# Oxychlordane: Accumulation in Rat Adipose Tissue on Feeding Chlordane Isomers or Technical Chlordane

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Oxychlordane storage in adipose tissue was studied in male and female rats fed pure *cis*- and/or *trans*-chlordane or technical chlordane at levels from 50 to 200 ppm for 15 days. *trans*-Chlordane resulted in greater oxychlordane storage than *cis*-chlordane in both sexes with lower accompanying parent isomer storage. Males fed either isomer stored less oxychlordane than females. Oxychlordane storage during simultaneous administration of both chlordane isomers, or of technical chlordane itself, showed additivity of oxychlordane formation with no interaction apparent. Results of incubating chlordane isomers with rat liver homogenates indicated that oxychlordane formation proceeds *via* a dehydrogenated intermediate, dichlorochlordene.

Oxychlordane is the trivial name of a recently described animal metabolite (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) derived from both principle octachloro isomers, *cis*(1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene; $\alpha$-chlordane) and *trans*(1-*exo*,2-*endo*,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene; $\gamma$-chlordane) in technical chlordane (Figure 1). This metabolite accumulates in animal tissues during administration of chlordane and has been found in cow's milk (Lawrence *et al.*, 1970; Schwemmer *et al.*, 1970). Its rate of accumulation in tissues has not yet been clearly defined and there is some evidence that oxychlordane residues have been previously misinterpreted as heptachlor epoxide on the basis of very similar glc and tlc characteristics under certain of the common chromatographic conditions (Lawrence *et al.*, 1970).

This paper presents a study of the accumulation of oxychlordane in rat adipose tissue during short-term controlled feeding of diets containing the pure chlordane isomers or technical chlordane together with a consideration of the mechanism of oxychlordane formation.

## PROCEDURE

In initial experiments male or female Holtzman rats were fed pure *cis*- or *trans*-chlordane isomers incorporated into a standard laboratory diet (Purina laboratory chow). These diets, which contained 50 to 200 ppm of the particular chlordane isomer, were offered for 11 to 15 days, and the rats were then sacrificed. Abdominal adipose tissue was exhaustively extracted with hexane, the extract cleaned-up with a florisil column, and the chlordane and oxychlordane present were analyzed by ec–glc. Either a DC-200 (10% on Gas Chrom Q, 200°C) or an XE-60 (5% on Gas Chrom Q, 200°C) column was used. The latter column allowed rapid resolution of oxychlordane from the chlordane isomers but did not resolve the *cis*- and *trans*-chlordanes. When such distinction was required, the slower DC-200 column was utilized. A small sample of oxychlordane (>90% purity) was used as the analytical standard. A slight impurity, apparently 1-*exo*-2-*endo*-dichlorochlordene-2, was evident in chromatograms of the oxychlordane standard. Its proportion in the oxychlordane was estimated to be 0.4% on the basis of an authentic dichlorochlordene sample. The data reported herein have not been corrected for the impurity in the standard.

In another experiment, *cis*- and *trans*-chlordane isomers were fed to female rats in various fixed ratio combinations from 9:1 trans:cis to 1:9. Each combination provided a total concentration of 100 ppm of chlordane isomers in the feed. Results from feeding these combinations for 15 days were compared to data obtained with technical chlordane at a level of 50 ppm in the diet.

Chlordane isomers were incubated with rat liver homogenates in the presence of NADP and oxygen. Liver from DDT-treated rats was used in some cases in order to achieve greater chlordane metabolism rates. Chlordane products were extracted from the incubation mixture with diethyl ether and directly injected onto the glc column for analysis.

## RESULTS

Residues of the parent chlordane isomers and of oxychlordane in rat adipose tissue following 15 days consumption of treated diets are listed in Table I. Both male and female rats stored less oxychlordane from the cis isomer than from *trans*-chlordane, while the reverse trend was observed for storage of the parent compounds. These observations imply greater reactivity of the trans isomer as the reason for the greater oxychlordane storage with that isomer.

Female rats stored markedly greater concentrations of oxychlordane in comparison to male rats (Table I). This sex difference was observed with both chlordane isomers, but was much greater with the more reactive *trans*-chlordane. At 100 ppm in the diet, storage ratios of oxychlordane:chlordane were 20.0:1 and 4.3:1 with *trans*-chlordane (female and male, respectively) and only 9.7:1 and 3.4:1 for *cis*-chlordane. The greater oxychlordane storage by female rats would seem to represent one more example of their well-known lesser capacity to metabolize foreign compounds. In this case, the metabolism of chlordane beyond oxychlordane is apparently slower in the female rat, just as seen with the metabolism of aldrin and heptachlor.

