Characterization of Oxychlordane, Animal Metabolite of Chlordane*

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Recognition that α- and γ-chlordane (CHL) comprise the principal terminal residues on plants treated with Technical CHL (1) prompted study of these isomers, the main components of Technical CHL -- as distinguished from the total composition. A surprising observation was made as a by-product of one toxicological study wherein massive doses of pure CHL isomers were fed to rats. It appeared from gas-liquid chromatographic analysis of the rat fat that heptachlor epoxide (HE) had been formed as a metabolite (2). The present study shows this conclusion to be an artifact resulting from the limitations of a widely used chromatographic column (DC-200) and that a heretofore unrecognized compound resulted from the metabolism of α- or γ-CHL.

Critical study disclosed means of distinguishing the newly recognized metabolite from HE and provided tools for study of a new aspect of CHL metabolism.

A source of the metabolite for suitable isolation was found in the fat of pigs ingesting for 90 days diets fortified with massive doses (300 ppm) of individual CHL isomers (3). Pure metabolites isolated from feeding of either α- or γ-CHL were compared and found to be identical by melting point, electron capture gas-liquid chromatography, thin-layer chromatography, infrared and nuclear magnetic resonance spectroscopy and p-values. The metabolite, now called oxychlordane (OXY), $C_{10}H_4Cl_8O$, appears to form from CHL in the reaction $C_{10}H_6Cl_8 + 2(O) \longrightarrow C_{10}H_4Cl_8O + H_2O$.

OXY has also been synthesized in vitro by oxidation of 1,2-dichlorochlordene-2 and through direct oxidation of the respective CHL isomers with chromic acid (3).

While the pure isolated metabolite from feeding separately two isomers of CHL and the synthetic compound are analytically equivalent, at least one of the in vivo isolates is optically active. Limited bio-assays with house flies may indicate that the synthetic compound is about double the potency of the isolates. This observation coupled with optical activity of one of the isolates indicates selective metabolism of one

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of the enantiomorphs by animals. Similar selective enantiomorphic enrichment has been reported in the metabolism of dieldrin in rabbits (4) and microsomal conversion of other cyclodienes (5).

ANALYTICAL CHARACTERIZATION OF OXYCHLORDANE (OXY)

Melting Point. α-isolate, 99.4-100.0°C.; γ-isolate, 99.0-101.0°C.; synthetic, 99.0-101.0°C. (all uncorrected).

Elemental Analysis. Theory for $C_{10}H_4Cl_8O$: 28.34% Cl, 0.95% H, 66.93% Cl, 3.78% O. Found: γ-isolate - 28.42% Cl, 1.00% H, 66.92% Cl, 3.66% O, (by difference); α-isolate - 66.83% Cl.

Nuclear Magnetic Resonance Spectrum (NMR). α-, γ-isolates and synthetic OXY exhibited the same NMR spectra showing a multiplet at 3.20 to 3.65 ppm (2H; H-C-C-H); a singlet, 3.85 ppm (1H; epoxy); and doublet, 4.30 to 4.40 ppm (1H; H-C-C-H).

Infrared Absorption Spectrum (IR). α-, γ-isolates and synthetic OXY exhibited identical infrared absorption spectra (Fig. 1).

