Bull. Environ. Contam. Toxicol. (1985) 34:518–526

Environmental Contamination and Toxicology

Rapid and Low-cost Method for Monitoring Determination of Selected Chlorinated Pesticides in Feed Mixtures

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Considerable quantities of organochlorine insecticides are still used world-wide and their persistent residues are widely distributed through the environment /Wahid and Sethunathan 1979/. On the other hand, there is still a huge need for feed mixtures and their components in animal breeding /Buchholz 1979/. As technical BHC and Lindane are still used in many countries and a substantial increase in contamination of the environment with DDT has been observed in the world the problem arises as to how determine precisely the contamination of animal feeds with the residues. For example, our control monitoring study showed that in Poland total DDT was 17.5 ppm in oat bran in 1981, and in 1982 it was 18.5 ppm in the same crop /unpublished data/. Therefore, a new rapid and low-cost method was developed for determination of these substances in feeds, making possible continuous monitoring of the level of contamination of feed mixtures.

MATERIALS AND METHODS

Feed mixtures and feed components were obtained from different feed factories in a routine monitoring programme.

The following equipment was used: Sorval homogenizer; rotary evaporator; gas chromatograph with ECD; round-bottomed flasks of 100 and 500 ml; separatory funnel of 1000 ml; measuring cylinders of 100 and 500 ml; calibrated tubes of 1 ml and 10 ml; pipettes of 1,2 and 5 ml.

The following reagents were used: acetonitrile purified and distilled; distilled petroleum ether bp. 40-50°C; sodium sulfate analytically pure, anhydrous, roasted at 600°C; distilled water; cottonwool; sul-

furic acid, analytically pure; mixture of acetonitrile and water /65+35/; 5% water solution of sodium sulfate.

150 ml of the acetonitrile-water mixture were poured over a 10 g sample in a mason jar of the OMNI MIXER® DuPont homogenizer and the content was left for 16 h. After that period it was mixed for 3 min, the supernatant was vacuum filtered through a layer of Celite 545® and transferred to a separatory funnel of 1000 ml; the sample was mixed additonally 2 times, each time with 100 ml of the mixture of acetonitrile and water.

500 ml of a 5% solution of sodium sulfate were added to the combined extracts in the separatory funnel and were extracted 3 times, each time with 100 ml of petroleum ether. The ether extracts were dried by filtering through sodium sulfate and condensed to approximately 5 ml in a rotary evaporator at the temperature below 40°C.

The condensed ether extract was transferred quantitatively with petroleum ether to a calibrated tube of 10 ml and the volume was adjusted to 10.0 ml. One ml of concentrated sulfuric acid was added and the content was vigorously shaken for about 1 min. Thereafter, it was left for 2-3 min, and 5.0 ml of the supernatant were pipetted out to the funnel with sodium sulfate. The sodium sulfate layer was washed several times with petroleum ether. The collected ether and ether washings in the round-bottomed flask of 100 ml were condensed in a rotary evaporator almost to dry /not entirely dry/. The remains were transferred in petroleum ether to a calibrated tube of 1.0 ml and the final volume was adjusted to 1.0 ml.

A Varian gas chromatograph Model 2100 with ECD was used. The column was glass, 360 cm x 2 mm, packed with 1.5% OV-17 + 1.95% OV-210 on 80-100 mesh Gas Chrom Q. The temperatures: column - 185°C, detector - 250°C, injector - 250°C. Carrier gas was nitrogen at the flow rate of 30 cm^3/min. The obtained recoveries and statistical analysis of the method are presented in Table 1.

The chromatograms were calculated by the following formula:

$$x = \frac{hpr \times C \times 1}{hst \times 5}$$

x - content in the studied sample in ppm
hpr - height or area below peak of sample

hst - height of area below peak of standard
C - concentration of the standard
1 - final volume of the sample extract
5 - weight of analyzed sample in g /only 5 g because after shaking with sulfuric acid only a half, i.e. 5.0 ml of the ether extract volume, was taken/.

