Bull. Environ. Contam. Toxicol. (1991) 46:803–809

Environmental Contamination and Toxicology

Persistent Organochlorine Pesticides in Blood Serum and Whole Blood

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Since organochlorine pesticides were introduced for plant protection and sanitation, they have been of great benefit in the control of pest populations and in combating the spread of infectious diseases. Unfortunately, they accumulate in the environment and this has resulted in a ban on their use. Nevertheless, they are still widely used in tropical countries as the insecticides of choice (Sant'Ana et al.1989; Steinberg et al.1989). Widespread use of these pesticides has contaminated large areas of the environment, including the human body, which forms a last link in the food chain (Griffith et al.1985; Pines et al.1987).

An analytical procedure was elaborated to find out the extent of contamination of the human body by persistent residues of organochlorine pesticides and to determine the gradient between adipose tissue and biological fluids, which correlates with bioaccumulation and dissipation processes. The method has two important advantages: it is a simple, low-cost semi-micro, and it makes it possible to determine free and bound pesticides. In the sample clean-up step sulfuric acid was used, which precipitates fats and hydrolized a complex of pesticides with endogenous compounds of human body origin (Maliwal et al.1981; Gupta et al.1978; Waliszewski et al.1982; 1983). The sulfuric acid also removed many compounds such as phthalate esters, which interfere in the GLC analysis by overlaping the peaks on many organochlorine pesticides (Waliszewski et al.1990). For analysis, the following persistent organochlorine pesticides whose residues exist in the environment were selected due to their use in agriculture and sanitation. They are: HCB, lindane and alfa-, beta-, delta-, epsilon-HCH isomers presented in technical HCH, DDT and its metabolite DDE, heptachlor and heptachlor epoxide, alfa-, beta-endosulfan and endosulfan sulfate, mirex and kepone. Chlordane and its isomers, dieldrin and methoxychlor, are degraded during clean-up of extracts with concentrated sulfuric acid.

Burdening of human body with industrial PCBs has been significant in industrialized countries (Skaare et al.1988). Generally, the contamination by organochlorine pesticides is much higher in the developing and tropical countries than it is in the industrialized

countries, caused by their common use in agriculture and sanitation, and limited use of industrial PCBs.

MATERIALS AND METHODS

The reagents were purchased by Baker S.A. de C.V. and Técnica Química S.A., Petroleum ether (boiling temp. 40-50°C) distilled, acetone distilled, acetic acid and sulfuric acid of analytical grade, anhydrous sodium sulfate heated overnight at 650°C. Analytical standards of organochlorine pesticides were obtained from Poly Science Corp. Before analysis, all reagents were tested for impurity presence. If such compounds were detected, the reagents were cleaned up additionally by fractional distillation and chemical purification to obtain a gas chromatogram without the peaks of impurities.

Glassware equipment was suitable for a laboratory of pesticide residues. All equipment was glass to prevent contamination by undesirable common substances. The glassware was washed with concentrated KOH solution and then with concentrated sulfuric acid, rinsed with distilled water, distilled acetone and petroleum ether.

Gas liquid chromatograph, Varian Model 2100 equipped with ^{63}Ni electron capture detector and glass column 185 cm x 2 mm id., packed with 1.5% OV-17 + 1.95% OV-210 on 80-100 mesh Gas Chrom Q. Operating conditions were as follows: nitrogen carrier gas at 30 ml/min; temperatures: column 185°C, injector 250°C, detector 300°C. Injection volume 1 ul.

To a 25 ml tube with glass stopper, containing the determined volume (maximum 5 ml of anticoagulated whole blood or blood serum sample), acetic acid was added in a ratio of (1 + 1). The tube was left for 30 minutes, except for occasional mixing. This time is needed to hydrolize and liberate pesticides from complexes with endogenous substances of the blood. Then, organochlorine pesticide residues were extracted three times with 10 ml portions of a mixture of petroleum ether and acetone (9 + 1). The extracts were collected in a 100 ml separatory funnel and washed twice with 25 ml portions of distilled water to remove traces of acetic acid, acetone and water soluble substances. The petroleum ether extract was dried by filtering it through a sodium sulfate layer. Then, sodium sulfate was rinsed with petroleum ether and rotary evaporated to about five mililiters. The concentrated extract was transfered quantitatively with petroleum ether to a calibrated 10 ml tube with glass stopper and 1 ml of concentrated sulfuric acid was added. The contents were vigorously shaken for about one minute and left for about three minutes to ensure good phase separation. The petroleum ether phase was taken out with a pipette and dried by passing it through a sodium sulfate layer to a 50 ml roundbottomed flask. The sodium sulfate layer was rinsed several times with petroleum ether. The combined extract and rinses were rotary evaporated to a few drops. The remains were transfered quantitatively with petroleum ether to a

1 ml calibrated tube and the final volume was adjusted to 1.0 ml. The qualitative and quantitative determination was performed by gas chromatography with electron capture detector.

