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Metabolism of DDT and Kelthane in Cell Suspension Cultures of Parsley (*Petroselinum hortense*, Hoffm.) and Soybean (*Glycine max* L.)

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Abstract. Cell suspension cultures of parsley and soybean were incubated for 44 to 48 h with ^{14}C-labeled DDT or Kelthane; autoclaved cultures were used as controls. Most of the radioactivity became associated with the cells, and metabolites were isolated by a sequential extraction procedure. The metabolites amounted to 0.6 to 2.2% of the applied pesticide. Relatively non-polar metabolites were identified as DDE in the case of DDT, and remained unidentified in the case of Kelthane. Polar metabolites were also isolated and are as yet unidentified. They were chromatographically different from the known and less polar metabolites of DDT and Kelthane reported from animal and insect studies. [DDT = 1,1,1-Trichloro-2,2-bis-(4-chlorophenyl)-ethane; Kelthane = (1,1-bis-(4-chlorophenyl)-2,2,2-trichloro-ethanol; DDE = 1,1-Dichloro-2,2-bis-(4-chlorophenyl)-ethylene.]

Key words: Metabolism – DDT – Kelthane – Cell suspension cultures – *Petroselinum hortense* Hoffm. – *Glycine max* L.

Introduction

Apparently, no studies on the metabolism of the insecticide, DDT, and the acaricide, Kelthane, in higher plants have so far been reported. The chemical structures of these compounds are shown in Figure 1. It has recently even been stated that plants seem to be unable to metabolize DDT (Khan et al., 1975). On the other hand, DDT is known to be degraded by bacterial, mammalian and insect systems to DDE and various other metabolites (Pfister, 1975; Khan et al., 1975; Klein and Korte, 1970). Certain algae have been found to convert DDT to DDE (Bowes, 1972; Rice and Sikka, 1973).

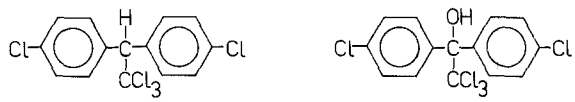


Fig. 1. Structures of DDT and Kelthane

Plant cell suspension cultures offer several advantages in the study of the metabolism of xenobiotics, viz., sterility, reproducibility and relative homogeneity of the plant material, easy manipulation of physiological status by medium composition or by light treatment and, in particular, reduced problems of uptake and transport. Suspension cultures of parsley and soybean are now reported to convert DDT and Kelthane to various metabolites. Some of these results have been briefly communicated (Scheel and Sandermann, 1976; Sandermann et al., in press).

Materials and Methods

Materials

(Ring-U-^{14}C)-labeled DDT was purchased from Amersham-Buchler, while (ring-U-^{14}C)-labeled Kelthane and non-radioactive FW-152 were gifts from Rohm and Haas Co., Philadelphia. Reference pesticides were purchased from Riedel-de-Haen, Hannover. The radioactive compounds were purified before use by two-dimensional thinlayer chromatography in solvent systems (A) and (B) (see below). Cell suspension cultures of parsley (*Petroselinum hor-*

Abbreviations: DDT, 1,1,1-Trichloro-2,2-bis-(4-chlorophenyl)-ethane; Kelthane, (1,1-bis-(4-chlorophenyl)-2,2,2-trichloro-ethanol; DDE, 1,1-Dichloro-2,2-bis-(4-chlorophenyl)-ethylene; DDA, 2,2-bis-(4-chlorophenyl)-acetic acid; DDOH, 2,2-bis-(4-chlorophenyl)-ethanol; DDD, 1,1-Dichloro-2,2-bis-(4-chlorophenyl)-ethane; DBP, 4,4′-Dichloro-benzophenone; DDMU, 1-Chloro-2,2-bis-(4-chlorophenyl)-ethylene; DDM, Bis-(4-chlorophenyl)-methane; FW-152, 1,1-Bis-(4-chlorophenyl)-2,2-dichloro-ethanol; SDS, sodium dodecylsulphate

tense Hoffm.) and soybean (*Glycine max* L) were grown in B 5-medium in the dark, as described by Hahlbrock (1975). Prior to administration of the labeled compounds, the parsley cultures were irradiated with white light from fluorescent lamps (Philips K 40W/18, 27,000 lx, 4 h, 25°).

