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# THIN-LAYER CHROMATOGRAPHY OF RAT BILE AND URINE FOLLOWING INTRAVENOUS ADMINISTRATION OF THE PESTICIDAL SYNERGIST OCTACHLORODIPROPYL ETHER

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SUMMARY

Chromatographic differences in rat bile and urine samples resulting from single intravenous administration of octachlorodipropyl ether were elaborated using Silica Gel DF-5 chromatoplates with toluene–acetic acid–water (10:10:1) as developer. Detection was accomplished by 2537 Å and 3660 Å ultraviolet as well as conc. sulfuric acid–*n*-butanol and silver nitrate–2 *N* alcoholic potassium hydroxide chromogenic reagents. Ten metabolites were detected in the bile and 5 in the urine. The relative rates of elimination of the major biliary and urinary metabolites were compared.

## INTRODUCTION

Previously we reported on the elimination of pesticidal synergists (piperonyl butoxide and tropital)[1], $^{14}$C-tropital[2] and related methylenedioxyphenyl derivatives, *e.g.*, safrole, isosafrole and dihydrosafrole[3] and their metabolites in rat bile and urine resulting from single intravenous administration of the above compounds.

It was found in these studies that the synergists piperonyl butoxide and tropital were altered chemically and although the rate of elimination was high, it did not reach a rapid peak and rapid subsequent decline but suggested *prolonged* elimination of the metabolites into the bile.

The purpose of this investigation was to follow by thin-layer chromatographic techniques the elimination of the synergist octachlorodipropyl ether** (I) and its metabolites in rat bile and urine following a single intravenous injection of the com-

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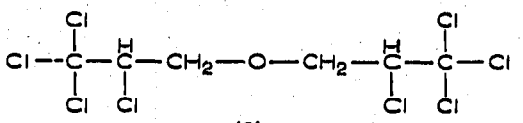
** Octachlorodipropyl ether (S-421) containing 1 % stabilizer (epichlorohydrin) was obtained from Badische Anilin & Soda Fabrik, A-G, Ludwigshaven, Germany; b.p. 144–150°/1 mm.

pound and to compare its elimination with that of the methylenedioxyphenyl synergists studied above.



(I)

Octachlorodipropyl ether was first prepared by BECKE AND SPERBER[4] *via* the reaction of bis(chloromethoxy-methane) and trichloroethylene and has been shown to be a synergist for pyrethrum[5], pyrethrins, allethrin and cyclethrin[6,7], DDT[8], carbamate insecticides[9], including Sevin[9,10] and insecticidal organic phosphoric acid derivatives[11].

## EXPERIMENTAL

### *Bile and urine sampling*

Single intravenous injections of 0.05–0.09 ml of octachlorodipropyl ether were given to adult rats of the Sprague-Dawley strain averaging 350 g in weight. Bile samples were collected by fistula and urine samples by cannulation from each rat. Details on the handling of the animals, anesthesia, surgery, sample colllection and timing have been previously described[12]. At least three urine samples were collected, one before i.v. injection, a second at an appropriate interval after injection and a third sample at the termination of the bile collection. All samples were kept frozen until ready for analysis.

### *Preparation of the plates*

Thin-layers (8 × 8 in.) 250 $\mu$ thick were prepared in the usual manner by mixing a slurry of Silica Gel DF-5* and water in a ratio of 30 g of absorbent to 72 ml water. They were air-dried for several hours, then oven-dried for half an hour at 75°, washed by ascending chromatography with chloroform–methanol (1:1) then oven activated at 75° for half an hour.

### *Solvent system*

(a) Toluene–acetic acid–water (10:10:1).

### *Detecting reagents*

*Chromogenic agents.* (a) Conc. sulfuric acid–*n*-butanol (15:85)[13]; (b) Silver nitrate–2 *N* alcoholic potassium hydroxide[14].

Plates were sprayed with 2 *N* methanolic potassium hydroxide, heated for 20–30 min at 120°, oversprayed with 1 % silver nitrate in 30 % nitric acid. Then the plates were exposed to unfiltered U.V. illumination for 15–20 min, or until spot intensity reached a maximum.

