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# Determination of chlorinated insecticides in blood samples of agricultural workers

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## ABSTRACT

Lindane, aldrin and *p,p'*-DDT were determined in blood samples from 71 farmers by means of an analytical method which combines a direct whole-blood extraction with *n*-hexane and gas chromatography (GC)–electron-capture detection (ECD), using a capillary column, applied to the organic extract. This technique allowed the determination of pesticides at levels varying from 0.1 to 180 µg per l of blood, the detection limit for every pesticide being 0.1 µg/l. GC–mass spectrometry was used to confirm the identity of each pesticide. The advantage of capillary column GC–ECD for pesticide determination is its sensitivity and high resolution, which makes it possible to separate pesticides from a complex *n*-hexane extract obtained in a very simple pretreatment of the blood sample, which is itself a very complex matrix.

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## INTRODUCTION

It has long been recognized that chlorinated insecticides may be stored in the body fat and exert a toxic action upon the central nervous system, and as a consequence the determination of such compounds in biological samples, specifically blood, from occupationally exposed populations is considered of high interest. In addition, lindane may produce kidney and liver changes in some experimental animals and, more importantly, there have been several cases of aplastic anaemia in which there was a clear association between erythropoietic depression of the individuals involved and their recent or, less frequently, remote exposure [1]. Occupational overexposure to aldrin produces excitation of the

nervous system. In cases of acute poisoning dysrhythmia in the electroencephalogram (EEG) and convulsive fits have occurred. Nevertheless, in these patients there is a marked trend of the EEG towards normality several months after removal of exposure, along with a decrease in blood dieldrin concentrations (also a metabolite of aldrin) [2], which correlate with both intensity and exposure time [3]. The exposure to *p,p'*-DDT may be of occupational origin or strongly related to environmental contamination. Nowadays pesticide blood levels are considered a good indicator of pesticide body burden as close correlations between the concentrations of pesticides in blood and fat have been established, even in the case of non-occupationally exposed individuals [4].

From the analytical point of view, two aspects require comment with regard to pesticide gas chromatography (GC) determination: (1) sample

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treatment and (2) column and analytical conditions. Dale *et al.*'s [5] extraction method with *n*-hexane has been considered a proper procedure for blood sample treatment by many authors [6–8].

Analysis of pesticides has dramatically improved with the use of capillary columns. With this system better sensitivity and resolution, have been achieved [9,10] and it is also a less time-consuming method. In accordance with this, the determinations of blood lindane, aldrin and *p,p'*-DDT in our laboratory were carried out using a method combining direct extraction with *n*-hexane, capillary column GC–electron-capture detection (ECD) and GC–mass spectrometry (MS).

The aims of this paper are: (1) to describe an analytical method that improves the separation of the peaks in the chromatograms and one that requires a shorter analytical time, about 30 min per sample, in comparison with the results obtained with a packed column; and (2) to assess the occupational exposure to pesticides of a group of agricultural workers and compare this group with a group of workers with no occupational exposure.

## EXPERIMENTAL

A population group of 71 farmers with mean age of 43 (median = 43) and mean exposure time to pesticides of 12.6 years (median = 12) was studied. The origin of the exposure was the preparation and use of pesticide mixtures on different types of crops: strawberry, large strawberry, market garden crops, flowers and other fruits. Seven unexposed workers of the same age range were studied as controls.

### Sample collection

A 5-ml venous blood sample was drawn from each worker under fasting conditions and before a work shift with a vacuum tube containing heparin. Blood samples were immediately frozen and kept at  $-30^{\circ}\text{C}$  until they were analysed.

### Chemicals

Pesticide-grade *n*-hexane used in the analytical procedure was obtained from Monplet & Es-

teban (Barcelona, Spain). Aldrin, lindane, *p,p'*-DDT and dieldrin used to prepare standard solutions were obtained from PolyScience (IL, USA).

