Trans-Nonachlor Residues in Human Adipose Tissue

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trans-Nonachlor, chemically known as 1,2,3,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane, is a component of technical chlordane and technical heptachlor. According to Interpretation No. 23 of the Federal Insecticide, Fungicide and Rodenticide Act, as amended, technical chlordane is defined as containing 60% octachloro-4,7-methanotetrahydroindane and 40% related compounds. trans-Nonachlor is one of several chemicals found as a part of these related compounds and has been detected in technical heptachlor (Cochrane et al. 1970). Chlordane has been registered with the federal government since 1948 as an economic poison. It has been widely used as an insecticide and to a lesser extent, as a herbicide. Since its insecticidal efficacy includes pests of turf and lawns as well as household pests, its usage tends to bring this chemical in close proximity to humans.

The objective of this article is to report the discovery of trans-nonachlor in human adipose tissue and to provide a preliminary assessment of its geographic distribution in the United States. Although this report ascribes no clinical or symptomatic repercussions to these residues in humans, the finding of trans-nonachlor is indicative of exposure to chlordane and heptachlor. Other chemicals which are representative of chlordane and heptachlor exposure have also been reported in human tissues. Oxychlordane, a mammalian metabolite of several chlordane constituents, has previously been reported (Biros and Enos 1973). Heptachlor epoxide, derived from heptachlor or possibly in part from the heptachlor in technical chlordane, has also been reported in a national survey of human adipose tissue (Kutz 1974). trans-Nonachlor has also been reported in some environmental samples (Law and Goerlitz 1974, Lichtenstein 1971).

trans-Nonachlor was first detected as an unidentified component of adipose tissue extracts at the Pesticide Laboratory, Michigan Department of Public Health, which performs chemical analyses under contract for the National Human Monitoring Program (Kazen et al. 1974). These extracts were sent to the Environmental Toxicology Division, Research Triangle Park, N.C., for chemical characterization. When it was determined

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that the component was trans-nonachlor (Sovocool and Lewis 1975), a special study was organized under the National Human Monitoring Program to provide some preliminary data on the chemical epidemiology of this residue.

The National Human Monitoring Program functions to determine, on a national scale, the incidence and level of exposure to pesticides experienced by the general population and to identify changes and trends in these parameters when they occur. Further program information is detailed by Yobs (1971).

MATERIALS AND METHODS

Human adipose tissue was collected at various geographic locations throughout the conterminous United States. These tissues were extracted and analyzed for organochlorine pesticides using gas liquid chromatography with electron capture detection (GLC-EC). The presence of trans-nonachlor was confirmed, where possible, with gas liquid chromatography combined with mass spectrometry (GLC-MS). Residue levels of selected extracts were further estimated by multiple ion detection mass spectrometry (MID-MS).

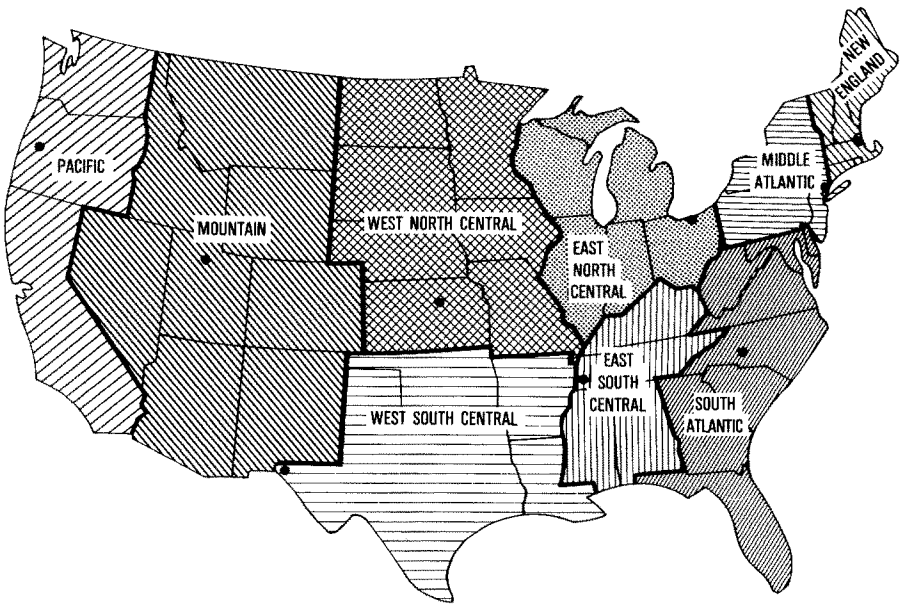

FIGURE 1

MAP OF THE UNITED STATES SHOWING THE LOCATION OF THE COLLECTION SITES IN THE 9 CENSUS DIVISONS

Five of the tissue specimens submitted from each of these sites during FY 1974 were extracted; these extracts were pooled to make a composite sample for each collecting site in each census division. The demographic (age, sex and race) description of the patients sampled to make up each composite are summarized in Table 1.