*Radiation sources.* (a) U.V. 3660 Å – Mineralight. Blak-Ray Model UVL-22**
(b) U.V. 2537 Å — Mineralight, Model UVS-11**.

* Obtained from Camag, Muttenz, Switzerland.
** Obtained from Allied Impex Corp., New York, N. Y.

*Chromatography*

Twenty microliters of all bile and urine samples were applied as half-inch streaks* on Silica Gel DF-5 plates and developed with toluene–acetic acid–water (10:10:1). Each developed plate was examined under visible light and 2537 Å and 3660 Å illumination, then photographed in color under 2537 Å illumination. Finally each plate was sprayed with the detecting reagent as described and photographed using equipment and procedures previously described[1].

## RESULTS AND DISCUSSION

Chromatographic differences in bile and urine samples resulting from i.v. administration of octachlorodipropyl ether are summarized in Table I. Table I lists the

TABLE I

SUMMARY OF RAT BILE AND URINE $R_F$ VALUES ON SILICA GEL DF-5 RESULTING FROM SINGLE INTRAVENOUS ADMINISTRATION OF OCTACHLORODIPROPYL ETHER

Solvent system: Toluene–acetic acid–water (10:10:1).
Detectors: (1) No spray; visible; (2) No spray; 2537Å; (3) Conc. sulfuric acid–butanol (15:85); (4) Conc. sulfuric acid–butanol (15:85)—3660Å; (5) Silver nitrate–2 *N* alcoholic potassium hydroxide.
Colors: Q = quench; B = blue; Bk = black; Bn = brown; G = green; Gr = grey; O = orange; V = violet; Y = yellow; T = tan.

| *Sample* | $R_F \times 100$ | *Detection agent* | | | | |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | *4* | *5* |
| Bile | 15 | — | — | Bk | Bk | — |
| | 20 | B-G | — | V | — | — |
| | 30 | — | — | — | — | Bn |
| | 32 | — | Q | — | — | — |
| | 37 | — | — | — | — | Bn |
| | 43 | — | — | — | — | Gr |
| | 45* | Y-G | G | G | Q | — |
| | 48 | — | — | — | — | Gr |
| | 60** | — | — | O-Bn | Y | — |
| | 65 | — | — | — | — | Gr |
| | 78 | — | — | T | Y-O | Gr |
| | 81 | — | — | T | Y-O | — |
| Urine | 15 | — | — | Bk | Bk | — |
| | 30 | — | — | — | — | T |
| | 62 | — | — | — | — | Gr |
| | 68 | — | — | — | — | Gr |
| | 78 | — | — | — | — | Gr |
| OCPE Std. | 92 | — | — | — | — | T |

* A bile pigment.
** Cholic acid or its conjugate.

$R_F$ value of each component obtained on Silica Gel DF-5 using toluene–acetic acid–water (10:10:1) as developer, and data regarding its characterization (means of detection and color). Other solvent systems screened for the resolution of biliary and

* Bile and urine samples were applied with a Radin-Pelids thin-layer sample streaker obtained from Applied Science Laboratories, State College, Pa., U.S.A.

TABLE II

RELATIVE CHANGES IN CONCENTRATION WITH TIME OF COMPONENTS APPEARING IN RAT BILE AFTER A SINGLE I.V. ADMINISTRATION OF OCTACHLORODIPROPYL ETHER*

Abbreviations: Bk = black; Bn = brown; G = green; O = orange; T = tan; V = violet; Gr = grey; tr = trace.