RESULTS AND DISCUSSION

In order to determine precision of the presented analytical method recovery studies were performed 10 times each for feed mixtures and feed components. A mixture of standards was added 1 h before the sample was flooded with the mixture of acetonitrile and water /65+35/. The obtained recoveries for HCB, DDE and isomers of HCH and DDT /Tab.1/ oscillated around values over 90% and standard deviation was rarely higher than $\pm$ 6.0. This indicates a very good recovery of the examined substances from the samples, and an excellent repeatability of the presented method. Besides, the detection limits presented in the Table point to a good sensitivity of the method which permits for determination of HCH, DDT and HCB in feeds at sub-ppm range. The presented analytical method for feed mixtures and feed components is useful not only for this purpose but also for analyzing residues of chlorinated insecticides in grains and grain products which are the main components of feed mixtures. The use of the mixture of acetonitrile and water /65 + 35/ and the period of initial maceration of 16 h resulted in a release of the residues of substances under study from the analyzed material as well as in an inconsiderable transition of fat substances to the extract. The application of concentrated sulfuric acid instead of conventionally used adsorbents not only gives substantial economical savings but the acid itself induces breakage of the complex of pesticide and endogenous substance, thus giving a truer image of contamination of feed mixtures with the residues of chlorinated compounds.

The analysis by the presented method of dry residues of extracts of 10 g of arachide meal and 10 g of soy meal /substances rich in oil/ gave respectively 6 and 29 mg of dry residue, measured gravimetrically. These data visualize positive aspects of clean-up by the presented method. Financial advantages of this technique and good chromatographic image /Fig. 1,2,3,4/ recommend it for routine monitoring of contamination of feeds and grains with persistent chlorinated pesticides.

Contamination of animal feeds with residues of chlo-

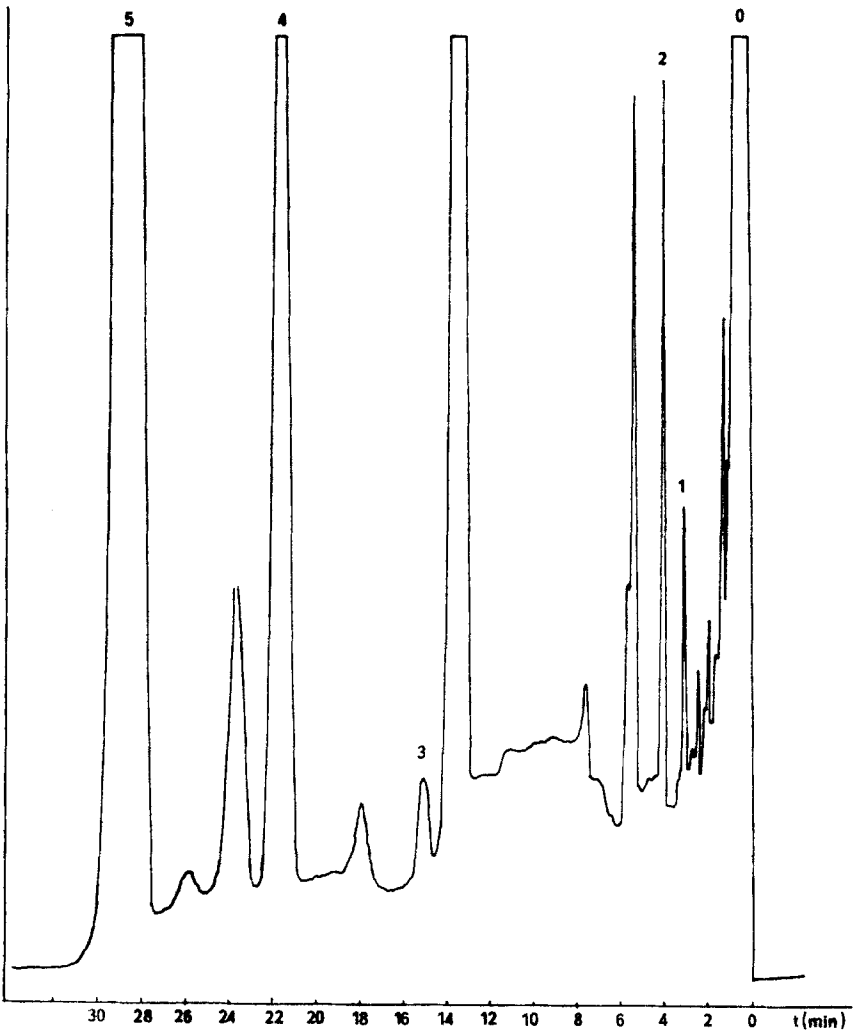

Figure 1
This figure represents a gas chromatogram of oat bran /in ppm/.
0 - solvent; 1 - alpha-HCH, 0.008; 2 - gamma-HCH, 0.088; 3 - pp′-DDE, 0.05; 4 - op′-DDT, 2.13; 5 - pp′-DDT, 15.323.

rinated pesticides result, in turn, in increased contamination of humans /Buchholz 1979/. Monitoring studies of feed mixtures, feed concentrates, grains and grain components of feed mixtures show distinctly different positive results in corn meal. But if one compares the results for feed concentrates and feed mixtures, evident increase is seen in the content of total DDT together with increase of the grain component. This, as well as increasing DDT levels in the whole ecosystem observed in recent years necessitate

