Investigation of Pesticide Residues in Human Adipose Tissue in the Northeast Louisiana Area

Earl S. Greer,* Donald J. Miller, Frank N. Bruscato, and Robert L. Holt

The presence of various organochlorine pesticides in human adipose tissue was investigated. Samples were obtained from northeast Louisiana, specifically Monroe, Louisiana, and the immediate surrounding area. Pesticide residue concentrations of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its analogues, mirex, dieldrin, and heptachlor epoxide were investigated and reported. Standard EPA extraction and cleanup techniques were employed, using hexane and acetonitrile for extraction and Florisil for cleanup. Analysis of the pesticide extracts was accomplished through the use of electron-capture gas chromatography. Concentration levels of some pesticides were found to be somewhat higher than the national average.

Organochlorine pesticides have received much attention in recent years since investigation has shown persistence of these pesticides in the environment and accumulated storage of these pesticides in the tissue of man and other animals. Residues of pesticides and their metabolites in human tissue have been reported by numerous investigators (Kutz et al., 1974, 1976a,b; Morgan and Roan, 1971; Owens, 1976).

Pesticides can gain entrance into the human body through a number of pathways: through the intestine by ingestion, through the lungs by the way of inhalation of airborne pesticides carried in dust, vapors, and possibly aerosols, and by direct penetration through the skin. In an agricultural environment all of the above entries of pesticides into humans are possible. This possibility must certainly be prevalent in the heavily agricultural northeast section of Louisiana where the samples of this study were taken. Pesticides have been used in this area for many years including heavy application of the well-known fire ant pesticide, mirex (Kutz et al., 1974, 1976a).

Since northeast Louisiana is a heavily agricultural area, a study of pesticide residues in people of this area was considered meaningful. Such a study has not been previously reported. This study could then provide at least two important functions: (1) alert public health officals if levels of the pesticides studied were found to be excessively high and (2) provide a base level at this time which could serve for future comparative studies of pesticide levels as in the case of an accidental contamination of the environment.

In this study the pesticides and pesticide residues examined for presence in human adipose tissue were DDT and its analogues (based on presence of *p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT), mirex, dieldrin, and heptachlor epoxide. DDT, while no longer in use, is a prevalent residue in the environment, persisting mainly as the metabolite *p,p'*-DDE. DDE is not effectively removed from the body, thus a gradual increase in the body tissues is generally noted (Kutz et al., 1976a; Morgan and Roan, 1971). Dieldrin gives a view of both dieldrin and aldrin accumulation since aldrin is rapidly converted to dieldrin by the body. Heptachlor epoxide can give insight to exposure to two pesticides, heptachlor and chlordane. Mirex is important not only because of its persistence in the environment but also because of its extensive use in northern Louisiana before its use was restricted.

Northeast Louisiana University, Department of Chemistry, Monroe, Louisiana 71209.

EXPERIMENTAL SECTION

Sampling. Tissue samples for this study were primarily obtained from hospitals in the Monroe, Louisiana area. Adipose tissue samples were taken at the time of surgery, placed in clean glass containers, and frozen until analysis. The size of tissue samples obtained ranged between 20 and 50 g. Postmortem samples were taken within 24 h after death and stored as above. For each sample various physical characteristics of the sample donor, residence, reason for surgery, and type of sample were obtained. These data are shown in Table I.

Extraction. Tissue extraction and cleanup were performed basically according to accepted EPA methods with slight modification (Thompson, 1974). Approximately 5 g of human adipose tissue were extracted four times with 50-mL portions of hexane to remove the oil containing the pesticides of interest. A 1–3-g sample of the oil obtained was subjected to a liquid–liquid partition process. The partitioning consisted of dissolving the weighed oil sample in approximately 15 mL of hexane and extracting four times with 30 mL of acetonitrile saturated with hexane. The acetonitrile fractions were combined and the pesticides were forced back into hexane by aqueous dilution (500 mL of hexane-extracted water and 100 mL of saturated sodium chloride solution) of the acetonitrile in the presence of hexane. The hexane fractions thus obtained were then subjected to subsequent cleanup on a standard Florisil (weight determined by standard lauric acid calculation) column utilizing elution fractions of 150 mL of hexane, 200 mL of 6% diethyl ether in hexane, and 200 mL of 15% diethyl ether in hexane. These fractions were collected and then concentrated to a suitable volume for gas–liquid chromatographic analysis.

Gas–Liquid Chromatography (GLC). All GLC analyses were conducted on a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a CDS-111c microprocessor integrator system. The gas chromatographic methods utilized have been previously reported (McCown et al., 1977). Gas chromatographic conditions used are outlined in Table II. Two columns were used for identification and quantitation of each residue reported.

Pesticide standards used in this work were of a known purity obtained from the U.S. Environmental Protection Agency, Health Effects Research Laboratory, Environmental Toxicology Division, Research Triangle Park, N.C. All solvents used were pesticide quality (distilled in glass) obtained from Matheson Coleman and Bell Manufacturing Chemists and checked for interferences. Pesticide residues were reported in units of micrograms/gram of oil (ppm).