| *Detector: sulfuric acid–butanol (15:85)* | | | | | | | | | *Detector: silver nitrate* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_F \times 100$ | *Spot color* | *Before i.v. injection* | *Color intensity hours after injection* | | | | | *Relative spot size* | $R_F \times 100$ | *Spot color* | *Color intensity hours after injection* | | | | | *Relative spot size* |
| | | | *0–1* | *1–2* | *4–5* | *6–7* | *7–8* | | | | *0–1* | *1–2* | *4–5* | *5–6* | *6–7* | |
| 15 | Bk | 0 | tr | 2 | 4 | 4 | 4 | 4 | 30 | Gr | 0 | 6 | 2 | 1 | 1 | 1 |
| 20 | V | 0 | 1 | 3 | 3 | 4 | 4 | 1 | 37 | Gr | 3 | 4 | 4 | 4 | 3 | 4 |
| **45 | G | 0 | 0 | 0 | 2 | 3 | 3 | 4 | 43 | Gr | 1 | 2 | 3 | 2 | 1 | 1 |
| ***60 | O-Bn | 2 | 2 | 3 | 3 | 3 | 3 | 4 | 48 | Gr | 0 | 3 | 3 | 2 | 1 | 1 |
| 18 | T | trace | tr | 2 | 2 | 2 | 2 | 1 | 65 | Gr | 0 | 1 | 1 | tr | tr | 1 |
| 81 | T | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 78 | Gr | 0 | tr | tr | tr | tr | 1 |

* One i.v. dose of 0.092 ml of octachlorodipropyl ether administered to a 325 g male rat of Sprague-Dawley strain. Bile samples were collected during the indicated intervals. Spot intensities and sizes were estimated visually using arbitrary increments (0–4).

** A bile pigment.

*** Cholic acid or its conjugate.

TABLE III

RELATIVE CHANGES IN CONCENTRATION WITH TIME OF COMPONENTS APPEARING IN RAT URINE AFTER SINGLE I.V. ADMINISTRATION OF OCTACHLORODIPROPYL ETHER*

Spot intensities and sizes were estimated visually using arbitrary increments (0–4).

| $R_F \times 100$ | Spot** color | Color intensity | | | | | Relative spot size |
|---|---|---|---|---|---|---|---|
| | | Before i.v. injection | Hours after i.v. injection | | | | |
| | | | 0–1 | 1–3 | 3–5 | 5–8 | |
| 30 | T | 0 | 1 | 4 | 3 | 2 | 4 |
| 62 | Gr | 0 | 0 | tr | 1 | 1 | 1 |
| 68 | Gr | 0 | 2 | 3 | 2 | 2 | 2 |
| 78 | Gr | 0 | 0 | 0 | tr | tr | 1 |

* One i.v. dose of 0.050 ml octachlorodipropyl ether administered to a 338 g male rat of Sprague-Dawley strain.

