PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 847-853 (1975)

Urinary Metabolites of 2, 5, 2', 5'-Tetrachlorobiphenyl in the Nonhuman Primate (38999)

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2, 5, 2', 5'-tetrachlorobiphenyl (TCB) is a major component of commercial polychlorinated biphenyls (PCBs) which are universally distributed pollutants and are present in the food chain primarily as a result of improper disposal (1, 2). The complexity of the commercial PCB mixtures has made it necessary to use pure isomers (e.g., TCB) for evaluation of metabolism of the compounds. The metabolic behavior of several pure PCB isomers in the pigeon, rat, and trout has been investigated by Hutzinger *et al.* (3). They found that pigeons and rats metabolized TCB to monohydroxy TCB, but they were unable to detect any metabolites in trout. Gardner *et al.* (4) found 3-hydroxy-TCB, 4-hydroxy TCB and *trans*-3,4-dihydro-3,4-dihydroxy TCB in the urine of rabbits fed TCB and postulated metabolism of TCB via an arene oxide intermediate. Van Miller *et al.* (5) reported that rats receiving ^{3}H TCB excreted about 66% of the ^{3}H in the feces by 72 hr with an additional 10% being recovered in the urine. Monohydroxy TCB was found to be the only metabolite in the ether extract of the urine (5, 6). Due to the observed species difference in metabolism of TCB, it became important to study TCB metabolism in a species more closely related to man. In the present study, four metabolites of TCB (Fig. 1) were isolated from the urine of infant nonhuman primates. Two of these four metabolites have not previously been reported as metabolites of TCB in any species.

Materials and methods. ^{3}H-labeled TCB was prepared from 2, 5, 2', 5'-tetrachlorobenzidine by the method of Hutzinger and Safe (7) and was dissolved in corn oil before use. Four male infant rhesus monkeys weighing 600–800 g were given ^{3}H-TCB at a dose of 500 mg/kg body wt (6.1 μCi/mg) by gastric intubation. The animals had unlimited access to water and were incubated at 24 and 48 hr with 20 ml of 5% glucose solution. The animals were sacrificed at 72 hr, at which time the urine was collected for analysis of TCB and its metabolites.

The collected urine was extracted three times with equal amounts of ether. The ether extracts were chromatographed by thin layer chromatography (TLC) on flexible, silica gel plates (J. T. Baker Chemical Co.) with methylene chloride (Solvent A) as the solvent. The radioactive bands were extracted with methanol and either further purified by TLC or converted to trimethylsilyl (TMS) derivatives in preparation for gas liquid chromatography (GLC), mass spectrometery (MS) or GLC-MS. Further purification for infrared spectroscopy (IR) was done on a high pressure liquid chromatograph.

Additional TLC purification was done as described above with hexane: ethyl acetate (7 : 2) (Solvent B) as the solvent. TMS derivatives were made by dissolving each compound in 0.1 ml of pyrridine and 0.06 ml of N_9O-bis-(trimethylsilyl)-acetamide (BSA). Dehydration of the hydroxylated derivative of *trans*-3,4-dihydro-3,4-dihydroxy TCB was done by dissolving the compound in 2 ml of methylene chloride and adding three to four drops of concentrated sulfuric acid. GLC was done on a Hewlett–Packard Model 7620A gas chromatograph fitted with an electron capture detector (EC) of flame ionization detector (FID). The GLC was equipped with a glass column (1/8 in. × 6 ft) containing 3% SE-30 on Gaschrom Q (100–200 mesh) and was run at 200° with N_2 (FID) or argon-methane (95 : 5) (EC) as the carrier gas (40 ml/min). Samples for MS were collected from GLC in a capillary tube which was then directly inserted into a MS-9 mass spectrometer

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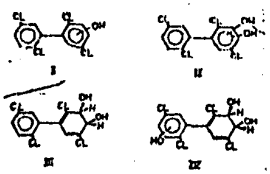


FIG. 1. Chemical structures of the metabolites of 2, 5, 2', 5'-tetrachlorobiphenyl (TCB) isolated in the urine of infant nonhuman primates 3 days after a single oral dose of ^{3}H-TCB. I = monohydroxy-TCB, II = dihydroxy-TCB, III = *trans*-3,4-dihydro-3,4-dihydroxy TCB, IV = hydroxy-3,4-dihydro-3,4-dihydroxy TCB.

(Associated Electrical Industries, Ltd.) or directly injected into a GLC-MS (Hewlett-Packard Model 3600). IR was done in a micro KBr disc on a Perkin-Elmer 247 IR spectrometer fitted with a beam condenser. High pressure liquid chromatography was done on a liquid chromatograph (Waters Associates Model M-6000) utilizing a C_{18} corasil column and eluting with methanol: water (3:2).

