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Residues of Chlorinated Pesticides in Eggs of the Gray Heron (*Ardea cinerea* L.): Contribution of Capillary Gas Chromatography Ion-Trap Mass Detection

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Introduction

Organochlorine pesticides, widely used during the 1950s and 1960s as insecticides, still cause many ecotoxicological problems today. Because of their poor degradation, they persist in the environment and accumulate all along the trophic chains. Aquatic ecosystems, and especially the freshwater environment, belong to the most sensitive bio to pes. During a pesticide control campaign on a lake (Lac de Granlieu, France), eggs of the gray heron have been used as indicators of pollution. In fact, the level of organochlorine pesticide residues in eggs can be closely correlated with effects on egg shell thinning and on reproduction [1- 5], and thus on the population dynamics of bird species. In this paper, we describe a sensitive GC-ECD method performed on two capillary columns, and developed to analyze simultaneously ten organochlorine pesticides in eggs of the gray heron. A gas chromatography-mass spectrometry (GC-MS) method is also described to clarify ambiguous results given by some compounds owing to coelution.

Experimental

1. Extraction Procedure

Eight eggs were analyzed; the collection of eggs was authorized by Ministère de l'Environnement. The content of the eggs (37- 46 g) was ground in a mortar with Hyflosuper cel (17-23 g). The homogenate was divided into two aliquots subjected to separate analyses. Both aliquots (10 g) were extracted with 100 ml acetonitrile. The acetonitrile extract was filtered over a Buchner funnel and

the residues were extracted from the acetonitrile fraction with 50 ml hexane, in the presence of 10 ml water saturated with sodium chloride (Prolabo, France) and 300 ml deionized water. Hexane extraction was repeated twice. The hexane extracts were combined and dried over anhydrous sodium sulfate (Prolabo, France) prior to concentration to 1 ml by using a rotary evaporator. The aliquot was cleaned on a Florisil column (Sep-Pak 20 cc - 5g, Waters, France). *p,p'*-DDE (fraction 1) was eluted with hexane (20 ml) and α -HCH, β -HCH, γ -HCH, δ -HCH, HCB, HE, dieldrin, *p,p'*-DDD and *p,p'*-DDT (fraction 2) were eluted with diethyl ether/hexane 5/95 (50 ml). Both fractions were concentrated to 1 ml by using a rotary evaporator and then made up to 5 ml with trimethyl-2,2,4-pentane before GC-ECD and GC-IT-MS analysis. Recoveries for the studied compounds in solution (50 pg/ μ l for each standard) after Florisil column chromatography were: α -HCH 92.3%, HCB 87.8%, γ -HCH 98.6%, β -HCH 90.8%, δ -HCH 85.4%, HE 97.5%, dieldrin 93.4%, *p,p'*-DDE 79.0%, *p,p'*-DDD 83.9%, *p,p'*-DDT 79.0%.

Accuracy and precision were assessed by use of blanks. Each sample was extracted in duplicate, gas chromatographic determinations (quantification of organochlorine pesticides standards and sample s) were performed in triplicate. Multiple determinations were all less than 10% different.

2. Gas Chromatography with ECD Detection

Gas chromatography was performed with an Auto System Perkin Elmer apparatus equipped with an ECD. The chromatograph was fitted with a DBS fused silica capillary column (30 m x 0.53 mm i.d., coated with a 1.5 μ m film thickness, J&W Scientific) and a SPB-608 fused silica capillary column (30 m x 0.53 mm i.d., 0.50 μ m film thickness, Supelco). The carrier gas was nitrogen at pressure 5 psi. The oven temperature was programmed as follows: initial temperature 190°C (11 min) after injection, increased at 2° min⁻¹ to 220°C, held there for 25 min, then increased at 5° min⁻¹ to 260°C, the final temperature being held for 3 min. Aliquots (1.0 μ l) of the extracts were injected in a wide bore glass injector liner (off column flash vaporization) at 260°C. The detector temperature was set at 300°C.

Total concentrations of organochlorine pesticides were determined by comparison of peak areas to those of authentic standards, regarding retention times relative to internal standard aldrin (RRT).

3. Gas Chromatography Mass Spectrometry

GC-MS analysis was performed on a Varian model 3400 capillary gas chromatograph equipped with a Saturn II ion-trap detector. Chromatographic conditions for these analyses were : a DBS fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μ m film thickness; J&W Scientific), with helium carrier gas at 10 psi. The oven program was as follows: initial temperature was 120°C (2 min) after injection, increased at 25° min⁻¹ to 190°C, held for 10 min, then at 2° min⁻¹ to 220°C, held

for 15 min, at $5^{\circ} \text{ min}^{-1}$ to 250°C , the final temperature being held for 5 min. The temperature program of the septum programmable injector (SPI) was 80°C to 190°C at $190^{\circ} \text{ min}^{-1}$, and then at $100^{\circ} \text{ min}^{-1}$ to 250°C .