Chromatography

Thinlayer chromatography was carried out on precoated silica gel G plates (Merck, No. 5554), using the following solvent systems:

(A) n-hexane.
(B) n-hexane/propionic acid, 95:5 (v/v).
(C) n-hexane/chloroform, 8:2 (v/v).

Descending paper chromatography was performed with Schleicher Schüll SS 2043b paper, using the following solvent system:

(D) butanol-1/acetic acid/water, 2:1:1 (v/v/v).

Pesticide derivatives were detected under UV-light (λ_{max}, 254 nm) or after exposure to iodine vapor under normal daylight conditions. Amino acids and peptides were detected by means of ninhydrin spray reagent.

Determination of Radioactivity

Liquid scintillation counting was performed with a scintillation fluid of the following composition: 5 g 2,5-diphenyloxazole/l toluene. Samples containing organic solvents were brought to dryness in vacuo prior to the addition of scintillation fluid. The distribution of radioactivity on thinlayer or paper chromatograms was determined by scanning (Berthold scanner LB 2723), or by scintillation counting of small sections of these chromatograms.

Feeding of Radioactive Compounds

The desired amount of [^{14}C]DDT or [^{14}C]Kelthane and 1.6 mg of a soybean lecithin preparation (Roth, Karlsruhe, Cat. No. 2-9812) were dissolved in 0.2 ml benzene. Solvent was removed under a stream of nitrogen, followed by the addition of 0.4 ml methanol and sonication (Bransonic sonic bath, No. 220; 1 min). The resulting dispersion was added to cell suspension cultures which had grown for 6 days and contained 8–10 g (wet weight) of cells. Parallel experiments were carried out under identical conditions with cell cultures which had been inactivated by autoclaving (20 min, 120°). The final concentration of soybean lecithin was about 50 µM. The amounts of DDT and Kelthane, respectively, are indicated in Table 1. Incubation was for 44–48 h in the dark at 27°, using a New Brunswick gyratory shaker at 120 rpm.

Isolation of Cells

Cells were harvested by filtration through a porous-glass filter. Propanol-2 (9 parts) was added to the culture fluid (1 part). The resulting precipitate was isolated by filtration. The filtrate was concentrated by means of a rotary evaporator (<25°), and examined by thinlayer chromatography (solvent system (A) or (B)). In all cases, unchanged DDT or Kelthane (<1% of the initial amount) was found as the only component. Less than 2% of the initial amount of DDT of Kelthane remained bound to the glass walls of the flasks used for the feeding of radioactive compounds (see above).

Extraction of Cells

The procedure used was essentially that of Bligh and Dyer (1959). The cell material (8–10 g wet weight) was suspended in 30 ml chloroform/methanol, 1:2 (v/v), and two cycles of freezing in liquid nitrogen and thawing were performed. Cell debris were isolated by filtration through a porous-glass filter, extracted with a mixture of 30 ml chloroform/methanol, 1:2 (v/v) and 8 ml water, and reisolated by filtration. The cell debris were finally washed with 15 ml chloroform/methanol, 1:2, (v/v). The various organic extracts were pooled. After addition of another 25 ml chloroform and 29 ml water the mixture was left for 12 h at 4°, to allow for phase separation.

Organic Phase of Lipid Extraction

Solvents were removed (<25°) by means of a rotary evaporator, and 3 ml n-hexane was added. The n-hexane solution was extracted with three portions, 9 ml each, of acetonitrile which had been saturated with n-hexane. The acetonitrile-phases were combined, 2 ml water was added and the mixture was extracted with three portions, 10 ml each, of n-hexane. The acetonitrile/water phases and the n-hexane extracts were brought to dryness (<25°), and examined by thinlayer chromatography after the addition of minimum amounts of n-hexane.

Aqueous Phase of Lipid Extraction

Solvents were removed (<25°) by means of a rotary evaporator, and the residue was dissolved in the minimum amount of water, followed by paper and thinlayer (solvent system B) chromatographic examination.