Interaction between the isomers could occur during their common metabolism because of the isomeric differences in oxychlordane storage. This possibility was investigated by feeding diets containing the isomers in several different fixed proportions. The results (Figure 2) showed that oxychlordane accumulation was essentially additive for each chlordane isomer with no interaction apparent. Likewise, storage of the unchanged parent isomers did not indicate any

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Table I. Oxychlordane and Parent Chlordane Isomer Storage in Rat Adipose Tissue

| Concentration and form of chlordane administered in diet, ppm | Sex | Residue in tissue (μg/g lipid)[a] | | Ratio: oxychlordane/ chlordane isomer |
|---|---|---|---|---|
| | | Parent chlordane isomer | Oxychlordane | |
| *trans*-Chlordane | | | | |
| 50 | F[b] | 5.73 ± 1.39 | 103.8 ± 5.6 | 18.1 |
| | M[c] | 1.09 ± 0.06 | 5.0 ± 0.9 | 4.6 |
| 100 | F | 10.08 ± 1.67 | 201.5 ± 18.4 | 20.0 |
| | M | 1.50 ± 0.11 | 14.6 ± 0.7 | 9.7 |
| 200 | F | 23.25 ± 0.70 | 470.5 ± 44.6 | 20.2 |
| | M | 2.20 ± 0.45 | 22.3 ± 2.6 | 10.1 |
| *cis*-Chlordane | | | | |
| 100 | F | 22.69 ± 1.02 | 100.2 ± 7.8 | 4.3 |
| | M | 1.63 ± 0.22 | 5.5 ± 1.0 | 3.4 |
| 200 | F | 48.04 ± 3.79 | 182.2 ± 7.8 | 3.8 |
| | M | 3.13 ± 0.12 | 8.5 ± 1.3 | 2.7 |
| Technical chlordane | | | | |
| 50 | F | 2.56 ± 0.31[d] | 17.1 ± 1.2 | 6.7[d] |

[a] Rats were fed the indicated chlordane levels incorporated into a commercial laboratory diet. Analysis was by glc method described in text. [b] Female rats were fed 15 days, five per treatment group, except those receiving Technical Chlordane (11 days). [c] Male rats were fed 11 days, four per treatment group. [d] Combined *cis*- and *trans*-chlordane.

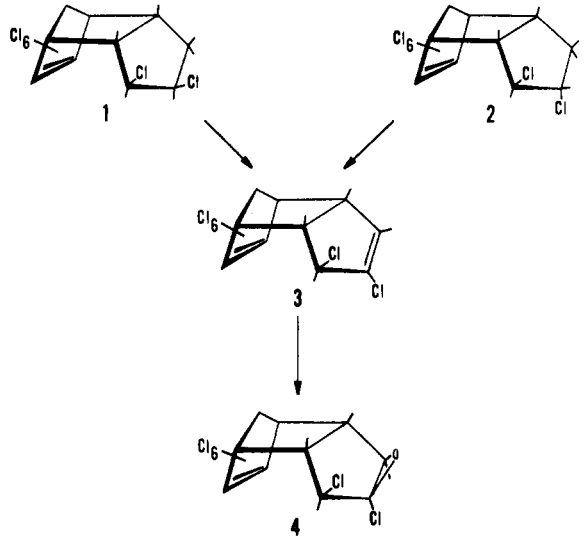


Figure 1. Structures of compounds discussed in the text. (1) *cis*-chlordane; (2) *trans*-chlordane; (3) 1-*exo*,2-*endo*-dichlorochlordene; and (4) oxychlordane

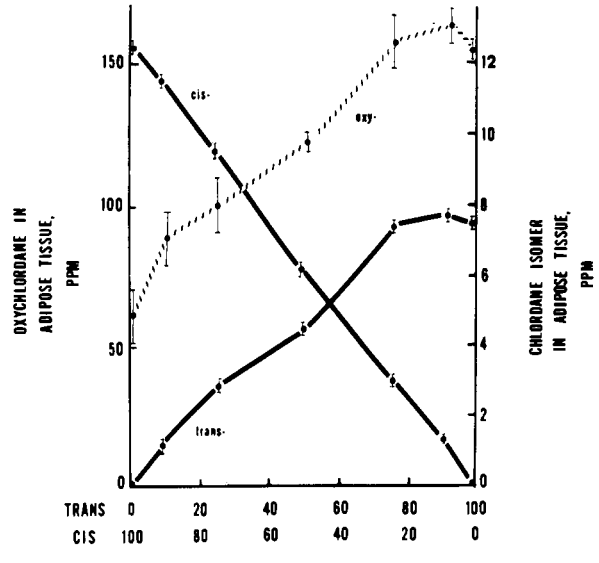


Figure 2. Oxychlordane and chlordane isomer storage in female rat adipose tissue after feeding fixed ratio combinations of *cis*- and *trans*-chlordane totaling 100 ppm in the diet

combined effects. The peak in oxychlordane storage observed with the 90:10 (trans:cis) isomer combination (Figure 2) was considered to be due to experimental error in diet formulation, but this point has not been reevaluated.

Since the oxychlordane storage increased in an essentially linear manner upon the feeding of combinations ranging from 100% cis isomer to 100% trans isomer, its storage upon feeding technical chlordane might be predictable. This was indicated in our trial when 17 ppm of oxychlordane resulted from feeding a diet containing 50 ppm of technical chlordane. That value corresponds fairly closely to a predicted storage level of 25 ppm, based on an approximately 1:1 chlordane isomer ratio which comprises 50% of the technical mixture.

