An Apparent Case of Chlordane Poisoning

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Abstract

The liver, kidney, stomach contents, blood from the heart, and the urine of an 8-week old poodle were analyzed for residues of chlordane. Results showed that high concentrations of technical chlordane were present in all samples. Chromatographs of the extracts from the liver, kidney, blood, and urine showed that epoxidation of the heptachlor peak had taken place, and that the retention times of some peaks were altered. The size of some peaks were different from those of the stomach contents and standard technical chlordane. Residues in the samples on a wet-weight basis ranged from 0.30ppm in the urine to 402ppm in the stomach contents. Results suggested strongly that the poodle died of chlordane poisoning.

Domestic animals come in contact with insecticides through consumption of contaminated feed or by direct application for the control of insect pests. This case involved an 8-week old poodle that had been chewing on and licking floor boards previously sprayed with an emulsion of chlordane.

History

In an attempt to rid his household of cockroaches, the owner had sprayed several areas of his household with an emulsion of chlordane. This was seven days prior to the death of the poodle. On the day of his death, the poodle ate normally in the morning and appeared healthy at 1500 hours. He started to convulse, salivate and then started to retch at 1530 hours. He was vomiting and had diarrhea. The poodle was severely depressed and his temperature was 95°F when presented to the Veterinary clinic at 1600 hours. The poodle died shortly after being presented.

Autopsy

The autopsy of the dog revealed that his lungs and liver were congested. The intestines and mucus membrane were pale. There was bloody froth in the trachea.

Samples

Samples received for analyses included the liver, kidney, stomach contents, blood from the heart, and urine.

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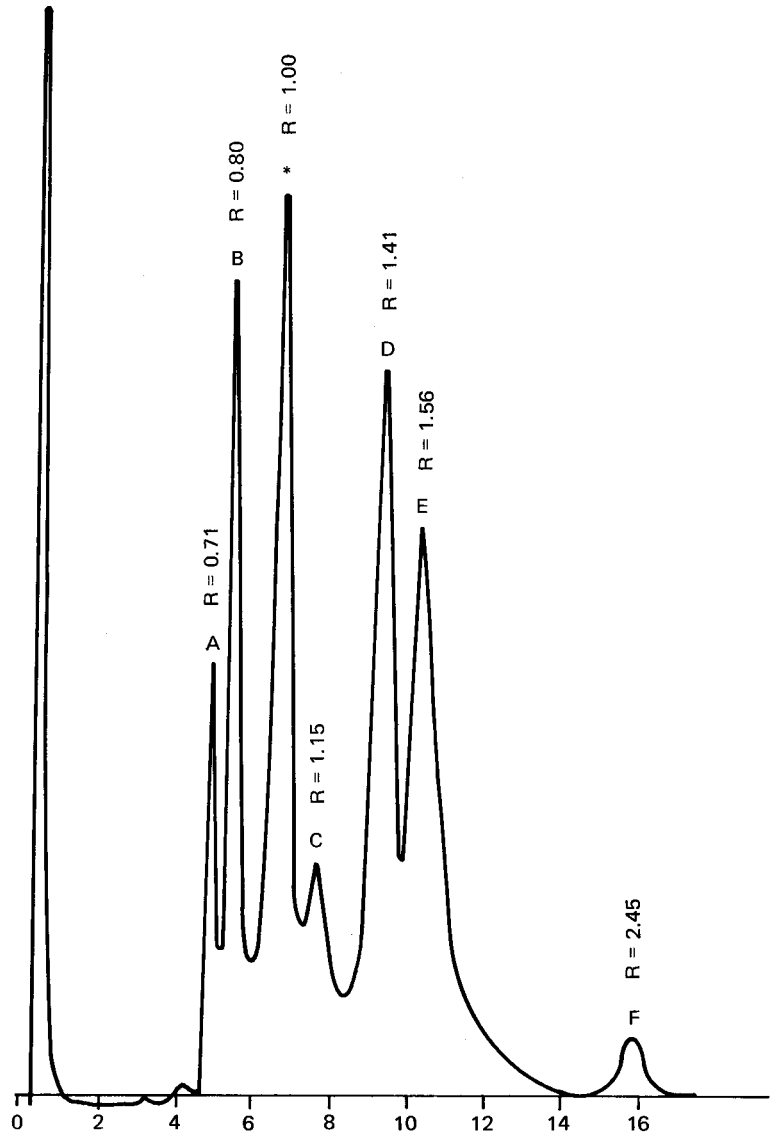
Method of Analyses
(a) The blood and urine were analyzed by the method of Dale and Miles(1970). Recovery of technical chlordane by this method was 82%. Replicate samples were within 0.25ppm.

