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Journal of Chromatography A, 719 (1996) 141–147

JOURNAL OF CHROMATOGRAPHY A

Determination of DDT and related compounds in blood samples from agricultural workers

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Abstract

An analytical method combining a solid-phase (C_{18}) clean-up and GC–electron-capture detection using a capillary column, was implemented to determine *p,p'*-DDT and its metabolites (*p,p'*-DDD and *p,p'*-DDE), as well as other organochlorine pesticides in whole blood samples from 30 farmers and 24 non-occupationally exposed workers. The average concentrations for the quantified pesticides, *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE, were 0.9, 1.5 and 8.0 μg/l whole blood for exposed workers and 0.3, 0.5 and 3.3 μg/l for unexposed workers, respectively. GC–MS was used to confirm the identity of the pesticides found. Solid-phase extraction and the protocol used give a cleaner analytical matrix, not only improving sensitivity and resolution, but also allowing analyses with smaller blood samples as compared to other methods.

1. Introduction

Since their first appearance organochlorine pesticides (OCPs) have been widely applied in agriculture all over the world and for a long time owing to their efficacy in pest control. In general terms, there are two consequences of their use. On the one hand, once in the environment these compounds enter the food chain and, as a consequence, animals and man are exposed. On the other hand, agricultural workers engaged in spraying pesticides were heavily exposed to OCPs. Thus, in some situations man may be exposed both environmentally and occupationally. Among the OCPs, DDT was the most widely used product until the mid 1970s when it was banned in many industrialized countries.

p,p'-DDT is metabolized via two main pathways. In the first, *p,p'*-DDT is metabolized to *p,p'*-DDA as the final product excreted in the urine, via *p,p'*-DDD, which itself is an insecticide (whose approved name is TDE). The second metabolic pathway in rats leads to different derivatives of the *p,p'*-DDE, which is the first metabolite and the only one of this route found in man [1].

In addition to its properties as a neurotoxicant, DDT is a potent hepatotumorogenic agent and allegations of a similar activity of DDE have also been made [2]. DDT, DDE, DDD (TDE), are highly lipophilic (partition coefficient between 5.69 and 5.98) accounting for the ease

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0021-9673/96/$15.00
SSDI 0021-9673(95)00284-7

with which they are bioconcentrated in fatty tissues of animals and man, including human milk [3].

Blood levels of pesticides and their metabolites are of interest since such levels reflect the body burden as there exist close correlations between their blood and fat concentrations, even in individuals not occupationally exposed [4,5]. Furthermore, in a study (1991–92) on agricultural workers occupationally exposed to pesticides, a clear differentiation of the blood levels of three OCPs (aldrin, lindane and DDT) by the kind of crop the farmers were engaged in, was established, as well as between occupationally and non-occupationally exposed individuals [6].

The present paper aims at: (1) describing a method that allows a quick determination of blood DDT, its metabolites and other OCPs, and which requires only half the sample volume to be treated (2 ml of whole blood, instead of 4 ml of serum in the original method of Saady and Poklis [7]), or (assuming an average haematocrit of 45%) roughly 1/4 the volume of blood sample to be drawn from every individual, with the additional advantage of avoiding serum separation clotting; and (2) assessing the occupational exposure to DDT of a group of agricultural workers by means of the blood levels of DDT itself and its metabolites in comparison with a control group of workers not occupationally exposed. This study (1992–1993) may be considered as part two of the initial study of Rosell et al. [6], both of which were conducted in the Maresme area (396 km^2, 200 000 inhabitants, Province of Barcelona, Autonomous Region of Catalonia, Spain).

2. Experimental

A group of 30 agricultural workers with a long occupational exposure to pesticides was studied. The exposure occurred through different routes, e.g. the preparation, mixing and use of, or contact with pesticide mixtures. These farmers were engaged in three different kinds of crops: strawberry and large strawberry (19 test persons), flowers, ornamental plants and market garden crops (4 test persons), and a group with different indoor jobs (workshop and office) at a flower market, all of them working at the same premises (7 test persons). A group of 24 non-occupationally exposed workers of a similar age range was studied as a control.

2.1. Sample collection

A 5-ml venous blood sample was drawn with a vacuum tube containing heparin as anticlotting agent from each worker under fasting conditions and before a work shift. Blood samples were immediately frozen and kept at −30°C until analysis.

