position (2,4,5-T) resulted in a further increase in acidic strength. Similar effects were observed with the 2-(phenoxy)propionic acid series.

It was not possible to determine the $pK_a$ values of MCPB and 2,4-DB because the difference in the optical densities of the anionic and molecular forms for both compounds was negligible. Determination of the $pK_a$ values for picloram and M-3723 also was not possible because the optical densities of the monoprotonated forms of these two pyridine derivatives could not be measured. The $pK_a$ value for TIBA could not be determined because of its limited solubility in 3.0 N HCl solution.

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# Urinary Metabolites of [$^{14}$C]Photodieldrin in Male Rabbits

Oral and intraperitoneal treatment of male rabbits with [$^{14}$C]photodieldrin resulted in the excretion of about 50% of the administered dose in urine. More than 98% of the radioactivity in urine was water soluble, about 30% of which was hydrolyzable with glucuronidase and about 6% with HCl. The remaining unhydrolyzable radioactivity was unextractable. Photodieldrin *trans*-diol was the major product followed by photodieldrin ketone and remaining minor unidentified products.

Photodieldrin, an environmental "terminal residue" of the commonly used insecticides aldrin and dieldrin (Rosen et al., 1966; Khan et al., 1974), is considerably persistent in the environment (Suzuki et al., 1974; Reddy and Khan 1975a). It is metabolized by some insects (Khan et al., 1969; Reddy and Khan, 1977) and mammals (Klein et al., 1969, 1970, 1973; Dailey et al., 1970, 1972; Reddy and Khan, 1974, 1975b) to lipophilic and hydrophilic metabolites. Oral and intraperitoneal treatment of male rabbits resulted in excretion of about 50% (55% when intraperitoneally and 48.5% when orally administered) of the administered dose in urine and only 3% in feces in 9 days (Reddy and Khan, 1975b). Less than 1% of the radioactivity in urine was extractable with ether, remaining being water soluble. These organosoluble metabolites included photodieldrin ketone, photodieldrin *trans*-diol, photodieldrin, and four other unidentified products (Reddy and Khan, 1975b). This report provides information about the nature of the water-soluble conjugated products of urine of male rabbits dosed with [$^{14}$C]photodieldrin.

MATERIALS AND METHODS

**Chemicals.** [$^{14}$C]Photodieldrin was prepared in this laboratory and was free of interferring chemicals as checked by thin-layer chromatography (TLC) and gas chromatography (GC) (Reddy and Khan, 1975b).

**Animals.** Male rabbits (Scientific Small Animals), 8 to 9 months old (about 3 kg body weight), were injected or fed about 30 $\mu$Ci (30 mg/kg) of [$^{14}$C]photodieldrin in corn oil (Reddy and Khan, 1975b). Urine and feces were collected separately for every 24 h for 9 days. A 0.1-mL aliquot of the urine was analyzed for total radioactivity by scintillation counting. About 50 mL of urine was extracted, three or four times, with 50 mL of ether. The pooled ether extract was evaporated to dryness and the residue redissolved in acetone and counted for organosoluble radioactivity. The acetone solutions of the urine extracts of 9 days were pooled, concentrated, and analyzed by TLC (Silica Gel F-254, 0.25 mm plates) using benzene–ethyl acetate (3:1). Plates were then exposed to x-ray film and autoradiographed (Reddy and Khan, 1975b,

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Table I. Nature of $^{14}C$ Radioactivity in Aqueous Phase of Urine[a]

| Nature of radioact. | % of administered dose ip | Oral |
|---|---|---|
| Total excreted in 9 days | 54.3 | 48.3 |
| Enzyme-released A | 13.1 | 10.0 |
| Acid-released B | 2.0 | 3.0 |
| Water-soluble C | 18.0 | 31.0 |
| Total | 33.10 | 44.0 |

[a] Aqueous phase of urine (9 days) after ether extraction was lyophilized and then extracted with methanol: A, radioactivity extracted with ethyl acetate from the aqueous phase after β-glucuronidase hydrolysis; B, radioactivity extracted with ethyl acetate from the aqueous phase of the urine after enzyme hydrolysis and treatment with 1 N HCl; C, radioactivity remaining in the water phase after extraction of enzyme (A) and acid-released (B) radioactivity (Reddy and Khan, 1975b).