RESULTS AND DISCUSSION

For the recovery study, a sample of bovine blood serum and anti-coagulated whole blood, obtained from a slaughterhouse, was analyzed to select the one which had the lowest organochlorine pesticide residue content. To a 25 ml tube, 1.0 ml of a standard solution of pesticide mixture in hexane was added. Hexane was evaporated, leaving the tube in laboratory condition. Then 5.0 ml of the sample was added. The content of the tube was shaken vigorously and placed in the refrigerator at +8°C for 24 hours, the time necessary for binding the pesticides to active biological blood compounds. After 24 hours the sample was acidified with acetic acid to hydrolize pesticides from complexes, extracted and cleaned up by the method described above.

The recovery values of ten repeated analyses and their statistical evaluation are presented in Table 1. The recovery values of fifteen researched pesticides were about 90%, except for alfa- and beta-endosulfan. The standard deviation (SD) and coefficient of variation (v%) were low, indicating excellent repeatability of the analytical procedure. Significant differences observed among the recovery values of alfa- and beta-endosulfan resulted from the partial conversion of beta-endosulfan to alfa-endosulfan under the influence of concentrated sulfuric acid during the clean-up step.

The use of acetic acid in the extraction of organochlorine pesticide residues from the blood samples, liberates the pesticides from complexes, that are bound to the plasma proteins. Moreover, acidic conditions prevent emulsion formation during the extraction step. The use of non-polar solvents, such as petroleum ether or hexane for the extraction of alkaline samples, rich in the protein complexes, results in emulsion formation, which causes many analytical difficulties (Gupta et al.1978). Usually, the emulsion should be broken up by sonication and centrifugation of the extracts, which adds to the analytical work.

Precipitation of fats from hexane extracts with concentrated sulfuric acid is recommended for its determination, precipitating quantitatively after being shaken with sulfuric acid. On the other hand, extracts rich in fats, cleaned up in the adsorption columns of florisil, aluminium oxide or silica gel never retain the total amount of fats, because the polarity of fats is similar to those of pesticides (Tonogai et al.1989). Our research of dry residues in the eluates of the adsorption columns of florisil, aluminium oxide deactivated with 4% water and silica gel deactivated with 10% water, always indicated the presence of dry residues, corresponding to fats. Gas chromatograms of the samples cleaned up in the adsorbent columns were characterized by the elevated and drifted base line. In contrast, those cleaned up

Table 1. Statistical evaluation of obtained results from the fortification study ($\bar{x}$=mean values; SD=standard deviation; v%=coefficient of variation; n=10

Compounds	Fortification levels ng/ml	Blood serum $\bar{x} \pm SD$	v%	Whole blood $\bar{x} \pm SD$	v%
HCB	0.5	93.8 ± 5.3	6.1	92.1 ± 4.9	5.8
alfa - HCH	0.5	92.7 ± 4.9	5.8	90.4 ± 5.1	6.0
beta - HCH	0.8	90.2 ± 6.8	7.9	89.3 ± 7.1	8.2
delta - HCH	0.7	91.8 ± 4.2	4.9	90.9 ± 4.7	5.8
epsilon - HCH	0.8	88.4 ± 6.6	7.6	87.2 ± 7.2	8.0
lindane	0.7	95.9 ± 4.8	5.6	94.8 ± 5.3	6.2
aldrin	0.7	89.9 ± 5.7	6.4	88.3 ± 6.1	7.2
heptachlor	1.0	90.3 ± 6.0	6.9	89.4 ± 6.5	7.4
heptachlor epoxide	0.8	92.3 ± 4.8	5.5	91.6 ± 5.1	5.9
pp' DDE	1.0	89.7 ± 6.4	7.0	88.1 ± 6.8	7.8
op' DDT	2.0	92.0 ± 5.1	5.8	90.9 ± 5.4	6.3
pp' DDT	2.0	93.1 ± 4.4	4.9	92.8 ± 4.7	5.3
alfa - endosulfan	2.0	134.6 ± 8.7	9.8	138.5 ± 8.6	9.7
beta - endosulfan	2.0	72.6 ± 8.6	9.3	71.9 ± 8.4	9.2
endosulfan sulfate	2.0	90.7 ± 5.9	6.7	90.1 ± 5.4	6.5
kepone	3.0	84.7 ± 6.2	6.9	85.1 ± 5.9	6.6
mirex	3.0	94.1 ± 4.7	5.6	93.3 ± 5.5	6.0