TABLE 1
Demographic Description of Patients Sampled
for *trans*-Nonachlor Analysis

Census Division	Geographic Location Sampled	No. of Patients Composited	Caucasian		Negro		Age Range	Samples Collected During
			Female	Male	Female	Male		
New England	Massachusetts	5	1	3	1	0	23-68	Postmortem
Middle Atlantic	New York	5	0	5	0	0	56-82	Postmortem
East North Central	Ohio	5	2	3	0	0	18-80	Postmortem
West South Central	Texas	5	4	1	0	0	10-65	Postmortem
South Atlantic	North Carolina	5	3	1	1	0	17-50	Postmortem
East South Central	Tennessee	5	4	1	0	0	25-77	Postmortem
West North Central	Kansas	5	3	2	0	0	41-67	Surgery
Mountain	Utah	5	2	2	1	0	22-73	Postmortem
Pacific	Oregon	5	4	1	0	0	29-43	Surgery

Sample Collection

Samples of human adipose tissue were obtained through cooperating medical pathologists and medical examiners at hospitals in cities selected according to a proportionate, stratified-random design. The conterminous 48 states were divided into 9 census divisions, according to the 1970 census of the United States. A city within each census division was selected from those already participating in the National Human Monitoring Program as the collection site for this special project (Figure 1).

Chemical Analysis

All tissues were extracted and cleaned-up according to a modified Mills-Olney-Gaither procedure (Thompson 1972) by laboratories under contract to the National Human Monitoring Program. Concentrated extracts, corresponding to the 6% diethyl ether in petroleum ether fraction from the florisil clean-up column were sent to the Environmental Toxicology Division for GLC-MS analysis.

Gas Chromatograph: Hewlett Packard 5700A with 1.8mm by 2mm i.d. X 4mm o.d. glass column packed with 1.5% OV-17, 1.95% OV-210 on 80-100 mesh Gas Chrom Q. The GC inlet temperature was 200°C. Helium flow rate was 42 ml/min. Temperature programmed runs were made from 80°C (2 min.) to 210°C at 8°C/min. Multiple ion detection runs were made isothermally at 190°C. The transfer line to the mass spectrometer was maintained at 210°C and the silicone membrane separator was kept at 180°C.

Mass Spectrometer: A Hewlett Packard 5930A quadrupole focusing mass spectrometer operated at 70eV electron energy and 400μ amps filament emission with target current of 310μ amps. MID runs used 100μ amps and high electron multiplier voltages. The ion source temperature was maintained at 180°C and the mass filter temperature at 110°C. Ions were detected using a Bendix continuous dynode electron multiplier with voltages varied between 1.5 and 2.5 Kv, as required for sufficient signal strength. Data were collected, stored and plotted using a Hewlett Packard 5932A Data System. Multiple ion detection was controlled by a suitable program provided by the manufacturer, and by the data system. The ions monitored were 405, 407, 409, 411 m/e. Data output also involved the use of a light beam oscillograph, as well as plotted mass spectra and reconstructed total ion chromatograms from the data system.

Using the periodic scanning mode of operation, with the Hewlett Packard 5932A Data System, mass spectra were generally scanned from 100 to 550 m/e at 160 m/e per sec., with scan cycle times of about 5.5 sec.

A standard sample of trans-nonachlor was secured from Velsicol Chemical Corporation, Chicago, Illinois. The melting point and mass spectrum of the standard were found to be in agreement with the literature (Damico et al. 1968 and Cochrane et al. 1970).

The technique of multiple ion detection (MID) was employed to estimate the trans-nonachlor levels in the samples from Massachusetts and Texas. Quantification by GC/MS was difficult to achieve, since no completely reliable method has yet been developed for trans-nonachlor.

Confirmation was based on the presence of the large M^+-Cl ion at 405 m/e (8Cl), and where possible, the much weaker molecular ion (M^+) at 440 m/e (9Cl). The weaker retro-Diels-Alder fragment ions were occasionally hidden under ions resulting from components in the adipose tissue. Gas liquid chromatographic retention times of standard trans-nonachlor were checked for all positive samples and were found to be identical to mass spectrum and GLC retention time of the compound identified in the human adipose tissue samples.

RESULTS AND DISCUSSION

The results of the analysis of human adipose tissue for trans-nonachlor are presented in Table 2. Of the nine composite samples analyzed, eight showed confirmable levels of trans-nonachlor. Estimates using the minimum detection limits indicated that levels in all positive samples were greater than 0.01 ppm. The MID estimates of residue levels in two samples are shown in parentheses.

TABLE 2

RESULTS OF ANALYSIS FOR TRANS-NONACHLOR IN HUMAN ADIPOSE COMPOSITE EXTRACTS

Census Division	GLC-Mass Spectrometry Confirmation
New England	Confirmed (0.04 ppm)[1]
Middle Atlantic	Confirmed
East North Central	Not Confirmed
West South Central	Confirmed (0.06 ppm)[1]
South Atlantic	Confirmed
East South Central	Confirmed
West North Central	Confirmed
Mountain	Confirmed at a lower level than others
Pacific	Confirmed

[1] Concentration estimated by multiple ion detection using the intensity of 409 m/e ion.

Positive samples were found both in widely diverse geographic areas as well as in widely diverse demographic distributions. The positive composites included samples of males and females, caucasians and negroes, different age groups and samples collected at both surgery and postmortem. Postmortem specimens were collected from hospital cases as well as from coroner/medical examiner sources. Therefore, residues were found in tissues received from patients having pathological conditions and from those killed by traumatic injuries.

One composite sample from Ohio did not contain confirmable quantities of trans-nonachlor. Although the composite sample from Utah contained confirmable quantities of trans-nonachlor, the level was lower than in the other positive samples. The exact explanation for the apparent absence of trans-nonachlor from the Ohio sample and for the low level in the Utah sample cannot be determined from this study. It is, however, important to note that this is a preliminary report containing data derived from a small subsample of the national human monitoring survey.

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