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Table I. Information on Human Tissue Donors and Location of Tissue in Body

sample no.	age	sex	location of tissue	ht	wt, lb	residence (LA)	% oil
1	88	F	leg	a	a	West Monroe	59.4
2	69	F	abdomen	5'4"	170	a	90.0
3	40	M	abdomen	5'3"	135	Monroe	38.6
4	63	F	breast	5'4"	144	Monroe	74.0
5	47	F	abdomen	5'4"	110	Mangham	63.7
6	61	F	breast	5'3"	135	Choudrant	72.8
7	98	F	leg	a	a	Monroe	84.3
8	65	F	breast	a	a	West Monroe	85.2
9	63	F	abdomen	5'2"	130	Monroe	70.9
10	29	F	a	5'4"	225	Bastrop	71.7
11	17	M	breast	6'2"	165	Farmerville	68.8
12	39	M	abdomen	a	a	Columbia	39.0
13	82	F	leg	a	a	Alexandria	68.3
14	62	F	breast	a	a	Monroe	69.4
15	47	F	breast	a	a	Monroe	68.3
16	72	M	a	6'3"	165	Monroe	63.5
17	49	F	breast	a	a	Monroe	75.1
18	51	F	breast	5'2"	118	Columbia	64.9
19	89	F	leg	5'6"	105	Monroe	74.0
20	46	M	abdomen	5'9"	200	West Monroe	85.9
21	67	F	breast	5'2"	150	Monroe	75.4
22	34	F	upper back	a	a	Monroe	88.0

[a] Information not available.

Table II

GLC parameters	column I	column II
liquid phase	1.5% OV-17/1.95% OV-210	10% QF-1
solid support	Chromosorb WHP 100/120 mesh	Gas-Chrom Q 80/100 mesh
column dimensions	6 ft × 2 mm i.d. glass	6 ft × 2 mm i.d. glass
inlet temperature	200 °C	200 °C
column temperature	180 °C	180 °C
detector temperature	310 °C	310 °C
flow rate, mL/min	35-40	40-50

Table III. Concentration of Organochlorine Pesticides in Individual Tissue Samples in Micrograms/Gram of Oil (ppm)

sample no.	p,p'-DDE	p,p'-DDD	p,p'-DDT	mirex	dieldrin	heptachlor epoxide
1	8.99	0.26	2.05	0.07	a	0.03
2	5.26	0.24	0.87	0.16	0.11	0.04
3	12.53	0.07	0.87	0.18	a	0.19
4	4.41	0.07	0.72	0.04	0.22	0.20
5	6.98	0.07	0.73	0.06	0.12	0.06
6	5.08	0.14	1.55	0.12	0.10	0.19
7	3.32	0.17	1.43	0.06	0.24	0.18
8	4.49	0.19	0.93	0.07	0.28	0.24
9	6.23	0.30	2.20	0.03	0.48	0.27
10	21.29	0.56	3.50	0.10	0.62	0.11
11	2.04	0.02	0.15	0.57	0.07	0.06
12	5.10	0.08	0.41	0.11	0.13	0.37
13	7.60	0.15	1.80	0.45	0.06	0.27
14	5.44	0.04	0.52	0.01	0.18	0.17
15	6.18	0.07	1.11	0.02	0.08	0.12
16	19.51	0.15	4.43	0.60	0.22	0.31
17	5.70	0.05	0.71	0.02	0.07	0.13
18	6.49	0.06	1.01	0.32	0.07	0.16
19	1.09	0.02	0.22	a	0.03	0.02
20	7.50	0.08	1.57	0.02	0.19	0.19
21	3.40	0.04	0.07	a	0.04	0.18
22	2.20	0.04	1.15	0.03	0.03	0.07

[a] Less than 0.01 μg/g.

RESULTS AND DISCUSSION

The concentration of the various organochlorine pesticide residues of interest found in each tissue sample is shown in Table III. These pesticides were selected because of their persistence in the environment and their heavy usage in this section of the country. It was hoped that establishment of base line levels for these residues could be used to establish proposed levels for other persistent chlorinated pesticide residues. Results of this work were compared with base line levels established at a national level by the EPA National Human Monitoring Program for Pesticides and are shown in Figure 1 (Kutz et al., 1976a). No comparison was available for mirex at this time.

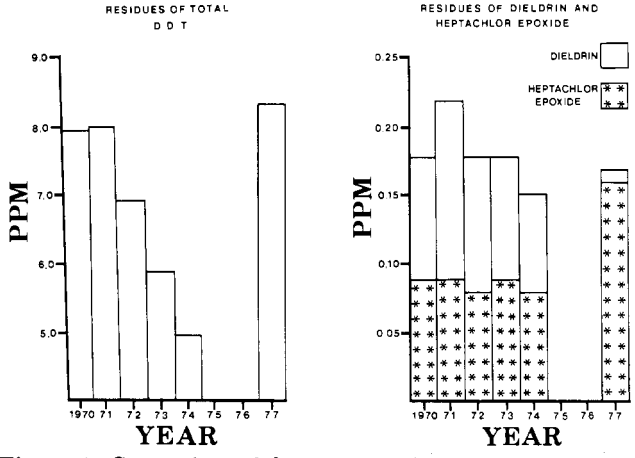

Figure 1. Comparison of the concentrations of organochlorine residues of DDT, dieldrin, and heptachlor epoxide from northeast Louisiana to a national survey of the United States, 1970–74 (Kutz et al., 1976a). Concentrations are expressed in ppm of lipid basis. Graph represents the geometric mean of the values obtained. Values listed for 1977 are the values obtained for northeast Louisiana. Other values are from the national survey. No national figures were available for 1975–77.