Results and discussion. The ether extracts were chromatographed into two major radioactive bands with R_f values (Solvent A) of 0.0 (a) and 0.45 (b) and a minor band with an R_f value of 0.9 (c). The radioactive substance in (c) was identified by GLC-MS as monohydroxy-TCB (I). The metabolite in the extract of (b) did not show any peaks typical of chlorinated compounds in GLC (EC) (even at 340° for 30 min). However, the TMS derivative of this compound gave a symmetric peak with a relative retention time (RRT) of 6.82 on GLC. The metabolite in this peak was identified by MS as dihydroxy TCB (II). The radioactive substance in (a) was further chromatographed with Solvent B resulting in two bands with R_f values of 0.05 (a_1) and 0.3 (a_2). The TMS derivative of the metabolite in (a_2) had a RRT of 2.76 and was identified by MS as a dihydro TCB diol. Further purification and silylation produced a purified radioactive substance which showed a single peak in GLC and had an infrared spectrum identical to *trans*-3,4-dihydro-3,4-dihydroxy-2, 5, 2', 5'-TCB (III) which has been isolated and characterized by Gardner *et al.* (4) from rabbit urine. The radioactive substance in the extract of (a_1) did not show a peak typical of chlori-

TABLE I. THIN LAYER CHROMATOGRAPHY DATA FOR THE HYDROXYLATED METABOLITES OF 2, 5, 2', 5'-TETRACHLOROBIPHENYL (TCB) ISOLATED FROM THE URINE OF INFANT NONHUMAN PRIMATES 3 DAYS FOLLOWING A SINGLE ORAL DOSE OF ^{3}H-TCB.

Metabolite	Thin layer chromatographic R_f values in solvents: Methylene chloride (Solvent A)	Hexane : ethyl acetate (7:2) (Solvent B)
TCB	0.9	—
I[a]	0.9	—
II[b]	0.5	—
III[c]	0	0.46
IV[d]	0	0.18
Dehydration product of IV	0.52	—

[a] Monohydroxy-TCB.
[b] Dihydroxy-TCB.
[c] *Trans*-3,4-dihydro-3,4-dihydroxy-TCB.
[d] Hydroxy-3,4-dihydro-3,4-dihydroxy-TCB.

TABLE II. RELATIVE RETENTION TIMES[a] AND MOLECULAR ION DATA[b] FOR THE TRIMETHYLSILYL DERIVATIVES OF THE HYDROXYLATED METABOLITES OF 2, 5, 2', 5'-TETRACHLOROBIPHENYL (TCB) ISOLATED FROM THE URINE OF INFANT NON-HUMAN PRIMATES 3 DAYS FOLLOWING A SINGLE ORAL DOSE OF ^{3}H-TCB.

Trimethylsilyl derivatives of metabolites	Relative retention time	Molecular ion
I[c]	2.55	378
II[d]	6.82	466
III[e]	2.76	468
IV[f]	7.85	556
Dehydration product of IV	7.35	466

[a] Retention of sample/retention time of TCB (69 sec) (gas liquid chromatography).
[b] Atomic mass units (a.m.u.) (mass spectrometry).
[c] Monohydroxy-TCB.
[d] Dihydroxy-TCB.
[e] *Trans*-3,4-dihydro-3,4-dihydroxy-TCB.
[f] Hydroxy-3,4-dihydro-3,4-dihydroxy-TCB.

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nated compounds in GLC (EC). The TMS derivative of this compound, however, exhibited a symmetric peak on GLC with a RRT of 7.85 and was identified by MS as dihydrotrihydroxy TCB (IV). The similarity of the fragmentation pattern of the TMS derivatives of III and IV indicates a probable structure for IV as a hydroxylated derivative of *trans*-3,4-dihydro-3,4-dihydroxy TCB with the additional OH group on the aromatic ring. Further confirmation of the structure for IV was obtained by dehydration of the metabolite and subsequent silylation of the dehydration product. The resulting compound had R_f 0.52 in Solvent A, RRT 7.35 on GLC (FID), and a molecular ion of 468 a.m.u. These values are consistent with dihydroxy TCB, which is the expected dehydration product if IV is a hydroxylated derivative of 3,4-dihydro-3,4-dihydroxy TCB.

The TLC R_f values of the four metabolites on Solvents A and B are summarized in Table I. The RRT and the molecular ion data for the TMS derivatives of the four metabolites and the dehydration product of IV are shown in Table II. The mass spectra for II, TMS derivative of I–IV, and TMS derivative of the dehydrated product of IV are shown in Fig. 2. Similar metabolites have been found in the urine of adult monkeys (unpublished observations).