1977). A 0.1-mL aliquot of the aqueous phase, remaining after ether extraction, was counted for total water-soluble radioactivity. The aqueous phases of the urine samples for all 9 days were pooled and lyophilized. The residue was extracted with 100 mL of methanol and the extract evaporated to dryness and used for further analyses. The methanol unextractable residue accounted for about 2% of the radioactivity in urine. The aqueous phase was also incubated with β-glucuronidase as well as hydrolyzed with 1 N HCl (Reddy and Khan, 1975b, 1977) and the released products extracted with ethyl acetate. The extracts were concentrated by evaporation. The above methods resulted in loss of recoverable radioactivity which was about 40 and 10% of the total aqueous radioactivity excreted in urine following intraperitoneal and oral administration, respectively (Table I, confirm Reddy and Khan, 1975b). The analyses by TLC followed by x-ray autoradiography were carried out. The spots corresponding to authentic standards as visualized by silver nitrate spraying were scraped off the plates, extracted, and analyzed by GC, infrared spectroscopy, and GC–mass spectrometry (Reddy and Khan, 1977).

## RESULTS AND DISCUSSION

The hydrolysis of the aqueous phase of the urine by β-glucuronidase and 1 N HCl released, respectively, 10–13 and 2–3% of the administered dose (Table I). Thin-layer chromatography of these products showed the presence of six metabolites (A, B, C, D, E, F) along with photodieldrin (G) (Figure 1). Photodieldrin was not found in the acid hydrolysate. These six metabolites were also present in the ether extract (Reddy and Khan, 1975). Metabolite A appeared to be more polar and was produced in higher amounts as compared with other metabolites. Infrared spectra of this metabolite (A) showed a peak at 3325 $cm^{-1}$, indicating the presence of an OH group. This was further confirmed by acetylating this compound (Reddy and Khan, 1975b, 1977) and then studying its behavior by TLC and GC. $R_f$ values of the acetate derivative matched with those of the authentic standards of *cis*- and *trans*-photodieldrin diacetate (Table II). The GC analysis showed the relative retention time of the acetylated metabolite to be identical with that of the *trans*-photodieldrin diacetate (Table II). The GC–mass spectra of the diacetate derivative of the metabolite A were identical with those of the standard *trans*-photodieldrin diacetate. Both compounds showed the highest $m/e$ value 445 *p*-Cl.

Metabolite F was found to be identical with the authentic photodieldrin ketone as checked by TLC and GC analysis (Table II). Infrared spectra of this metabolite showed the peak at 1720 $cm^{-1}$, indicating the presence of a carbonyl group, and at 875 $cm^{-1}$, indicating the presence of an epoxy group.

The metabolite G was found to be the parent compound photodieldrin (Table II) as analyzed by TLC and GC.

The water-soluble metabolites which amount to about 50% of the administered dose appear to be similar to the organosoluble metabolites (amounting to less than 2% of the administered dose) that we reported earlier (Reddy and Khan, 1975b). In rabbits treated (intravascular injection) with [$^{14}C$]photodieldrin, the radioactivity excreted in urine was ~15% of the administered dose (in the form of water-soluble products) while that in feces was about 1.6% of the administered dose (Klein et al., 1969). One of the metabolites in rat urine, which was orally treated with [$^{14}C$]photodieldrin and showed higher activity in feces than in urine, was identified as photodieldrin ketone (Klein et al., 1970; Dailey et al., 1972). Insects such as houseflies (Khan et al., 1969; Reddy and Khan, 1977), mosquitoes (Khan et al., 1969; Klein et al., 1969), and cabbage looper (Klein et al., 1969) have also been reported to metabolize photodieldrin to photodieldrin ketone and hydrophilic metabolites. The housefly metabolites have been identified as photodieldrin ketone and *trans*-photodieldrin diol (Reddy and Khan, 1977). These two metabolites have also been identified in soils treated with photodieldrin (Weisgerber et al., 1975). Treatment of mosquitoes, rabbits, and rats with dieldrin has been reported to produce *trans*-