### Calibration

A calibration curve was prepared for every pesticide with standard solutions obtained by weighing and dissolving appropriate quantities of each pesticide in *n*-hexane containing internal standard (dieldrin 40  $\mu\text{g/l}$ ) (see Fig. 1).

### Analytical conditions

The chromatographic study was carried out using a Hewlett-Packard Model 5890 gas chromatograph equipped with a  $^{63}\text{Ni}$  electron-capture detector and a SPB-5 capillary column, 30 m  $\times$  0.25 mm I.D., 0.25  $\mu\text{m}$  film thickness, from Supelco (Bellefonte, PA, USA). Temperatures were: column, initial  $180^{\circ}\text{C}$  (1 min), final  $270^{\circ}\text{C}$ ; rate  $2^{\circ}\text{C}/\text{min}$ ; injector  $270^{\circ}\text{C}$ ; detector  $300^{\circ}\text{C}$ . Helium was used as the carrier gas at a linear speed of 19 cm/s and 25 cm/s, and nitrogen as the auxiliary at 45 ml/min. The injector was in split mode (1:60) and the injected volume was 2  $\mu\text{l}$ .

The MS study was carried out with a Hewlett-Packard Model 5995 gas chromatograph–mass spectrometer using an SPB-5 capillary column, 30 m  $\times$  0.25 mm I.D., 0.25  $\mu\text{m}$  film thickness, from Supelco. Temperatures were: injector,  $200^{\circ}\text{C}$ ; transfer line,  $220^{\circ}\text{C}$ ; ionization source,

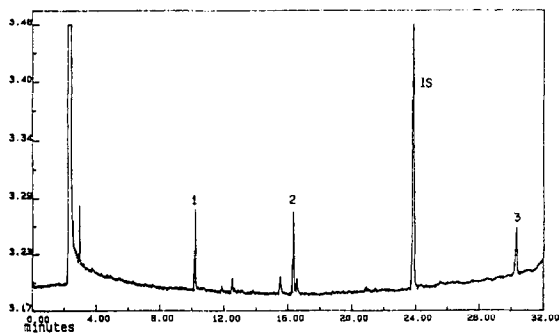


Fig. 1. Chromatogram of a mixture of standards. 1 = Lindane (0.14  $\mu\text{g}$ ); 2 = aldrin (0.13  $\mu\text{g}$ ); and 3 = *p,p'*-DDT (0.16  $\mu\text{g}$ ); IS = internal standard. Linear velocity of helium: 19 cm/s. Detector response: arbitrary units.

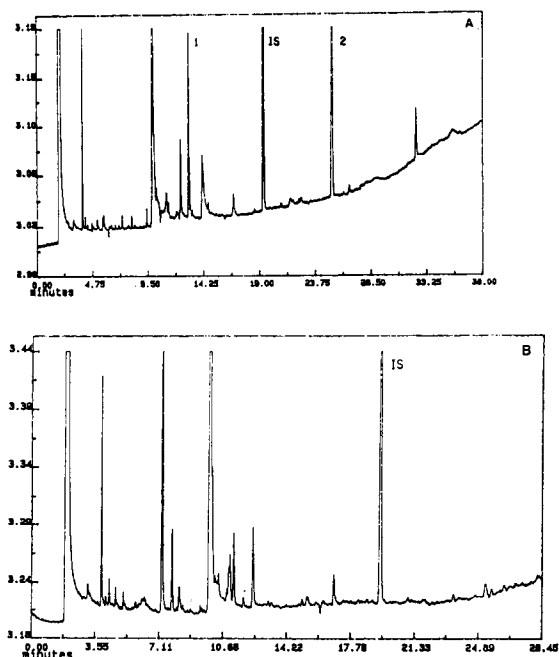


Fig. 2. Chromatograms obtained from blood of (A) an exposed worker and (B) an unexposed worker. 1 = Aldrin; 2 = *p,p'*-DDT; IS = internal standard. Linear speed of He: 25 cm/s.