Comparisons of DDT and heptachlor epoxide from northeast Louisiana to the national levels showed a much higher trend for Louisiana than the nation. DDT, in this study, had a mean value of 8.29 ppm compared to 4.99 ppm for the nation in 1974, some 66% higher. Heptachlor epoxide had a mean value of 0.16 ppm compared to 0.08 for the nation in 1974, a rise of 100%. The data were further assessed to be accurate by a percentage of 82.75% for the total DDT present as the metabolite p,p'-DDE, as compared to a national value of 83.00%—the expected percentage of p,p'-DDE. At this time it is difficult to determine the reason for higher levels of DDT and heptachlor epoxide in northeast Louisiana than in the nation.

It is thought, however, to be related to the fact that northeast Louisiana is an extensively agrarian community, therefore providing more exposure for its residents to pesticides than urban communities and the nation as a whole. Future studies should show the same dropoff in levels as the nation shows, which would not explain the higher levels, but would show only that a higher level existed in this area.

Dieldrin, in this study, did not show the same higher trend when compared to national values as did DDT and heptachlor epoxide. This conflict in data presents some problems for proposing base lines for other residues. However, in examining national data for dieldrin there appears to be a definite trend of consistent levels over a period of years rather than a drop. The close correlation of Louisiana values to national values (Figure 1) might indicate that the base line level for dieldrin has been reached in northeast Louisiana and as with the nation as a whole this level will probably remain consistent. On the other hand, dieldrin may not have been used as extensively in this area as was DDT and heptachlor epoxide. Also, national data was only available through 1974 for comparison and later data comparison for 1976–1978 might prove more useful. In any event, other pesticide residues will have to be monitored before definite base line correlations for northeast Louisiana can be established. The establishment of base line pesticide levels in an agricultural environment will be very useful to agencies involved in monitoring pesticides.

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Derivatization of Several Carbamate Pesticides with Methanesulfonyl Chloride and Detection by Gas–Liquid Chromatography with the Flame Photometric Detector: Application to Residues of Carbaryl on Lentil Straw

Jay C. Maitlen* and Leslie M. McDonough

The mesylate derivatives of the carbamate pesticides propoxur, MCA-600 (benzo[*b*]thien-4-yl methylcarbamate), Landrin, Carbaryl, carbofuran, 3-hydroxycarbofuran, 3-oxocarbofuran, methiocarb, methiocarb sulfoxide, and methiocarb sulfone were prepared and found to be suitable for the gas chromatographic detection of these pesticides. The carbamate pesticide is hydrolyzed with methanolic KOH, and the resultant phenol reacted with methanesulfonyl chloride and pyridine to form the mesylate derivative, which is determined by a gas chromatograph equipped with a flame photometric detector in the sulfur mode. This method is highly specific and free from interference of extracted crop materials. This procedure was applied to the determination of carbaryl residues in lentil straw. Recoveries of carbaryl from lentil straw samples fortified with the pure pesticide at the rate of 0.1 ppm averaged 103.0% (range 91.0–118.0%).

The determination of carbamate pesticide residues in crops is often difficult. Because direct analysis of carbamates by gas–liquid chromatography (GLC) is difficult (since most carbamate pesticides degrade on the GLC column), the most common approach is to hydrolyze the carbamate ester linkage and derivatize the resultant phenol with a chloro, fluoro, or bromo compound to yield a product that can be readily detected by a GLC equipped with an electron-capture detector (ECD). Many of these procedures are discussed in reviews by Williams (1971) and by Dorough and Thorstenson (1975).

In general, the methods are quite sensitive, but problems may arise because of the lack of specificity of the ECD. Thus, complicated cleanup procedures of the crop extract may be necessary to remove plant materials that inhibit the derivatization of the carbamate or to remove plant materials that produce GLC peaks with the same retention times as the carbamate derivative being analyzed. In 1966, Brody and Chaney introduced the flame photometric detector (FPD), which is specific for compounds containing only phosphorus or sulfur, so many problems caused by inefficient cleanup of unwanted plant extractives were eliminated. Subsequently, Bowman and Beroza (1967) took advantage of the specificity of the FPD in the phosphorus mode and developed a method based on the thiophosphoryl derivative of several carbamate pesticides. The disadvantage was that the GLC analysis of this derivative had to be completed the same day it was prepared or an interfering compound formed that masked the derivative being determined. Later, Moye (1975) developed a procedure based on the derivatization of carbamate

Yakima Agricultural Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Yakima, Washington 98902.