2.2. Chemicals

The reagents used in the analytical procedure were purchased from different firms: 2,2,4-trimethylpentane (isooctane) was from Romil Chemicals (UK); methanol, reagent grade, from Merck (Darmstadt, Germany); ammonia solution, reagent grade, from Ferosa (Barcelona, Spain). The pesticides used as standards were: *trans*-nonachlor, *p,p'*-DDD, *o,p*-DDT and heptachlor epoxide from the Institute of Organic Chemistry, (Warsaw, Poland); *o,p*-DDD from Labor. Dr. Ehrenstorfer (Augsburg, Germany); dieldrin, chlordane, lindane, endrin (internal standard), endosulfan, *p,p'*-DDE and *p,p'*-DDT from PolyScience (Niles, IL, USA). Identification was confirmed by means of a standard reference material kit, 1583 Chlorinated Pesticides in Isooctane, from NBS (Washington, DC, USA).

2.3. Sample treatment

Each blood sample was extracted with methanol and centrifuged after thawing at room temperature and once homogenised. The extract was next passed through a solid-phase extraction cartridge packed with 500 mg of C_{18} bonded porous silica, Analytichem Bond Elut LRC from Varian (Harbour City, CA, USA), to separate and concentrate the analytes, according to the scheme shown in Fig. 1. To carry out this step,

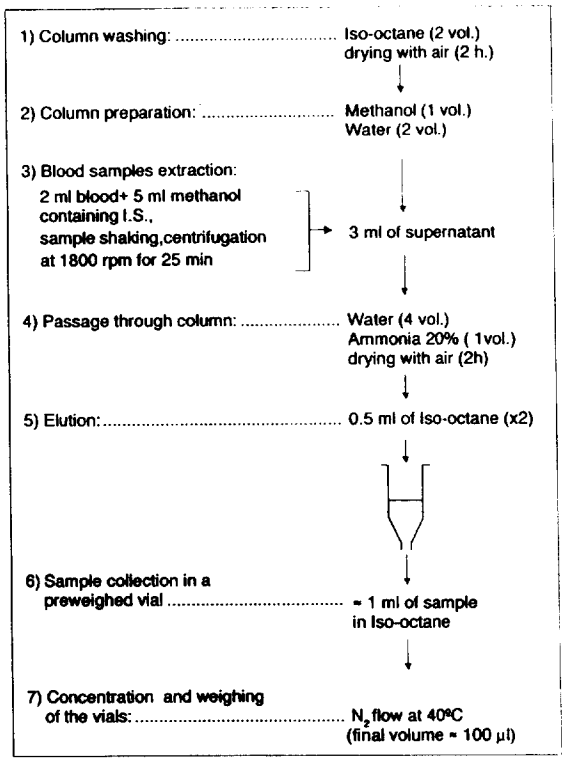

Fig. 1. Sample treatment scheme.

the extracts were grouped in batches of 10 each and every batch was treated by means of a vacuum processing station (Analytichem Vac Elut SPS24, Varian) at a standardized flow-rate of 2 ml/min. The extracts were processed simultaneously, but the flow was controlled individually and, for each sample, the process was stopped by means of a valve when the extract came level with the surface of the solid phase inside the cartridge. The eluates were collected in preweighed vials in order to control the final volume, after concentration using a nitrogen flow. Every sample batch was analyzed along with a blank of methanol following the same process.

2.4. Calibration

The linearity of the electron-capture detector (ECD) was studied and a calibration curve plotted for every quantified pesticide. Standard solutions obtained by weighing and dissolving appropriate quantities of each pesticide in iso-octane containing endrin as internal standard were used (see Fig. 2a).

Linearity ranges for quantified pesticides, calculated from an area/mass vs. injected mass graph, were: *p,p'*-DDT, 120–600 pg, *p,p'*-DDD, 120–600 pg and *p,p'*-DDE, 100–400 pg.

Calibration equations for these pesticides were the following: *p,p'*-DDT, $y = 1.49 \cdot 10^{-3} + 1.03 \cdot 10^{-2}x$, $r^2 = 0.997$; *p,p'*-DDD, $y = 1.78 \cdot 10^{-2} + 1.23 \cdot 10^{-2}x$, $r^2 = 0.999$ and *p,p'*-DDE, $y = 5.71 \cdot 10^{-3} + 2.31 \cdot 10^{-2}x$, $r^2 = 1.000$, where x is the concentration of pesticide in µg/l whole blood, and y is the area of standard/area of internal standard. Nevertheless, direct quantification, using an external standard of concentration very close to that of the sample (20% maximum difference between areas), was preferred because the pesticide concentrations in the samples studied were usually below the lower limit of the linear range.