** As detected with the silver nitrate–2*N* alcoholic potassium hydroide reagent. Gr = grey; T = tan; tr = trace.

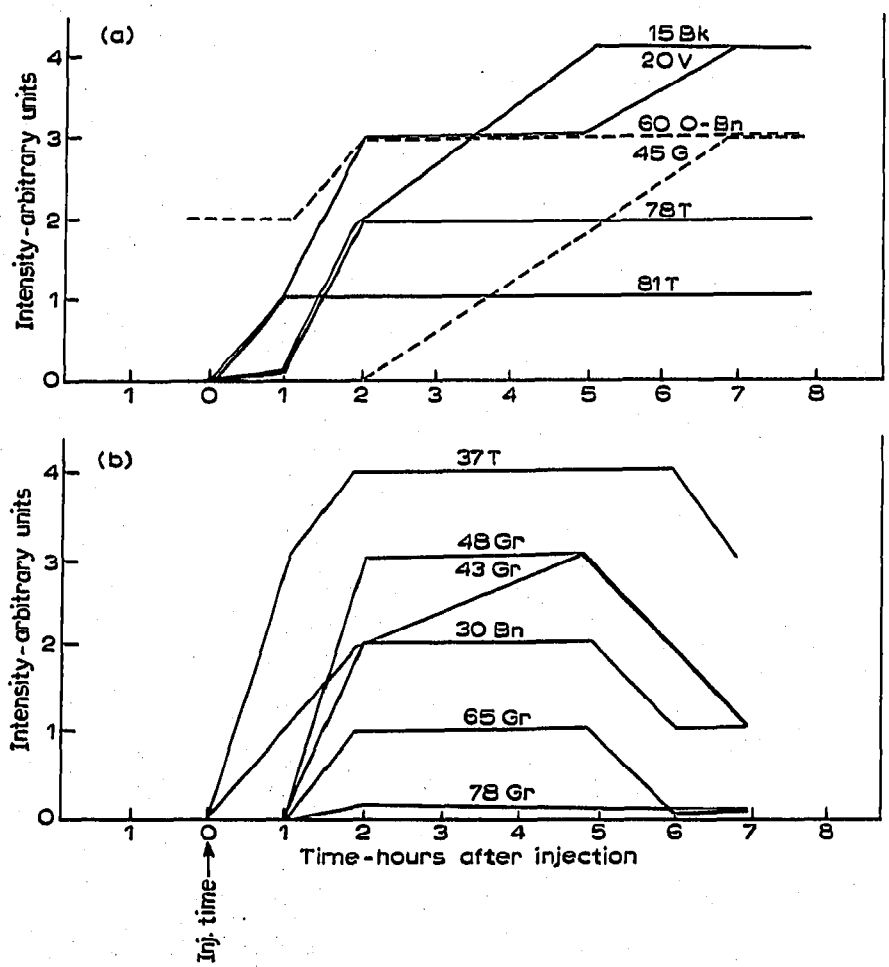



Fig. 1. Relationship of various bile components following intravenous administration of octachlorodipropyl ether. The data shown were taken from bile chromatograms developed with toluene–acetic acid–water (10:10:1). Concentrations were estimated visually as arbitrary degrees of intensity. Top and bottom figures illustrate biliary metabolites detected with (a) the sulfuric acid–butanol (15:85) and (b) silver nitrate–2 *N* alcoholic potassium hydroxide, respectively. Solid lines represent octachlorodipropyl ether metabolites and dotted lines depict bile components (*e.g.*, $R_F$ 0.45 bile pigment, $R_F$ 0.60 cholic acid or conjugate).

urinary metabolites, *e.g.*, (b) benzene–acetone (39:1) and (c) ethyl acetate–acetic acid–methanol (70:10:20) were found to be very much less effective than the toluene–acetic acid–water (10:10:1) system. Solvent system (c), however, effected the separation of two additional bile metabolites at $R_F$ 0.40 and 0.84 (as revealed by the sulfuric acid–butanol treatment).

The sulfuric acid–butanol reagent detected four possible metabolites in the bile and one in the urine, while the silver nitrate reagent revealed five possible metabolites in the bile and four in the urine.

Table II depicts the change in concentration with time of each component in the bile detected by the sulfuric acid–butanol and silver nitrate reagents. Table III shows the change in concentration with time of each component in the urine after detection with silver nitrate. No detectable free octachlorodipropyl ether has been found in either bile or urine by the methods utilized above.

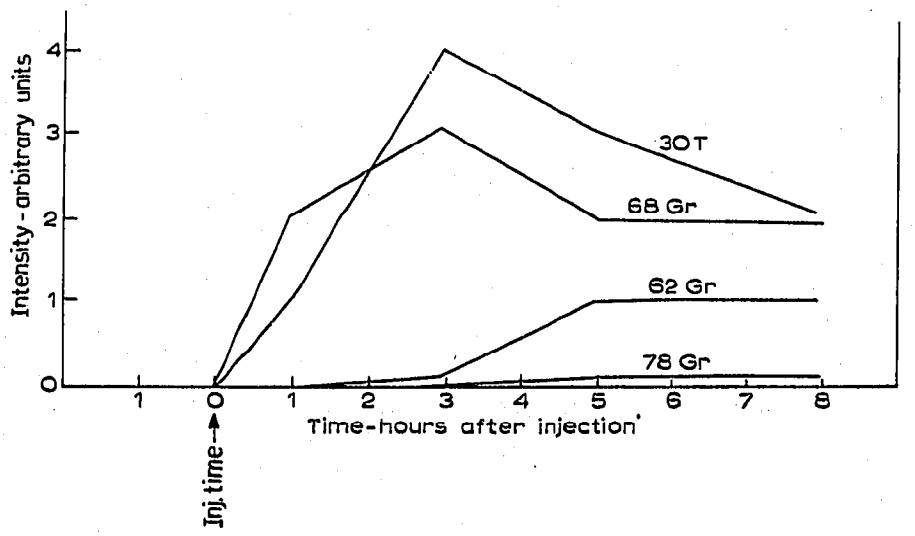



Fig. 2. Relationship of various urinary components following intravenous administration of octachlorodipropyl ether. The data shown were taken from urine chromatograms developed with toluene–acetic acid–water (10:10:1) and detected with the silver nitrate–2 *N* alcoholic potassium hydroxide reagent. Concentrations were estimated visually as arbitrary degrees of intensity.