Silylation of the metabolites of TCB made the identification of II and IV possible in addition to improving the resolution. Without silylation monohydroxy TCB usually shows a broad tailing peak on GLC and dihydro

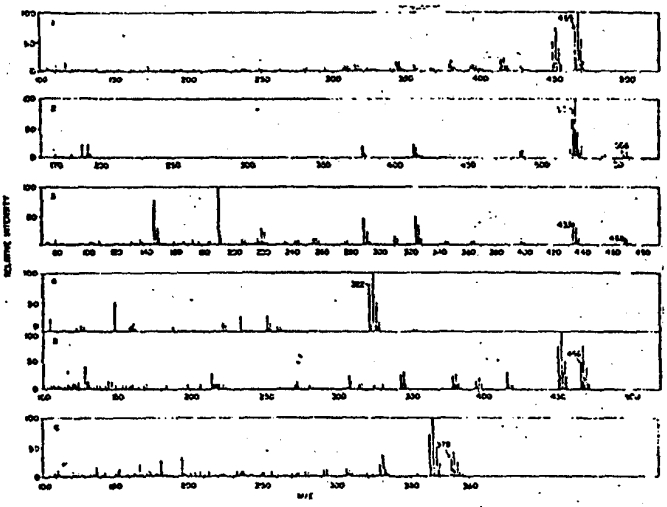


FIG. 2. Mass spectra of the hydroxylated metabolites or their trimethylsilyl (TMS) derivatives of 2, 5, 2', 5'-tetrachlorobiphenyl (TCB) isolated in the urine of infant nonhuman primates 3 days following a single oral dose of ^{3}H-TCB. (1) TMS derivative of the dehydrated product of hydroxy-3,4-dihydro-3,4-dihydroxy TCB; (2) TMS derivative of hydroxy-3,4-dihydro-3,4-dihydroxy TCB; (3) TMS derivative of *trans*-3,4-dihydro-3,4-dihydroxy TCB; (4) dihydroxy-TCB; (5) TMS derivatives of dihydroxy-TCB; and (6) TMS derivative of monohydroxy-TCB.

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TCB diol decomposes inside the column at the temperatures employed.

Identification of metabolites in this experiment, particularly II and IV, suggests that the mechanism of metabolism is through arene oxide intermediates which are then converted to the hydroxylated metabolites. Aromatic hydrocarbons that have been reported to be metabolized through arene oxide intermediates often exhibit carcinogenic, teratogenic, mutagenic, and necrogenic potential in experimental animals (8). These arene oxide intermediates are potent alkylating agents of nucleic acids and proteins; hence the alkylation of the macromolecules is suspected to be the cause of these wide ranging effects. The strong evidence reported here that TCB is metabolized through arene oxide intermediates suggests the possibility that similar macromolecular alterations may occur in the nonhuman primate subjected to PCB intoxication.

Summary. The metabolism of 2, 5, 2', 5'- tetrachlorobiphenyl (TCB) in nonhuman primates was found to be different from that previously reported in lower species. Monohydroxy TCB (I), the only metabolite in the ether extracts of rat urine, is a minor metabolite in the urine of nonhuman primates. The two major metabolites identified in the urine were dihydroxy TCB (II) and trans-3,4-dihydro-3,4-dihydroxy TCB (III). A second minor metabolite was identified as hydroxy-3,4-dihydro-3,4-dihydroxy TCB (IV). None of the above mentioned metabolites have been reported in primates and only I and III have been identified in lower animals. It is concluded that a likely mechanism for metabolism of TCB in primates is through arene oxide intermediates. This observation is of particular importance in that these types of intermediates are known to alkylate cellular components causing carcinogenic, mutagenic, necrogenic and teratogenic effects.

We thank Dr. Bruce M. Johnson, Department of Clinical Oncology, University of Wisconsin Medical School for the use of the mass spectrometer. This investigation was supported in part by U.S. Public Health Service Grant Nos. ES-00472, HL-10941, CA-13288 and RR-00167 from the National Institutes of Health. Primate Center publication no. 00-000.

1. Risebrough, R. W., Reiche, P., Peakall, D. B., Herman, S. G., and Kirven, M. N., Nature (London) 220, 1098 (1968).
2. Kolbye, A. C., Environ. Health Persp. 1, 85 (1972).
3. Hutzinger, O., Nash, D. M., Safe, S., Defreitas, A. S. W. Norstrom, R. J., Wildish, D. J., and Zitko, V., Science 178, 312 (1972).
4. Gardner, A. M., Chen, J. T., Roach, J. A. G., and Ragelis, E. P., Biochem. Biophys. Res. Commun. 55, 1377 (1974).
5. Van Miller, J. P., Hsu, I. C., and Allen, J. R., Proc. Soc. Exp. Biol. Med. 148, 682 (1975).
6. Hsu, I. C., Van Miller, J. P., and Allen, J. R., Bull. Environ. Contam. Toxicol. in press (1975).
7. Hutzinger, O., and Safe, S. S., Bull. Environ. Contam, Toxicol. 7, 314 (1972).
8. Jerina, D. M., and Daly, J. W., Science 185, 573 (1974).

Received March 3, 1975. P.S.E.B.M. 1975, Vol. 15

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