BUTACHLOR, CHLORMETHOXYNIL AND BIFENOX IN SURFACE WATER AND TAP WATER IN 1987

ND is below the detection limit, which is 0.01 μg/l for butachlor and 0.005 μg/l for chlormethoxynil and bifenox.

Compound	*Sample*	*22.1.87*	*19.3.87*	*17.4.87*	*21.5.87*	*19.6.87*	*28.7.87*	*21.8.87*	*22.9.87*	*21.10.87*
Butachlor	Onga R.	ND	ND	ND	0.01	2.26	0.15	0.07	ND	ND
	Higashitani R.	ND	ND	0.05	ND	3.44	0.08	0.03	ND	ND
	Nishitani R.	ND	ND	ND	ND	1.32	0.02	ND	ND	ND
	Murasaki R.	ND	ND	ND	ND	2.10	0.04	0.04	ND	ND
	Chikuma R.	ND	ND	ND	ND	1.28	0.07	0.06	ND	ND
	Nuki R.	ND	ND	ND	ND	0.75	0.01	ND	ND	ND
	Tap water	ND	ND	ND	ND	0.59	0.04	0.01	ND	ND
Chlormethoxynil	Onga R.	ND	0.010	ND	ND	0.254	0.017	ND	ND	ND
	Higashitani R.	ND	ND	ND	ND	0.049	ND	ND	ND	ND
	Nishitani R.	ND	ND	ND	ND	0.027	ND	ND	ND	ND
	Murasaki R.	ND	ND	ND	ND	0.020	ND	ND	ND	ND
	Chikuma R.	ND	0.007	ND	ND	0.012	ND	ND	ND	ND
	Nuki R.	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Tap water	ND	ND	ND	ND	0.095	0.006	ND	0.009	ND
Bifenox	Onga R.	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Higashitani R.	ND	ND	ND	ND	0.019	ND	ND	ND	ND
	Nishitani R.	ND	ND	ND	ND	0.009	ND	ND	ND	ND
	Murasaki R.	ND	ND	ND	ND	0.006	ND	ND	ND	ND
	Chikuma R.	ND	ND	ND	ND	0.064	ND	ND	ND	ND
	Nuki R.	ND	ND	ND	ND	0.032	ND	ND	ND	ND
	Tap water	ND	ND	ND	ND	ND	ND	ND	ND	ND

water samples residues of butachlor and chlormethoxynil could be detected in addition to HCH isomers, oxadiazon and CNP. The RRTs of the pesticides with respect to heptachlor epoxide were in fair agreement with those of standards. The residue concentrations of butachlor, oxadiazon, CNP and chlormethoxynil were 2.26, 0.66, 0.02 and 0.25 μg/l, respectively; α-, β-, γ- and δ-HCH isomers were detected at 0.005, 0.013, 0.006 and 0.002 μg/l, respectively.

Fig. 5b shows a typical chromatogram of a sample taken from the Onga River in April 1987, when the concentrations of herbicides were expected to be very low. The extract was concentrated to 1.0 ml. Although more peaks were monitored than in Fig. 5a, separation of most of them was accomplished. HCH isomers except δ-HCH were detected. However, none of the peaks derived from herbicides, butachlor, oxadiazon, CNP, chlormethoxynil or bifenox could be detected. The three large peaks eluting between 25 and 35 min were not identified. They do not seem to be CCHs because they appeared without regard to changes of season.

Fig. 5c shows a chromatogram of a sample of tap water taken in our laboratory in June 1987. The extract was concentrated to 10 ml. The chromatogram was similar to that in Fig. 5a, although the levels of CCHs were slightly low. The residue concentrations of butachlor, oxadiazon, CNP and chlormethoxynil were 0.59, 0.28, 0.01 and 0.095 μg/l, respectively. These results indicate that the conventional water purification process had not removed these compounds.

Table II gives the residue levels of butachlor, chlormethoxynil and bifenox in river surface water and tap water in 1987. Residues of butachlor and chlormethoxynil were frequently detected from June to August and occasionally from March to September. However, residues of bifenox could be measured only in June. Of the three CCHs, butachlor was most often detected and its residual concentrations were the highest. In the Kitakyushu District, herbicide formulations containing these components are applied to control the weeds in flooded paddy fields in mid-June after rice-seedling transplantation. Herbicide application is terminated after the rice-plants have grown to a moderate height, and residues then could not be detected. These results indicate that the herbicides under study are not persistent in the aquatic environment.

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