(b) The stomach contents were analyzed by the method in PAM(1968-1971), section HE 211.3. Extracts were cleaned up on Florisil columns and eluted with 200cc of 6% ethyl ether in petroleum ether. Replicate samples were erratic. This was probably due to the fact that the sample was not homogenous. Portions taken for analysis may or may not have contained fatty foods or non-fatty foods.

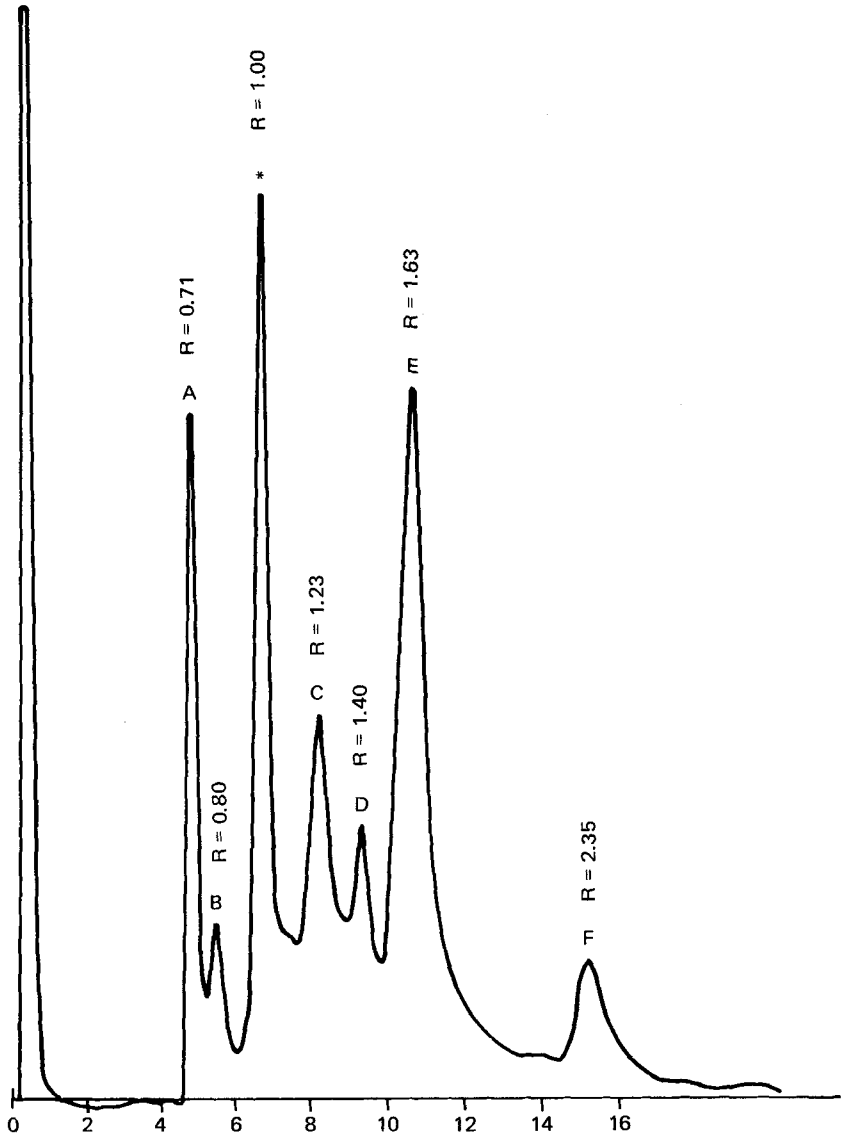
(c) The liver and kidney samples were analyzed by the methods of PAM(1968-1971), sections HE 211.3 and HE-212.31 with some modifications. Method HE 211.3 gave erratic results depending on the amount of lipid extracted. Depending how well the sample was minced, the range of lipids extracted was between 0.1% to 3.30%. The liver was large enough to be ground in a micro-grinder and replicate samples were within 0.5ppm. Method HE 212.31 gave better results by the use of a tissue grinder using acetonitrile to extract. This extract was diluted with 700cc of 2% NaCl and 100cc of petr. ether. The extract was partitioned into the petr. ether and washed two times with distilled water and cleaned up on a Florisil column using 200cc of 6% ethyl ether in petr. ether. Replicate samples were within 0.06ppm. Recovery from the liver was 88%. All results are given in Table 1.