2.5. Analytical conditions

Quantitative analyses were carried out using a Hewlett-Packard Model 5890 GC equipped with a ^{63}Ni ECD and a SPB 608 silica capillary column, 30 m × 0.25 mm I.D., 0.25 µm film thickness, from Supelco (Bellefonte, PA, USA). Temperatures were: column, initial 170°C (1 min), final 290°C (rates: 1°C/min until 180°C, 2°C/min until 250°C, and 3°C/min until 290°C); injector, 250°C; detector, 300°C. Helium was used as the carrier gas at a linear speed of 25 cm/s, and nitrogen as the auxiliary gas at 45 ml/min. The injection was in the split mode (1:60) and the injection volume was 2 µl.

The MS study was carried out with a Hewlett-Packard Model 5995 GC–MS using a SPB 5 silica capillary column, 30 m × 0.25 mm I.D., 0.25 µm film thickness, from Supelco. Temperatures were: column, initial 100°C (1 min), final 250°C (rates: 30°C/min until 180°C and 4°C/min until 250°C); injector, 250°C; transfer line, 270°C; ionization source, 270°C; analyzer, 270°C. The electron energy was 70 eV.

Qualitative analyses of the samples were carried out in the selected-ion monitoring (SIM)

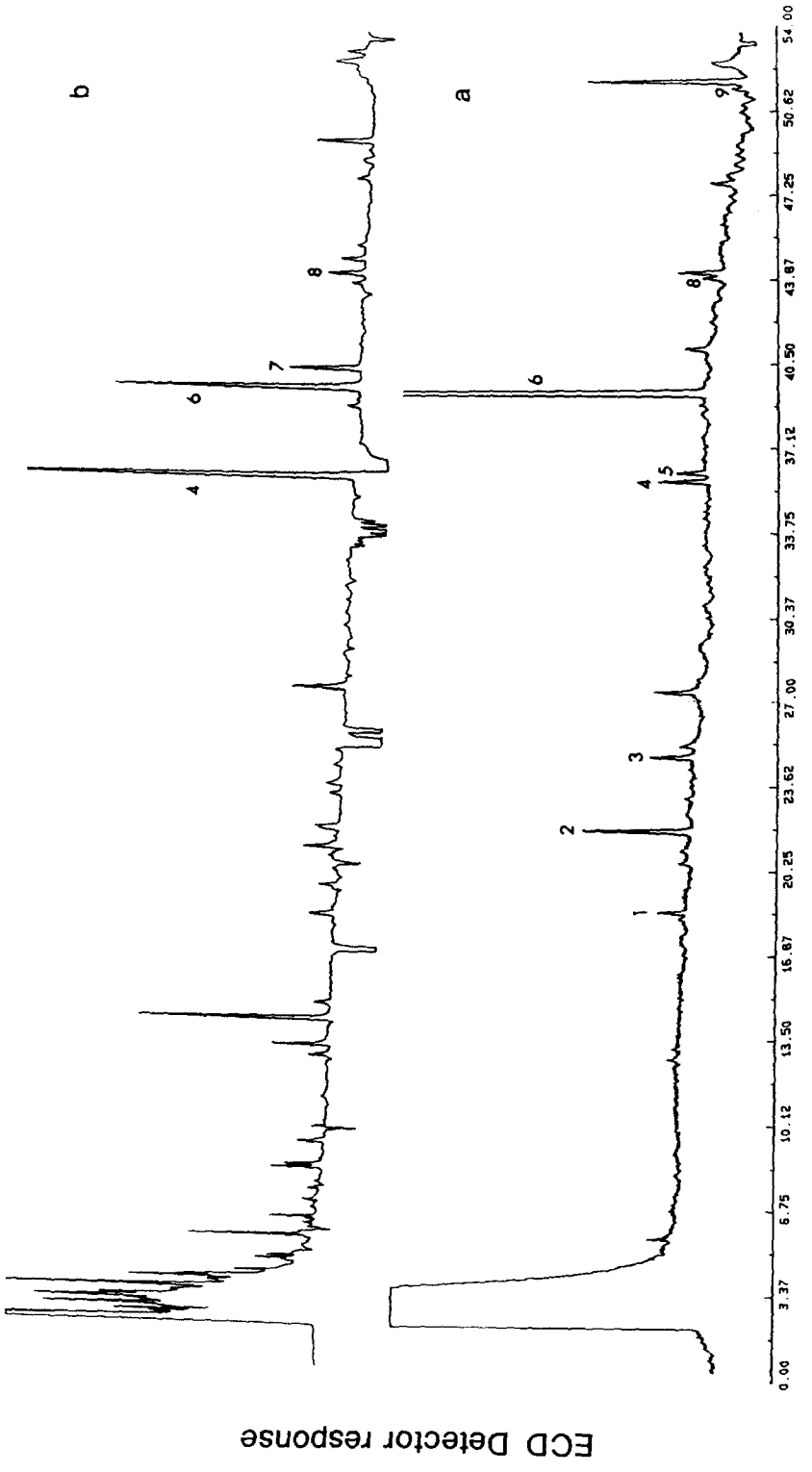

Fig. 2. (a) Chromatogram of a mixture of standards. Peaks: 1 = lindane, 2 = heptachlor, 3 = aldrin, 4 = *p,p'*-DDE, 5 = dieldrin, 6 = endrin, 7 = *p,p'*-DDD, 8 = *p,p'*-DDT, and 9 = methoxychlor. (b) Chromatogram from blood of exposed worker.

Table 1
OCPs and metabolites in blood studied with GC–MS

Compounds	t_R (min)	Monitored ions (m/z)
Identified		
Chlordane	24.1	371, 373 and 375
trans-nonachlor	24.2	407, 409 and 411
p,p'-DDE	26.3	246, 248 and 318
o,p-DDD	27.6	165, 235 and 237
p,p'-DDD	31.5	165, 235 and 237
o,p-DDT	31.8	165, 235 and 237
p,p'-DDT	35.4	165, 235 and 237
Methoxychlor	41.2	152, 227 and 274
Dubious		
Aldrin	16.4	261, 263 and 293
Heptachlorhepoxide	19.8	351, 353 and 355
Not identified		
Lindane	9.6	151, 217 and 219
Heptachlor	13.8	237, 272 and 274
Dieldrin	26.7	263, 345 and 380
Endosulfan (isomers)	23.5, 29.9	239, 241 and 339

mode at the m/z rates for different pesticides shown in Table 1.