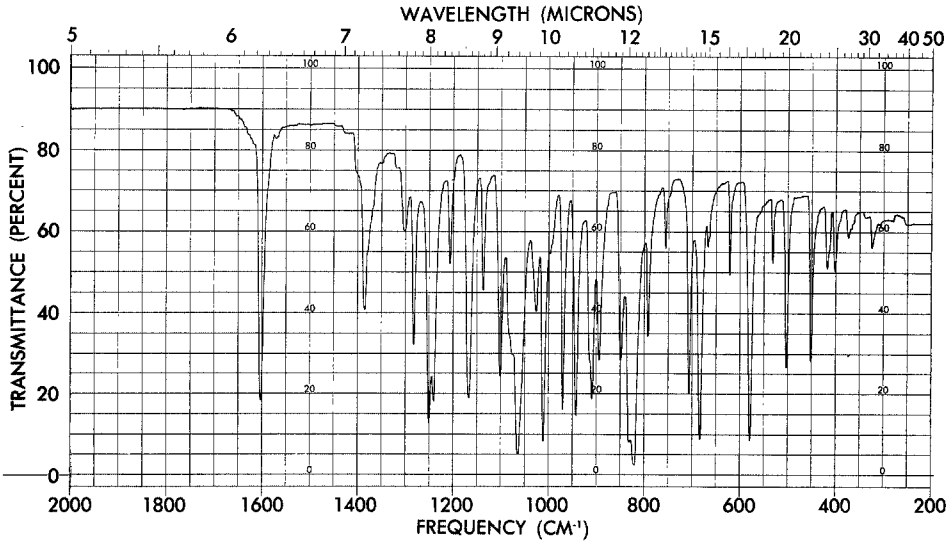

Figure 1. IR Spectrum of OXY in KBr Pellet

Optical Rotation. OXY isolated from γ-CHL feeding of pigs, $(\alpha)_D^{25}$ + 2.7 deg. dec. $^{-1}$g. $^{-1}$ml.; α-CHL feeding isolate and synthetic product, no optical rotation observed.

Gas Liquid Chromatography (GLC). Some columns fairly widely used for GLC analysis of organochlorine pesticides do not resolve OXY from HE. Clues to the presence of OXY with HE are broader peaks and slight discrepancy of RT compared with pure HE standards. For example, on DC-200/Epon 1001 columns the broadened, unresolved peaks for OXY-HE mixtures have retention time (RT) 0.92-0.98 relative to HE standards, depending on ratio of constituents.

Good resolution into 2 peaks is observed with either EC or micro-coulometric detectors and OV-17/QF-1 (various ratios) columns at 180°-225°C.: RT for OXY relative to HE -- 0.87-0.90. (RT for OXY relative to aldrin is 1.30-1.35). The RT for OXY on a 4-foot, 3% QF-1, 80/100 mesh Gas Chrom Q column at 165° with flame ionization detector is 0.82 relative to HE.

p-Values. Measured according to Beroza and Bowman (6), observed p-values are: In heptane/CH_3CN, 0.44-0.49 for OXY, 0.26-0.28 for HE; in heptane/85% aqueous dimethyl formamide, 0.70-0.78 for OXY, 0.44-0.55 for HE.

Thin layer Chromatography (TLC). Rf's observed on EK 100μ plates for 95% heptane, 5%, acetone solvent system are for OXY and HE respectively: Alumina 0.54, 0.40; Silica Gel 0.41, 0.34.

Response to Acid and Alkali. Stable to acid. No appreciable change in GLC peak height upon 18 hour contact of a 0.1μg. OXY per ml. n-heptane solution with H_2SO_4/fuming H_2SO_4 (1:1).

Unstable to alkali. No visible GLC peaks from direct pentane extract of reaction mixture: 20% ethanolic KOH at 55°C. for 30 min.; H_2O added. Extraction of same mixture, after acidification with H_2SO_4, yielded 3 peaks at 7.5, 10.2, and 12.0 minutes. The RT for OXY under identical conditions is 4.7 minutes.

Response to Chromogenic Reagents. OXY treated with Polen-Silverman (7, 8) or Davidow (9) reagents produces yellow solutions, visually of the same hue as those from HE but less intense. These reagents provide no self-revealing qualitative distinction between OXY and HE.

STORAGE OF OXY IN FAT OF MAMMALS

Rats. White rats, which for one year ingested diets fortified with either the α- or γ-CHL isomers of 50-50 mixtures, formed and stored OXY in their fat at levels shown in Table 1. The ratio of OXY concentration found in fat to that of CHL consumed is given as Storage Concentration Ratio.

To aid in assessing these observations it is interesting to note that the "no effect" level for CHL in rats is estimated to be 20 ppm in the diet, equivalent to 1 mg/kg/day (5).