Detection
Two Barber Coleman model 5360 gas chromatographs equipped with tritium electron capture detectors were used for detection. 8' x 4mm i. d. coiled glass columns used were 10% DC-200 on Gas Chrom Q, 80/100 mesh and a mixed column of 8% QF-1 and 2% OV-17 on Gas Chrom Q, 80/100 mesh. Col. temp.-200°C, detector temp.-210°C, injection port-250°C, and N_2 flow of 120ml/min. Results were confirmed by thin-layer chromatography.

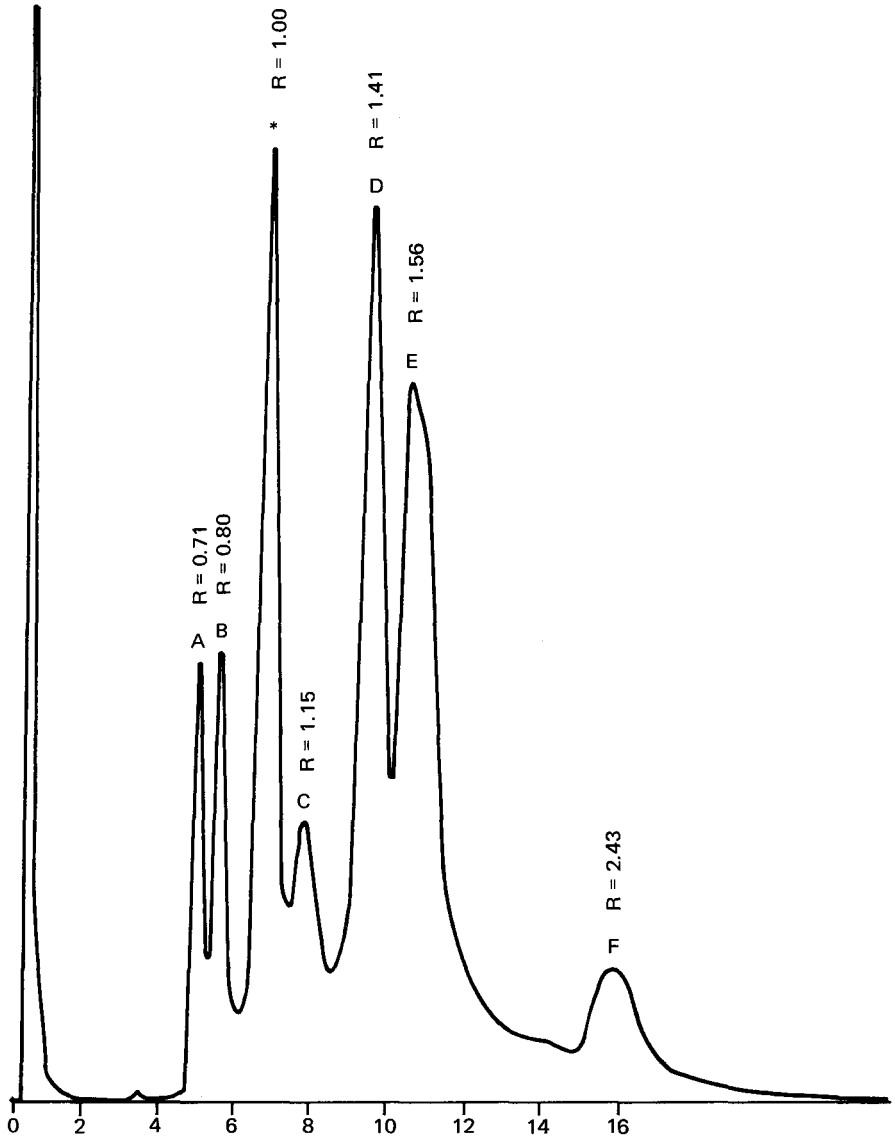
Discussion and Results
Several studies have been compiled on chlorinated hydrocarbon insecticides in animal tissues(Marth, 1962b and 1965). Data indicated that domestic as well as wild animals stored chlorinated insecticides in their liver, fat, kidney, heart, and brain. Heptachlor epoxide was produced by dogs fed heptachlor, and aldrin was converted to dieldrin. Data showed that the polycyclic chlorinated insecticides such as chlordane, heptachlor, aldrin, dieldrin, and endrin are more toxic than that of the chlorobenzene derivatives. Studies by DeWitt (1955,1956) showed that aldrin, dieldrin, and endrin