2.6. Detection limits

The instrumental detection limits (IDL) and the method detection limit (MDL), as well as the limits of quantitation (LOQ), were calculated for every compound using the above-mentioned analytical conditions. The short-term noise (N) was calculated by Pool's procedure [8]. The IDL and MDL were calculated at a signal-to-noise ratio of 3, and the LOQ as the amount of analyte that gives a signal-to-noise ratio of 10. Detection limits obtained using this procedure are summarized in Table 2. *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT concentrations in all samples were above the limits of quantitation.

2.7. Pesticide recovery

The method of addition of different volumes of a mixture of the pesticides to be analyzed to blood pool aliquots from workers non-occupationally exposed was used to generate samples that were analyzed according to the described method. The additions corresponded to three specific levels for each pesticide: 5, 10 and 20 μg/l. The percentages of recovery found for each pesticide showed a wide range of variation, between 50% and 110%. The best results (90–110%) were for the pesticides with the highest retention times (DDE, DDT and methoxychlor).

Table 2
Instrument (IDL) and method (MDL) detection limits and limits of quantification (LOQ) for every organochlorinated pesticide determined

Pesticide	IDL (pg)	MDL (μg/l whole blood)	LOQ (μg/l whole blood)
Lindane	0.5	0.03	0.10
Heptachlor	0.2	0.01	0.03
Aldrin	0.3	0.02	0.07
p,p'-DDD	0.6	0.03	0.10
p,p'-DDE	0.3	0.06	0.19
Dieldrin	0.5	0.03	0.10
p,p'-DDT	0.7	0.04	0.13

2.8. Interferences

Obtaining cleaner analytical matrices is the main rationale for the use of a solid-phase extraction system in the treatment of the samples. Nevertheless, this brings about an increase in the risk of contamination with a number of compounds, contamination that actually occurred when the first tests were carried out. This necessitated studying the origin of the contamination that occurred both in samples and blanks. Thus, a GC–MS study was carried out to identify the interferences.