TABLE 1

OXY Storage Levels in Fat of Rats*
Fed One Year on Diets Dosed with
Chlordane Isomers

Isomer	PPM in Diet (D)	OXY in Fat, PPM - (θ)**	Storage Conc. Ratio, θ/D
Control	0	0.2	--
α	5	8.	1.6
"	15	7.	0.5
"	15	13.	0.9
"	15	12.	0.8
"	45	22.	0.4
γ	75	72.	1.0
"	75	93.	1.2
"	75	105.	1.4
"	150	150.	1.0
50-50 α & γ	45	55.	1.1
"	75	75.	1.0

*Both sexes included. Each line represents one individual, but identity of sex was not annotated.

**Calibrated against HE standard; not corrected for value observed for control.

Dogs. Beagle dogs continually consuming diet spiked with Technical CHL stored, after two years, OXY at levels as shown in Table 2. The fat also gave chromatographic responses related to other constituents of Technical CHL.

The evaluation of these results may be aided by noting that the "no effect" level for CHL in dogs has been estimated to be 3.0 ppm in the diet, equivalent to 0.075 mg/kg/day (5).

TABLE 2

OXY Storage Levels in Fat of Dogs
Fed 2 Years on Diet Dosed with
Technical Chlordane

CHL Fed, ppm -- (F)	Sex	OXY in Fat, ppm -- (θ)*	Storage Conc. Ratio -- (θ/F)
3 ppm	M	3.0	1.0
3 ppm	F	3.7	1.2
30 ppm	M	24.	0.8

*Analyses are made on fat of individual animals.

Pigs. Pigs administered high levels of CHL isomers in their diets for 90 days provided an adequate source for isolating the metabolite. Six pigs were fed diets fortified at 300 ppm (equivalent to 3-9 mg/kg, decreasing as body weight increased) with individual CHL isomers. No visible gross side reactions were observed in any of the experimental subjects. Analyses of fat in animals sacrificed at 60 and 90 days are given in Table 3. The fat of these animals provided the source for isolation of pure metabolite (3) which was subsequently determined to be OXY formed from both CHL isomers.

TABLE 3

Residue Storage Levels in Fat of Pigs
On Feed Dosed at 300 ppm with Chlordane Isomers

Isomer	Days	Found in Fat, ppm		Storage Conc. Ratio	
		OXY (θ)*	CHL Isomer Fed (C)	OXY (θ/300)	CHL (C/300)
$\frac{\alpha}{\pi}$	60	12.	8.	0.03	0.027
	90	36.	9.	0.12	0.030
$\frac{\gamma}{\pi}$	60	69.	9.	0.23	0.030
	90	71.	4.	0.24	0.013

*Calibrated against HE standard.

Briefly, isolation of the metabolite resulted from successive partitions between hexane and acetonitrile, acid and chromatographic clean-up, and recrystallization from pentane. Experimental details and proposal of structure for OXY are given elsewhere (3).

Assuming 20% fat in pigs, an estimate of the fraction of ingested CHL isomers stored as residues is given in Table 4. The magnitude is substantially in agreement with the findings of Korte (10) that CHL is largely dissipated in urine and feces as hydrophilic metabo-

lites; he estimated storage in fat of rabbits at about 4% of γ-CHL (called α-CHL in his nomenclature) consumed for 10 weeks. Our observations indicate that the formation and storage of metabolite is smaller for α-isomer than for γ-CHL.

TABLE 4

Propensity for Residue Storage
As Percent of CHL Consumed (Pigs)

90 Days, 300 ppm CHL in Diet

Isomer	CHL in Diet mg.	Stored in Fat, mg.		Stored, as % of CHL Fed	
		OXY	CHL	OXY	CHL
α	83,100	770	190	0.9	0.2
γ	82,200	1780	100	2.2	0.1

Cows (Beef and Dairy). To learn if OXY occurs in meat or milk as a result of feeding CHL at tolerance level proposed by FAO/WHO (11), 4 beef cattle and 12 dairy cattle were given feed dosed with pure chlordane isomers. Pure α-, γ-CHL and 50-50 mixtures of both were fed at 0.1 ppm and 0.3 ppm for 30 days.

