A Method for the Determination of Chlorinated Pesticides in Human Semen

GRZEGORZ A. SZYMCZYNSKI AND STEFAN M. WALISZEWSKI

From the Department of Andrology, Medical School of Poznań, Poznań, Poland

A method is described for the quantitative and qualitative determination of BHC and DDT isomers and HCB and Methoxychlor (DMDT) in human semen. Pesticide residues were extracted with petroleum ether, separated from other co-extractives on Florisil column, and analyzed by electron-capture gas chromatography. Recoveries were 96.4 to 110.8% and fortification levels were 0.02 to 0.20 μg/g, respectively.

Key words: chlorinated pesticide, human semen, gas chromatography.

There are many reports dealing with the determination of residues of chlorinated pesticides in all environmental elements, as well as in blood and other human tissues. In addition, much has been written about the toxic effects of these substances, and their influence upon genetic material (Caplan, Thompson, and Hebb, 1979; Puiseux-Dao et al, 1977). Because of the possible effects of chlorinated pesticides on reproductive functions, we decided to design an analytical method for their detection and measurement in human semen.

Materials and Methods

Reagents and Equipment

The following reagents and equipment were used: petroleum ether (boiling temperature 40–50 C); diethyl ether (peroxide-free, with 2% ethyl alcohol); sodium sulfate (anhydrous); Florisil (Floridin Company, Berkeley Springs, West Virginia, 25411, USA; activated for 16 hours at 135 C); gas chromatograph (Varian Model 2100, Walnut Creek, California, USA); evaporator (Büchi, Switzerland).

Pesticide Standards

1,2,3,4,5,6-hexachlorobenzol (HCB) was obtained from the Institute of Physical Chemistry (Warszawa, Poland); alpha-1,2,3,4,5,6-hexachlorocyclohexane (alpha-BHC), beta-1,2,3,4,5,6-hexachlorocyclohexane (beta-BHC), gamma-1,2,3,4,5,6-hexachlorocyclohexane (gamma-BHC), and delta-1,2,3,4,5,6-hexachlorocyclohexane (delta-BHC) from Celamerck (Ingelheim am Rhein, West Germany); epsilon-1,2,3,4,5,6-hexachlorocyclohexane (epsilon-BHC) from Merck (Darmstadt, West Germany); 1,1-dichloro-2,2-di(4,4́-chlorophenyl)-ethylene (DDE), 1,1,1-trichloro-2,2-di(2,4́-chlorophenyl)ethane (oṕ-DDT), and 1,1,1-trichloro-2,2-di(4,4́-chlorophenyl)ethane (pṕ-DDT) from Analabs (North Haven, Connecticut, 06473, USA); and 1,1,1-trichloro-2,2-di(4,4́-methoxyphenyl)ethane—Methoxychlor (DMDT) from Poly Sciences Corp. (Evanston, Illinois, USA).

Sample Preparation

The pool of human semen was formed by collecting randomly chosen ejaculates. Ten separate human semen specimens, each of 5 g, were weighed into glass beakers that contained a dry residue of the evaporation of a hexan standard pesticide solution. The contents in beakers were mixed and left for an hour in room conditions. After that time they were processed according to the extraction and purification method described below.

Extraction and Purification

Each semen sample was weighed and ground with enough anhydrous sodium sulfate to form a coarse powder. Thereafter, samples were quantitatively transferred to the chromatographic column and the pesticides were extracted with 150 ml of petroleum ether. The eluate was evaporated at <40 C in the rotary evaporator to a volume of approximately 5 ml. The chromatography column clean-up on Florisil was done according to Stimac (1979). Petroleum ether was poured into a chromatographic column. A plug of glasswool was placed just above the stopcock and approximately 20 ml of petroleum ether and 20 g of Florisil were added. When the adsorbent had settled, a 2 to 3 cm layer of sodium sulfate was added. The stopcock was turned on and the solvent was allowed to run through the column.

Reprint requests: G. A. Szymczynski, M.D., Department of Andrology, Medical School of Poznań, Jackowskiego 41, PL-60-513, Poznań, Poland.

Submitted for publication December 3, 1980; revised version received April 7, 1981; accepted for publication June 17, 1981.

