J. Pharm. Pharmacol., 1965, **17**, 362–367 Received February 1, 1965

Simplified gas chromatographic analysis of pesticides from blood

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A rapid method for the extraction and identification of 23 pesticides present in blood is described. The pesticides are extracted from blood with a mixture of acetone and ethyl ether in equal volumes. The extracts are evaporated to dryness, the residue dissolved in a known quantity of hexane and an aliquot injected directly into the gas chromatograph equipped with an electron capture detector. No purification of the acetone-ether extract is required. A glass column packed with 5% SE 52 on hexamethyldisilizane-treated chromosorb W 60/80 is used at a temperature of 190°. The method was successfully applied to blood from laboratory animals to which acute doses of six of the most common pesticides were given orally. The quantity of pesticides found is reported.

SYSTEMATIC schemes for the isolation and identification of toxic substances are largely confined to conventional poisons. With the advent of many new compounds having toxic properties, the deficiencies of the classical procedures have been made more obvious. This is especially true of the group of pesticides which includes chlorinated hydrocarbons and organophosphorus compounds. Symptoms associated with these have been treated as chronic rather than acute effects, and analysis has largely been directed towards the control of pesticide residues on foodstuffs.

Acute poisoning from these materials is increasing and the toxicologist needs to be able to identify rapidly and with certainty the presence in physiological materials of acutely toxic quantities of such materials. The quantities of pesticides in such cases are so small, and the conventional tests so insensitive and non-specific, that these poisons may readily be overlooked. It is therefore essential to make available simple and rapid methods of extraction to be used with detection methods to ensure that toxic material is not overlooked. Also the procedure should not require extensive clean up of the extracts before it can be applied.

Various papers have been published (Clifford, 1947; Schechter, Pogorelskin & Haller, 1947; Fairing & Warrington, 1950; Jones & Riddick, 1951, 1952; Krzeminski & Landmann, 1963) describing the isolation of minute amounts of pesticides from biological specimens by means that are usually complex and tedious. Extensive cleanup is required, and is accomplished for example by hexane: aceto-nitrile partitioning (Jones & Riddick, 1952; McKinley & Savary, 1962), paper chromatography (Mitchell, 1957; Müller, Ernst & Schock, 1957; MacRae & McKinley, 1961), thin layer chromatography (Walker and Beroza, 1963), silicic acid (Moats, 1962) and florisil column chromatography (Moddes, 1961; Moats, 1963). A rapid and simple general method for isolating and detecting a group of pesticides from blood is now described.

The method employs the acetone-ether extraction procedure for the gas chromatographic analysis of barbiturates described by Jain, Fontan

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& Kirk (1964). Because of the small amount of impurities present in blood, as compared to tissue, blood was chosen for study.

The method does not require cleanup of the extract, contamination of the detector is negligible, and low levels of organophosphorus compounds and chlorinated hydrocarbons can be detected. Most of the glassware used is disposable, which also eliminates much trouble and inconvenience.

Experimental

MATERIALS

The Hy-Fi gas chromatograph, Aerograph Model 600 (Wilkens Instrument and Research, Inc., Walnut Creek, California), with an electron capture detector, and the Leeds and Northrup Speedomax H, zero to 1 mV recorder, model S, were used. The chromatographic column was a spiral Pyrex glass tube of 0·125 inch outside diameter, 0·070 inch inside diameter, and 4½ feet in length. The solid support material was acid washed Chromosorb W 60 to 80 mesh which was treated with hexamethyldisilizane. This was coated with 5% (w/w) SE 52*.

The stationary phase was applied by forming a slurry of SE 52 in chloroform with the solid support and evaporating the excess solvent rapidly on a steam-bath under vacuum. When dry, the material was packed in the glass column by applying vacuum to one end and gently tapping the column mechanically. Occasionally, nitrogen pressure was applied at the other end to ensure uniform and tight packing.

Standard solutions. Pesticide solutions for use in testing recovery were prepared by dissolving the appropriate quantity of various compounds as listed under Results, in 90 to 100% purity in hexane (Reagent A.C.S.) to give a concentration of 1 mg/ml. These solutions were diluted further as needed; e.g., lindane was injected at concentrations down to 0·2 ng/μl. Standard solutions were chromatographed to determine the retention times of the pesticides studied.

Extraction solvent. The extraction solvent was made by mixing equal volumes of acetone and ether (A.R.). This was used to extract the pesticides from blood samples in all instances before chromatographing the material. Samples examined included 16 compounds added in known quantities to human blood containing EDTA as anticoagulant, and blood from 17 rats which had been given one of six different pesticides as detailed under Results, and blood from 3 rats used as controls.