Insoluble Material of Lipid Extraction

Radioactivity was not eluted from the insoluble fraction by treatment with 20 ml 100 mM Tris-HCl, pH 7.5 in any of the experiments. This material was therefore treated (100°, 5 min) with 20 ml 100 mM Tris-HCl, pH 7.5, containing 1% (w/v) sodium dodecylsulphate, followed by filtration. The filtrate was extracted with 10 ml ether, and the ether and water phases were further examined by thinlayer and paper chromatography. The ether phases contained only small amounts of unchanged substrate.

Results

A procedure for the isolation of pesticide metabolites from plant cell suspension cultures is outlined in Figure 2. Standard procedures for lipid extraction (Bligh and Dyer, 1959) and for pesticide clean-up (Jones and Riddick, 1951, 1952) were used. The extraction step with boiling 1% sodium dodecylsulphate appears to be useful for a differentiation between lignin-like and other buffer-insoluble substances. This reagent will extract proteins, certain polysaccharides and lipophilic substances. A Björkman-type spruce lignin, however, remained insoluble under these conditions.

Relatively non-polar, but also a number of polar metabolites were formed from DDT and Kelthane in total yields between 0.6 and 2.2%. The fractions of the work-up procedure (cf. Fig. 2), containing metabolites are listed in Table 1, where the amounts of the various metabolite fractions are also shown.

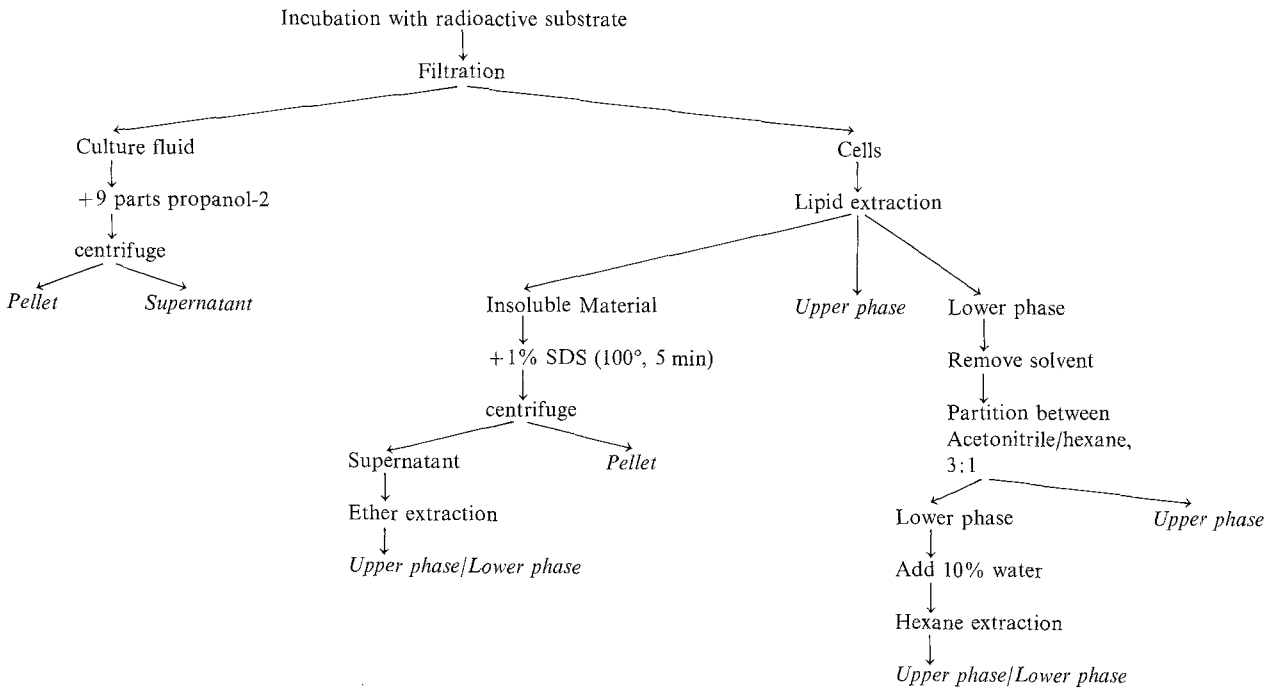
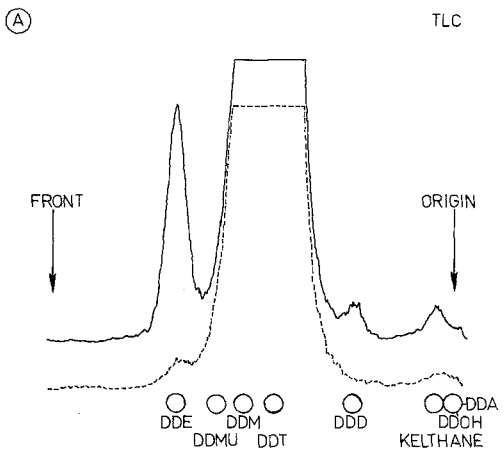


