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Note

Gas chromatographic determination of endosulfan in fish and water samples

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The broad-spectrum chlorinated hydrocarbon insecticide endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide; Thiodan®) is widely used in fruit and vegetable production. In technical formulations two stereoisomers, designated α and β, are present in a ratio of 7:3. Endosulfan is a chlorinated hydrocarbon with a relative short persistence in the environment and low mammalian toxicity. However, to fish it is one of the most toxic pesticides known: the 24-h LC_{50} values range between 0.09 and 11.2 $\mu g/l$[1].

A routine gas chromatographic method for the determination of endosulfan and its metabolite endosulfan sulphate in *post mortem* material and water was developed to investigate some actual cases of fish poisoning. Previously published methods involve time-consuming sample pre-treatments[1–5] or do not include the metabolite endosulfan sulphate[2,5–9]. Other methods have the disadvantage of using clean-up columns packed with Florisil, which is difficult to standardize and can cause partial hydrolysis to endosulfan alcohol during elution[1,3,8,10,11]. The present method does not have these disadvantages and was used in diagnosing four actual cases of massive fish poisoning in The Netherlands during 1984–85. These cases were a result of illegal spraying of willow-trees, the use of endosulfan in warehouses with concomitant contamination of adjacent ditches and spraying fruit trees followed by pollution of adjacent streams by drift and run-off.

EXPERIMENTAL

Sampling

At selected locations, samples of dead fish and frogs and water samples were collected by officers of the Dutch Ministry of Agriculture and Fisheries in cooperation with employees of the local Waste Water Authority. The fish and frog samples were dissected on receipt. Tissues were stored at −20°C and water samples at 4°C until taken for analysis.

Extraction and clean-up

Amounts of 2–5 g of accurately weighed tissue were ground in a mortar with anhydrous sodium sulphate to a free flowing powder. The powder was extracted with 25 ml of toluene in a conical flask by mechanical shaking for 1 h. After filtration

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through Whatman 41 paper, the filtrate was transferred into a glass chromatographic column (15 × 1 cm I.D.) filled successively with sodium sulphate (1 g), freshly prepared neutral alumina with a water content of 7.5% (w/w) (5 g) and sodium sulphate (1 g). The eluate was collected in a 50-ml calibrated flask until the meniscus reached the top of the column. The conical flask and filter were washed with 25 ml of toluene, which was also transferred into the column. The calibrated flask was filled to the mark with toluene.

Water samples (100 ml) were extracted twice with 50 ml of toluene by mechanical shaking for 1 h in conical flasks. After separation of the layers in a separating funnel, the collected organic phases were adjusted to contain exactly 100 ml. An aliquot of the extract was dried over anhydrous sodium sulphate for gas chromatographic analysis.

Determination

Gas chromatographic analysis was performed using a Varian 3700 gas chromatograph equipped with an electron-capture detector without make-up gas, autosampler, integrator and recorder. The glass column (6 ft. × 1/4 in. O.D. × 2 mm I.D.) was packed with 3% OV-101 on Chromosorb W HP (80–100 mesh). The temperatures of the column, injector and detector were 175, 220 and 350°C, respectively. The flow-rate of the carrier gas (nitrogen) was 24 ml/min. The injection volume was 1.7 μl.

For quantitation, standard solutions of α-endosulfan, β-endosulfan and endosulfan sulphate in toluene (Nanogen) for external standard calibration were used.

RESULTS AND DISCUSSION

Toluene was chosen as an extraction solvent because especially for endosulfan sulphate it appeared that the recoveries were unacceptable low when using hexane. Hexane apparently has too low a polarity for the relative polar endosulfan sulphate.

Table I summarizes the results of recovery experiments with spiked water and liver samples. Table II reports the lowest concentrations of the compounds that can be determined with reliable accuracy. The response of the electron-capture detector was linear in the range 1–10 ng/ml for α- and β-endosulfan and 5–50 ng/ml for endosulfan sulphate. The detection limits, based on three times the noise level, were 0.018 pg for α- and β-endosulfan and 0.090 pg for endosulfan sulphate.

TABLE I

MEAN RECOVERIES OF ENDOSULFAN IN SPIKED WATER AND LIVER SAMPLES

Sample	*Endosulfan*	*Concentration range (μg/kg)*	*Mean recovery ± S.D. (%)*	*No. of samples analysed*
Water	α	0.5–10	99 ± 2	9
Water	β	0.5–10	99 ± 6	9
Liver	α	10–10 000	84 ± 3	12
Liver	β	10–10 000	79 ± 4	12
Liver	Sulphate	500–50 000	86 ± 3	9

TABLE II

PRACTICAL DETECTION LIMITS (μg/kg)

Based on 5 g of tissue or 100 ml of water.