0196-3635/82/0300/0149/$00.60

TABLE 1. Recovery of Chlorinated Pesticides from 5.0 g of Human Semen

Analyzed Compound	Fortification Levels (μg/g)	Mean Recovery (%)	Standard Deviation (±SD)	Coefficient of Variation (%)
HCB	0.02	96.4	1.9	2.0
Alpha-BHC	0.02	97.1	3.5	3.6
Beta-BHC	0.04	98.8	4.8	4.9
Gamma-BHC	0.04	98.1	2.6	2.7
Delta-BHC	0.04	99.7	2.1	2.1
Epsilon-BHC	0.04	97.1	1.7	1.8
DDE	0.04	99.4	2.6	2.6
Op'-DDT	0.06	103.0	4.3	4.2
Pp'-DDT	0.10	110.8	4.5	4.1
DMDT	0.20	103.7	2.9	2.8

Florisil was prewashed with 50 ml of a mixture of diethyl ether and petroleum ether (30:70), then with 35 ml of petroleum ether, and approximately 1 cm of liquid was left above the sodium sulfate layer in the column. The solvent was discarded. The concentrated sample was quantitatively transferred to the Florisil column and the pesticides were eluted with 250 ml of a mixture of petroleum ether-diethyl ether (94:6). The eluate was concentrated at <40 C in the evaporator to approximately 2 ml. This concentrate was quantitatively transferred to a 5.0 ml volumetric tube. The volume was adjusted to 5.0 ml and the sample was thoroughly mixed by shaking. The gas chromatographic analysis was performed using gas chromatograph (Varian Model 2100) with electron capture detector $Sc^{3}11$. The column was glass, U-shaped, 360 cm × 2 mm, and was packed with 1.5% OV-17 and 1.95% OV-210 on 80 to 100 mesh Gas Chrom Q. The following operating conditions were used: carrier gas, nitrogen at the flow rate of 30 ml/minute; column temperature, 185 C; temperature detector, 210 C; temperature injector, 250 C.

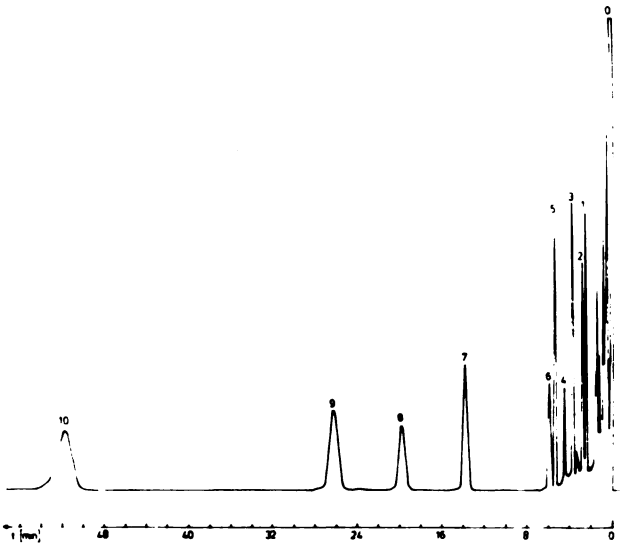

Fig. 1. Gas chromatogram of pesticide-fortified human semen: O = petroleum ether; 1 = HCB; 2 = alpha-BHC; 3 = gamma-BHC; 4 = beta-BHC; 5 = delta-BHC; 6 = epsilon-BHC; 7 = DDE; 8 =op'-DDT; 9 = pp'-DDT; 10 = DMDT.

Results and Discussion

The recoveries of chlorinated pesticides in ten samples of human semen are presented in Table 1. The amount of pesticide added ranged from 0.02 μg/g for HCB and alpha-BHC to 0.20 μg/g for DMDT. For most compounds, the average recoveries were close to 100%. Average recoveries ranged from 96.4 to 110.8%. The chromatogram of the samples of human semen with pesticide residues appeared as clean as those obtained for the standard samples of this product. A typical chromatogram of a 5 g sample of human semen with added pesticide is shown in Fig. 1. The chromatogram is relatively free from extraneous peaks. All pesticides examined were easy to identify and could be quantitatively determined.

Using this procedure, we have examined the pesticide residues in human semen in a random population and found that the average values of pesticides obtained ranged from 0.001 to 0.005 μg/g (Szymczynski and Waliszewski, in press). We expect that because of excellent recoveries, this method may be used for the determination of the residues of dieldrin and its derivatives, and of heptachlor and heptachlor epoxide in human semen.

References

Caplan YH, Thompson BC, Hebb JH, Jr. A method for the determination of Chlordecone (Kepone) in human serum and blood. J Anal Toxicol 1979; 3:202–205.

Puiseux-Dao S, Jeanne-Levain N, Roux F, Ribier J, Borghi H, Brun C. Analyse des effets du lindane, insecticide organochloré au niveau cellulaire. Protoplasma 1977; 91:325–341.

Stimac RM. Rapid florisil clean-up method for analysis of chlorinated pesticides residues. J Assoc Off Anal Chem 1979; 62:85–88.

Szymczyński GA, Waliszewski SM. Content of chlorinated pesticides in human semen of random population. Int J Androl, in press.