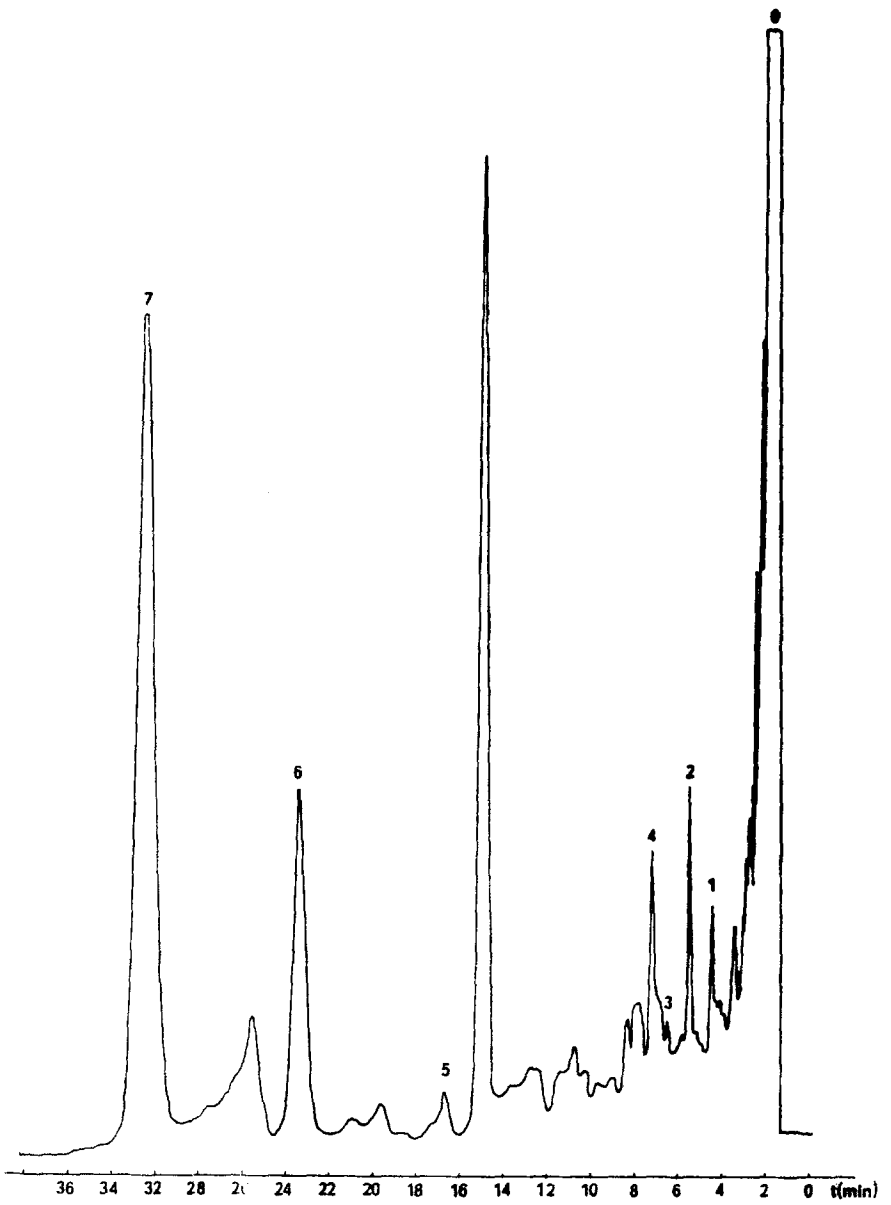

Figure 2

This figure represents a gas chromatogram of feed concentrate /in ppm/.

0 - solvent; 1 - alpha-HCH, 0.004; 2 - gamma-HCH, 0.014; 3 - beta-HCH, ".008; 4 - delta-HCH, 0.016; 5 - pp′-DDE, 0.02; 6 - op′-DDT, 0.120; 7 - pp′-DDT, 1.120.