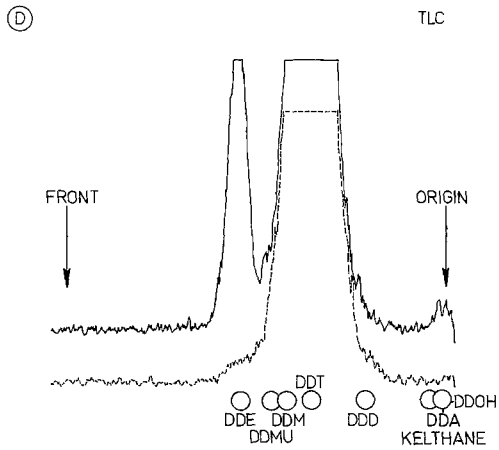
Fig. 2. Outline of the procedure for the isolation of pesticide metabolites from plant cell suspension cultures. The various fractions were examined by chromatography (cf. Figs. 3, 4) when they contained radioactive material

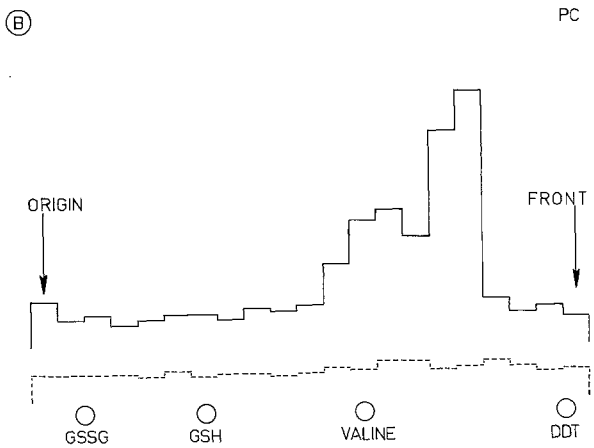
Table 1. Metabolite fractions isolated after administration of [^{14}C]DDT or [^{14}C]Kelthane to cell suspension cultures of parsley or soybean

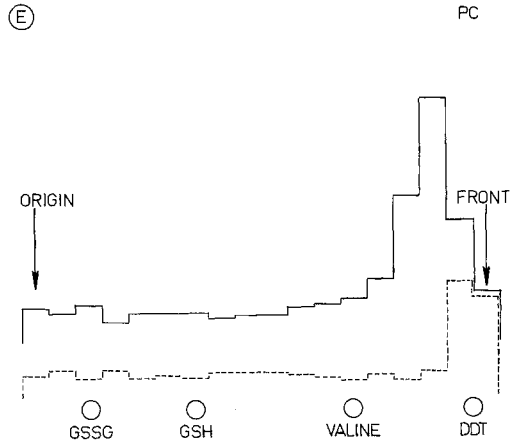
Experiment (Plant, substrate, amount applied)	Incubation period (h)	Metabolite fraction (cf. Fig. 2)								Total amount of metabolites (nmol, %)
		Lipid extract						SDS extract		
		Upper phase		Lower phase						
				Final n-hexane phase		Final acetonitrile phase				
		nmol	Fig.	nmol	Fig.	nmol	Fig.	nmol	Fig.	
Parsley, [^{14}C]DDT, 50 nmol, 1.5 μCi	48	0.05	3B	0.1	3A	–	–	0.15	3B	0.3 (0.6%)
Soybean, [^{14}C]DDT, 20 nmol, 0.6 μCi	44	0.12	3E	0.24	3D	0.04	3C	–	–	0.4 (2.2%)
Parsley, [^{14}C]Kelthane, 710 nmol, 0.45 μCi	44	6.5	4D	0.4	4C	1.3 and 5.7	4A 4B	–	–	13.9 (2.0%)