220°C; analyser, 220°C. The electron energy was 70 eV.

Qualitative analyses of the samples were carried out with selective ion monitoring (SIM) at the following *m/e* rates: lindane, 219, 217 and 181; aldrin, 291, 263 and 261; and *p,p'*-DDT, 237, 235 and 165.

#### Detection limit

Using the above-mentioned analytical conditions, the detection limit for every pesticide was 0.01 µg per l of whole blood. Fig. 2 shows chromatograms from (A) an exposed worker and (B) an unexposed worker.

#### RESULTS AND DISCUSSION

Lindane, aldrin and *p,p'*-DDT were identified in all the samples from the occupationally exposed group. The overall results of the 71 farmers involved in different kinds of exposure are presented in Table I. These results have been divided into two groups in accordance with the

TABLE I  
OVERALL RESULTS

Compound	µg per l whole blood			
	Mean <sup>a</sup>	S.D.	Median <sup>a</sup>	Range
Lindane	0.021	0.021	0.010	<0.01–0.12
Aldrin	0.448	0.817	0.030	<0.01–3.32
<i>p,p'</i> -DDT	3.63	3.54	2.84	0.08–18.0

<sup>a</sup> Mean and median of results obtained from blood samples of 71 farmers.

type of crops and are presented in Table II along with the results from non-occupationally exposed workers. Samples from non-occupationally exposed people showed neither lindane nor aldrin but *p,p'*-DDT was present in almost all of them, as is shown in group 3 of Table II.

The levels of lindane and aldrin in the samples from the farmers were very low. The lindane concentrations found should be considered as trace levels, with no differences between groups, while in the case of aldrin the concentrations were also very low but a difference could be established according to the type of crop. The highest values were found in the group of straw-

TABLE II  
RESULTS ACCORDING TO CROPS

Compound	µg per l whole blood			
	Mean	S.D.	Median	Range
<i>Group 1 (strawberry, large strawberry, mixed crops)</i>				
Lindane	0.026	0.037	0.010	<0.01–0.12
Aldrin <sup>a</sup>	0.525	0.778	0.040	<0.01–3.19
<i>p,p'</i> -DDT <sup>a</sup>	2.11	2.01	1.06	0.12–8.51
<i>Group 2 (vegetables, ornamental plants, flowers, fruits and mixed crops)</i>				
Lindane	0.015	0.025	0.010	<0.01–0.12
Aldrin <sup>a</sup>	0.015	0.017	0.010	<0.01–0.05
<i>p,p'</i> -DDT <sup>a</sup>	4.58	3.59	3.75	0.08–14.0
<i>Group 3 (non-exposed population)</i>				
Lindane and aldrin	<0.01 µg/l			
<i>p,p'</i> -DDT	0.13	0.14	0.04	<0.01–0.41

<sup>a</sup> Differences of concentration between group 1 and group 2 are statistically significant.

berry farmers. The highest pesticide concentrations found in the samples from the farmers are those of *p,p'*-DDT, and they also allow us to distinguish between two groups, strawberry farmers and other crop farmers, the levels found in the former group being higher.

As far as lindane is concerned, it should be considered as a trace compound of uncertain origin (environmental contamination), while aldrin concentrations are higher in the strawberry group as it might have been used for the protection of this crop. *p,p'*-DDT concentrations are markedly higher and also allow us to establish a difference between the two above-mentioned groups. Nevertheless, a full assessment of both findings, *i.e.* higher *p,p'*-DDT concentrations and group differences, could not be carried out because comprehensive information about the impact of environmental contamination upon the studied groups was not available to us.

Whole-blood sample extraction with 5 ml of *n*-hexane is an easy, fast and accurate method, and when used under the conditions described herein allows us to achieve very low detection limits, in agreement with those reported by

Mussalo-Rauhamaa *et al.* [11] with a similar, but not fully described extraction procedure.

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