The most common compounds identified as interferences were aliphatic hydrocarbons, mainly alkanes and alkenes C_8–C_{12}, but not the cyclic ones. These compounds could originate from the isooctane used as eluent. Likewise, longer chain hydrocarbons C_{18}–C_{36} were detected as interferences from the octadecylsilane cartridges as Junk et al. have previously described [9]. Other identified interfering compounds came from the chemical breakdown of the octadecylsilane

chains of the cartridge. For instance, the use of methanol in the process gives rise to methyl ethers. In GC–SIM-MS, using the m/z 89 ion corresponding to $[(CH_3O)Si(CH3)_2]^+$ [10], more than 40 peaks were detected. Finally, the major group of interfering compounds, giving a substantial ECD response, were the phthalates, which are used as plasticizers, and which come from the repeatedly used glassware (surface adsorption), an other laboratory material. Among them, diethyl, dibuthyl and bis(2-ethylhexyl) phthalates were identified.

Setting up a stringent analytical guideline with specific reference to laboratory glassware cleaning and avoiding the use of polymers, has proven to be of great practical importance, as the interferences were minimized after this protocol was implemented.

3. Results and discussion

p,p'-DDT, p,p'-DDD, and p,p'-DDE were found in appreciable amounts, so that they could easily be quantified in all blood samples. o,p-DDT, o,p-DDD and methoxychlor could be detected only in a few samples. The presence of aldrin and heptachlor epoxide could not be definitively confirmed in any sample because they were found only in a few samples at the detection limit level (MDL). The overall results of the 30 agricultural workers using pesticides along with those from non-occupationally exposed workers are presented in Table 3, in which total DDT stands for the sum of p,p'-DDE, p,p'-DDD and p,p'-DDT in each sample. The mean levels of these compounds found in the farmers were significantly higher than the mean levels in the control group.

Table 3
Pesticide mean values (μg/l) whole blood and standard deviations of samples from occupationally exposed workers and a control group

	Exposed (n = 30)		Control (n = 24)	
	Mean	S.D.	Mean	S.D.
Total DDT	10.4	7.5[a]	4.1	4.4
p,p'-DDE	8.0	6.3[a]	3.3	3.8
p,p'-DDD	1.5	1.1[a]	0.5	0.3
p,p'-DDT	0.9	0.6[a]	0.3	0.3

[a] Values significantly higher than those of the control group.

The ratio of the blood concentrations of the above-mentioned last three compounds is roughly 8:1.6:1 in the group of occupationally exposed workers, which is similar to the ratio found in the control group (10:1.6:1). From these results it is obvious that the preponderant compound in both groups is p,p'-DDE. This is a finding common to similar studies. However, this ratio shows only little variation between two groups of occupationally exposed workers (3.3:0.7:1 and 2.1:0.6:1), while a wide variation is seen among general population groups (from 1.5:0.5:1 to 6.75:1:1) from the same country (1975–1979), former Yugoslavia [11]. The ratio of the serum concentrations of p,p'-DDE and p,p'-DDT in individuals from three farming cooperatives in southern Honduras with heavy aerial pesticide spraying was 15.8:1, and 18.3:1 among the controls [12].

The level of p,p'-DDE in the control group of our study (Table 3) is slightly lower than that found in four population groups from Zagreb (medians: 7, 4, 8, and 2 μg/l serum, period 1985–1990), but the corresponding level in the occupationally exposed group is of the same order. Similar considerations may be applied to p,p'-DDD and p,p'-DDT, except for the levels in the occupationally exposed group from the Maresme area, which are higher than those in the Yugoslavian population groups. These results also showed a marked downward trend for the p,p'-DDE levels among the population groups in Yugoslavia over the period 1975–1990. This decrease may be attributed to restrictions in the use of organochlorine pesticides introduced almost twenty years ago [13]. In spite of the similarity of the blood p,p'-DDE concentrations in both Catalan and Croatian non-occupationally exposed groups, such a hypothesis cannot be extended to the population of the Maresme area owing to the lack of reliable data from the past. Moreover, the current relatively high levels of p,p'-DDT, p,p'-DDD and p,p'-DDE among

the farmers in the Maresme area suggest that they were subjected to a moderate pesticide exposure at least not long ago, otherwise these values would probably be lower than they actually are.

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