Figs. 1 and 2 graphically depict the changes in concentration with time of components appearing in rat bile and urine respectively following a single i.v. administration of octachlorodipropyl ether. (The $R_F \times 100$ values and the spot colors correspond to those in Table I.) It can be seen from these figures that the rate of elimination of the biliary and urinary metabolites, although high (generally within 2 hours after administration of the drug), did not rapidly decline thereafter, but suggests slow prolonged elimination of the metabolites in the bile. This is analogous to the earlier experiments carried out with the methylenedioxyphenyl synergists piperonyl butoxide and tropital[1]. As suggested earlier, delayed elimination from the body of pesticides and other compounds coupled with inhibition of certain detoxification mechanisms could constitute a hazard to man when exposed to these compounds.

Although the metabolites found in the bile and urine chromatograms have not been identified thus far, it is of interest to speculate at this point as to their possible identity.

Fig. 3 depicts the possible routes of metabolism for octachlorodipropyl ether *via* successive steps of dehydrohalogenation, ether cleavage and hydrolysis. The possibility of hydroxy derivatives being formed and excreted in the urine as the corresponding glucuronide and/or sulfate conjugates exists, and is being currently explored. Similarly the possibility exists of formation of small fragment metabolites such as chloroform, methylene chloride or ethers (*e.g.* vinyl ether, etc.).

Fig. 4 depicts the possible formation of chlorinated acids and cyclic products formed from octachlorodipropyl ether *via* the sequences shown.

The reactivity of a number of linear and cyclic theoretical metabolites above

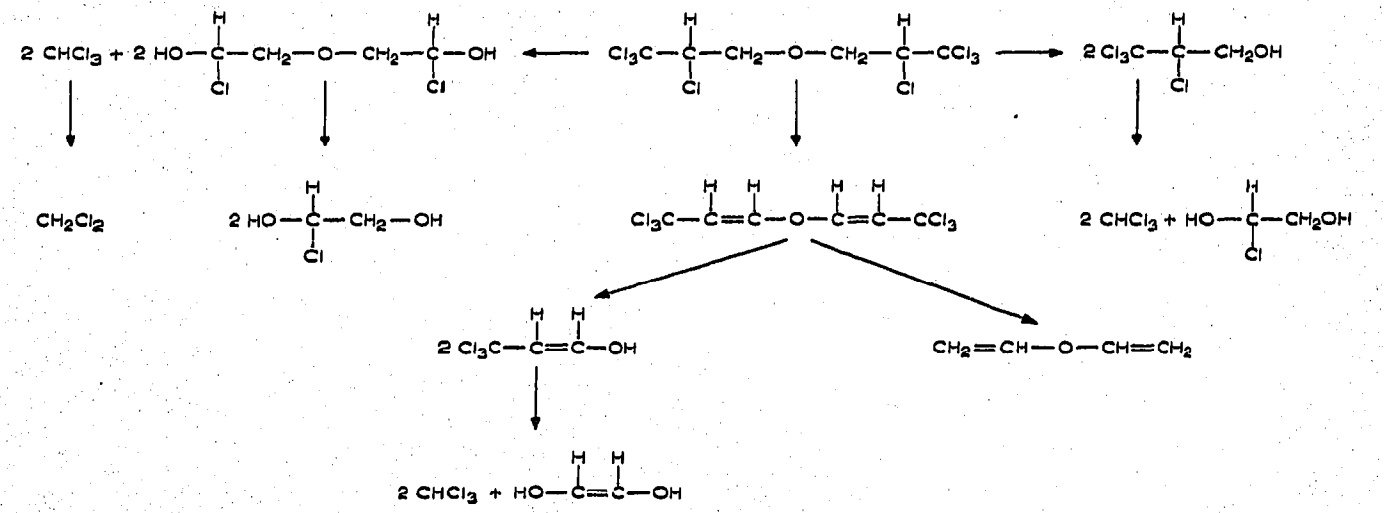


Fig. 3. Possible routes of metabolism of octachlorodipropyl ether to hydroxy derivatives, chlorinated hydrocarbons and ethers.