(a) 10ng technical chlordane. *1ng standard aldrin.



(b) Typical chromatograph of technical chlordane found in the liver, kidney, blood, and urine of the poodle. *1ng standard aldrin.



(c) Chromatograph of extract from stomach contents. *1ng standard aldrin.

resulted in a higher mortality rate than DDT or strobane. L. Stickel and W. Stickel(1969a,1969b,1970) studied the distribution of DDT and dieldrin residues in birds and mammals. The similarity in structure of chlordane to aldrin, dieldrin, heptachlor, and endrin places this insecticide in the more toxic class of chlorinated hydrocarbons. The single oral LD_{50} for chlordane in dogs is between 90 and 140 mg/kg (Radeleff,1970).

Table 1 contains the results found in the various tissues of the dog. Residue concentrations expressed as ppm wet-weight were more suitable for replicate samples. Lipid weight extracted fluctuated and replicate samples were erratic. Stickel and Stickel(1969b) found that fat declined in the bodies of birds at death and increased in their livers, and that different extraction solvents and procedures can materially alter the amount of lipid extracted. The lipid percentages extracted from the liver were 2.09 and 2.18%. The lipid percentages in the kidney were 1.99 and 3.30%. These were the best lipid extractions for replicate samples.

Three analyses by method HE 211.3, PAM , were performed on the stomach contents. Results were 50, 105, and 402 ppm. This indicated that the sample was not homogenous. Total contents weighed 16 grams. A spatula was used to mix the sample.

Figure 1 shows the chromatograph of standard technical chlordane and chlordane extracted from the samples. Chromatograph(a) represents 10 ng of technical chlordane. Chromatograph(b) represents a typical chromatograph of the liver, kidney, blood, and urine on the DC-200 column. All chromatographs were the same for the samples except the concentration of chlordane was different. Note the difference in the size of peaks D and E, and the slight difference in the retention times of peaks E and F. Note the reduction of the heptachlor peak(R= 0.80) and the appearance of an additional peak with retention time of heptachlor epoxide(R= 1.23) in the sample(b). This indicated that epoxidation had taken place. This peak was confirmed on another column of 8% QF-1 and OV-17, which indicated the reduction of the heptachlor peak and the appearance of the heptachlor epoxide peak. Other authors(Thurston, 1965;PAM,1968-1971) have noted changes in the number and size of chromatographic peaks for chlordane in a variety of samples.

In the chromatograph of the stomach contents(c), all retention times were identical to the standard chlordane , and peaks A, C, D, E, and F had almost the same relative peak height as the standard chlordane. There

was no indication of the heptachlor epoxide peak. In all of the samples standard aldrin was injected to insure that all retention times were accurate. Standard technical chlordane normally has another small peak with a retention time of 0.97 on the DC-200 column.

Since it is well documented that toxicity studies (DeWitt,1955,1956; Marth,1965; Stickel,et al,1969a, 1969b,1970; Thurston,1965) do not correlate between different species, and not necessarily within the same species, only the results of Table 1 can be examined. The symptoms of vomiting, convulsions, salivation, retching, diarrhea, and depression combined with death and high residues in various tissues, leads to the conclusion that this dog died of chlordane poisoning.

Table 1
Technical chlordane residues in an 8-week old poodle

Sample	ppm wet weight	ppm lipid weight
Liver	16[a]	893
Kidney	45[a]	368
Stomach contents	50-402[b]	
Blood from heart	1.21	
Urine	0.30	

(a) Method HE 212.31 PAM
(b) Method HE 211.3 PAM

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