Table II. $R_f$ Values (TLC) and Relative Retention Time (GC) of Photodieldrin and Its Metabolites Obtained from Rabbit Urine as Compared with the Authentic Reference Compounds

| Compound or metabolites | $R_f$ values in indicated solvent system[a] Benzene–ethyl acetate (3:1) | Chloroform–methanol (9:1) | Benzene | rel retention time[b] Column A | Column B |
|---|---|---|---|---|---|
| Photodieldrin | 0.51 | 0.77 | 0.29 | 1.93 | 2.28 |
| Photodieldrin *cis*-diacetate | 0.42 | 0.68 | 0.00 | 6.00 | 7.04 |
| Photodieldrin *trans*-diacetate | 0.42 | 0.68 | 0.00 | 4.68 | 5.33 |
| Metabolite A | 0.00 | 0.24 | 0.00 | | |
| Metabolite A diacetate derivative | 0.42 | 0.68 | 0.00 | 4.68 | 5.53 |
| Photodieldrin ketone | 0.46 | 0.74 | 0.21 | 1.18 | 1.23 |
| Metabolite F | 0.46 | 0.74 | 0.21 | 1.18 | 1.23 |
| Metabolite G | 0.51 | 0.77 | 0.39 | 1.93 | 2.28 |

[a] Average values of two or three experiments. [b] Column A (6 ft × 0.125 in.) was 6% DC-200 on Varaport-30; Column B was 5% SE-52 on Chromosorb W (acid-washed DMCS treated). Relative retention time calculated with dieldrin as 1. Values are averages of three experiments. Operating conditions were: temperature, inlet 220 °C, column 210 °C, detector 220 °C; nitrogen flow 25 mL/min; sensitivity $1 \times 10^{-9}$.

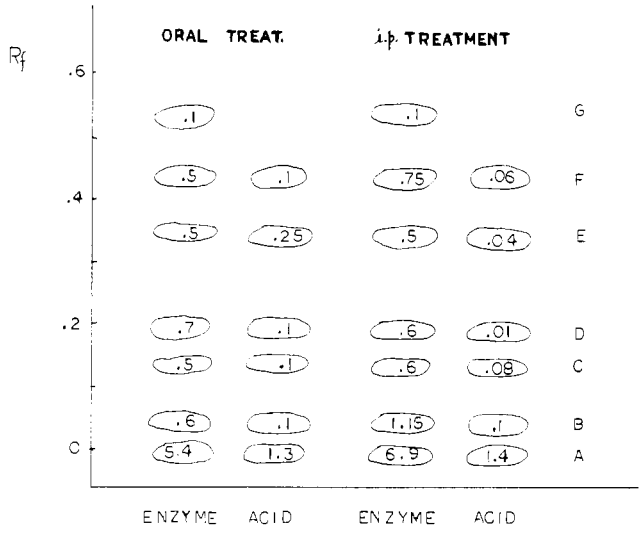


**Figure 1.** Thin-layer chromatographic presentation of [$^{14}$C]-photodieldrin and its metabolites in the aqueous phase of the rabbit urine released after enzymic and acid hydrolyses: spot A, photodieldrin diol; B, C, D, E, unidentified metabolites; F, photodieldrin ketone; G, photodieldrin. Various fractions of aqueous phase of urine extracts were applied on to TLC plates and developed in the solvent benzene–ethyl acetate (3:1) and exposed to x-ray film for 2 to 3 weeks. The number in each spot represents the amount of the metabolite as a percent of the administered radioactivity.

aldrin diol as major metabolite (Oonithan and Miskus, 1964; Korte and Arent, 1965; Matthews et al., 1971).

The major metabolites of [$^{14}$C]photodieldrin in the urine of rabbits are photodieldrin *trans*-diol and photodieldrin ketone. Only small amounts of these metabolites and other excreted metabolites remain free as organosoluble, most of these become further conjugated and water soluble. The conjugation with glucuronic acid seems to be the major mechanism. The data presented here and in other communications (Reddy and Khan, 1974, 1975b, 1977) indicates that photodieldrin, unlike dieldrin, is less stable in rabbits and houseflies.

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