These data are supplemented by Figures 3 and 4 where radioactivity profiles of chromatograms of these metabolite fractions, and of the corresponding fractions from the control incubations are shown. Further characterization has only been possible for the DDE-like metabolite of Figure 3A and 3D. This material co-chromatographed with authentic DDE in solvent systems A, B and C, and, like DDE, had λ_{max}, 277 nm (in benzene.) On the basis of their chromatographic behaviour, the major metabolites of Figure 4A–C might be the 2-hydroxylated analogues of DDOH (Fig. 4A) and DDA (Fig. 4B, C). However, no reference substances were available. All other metabolites isolated are as yet unidentified, but were chromatographically different from the known DDT and Kelthane metabolites which were used as reference compounds (cf. Figs. 3, 4). Several metabolite fractions appeared to be unusually polar because they failed to migrate on thinlayer chromatography in solvent systems A–C. On paper chromatography, they









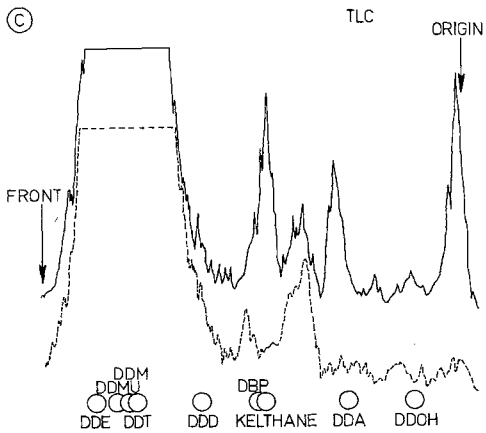
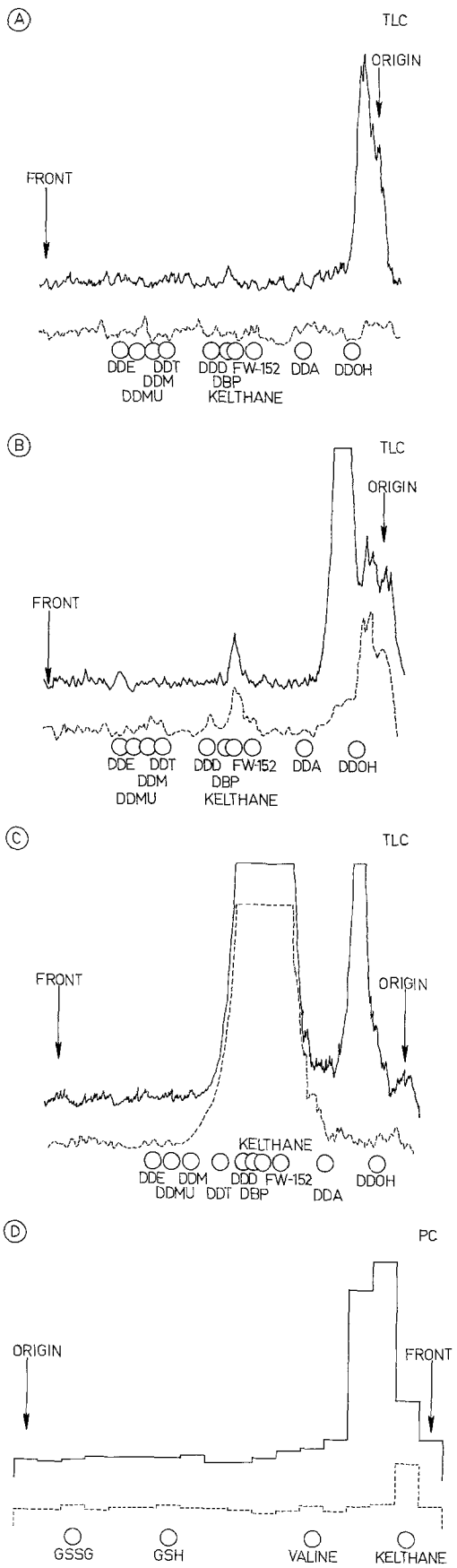