## DISCUSSION

In this study, oxychlordane accumulation in rat adipose tissue was shown to exceed that of the parent chlordane isomers for both sexes. It, therefore, appears to be the major terminal chlordane residue in this, and possibly other, mammalian species (Polen *et al.*, 1971). The ratio of oxychlordane stored to level of dietary chlordane (the so-called "storage concentration" ratio) ranged from 2.0–2.3 and 1.0 for *trans*-chlordane (female and male, respectively) to 0.10–0.15 and 0.04–0.06 for *cis*-chlordane. This ratio for females receiving *trans*-chlordane was considerably higher, and those for both sexes receiving *cis*-chlordane were far lower than the values recently reported by Polen *et al.* (1971) who also fed Holtzman rats similar levels of chlordane. Our values may differ because of the short-term feeding involved (2 weeks) compared to 12 months by Polen and coworkers. Our animals probably did not reach a steady state condition of oxychlordane formation and storage during the experiment.

The tissue storage studies indicate an appreciably greater rate of oxychlordane formation from the *trans*-chlordane isomer. Other workers have presented evidence that oxychlordane is a common metabolite of both *cis*- and *trans*-chlordane (Schwemmer *et al.*, 1970). One feasible metabolic pathway of oxychlordane synthesis is that *via* the dehydro-

Table II. Metabolic Conversions of Chlordane Isomers and Dichlorochlordene by Rat Liver Homogenates[a]

| Animal pretreatment | Incubation time, min | Male: Chlordane, μg | Male: Dichlorochlordene, μg | Male: Oxychlordane, μg | Male: Total recovery, % | Female: Chlordane, μg | Female: Dichlorochlordene, μg | Female: Oxychlordane, μg | Female: Total recovery, % |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *trans*-Chlordane[b] | | | | | |
| None | 60 | 8.0 | 0.8 | 2.2 | 58 | 18.0 | 0.5 | 0.4 | 99 |
| DDT, 50 ppm | 16 | 3.4 | 4.2 | 6.4 | 81 | | | | |
| | 60 | 0.2 | 0.0 | 6.6 | 36 | 4.1 | 0.6 | 6.5 | 59 |
| | | | | *cis*-Chlordane[c] | | | | | |
| None | 60 | 9.8 | 0.0 | 0.3 | 65 | 15.4 | | | 99 |
| DDT, 50 ppm | 16 | 9.5 | 0.9 | 1.5 | 59 | | | | |
| | 60 | 0.6 | 0.0 | 2.2 | 18 | 5.2 | | 1.4 | 42 |
| | | | | Dichlorochlordene[d] | | | | | |
| DDT, 50 ppm | 15 | | 3.8 | 3.5 | 86 | | | | |
| | 60 | | 1.1 | 4.9 | 70 | | | | |

[a] Substrates were incubated with 10,000 × *g* supernatant of homogenized liver (1 + 4 in 1.15% KCl + 0.025% nicotinamide solution), 1.0 ml in a system containing 0.013 m*M* $MgSO_4$, 2.5 μ*M* NADP, 0.085 m*M* nicotinamide, 0.029 m*M* glucose 6-phosphate in phosphate buffer, pH 7.4. Total incubation volume was 4.5 ml. Each substrate was added in 0.05 ml of methylcellosolve. [b] Substrate level: 17.5 μg/flask (0.043 μ*M*). [c] Substrate level: 15.6 μg/flask (0.038 μ*M*). [d] Substrate level: 8.5 μg/flask (0.021 μ*M*).

genated intermediate, dichlorochlordene, as depicted in Figure 1. From that model, the rate-limiting step should be the dehydrogenation reaction favoring the trans isomer. Preliminary studies of oxychlordane production by rat liver homogenates in our laboratory confirm the much greater rate with *trans*-chlordane as substrate (Table II). These *in vitro* studies also revealed the formation of another metabolic product which appears to be identical with 1-*exo*,2-*endo*-dichlorochlordene-2. The dichlorochlordene itself was also found to yield oxychlordane on incubation with liver homogenate (Table II). These results indicate the plausibility of the model pathway presented.

The structural similarity of oxychlordane to other chlorinated cyclodiene epoxides suggests that oxychlordane should be more toxic than chlordane isomers. Preliminary investigation (Velsicol Chemical Corporation, 1971) has indicated a somewhat greater toxicity of oxychlordane over *trans*-chlordane in both acute and chronic tests. If, however, oxychlordane were the primary toxicant formed from the chlordane isomers, a greater difference in their toxicities would be expected since we found that *trans*-chlordane feeding resulted in 2.6× more oxychlordane stored than was observed with *cis*-chlordane. Recent toxicity evaluations with male rats showed *cis*-chlordane to have only a slightly higher $LD_{50}$ than *trans*-chlordane (392 *vs.* 327 mg/kg) and the difference was not statistically significant (Velsicol Chemical Corporation, 1971).

Oxychlordane formation, however, is clearly not the predominate mode of chlordane metabolism in liver, as shown by the progressive loss of identifiable material in the liver incubates (Table II). Oxychlordane, thus, may represent a relatively stable storage product from a minor pathway of chlordane metabolism.

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