No OXY was detected in milk from the dairy cattle (sensitivity 0.005 ppm) after 30 days of feeding at these rates. OXY was occasionally detected in omental and back fat of dairy cattle at levels up to 0.02 ppm. The maximum ratio of metabolite level in fat to level of CHL fed was 0.07; 67% of the observations resulted in a ratio at or below 0.03. Analysis of brain and liver tissues from the same animals showed no detectable OXY.

In beef cattle, OXY was detected in omental and back fat at levels up to 0.03 ppm, representing a maximum Storage Concentration Ratio of 0.10. None of the metabolite was detected in liver or brain of these animals.

ABSENCE OF OXY IN PLANTS AND SOIL

Thus far we have observed no evidence of occurrence of OXY in plants or in soil from supervised field trials with Technical CHL, even where substantial other residues are expected and detected. Fig. 2 is a gas chromatogram (11% OV-17, QF-1 column) from residues on sugar beets. This is typical of observations on 22 samples of sugar beets, soybean stalks, flax straw and soil from either foliar or soil treatments at 1-12 lb. per acre.

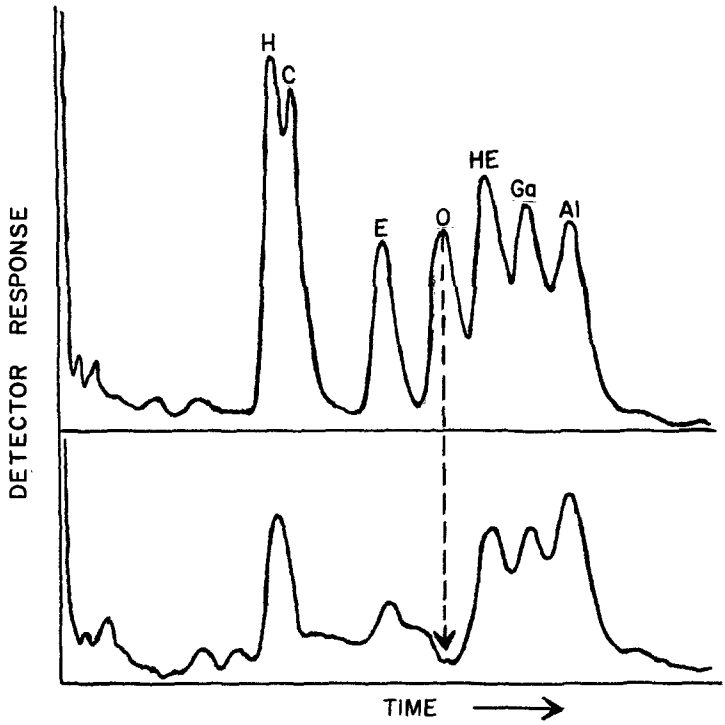

Fig. 2. Chromatograms: Upper. Standards: H, heptachlor; C, E, minor constituents of Tech. CHL; O, OXY; HE, heptachlor epoxide; Ga, γ-CHL; Al, α-CHL. Lower. Residue on sugar beets grown in soil treated with Tech. CHL, 12 lb/A.

SUMMARY AND DISCUSSION

Oxychlordane, $C_{10}H_4Cl_8O$, (OXY) has been detected in and isolated from the fat of animals fed pure isomers of chlordane (CHL). The propensity for storage is low. The ratio of storage level in fat to feeding level, an index of probability of amplification, is about 0.1 in a 30-day feeding of CHL and approaches roughly unity for chronic 2-year feeding of CHL. α-CHL appears to exhibit a somewhat smaller propensity of formation and storage of OXY than does γ-CHL.

OXY has not been detected in plants or soil treated with Technical CHL in supervised trials.

At feeding levels corresponding to proposed international tolerances, OXY is not detected in milk but may be found in the fat of cattle at or below 0.03 ppm.

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