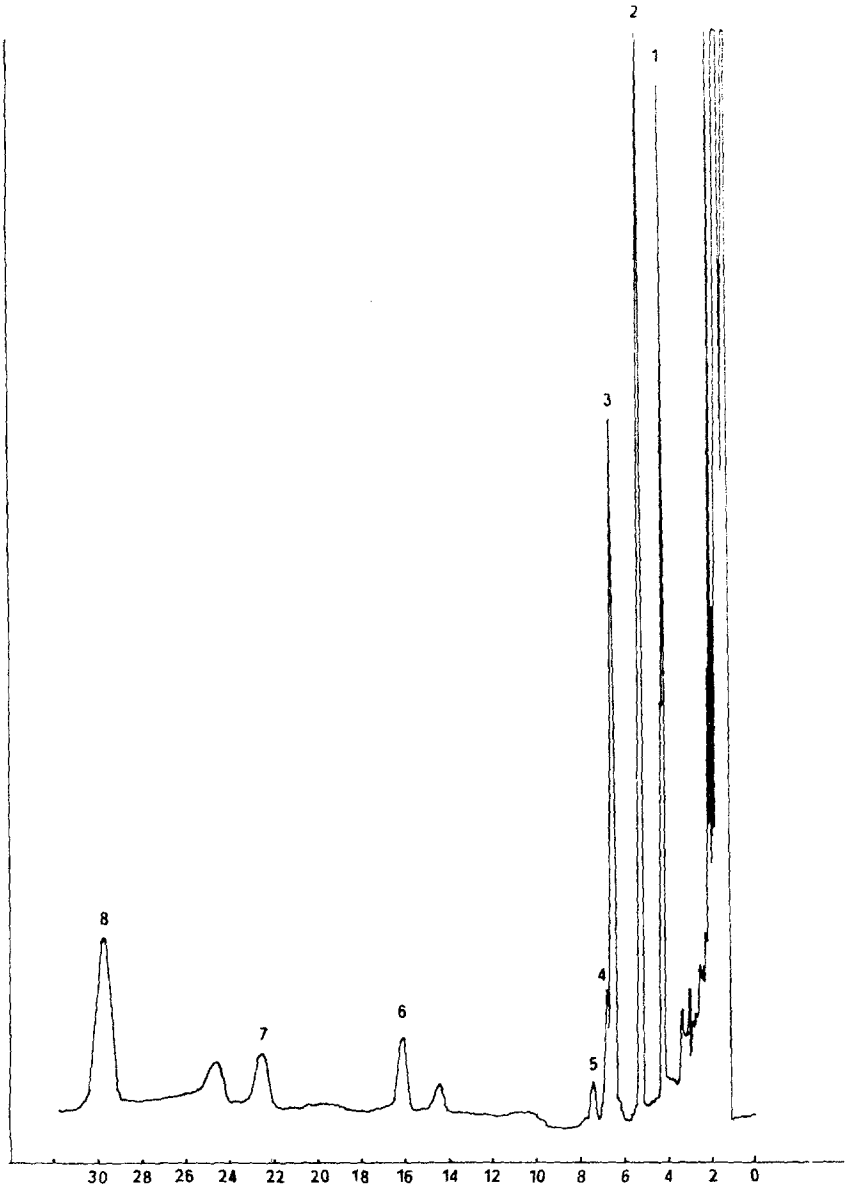

Figure 3
This is a gas chromatogram of soy bran /in ppm/.
0 - solvent; 1 - alpha-HCH, 0.030; 2 - gamma-HCH, 0.154; 3 - unknown compound; 4 - delta-HCH, 0.010; 5 - epsilon-HCH, 0.008; 6 - pp´-DDE, 0.020; 7 - op´-DDT, 0.035; 8 - pp´-DDT, 0.150.