The rats were healthy females of the Long Evans strain, 35 to 40 days old, weighing between 106 and 137 g; they were separately caged and were reared on White Diet.†

PROCEDURES

All pesticides, both standard solutions and extracts, were dissolved in hexane for gas chromatography. The operating conditions were as

*Phenylmethyl silicone.

†White Diet consists of: ground whole wheat 67·5, casein, tech. 15·0, skim milk powder 7·5, sodium chloride 0·75, calcium carbonate 1·5, melted fat 6·75, fish oil 1·0% and KI solution 0·9 mg iodine/g diet (in solution).

follows: Oven temperature, 190°, injector temperature, 230°, and flow rate of carrier gas (nitrogen), 70 ml. Input impedance was 10^7 ohm and output sensitivity 1X, corresponding to $3{\cdot}3 \times 10^{-9}$ A for full scale recorder deflection. Detector voltage was −90 V. All samples, dissolved in hexane, were injected into the sampling port with a Hamilton syringe of suitable capacity.

Extraction. All blood samples were extracted and gas chromatographed to observe the normal background response and the recovery of pesticide. For convenience, 500 μl of each blood sample was ordinarily taken in a 1/2 dram shell vial. A quantity of mixed solvent (acetone-ether) was added and the mixture stirred for a few sec with a melting-point capillary of 1·3 to 1·5 mm diameter with sealed end, after which the clear supernatant was transferred to another 2 dram shell vial with a Pasteur pipette. This process was repeated three times, fresh solvent being used each time and all supernatants being combined in the second shell vial. The combined extracts were evaporated to dryness at room temperature with a fine jet of air impinging on the surface of the sample. This was conveniently achieved by using a bell jar with a side arm connected to the vacuum line. A glass capillary was mounted through the top opening of the jar to provide the air flow.

The residue was dissolved in 500 μl of hexane, the suspended material was allowed to settle for a few sec, and 1 μl of the clear supernatant was injected into the gas chromatograph. To test the applicability of the method to very small samples of blood, the quantity of sample was reduced to 10 μl for blood from rats fed lindane in LD50 doses (150 mg/kg). The residues of the 10 μl samples, after extraction and evaporation, were dissolved in 100 μl of hexane, and 1 μl of this was chromatographed in the usual manner.

Animals. Pesticides were administered in olive oil solution by stomach tube to 17 animals after they had fasted for 24 hr. One control rat received 1·5 ml of olive oil, and two were given nothing. Pesticides were administered at the LD50 dosage quoted by Negherbon (1959). At varying periods from 0·4 to 9 hr, blood was collected from the descending aorta while the animals were under ether anaesthesia. After as much blood as possible was withdrawn into a heparinised syringe, the rats were bled to death by severing the aorta. The samples were transferred to clean bottles and stored under refrigeration until required for extraction.

Results and discussion

The relative retention values of 23 pesticides, 10 of them organophosphates, 12 of them chlorinated hydrocarbons, and 1 nitro-compound (Morocide) were determined relative to lindane. The reproducibility was good; shorter retention times could be reproduced accurately and the longer ones with errors of 5–7%, providing other conditions remained constant. These values are shown in Table 1, along with the approximate recovery values of 16 of the materials which were added to blood at a level of 1 ppm, extracted, and subjected to gas chromatography.

TABLE 1. RELATIVE RETENTION VALUES AND RECOVERY OF PESTICIDES

Pesticides	Relative retention values	Approximate recovery from blood %*
Aldrin	2·05	100
Chlordane	1·7, 3·3, 3·6	—
DDT (or TDE)	4·7, 6·2	—
DDT	4·3, 6·1, 7·9	—
p-p' DDT	7·9	85-90
Diazinon	1·15	—
Dibrom	0·15	—
Dieldrin	4·5	90–95
Di-syston	1·2	85
Endrin	5·1	100
EPN	12·5	100
Ethion	6·6	95
Heptachlor	1·6, 3·3	100
Heptachlor epoxide	2·8	90
Lindane	1·0	100
Malathion	2·7	95
Methoxychlor	12·9	85
Methyl trithion	5·4	90
Morocide	5·8	75
Parathion	2·4	92–95
PCNB tetrachlor	0·95	—
Thimet	0·8	—
Trithion	7·6	90

Conditions: Oven temperature 190°; injector 230°.
4½ ft. glass column packed with 5% SE 52 on hexamethyldisilizane-treated Chromosorb W, 60–80 mesh.
Flow rate of carrier gas (nitrogen) 70 ml/min.

* The % recoveries reported are calculated to the technical grades of pesticides used as standards.