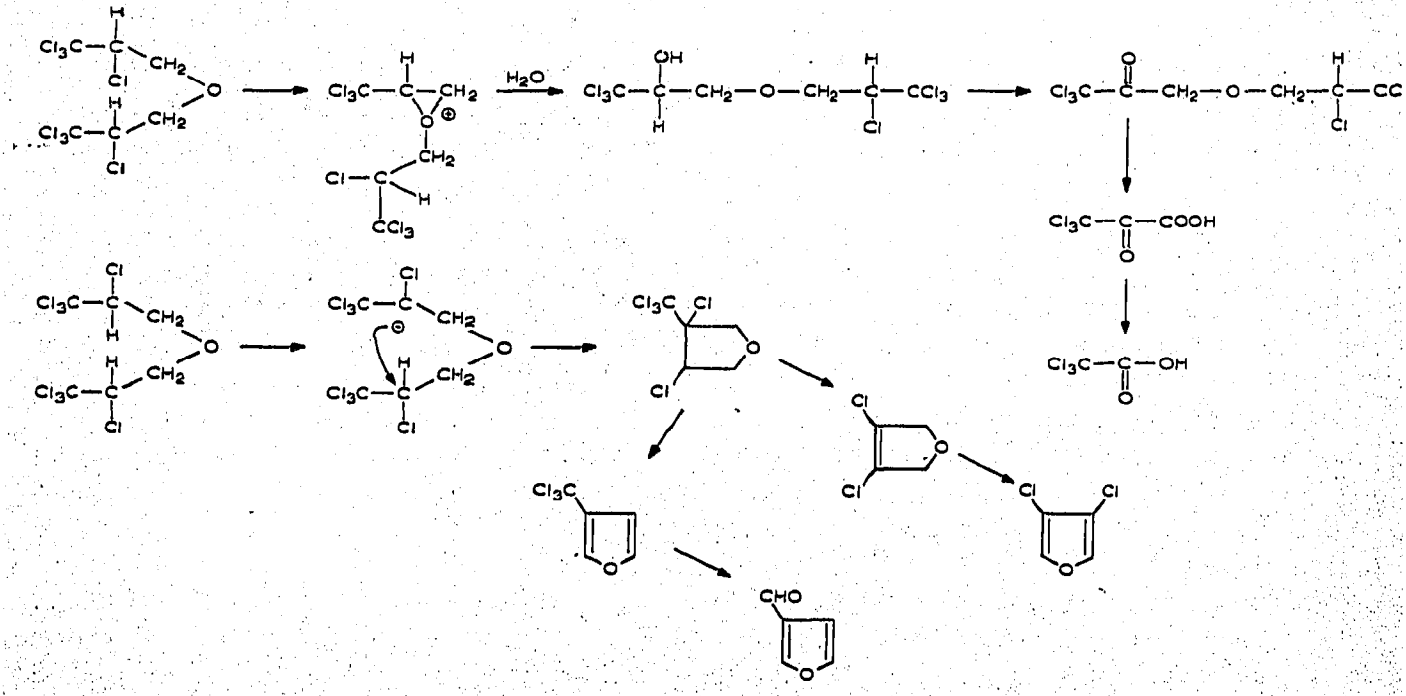


Fig. 4. Possible routes of metabolism of octachlorodipropyl ether to acids and cyclic derivatives.

in an aqueous milieu (*e.g.*, urine extract and the developing solvent for TLC, toluene–acetic acid–water (10:10:1)) could preclude their detection *per se*.

In this regard, gas chromatographic and gas chromatographic–mass spectrographic procedures are currently being explored for their utility in the separation and elaboration of the biliary and urinary metabolites of octachlorodipropyl ether following i.v. administration in the rat.

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