Fig. 3A–E. Radioactivity profiles of metabolite fractions isolated after feeding of [^{14}C]DDT to cell suspension cultures of parsley (**A, B**) or soybean (**C, D, E**). The dashed curves were obtained with corresponding fractions from the autoclaved control incubations. **A** Non-polar metabolite fraction, recovered from the final n-hexane phase of the acetonitrile/n-hexane/water extraction. Tlc; solvent system (A). **B** Polar metabolite fractions, obtained from the aqueous phase of the initial lipid extraction and from the SDS-extract. Only one radioactivity profile is shown because both metabolite fractions were chromatographically very similar. DDT, Kelthane and the other DDT derivatives used in Figure 3A ran close to the solvent front. PC; solvent system (D). **C** Metabolite fraction of the acetonitrile/water-phase of the final acetonitrile/n-hexane/water extraction. Tlc; developed first in solvent system (A), then in the same direction in solvent system (B). **D** Metabolite fraction of the final n-hexane-phase of the acetonitrile/n-hexane/water extraction. Tlc; solvent system (A). **E** Metabolite fraction of the aqueous phase of the initial lipid extraction. PC, solvent system (D). When examined by tlc (solvent system (B)) the radioactive material of the control incubation (---) migrated like authentic DDT, whereas the radioactive metabolite formed by the undenatured cells (—) remained at the origin. Abbreviations: Tlc = thin-layer chromatography; PC = paper chromatography; GSH = glutathione; GSSG = oxidised glutathione



migrated close to the arbitrary valine standard, while the known DDT metabolites ran near the solvent front (Figs. 3B, E; 4D).

Discussion

Most of the metabolites of DDT and Kelthane found in the present paper have apparently so far not been described in the literature, and remain to be identified. Nevertheless, their formation is of interest, because of the stated inability of plants to metabolize DDT (Khan et al., 1975), and because of the persistence of DDT. A calculation of the global fate of the total amount of DDT released so far into the environment has indicated that the biota contain much less DDT than expected (Woodwell et al., 1971). Plants constitute an enormous biomass (Woodwell et al., 1971; Hall, 1976), and therefore part of the released DDT might have been metabolized by plants. It appears to be interest to study the metabolism in plants of other persistent xenobiotics, such as polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). Plant cell cultures have been found to convert the carcinogen, benzo(α)pyrene, to several polar and so far unidentified metabolites (Harms, 1975; v.d. Trenck and Sandermann, unpublished).

The present work was done under special conditions because undifferentiated plant cells were used and soybean lecithin was employed as a mild emulsifying agent. More work is required to determine whether there are restrictions in the uptake or metabolism of xenobiotics by intact plants.

It has recently been suggested that plants might be considered as 'green livers' because enzyme sys-

Fig. 4A–D. Radioactivity profiles of metabolite fractions isolated after feeding of [^{14}C]Kelthane to cell suspension cultures of parsley. The dashed curves were obtained with corresponding fractions from the autoclaved control incubations. **A** and **B** Metabolite fraction of the acetonitrile/water-phase of the final acetonitrile/n-hexane/water extraction. This material was first chromatographed by tlc, solvent system (B). Two overlapping peaks of radioactivity were observed near the DDA and DDOH standards, and these peaks were eluted separately. The slower-running material is shown in (A), the faster-running material is shown in (B). Rechromatography was by tlc, solvent system (B). **C** Metabolite fraction of the final n-hexane-phase of the acetonitrile/n-hexane/water extraction. Tlc; solvent system (B). The radioactive metabolite located near the DDOH standard appeared to be chromatographically similar to the metabolite of Figure 4B. **D** Metabolite fraction of the aqueous phase of the initial lipid extraction. PC, solvent system (D). When examined by tlc (solvent system (B)) the radioactive material of the control incubation (---) migrated like authentic Kelthane, whereas the radioactive metabolite formed by the undenatured cells (—) remained at the origin

tems which are biochemically similar to detoxifying activities of animal liver have in recent years also been discovered in plants (Sandermann et al., in press). It will therefore be examined whether the unknown polar metabolites of the present paper are related to the hydroxylated derivatives of o,p′-DDT and DDE recently isolated from animal feces (Feil et al., 1975; Sundström et al., 1975). Preliminary experiments have indicated that Kelthane is converted to polar metabolites by a microsomal preparation from parsley cells (Scheel and Sandermann, 1976).

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