The transfer line and the manifold were set at 240°C and 250°C , respectively. Injection volumes of $1 \mu\text{l}$ were used in each analysis.

The Mass Spectrometer (MS) was operated in the Electron Impact (EI-MS) energy set at 70 eV, and Selected-Ion Monitoring (EI-MS-SIM) was performed according to characteristic ions of standard pesticides.

Results and Discussion

GC-ECD and GC-IT-MS Results

Analysis by GC-ECD shows that compounds with RRTs identical to those of the ten organochlorine pesticides are present in variable amounts in the eight eggs analyzed (Table 1). Dieldrin and *p,p'*-DDE, eluted in separate fractions, show identical RRT when analyzed with both DB-5 and SPB-608 column of distinct polarity. After GC-ECD analysis, samples containing possible residues were analyzed by EI-MS-SIM. With helium as carrier gas, *p,p'*-DDE and dieldrin also exhibit identical RRTs (Table I). On analysis by GC-ECD, some compounds were detected in eggs at concentrations higher than or equal to LODs established for EI-MS-SIM. Yet some of them could not be identified by using this last technique.

This difference can be explained by possible coelution of some interfering compounds. The compound eluted by GC-ECD as *p,p'*-DDE is also identified by EI-MS-SIM in all eggs analyzed. The structure of the compound expected as dieldrin according to GC-ECD analysis is not confirmed by EI-MS-SIM. In fact, its mass spectrum does not conform to that of the dieldrin standard (Figure 1a and 1b). This chromatographic peak was found to give mass spectra of two different compounds. The presence of ions at m/z 316, 318, 320 (Figure 1b) suggest that one of them is a multichlorine component in agreement with GC-ECD detection. In all eggs examined, dieldrin was not authenticated by EI-MS-SIM.

Conclusion

Despite the use of columns with distinct polarity, GC-ECD, a selective and sensitive method for organochlorine analysis may nevertheless yield ambiguous results in some cases. GC-MS excludes such ambiguities by distinguishing between authentic pesticides residues and coeluted interfering

compounds found in the eggs of the gray heron. Results were obtained with sufficient sensitivity (lower than one $\mu\text{g kg}^{-1}$ (ppm)), corresponding to the concentration range previously described to be without toxic effects on many birds [7,8). This technique affords additional structural confirmation beyond relative retention time matching with a reference standard.

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Table 1. GC-ECD and GC-IT-MS results.

Compound	GC-ECD		LOD $\mu\text{g kg}^{-1}$	Eggs number ^{a)}	Concentration $\mu\text{g kg}^{-1}$ ^{b)}	EI-MS-SIM		NbE>LOD	NbE identified
	RRT DB-5	RRT SPB-608				RRT DB-5	LOD $\mu\text{g kg}^{-1}$		
α -HCH	0.438	0.510	0.05	8	1 to 4	0.587	10	0	0
HCB	0.472	0.469	0.25	6	7 to 264	0.600	50	5	4
β -HCH	0.506	0.673	0.50	8	1 to 108	0.652	10	7	2
γ -HCH	0.534	0.643	0.50	3	1 to 2	0.658	10	0	0
δ -HCH	0.609	0.847	0.50	1	4	0.723	10	0	0
HE	1.191	1.388	0.05	8	3 to 41	1.189	10	6	2
dieldrin	1.534	1.887	0.10	8	17 to 229	1.508	10	8	0
<i>p,p'</i> -DDE	1.538	1.892	0.10	8	87 to 954	1.508	50	8	8
<i>p,p'</i> -DDD	1.773	2.356	0.25	6	2 to 14	1.563	50	0	0
<i>p,p'</i> -DDT	2.027	2.613	0.25	4	1 to 19	1.956	50	0	0

RRT (DB-5): Relative retention time to internal standard aldrin.

RRT (SPB-608): Relative retention time to internal standard aldrin.

LOD: Limit of detection reported in $\mu\text{g kg}^{-1}$ (ppb)

^{a)} Number of eggs where compounds have been detected.

^{b)} Concentration reported in $\mu\text{g kg}^{-1}$ (ppb) of the wet weight.

NbE > LOD: Number of eggs (NbE) present GC-ECD concentrations greater than or equal to LOD obtained by EI-MS-SIM.

NbE identified: Number of eggs for which the pesticide was identified by EI-MS-SIM.

Figure 1. (a) GC-IT-MS spectrum for the standard dieldrin (7); (b) GC-IT-MS spectrum for the peak chromatographed by GC-ECD as dieldrin.