Compound	*Tissues*	*Water*
α-Endosulfan	5.0	0.5
β-Endosulfan	5.0	0.5
Endosulfan sulphate	25.0	2.5

Table III gives the results of endosulfan determinations using the present method in four cases of endosulfan fish and frog poisoning. Figs. 1 and 2 represent typical chromatograms of an endosulfan standard solution and a fish liver extract, respectively.

The results in Table I indicate a good recovery of endosulfan in both spiked water and liver samples. Figs. 1 and 2 show a good separation between the three components of interest. No interference from matrix components or other chlorinated hydrocarbons was observed.

In addition, the sensitivity of the method is satisfactory, as can be seen in Table II. The residue levels in Table III indicate lethal poisoning of fish and frogs.

CONCLUSION

The described method provides a sensitive and rapid means for the simultaneous determination of α- and β-endosulfan and the toxicologically important metabolite endosulfan sulphate in tissues of fish and frogs and in water samples. It proved to be a valuable tool for diagnosing lethal fish poisoning by endosulfan. Further, the

TABLE III

RESIDUES OF ENDOSULFAN IN FOUR CASES OF FISH AND FROG POISONING

Case No.	*Sample*	*No. of samples analysed*	*Concentration (μg/kg wet weight)*					
			Mean			*Range*		
			α	*β*	*Sulphate*	*α*	*β*	*Sulphate*
1	Livers (fish)	4	990	490	<25	440–1400	250–820	–
	Gills (fish)	4	1000	260	125	360–1600	160–370	100–150
	Water	2	1.1	1.8	<2.5	0.2–2.0	0.3–3.4	–
2	Gills (fish)	13	490	300	108	270–750	140–650	0–1400
	Livers (frogs)*	1	7400	14 000	18 000	–	–	–
3	Gills (fish)	4	1100	560	<8.0	230–2800	190–1200	–
	Water	2	<0.5	<0.5	<2.5	–	–	–
4	Gills (fish)	3	109	32	<15	87–150	15–43	–

* Mixture of five livers.

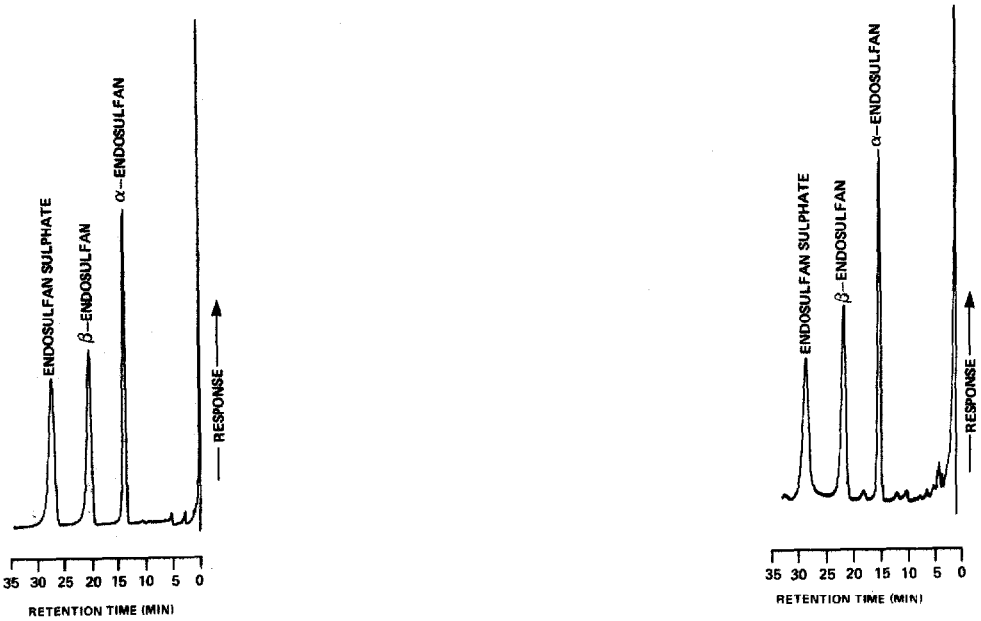

Fig. 1. Gas chromatogram of a standard solution containing 2.5 ng/ml of α-endosulfan, 5 ng/ml of β-endosulfan and 25 ng/ml of endosulfan sulphate on a 3% OV-101 on Chromosorb W HP (80–100 mesh) packed glass column (6 ft. × 1/4 in. O.D. × 2 mm I.D.). Injection volume, 1.7 μl; carrier gas (nitrogen) flow-rate 24 ml/min.

Fig. 2. Gas chromatogram of 5 g of a cleaned liver extract of poisoned fish. Conditions as in Fig. 1.

results indicate that the use of endosulfan as an insecticide should be avoided near surface waters, in order to minimize its destructive effects on aquatic ecosystems.

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