Oxychlordane Residues in Human Adipose Tissue

by
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Recent investigations have demonstrated that the octachloro isomers of the insecticide chlordane are oxidatively metabolized in mammalian systems to oxychlordane (1-exo-2-endo-4,5,6,7,8,8-octachloro-2,3-exo-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene), probably via a 1,2-dichlorochlordene-2 intermediate (SCHWEMMER, et al., 1970 and STREET, et al., 1971). Residue storage levels of this non-polar, lipid soluble metabolite have been determined in several species including rats, dogs, swine, and cattle under controlled feeding conditions (POLEN, et al., 1970). In addition, residues of oxychlordane have been found in milk from cows which had been feeding on alfalfa contaminated with chlordane (LAWRENCE, et al., 1970). In this communication, we wish to report the confirmation and consistent finding of oxychlordane residues in general population human adipose tissue samples obtained through the National Human Monitoring Program (YOBS, 1971) for pesticides administered by the Office of Pesticides, U. S. Environmental Protection Agency.

Adipose tissue specimens are routinely collected from post-mortem examinations and therapeutic surgery procedures and analyzed for a series of organochlorine pesticide residues using a modified Mills-Onley-Gaither procedure (MILLS, et al., 1963 and THOMPSON, 1971). The results of a typical electron capture gas chromatographic determination of a purified tissue extract (Florisil column chromatography, fraction 1 eluate) illustrating the elution characteristics of oxychlordane are presented in Figure 1.

Analytical conditions are also indicated. A representative series of twenty-seven tissue specimens were analyzed and oxychlordane residue determined. Mean values, standard deviations and ranges for this group are presented in Table 1.

TABLE 1

Oxychlordane Residues in General Population Human Adipose Tissue Samples

Mean and Std. Deviations (ppm)	No. of Specimens	Range (ppm)	% Specimens Positive
0.14 ± 0.09	27	0.03 - 0.40	21/27 (77.8%)

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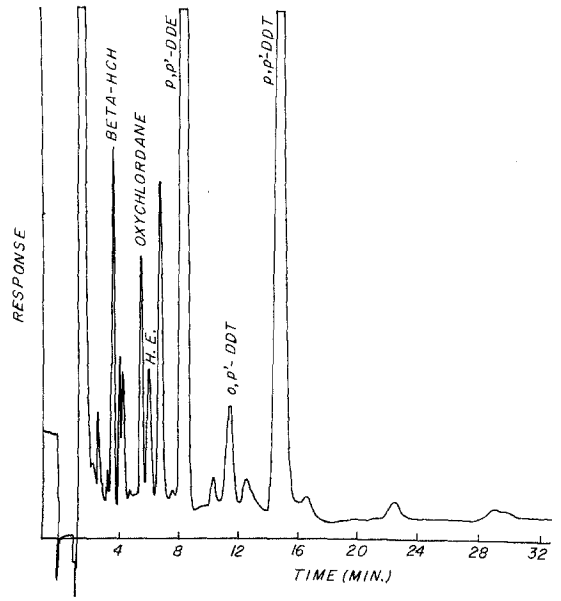

Figure 1. Organochlorine pesticide residues, including oxychlordane, in human adipose tissue extract. Electron capture gas chromatographic determination on column of 1.5% OV-17/1.95% QF-1 on 80/100 mesh silanized support at 200°C oven temperature and nitrogen carrier flow of 60 ml/min.

Routine monitoring laboratory procedure provided for the confirmation of the organochlorine pesticide residues including oxychlordane by comparison of qualitative and quantitative results employing two gas chromatographic columns (1.5% OV-17/1.95% QF-1, RRT_{aldrin} of oxychlordane, 1.38 at 200°C; 5% OV-210 RRT_{aldrin} of oxychlordane, 1.56 at 180°C) and thin layer chromatography. In this instance, additional instrumental confirmation and characterization of the oxychlordane was obtained using infrared spectroscopy and mass spectrometry. Quantities of residue sufficient for confirmation by these techniques were obtained using the following procedure. Human adipose tissue (500 g total) was extracted in the usual manner (THOMPSON, 1971) with petroleum ether. The extract was filtered, partitioned with acetonitrile, dried, concentrated, and fractionated and purified by Florisil column chromatography. The eluate from the first fraction (6% diethyl ether in petroleum ether eluate, Figure 1), containing the oxychlordane as well as

remaining organochlorine pesticides except dieldrin, was concentrated and additionally purified by chromatography through a second Florisil column employing 200 ml petroleum ether (fraction 1) and 250 ml 1% (v/v) methanol in petroleum ether (fraction 2) as eluates. The second eluate containing oxychlordane and the more polar polycyclic chlorinated pesticides including the hexachlorocyclohexane isomers, was concentrated and finally fractionated and purified by silica gel column chromatography to isolate the chlordane metabolite residue. Elution with 15% (v/v) benzene in hexane provided material sufficiently free of interfering residues and tissue coextractives for infrared analysis. For mass spectral analysis, however, a sample of oxychlordane was obtained by gas chromatographic trapping of the material in this eluate.

The infrared spectrum of the isolated residue compared well with that obtained for an authentic sample (FDA analytical reference standard) and available literature data (SCHWEMMER, et al., 1970, POLEN, et al., 1970, and LAWRENCE, et al., 1970). The following observed frequencies of absorption (cm^{-1}) were considered characteristic: 1605, fully substituted olefinic bond; 1240, 1250, epoxide C-O bond stretch; 823, 897, 910, epoxide ring vibrations; and finally, 690, 713, C-Cl bond stretching.

Mass spectral analysis at 80 EV of the oxychlordane residue employing direct probe introduction techniques revealed a relatively weak molecular ion of m/e 420. Further fragmentation of the parent molecule resulted in major daughter ions of m/e 385, formed by loss of a single chlorine atom (seven chlorine atom isotope intensity distribution); m/e 235, 270, retro Diels-Alder fragments of probable $C_5Cl_5^+$ and $C_5Cl_6^+$ composition, respectively; m/e 149, 115 (base peak) fragments corresponding to the cyclopentane oxide moiety of the parent molecule formed by retrodiene decomposition with possible $C_5H_3Cl_2O^+$ and $C_5H_4ClO^+$ composition; and finally, a major isotope group of m/e 185 corresponding to a fragment species containing three chlorine atoms (SCHWEMMER, et al., 1970, and LAWRENCE, et al., 1970).

The occurrence of storage of oxychlordane residues in general population human adipose tissue may indicate consistent previous exposure to chlordane insecticide and/or oxychlordane from many possible sources. With the inclusion of this pesticide metabolite in the human tissue residue monitoring program, significant trends and possible sources of exposure, as well as potential health effects may be assessed more accurately. Human toxicity, dose storage and response relationships, extent and distribution in humans, long term low level exposure effects and other factors including the determination of oxychlordane as a human metabolite of chlordane remain to be determined.

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Note added in proof: Independent studies performed by Food and Drug Administration laboratories (BURKE and McMAHON) have also confirmed oxychlordane residues in human adipose tissue. Identification in this instance has been supported by gas and thin layer chromatographic data.

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