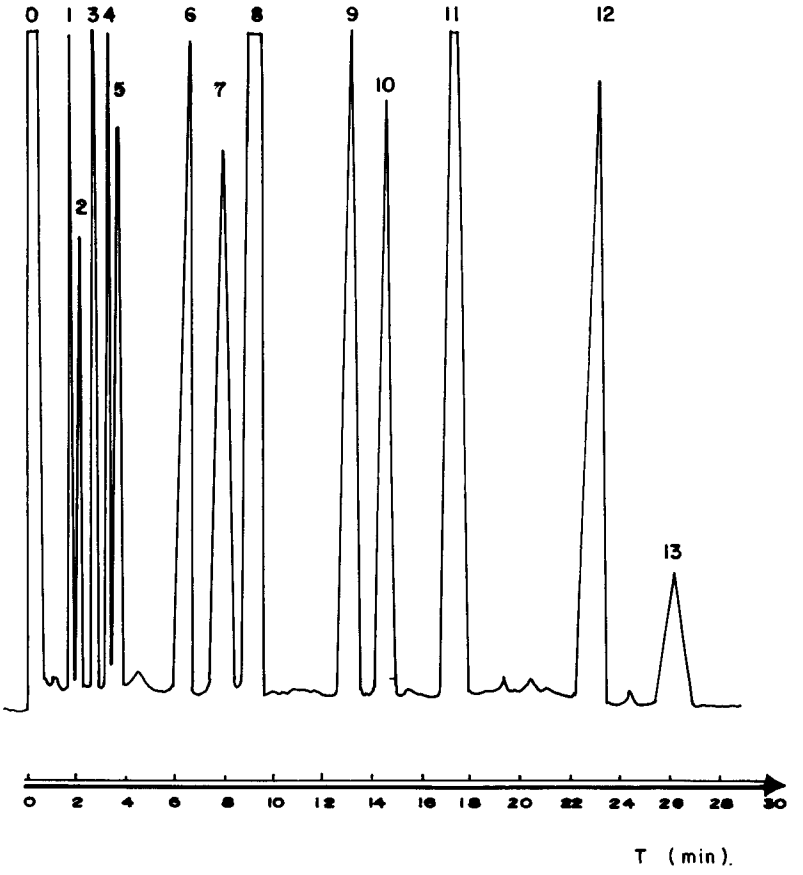

Figure 1. A gas chromatogram one of the human whole blood sample. 0-solvent, 1-HCB, 2-alfa-HCH, 3-gamma-HCH, 4-beta-HCH, 5-heptachlor, 6-heptachlor epoxide, 7-alfa-endosulfan, 8-pp'DDE, 9-op'DDT, 10-beta-endosulfan, 11-pp'DDT, 12-endosulfan sulfate, 13-mirex

with sulfuric acid presented a stable base line during the prolonged time (Fig.1). Moreover, after analysis of a few fat samples cleaned up in the adsorption columns, the ECD characteristics indicate contamination of the radioactive foil. This was not observed during analysis of extracts cleaned up with concentrated sulfuric acid, which indicates that this technique is superior.

The use of concentrated sulfuric acid in the clean-up step does not permit the separation of PCBs from organochlorine pesticides. The latest monitoring study of the burdening of the human body with PCBs indicates a tendency to decrease (Pines et al.1988; Mes 1990). Thus, if the characteristic image of PCBs on the gas chromatogram is presented, a column chromatographic separation of PCBs from organochlorine pesticides should be made after clean-up of extracts with sulfuric acid.

In a previous monitoring study of human fat tissues, concentrated sulfuric acid as a clean-up medium was used, resulting in elevated residues (Szymczyński et al.1986). These and other observations made by Páldy et al.(1988) confirm that the pool of organochlorine pesticides in the human body was found as free-easily detected and bound, requiring hydrolytic conditions to show its presence in the analyzed samples (Waliszewski et al. 1983).

An important contamination of the environment by the phthalate esters was recently observed (Thurén 1988; Wams 1987). During the clean-up of the extracts in the adsorption columns, they were eluted by the same volume as the pesticides. Moreover, during the GLC determination they overlap the peaks of some organochlorine pesticides. The clean-up of extracts with concentrated sulfuric acid removed the ubiquitous phthalate esters, thus eliminating the disturbance in the GLC determination and giving a truer determination of organochlorine pesticide residues (Waliszewski et al.1990).

Under the sponsorship of CONACYT and the University of Veracruz, the described method has been introduced for monitoring the study of human fluids contamination by residues of persistent organochlorine pesticides. This method is rapid, low cost and uses micro glassware. It makes possible the determination of bound and free residues and provides a truer measure of the contamination of human body fluids by persistent organochlorine pesticides.

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Received June 15,1990; accepted December 20,1990.