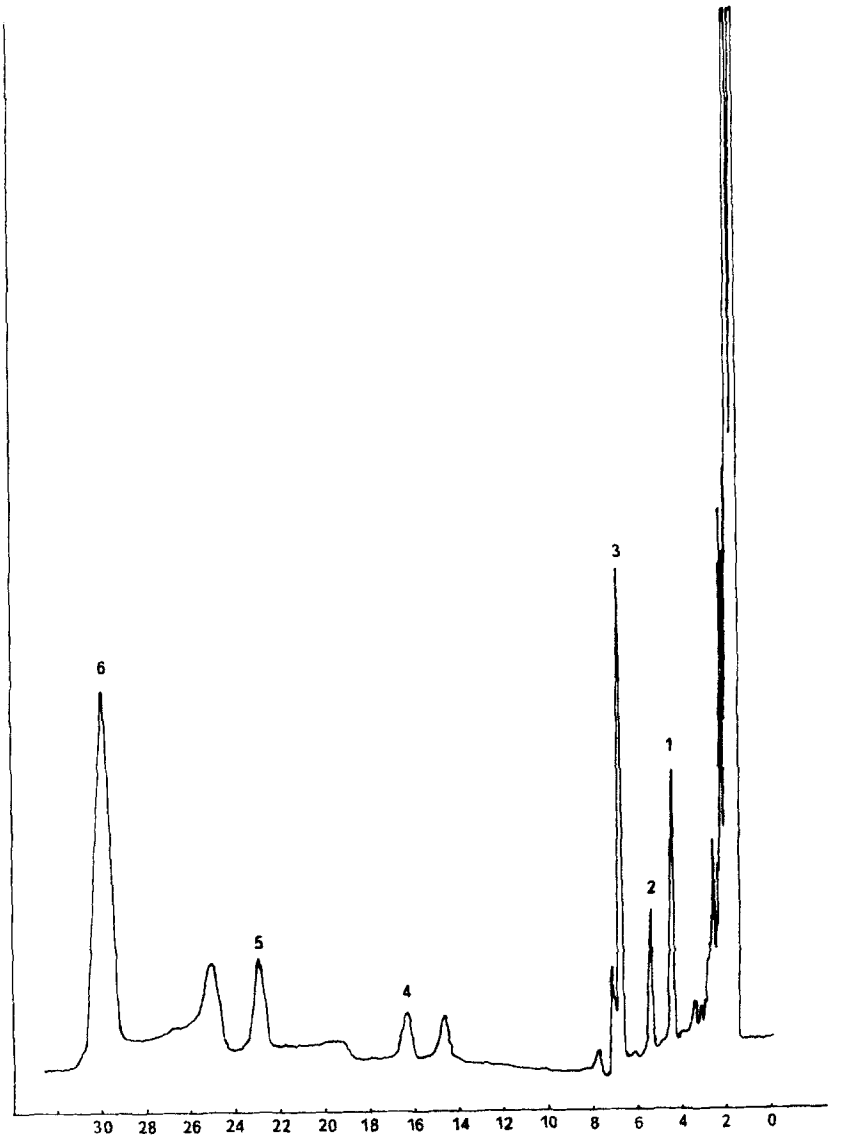

Figure 4
This figure represents a gas chromatogram of peanut bran /in ppm/. 0 - solvent; 1 - alpha-HCH, 0.008; 2 - gamma-HCH, 0.009; 3 - unknown compound; 4 - pp'-DDE, 0.02; 5 - op'-DDT, 0.06; 6 - pp'-DDT, 0.305.

routine monitoring control. This low-cost, rapid and adequate analytical method /presented in an algorithm on Fig. 5/ is offerred for the evaluation of these types of xenobiotics. It introduces a significant reduction of costs and time to the monitoring practice.

Table 1. Statistical analysis of recovery, fortification levels and detection limits in feed mixtures and feed components

compound studied	feed mixtures		feed components		fortification level/ppm/	detection level/ppm/
	$\bar{x}$	V%	$\bar{x}$	V%		
HCB	89.5 ± 3.77[1,2]	4.21	87.4 ± 2.92	3.34	0.0215	0.002
alpha-HCH	96.5 ± 2.78	2.87	95.7 ± 3.45	3.61	0.0212	0.002
beta-HCH	83.9 ± 6.12	7.29	91.5 ± 4.32	4.73	0.0428	0.008
gamma-HCH	94.4 ± 3.83	4.05	97.9 ± 5.39	5.51	0.0452	0.004
delta-HCH	93.4 ± 5.64	6.03	93.1 ± 5.21	5.61	0.0444	0.006
epsilon-HCH	84.0 ± 4.93	5.87	96.8 ± 4.46	4.60	0.0432	0.008
pp´-DDE	93.3 ± 7.69	8.24	89.7 ± 5.08	5.67	0.1000	0.010
op´-DDT	94.2 ± 8.67	9.20	91.6 ± 5.22	5.70	0.1500	0.020
pp´-DDT	94.1 ±10.95	11.63	95.7 ± 3.58	3.74	0.2500	0.030

[1] mean ± S.D.; the value of sample taken for fortification was subtracted from the value of each fortified sample

[2] n=10

sample of 10 g	5 min
maceration of mixture of acetonitrile + H_2O /65+35/ 150 ml + 100 ml + 100 ml	15 min
I filtration after 16 h	
add 500 ml of 5% solution of Na_2SO_4 and extract 3 x 100 ml of petroleum ether	15 min
condense to 10.0 ml	15 min
clean-up with 1 ml of concentrated H_2SO_4	10 min
condense 5.0 ml of cleaned up extract to 1.0 ml	15 min
analyze qualitatively and quantitatively GLC-ECD	60 min
	135 min

Figure 5. Flow chart and timing of the method.

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Received August 23, 1983; accepted September 15, 1983