Certain members of similar groups which were also chromatographed were not extracted, since their behaviour essentially should be identical with those studied. Recoveries were calculated, as were the concentrations of pesticides in the bloods of rats (Table 2), by peak areas based on

TABLE 2. DETAILS OF ANIMAL EXERIMENTS

No. of rats	Pesticides	LD50 dosage mg/kg	Time between dosing and drawing blood (hr)	ppm of pesticide found in the whole blood
3	DDT	200	4	DDE 50 *o-p'* 7 *p-p'* 6–7
1	Dieldrin	50	4	0·67
1	Dieldrin	75	9	2·2
1	Endrin	5	4	None
1	Endrin	10	4	Trace
1	Endrin	20	4	Trace
1	Endrin	50	9	1
2	Lindane	150	4	10
3	Malathion	1000	4	6·6
3	Parathion	5	0·4	3·3

a simple triangulation procedure. To take into account the change in the detector response with time, standards were run frequently, preferably with each injection. During the time of this study such detector response did not vary significantly. Both unknown and known samples were chromatographed in about the same concentration to maintain the linearity of response and to facilitate the quantitative work.

To interpret the results of pesticide extracts from blood, it was essential to determine what effect, if any, normal blood would exert when extracted and the material chromatographed. With the extreme sensitivity of the

electron capture detector for certain types of compounds, it was conceivable that some normal blood constituents might be extracted and recorded by the instrument. Similar considerations also applied to possible impurities present in the solvents. Results showed that no significant response was observed from normal blood, and by using A.R. grade solvents the response from solvent residue was also minimised.

Certain additional experimental points were found to be of significance. Initially, the extracts were evaporated at raised temperature to dry them rapidly, but this was found to lead to loss of most of the materials studied, and was abandoned in favour of the slower procedure described, in which loss did not occur. This procedure was much faster than evaporating spontaneously in the air, which was also tested and gave equivalent results. Another point of interest was the requisite number of extractions needed to obtain quantitative recovery. Several experiments were run with three, as opposed to six, extractions. No significant differences were found, and the procedure of using three extractions was adopted.

Female rats were administered LD50 doses of six of the most commonly used pesticides, two organophosphorus compounds, and four chlorinated hydrocarbons, as described. The general results of these experiments are shown in Table 2. One of three rats which was given parathion showed severe tremors and convulsions within 20 to 25 min of ingestion. One of three that received malathion showed violent convulsions 3 hr after feeding, the other two showed no symptoms up to 4 hr after dosing. No physiological effects were noted with endrin, and the blood contained minimal quantities of the material, even after 9 hr. All other pesticides were detected in significant amounts in the blood at levels insufficient to produce death. The failure to obtain an approximate 50% death rate may be because of the species used, to the small number of animals, and to the fact that technical rather than purified grades of pesticide were used.

Blood was examined rather than fat which is usual for chlorinated hydrocarbons, because we were interested in acute rather than in chronic poisoning. The limited number of experiments did not allow determination of the time at which each pesticide reached maximum concentration in blood. However, in acute poisoning, time is more vital than maximum analytical response, and the times used are suitable for rapid screening tests of affected persons.

The amount of blood analysed, 0·5 ml, was chosen for convenience, and would be readily available in an acute poisoning; with lindane much smaller samples from the finger or ear lobe, were found sufficient. Only a small part of each sample extracted was injected into the gas chromatograph and therefore much smaller quantities of blood could suffice. It would also make experimentation with animals smaller than rats practicable.

Dale, Gaines, Hayes & Pearce (1963) showed that DDT occurs in the plasma of rats fed with this material in peanut oil at the rate of 150 mg/kg of body weight. No similar data have been noted for the organophosphorus pesticides. Our findings demonstrate that the pesticides are to be found in blood in significant quantity in acute poisoning.

The analytical procedure has some advantages over more complex methods. The solvent system is effective, may be used rapidly and is applicable to a wide variety of toxic substances other than pesticides. With blood there is no significant blank readings nor is the electron capture detector disturbed by accumulation of materials. SE52 on a support of hexamethyldisilizane-treated Chromosorb W 60/80 caused little contamination of the detector and the solid support showed little adsorption capacity compared with other supports tested.

Acknowledgements. This investigation was supported by Public Health Service Research Grant, AC 00185, from the Division of Environmental Engineering and Food Protection, and by the Committee on Research, University of California. The various pesticides used in this study were donated by the California Chemical Co. (Ortho Division), Richmond, California; the E.I. Du Pont de Nemours and Co., Wilmington, Delaware; the Geigy Chemical Corporation, McInthosh, Alabama; and the Stauffer Chemical Co., Richmond, California, to whom we extend our thanks. We wish also to acknowledge the technical assistance of Miss Lisa Diamond.

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