

### Metabolic Studies on Polychlorinated Biphenyls. I. Metabolic Fate of 3,4,3',4'-Tetrachlorobiphenyl in Rats

Polychlorinated biphenyls (PCB) have a wide range of industrial application and are widespread in the environment. Because of their high degree of chemical stability and lipid solubility, they have been accumulated even in human tissues through food chains.<sup>1)</sup> Particularly in Japan, attention has been paid to one of the commercial preparations of PCB, Kanechlor-400 (KC-400), because, in addition to above ecological problems, there have been experienced outbreaks of KC-400 intoxication (so-called Yusho) in the southwest part of Japan in summer of 1968.<sup>2)</sup>

In the first attempt to explore the toxicological properties of KC-400, we recently performed the distribution and elimination studies in animals using KC-400.<sup>3a,b)</sup> Although several important findings were obtained by these studies, exact features on individual chlorobiphenyls were not ascertained, since KC-400 is a complex mixture consisting mainly of tetrachlorobiphenyls, and also of tri, penta and hexachloro derivatives as minor components.<sup>2,4)</sup> Therefore, we have initiated a series of studies to elucidate chemical structures and establish synthetic methods of major components of KC-400 for facilitating studies on their metabolic fate.<sup>4)</sup>

The present paper will communicate shortly the metabolic fate of 3,4,3',4'-tetrachlorobiphenyl (3,4,3',4'-TCB), one of the major components of KC-400, in rats.

3,4,3',4'-TCB (25 mg/body) in 1.0 ml of soybean oil was administered orally to adult male Wistar rats (180—270 g) every third day for 9 days. Feces collected for 12 days after the first administration were dried and extracted with  $\text{CHCl}_3$  by Soxhlet extractor for 14 hr. The urine sample collected for the same days as feces was adjusted to pH 2.0 and extracted continuously with  $\text{CHCl}_3$  for 6 hrs. Examination of these extracts from 12 days urine and feces by thin-layer (TLC) and gas-liquid chromatography (GLC) indicated that only the fecal extract contained at least 3 metabolites along with unchanged 3,4,3',4'-TCB.

These metabolites seemed to be phenolic in nature since they colored blue or red with Folin-Ciocalteu or diazotized benzidine reagent, respectively, on TLC and were detectable as respective trimethylsilyl derivatives on GLC.<sup>5)</sup> The metabolites in the fecal extract were then separated from unchanged 3,4,3',4'-TCB by column chromatography using activated aluminum oxide (Wako, 200—300 mesh) as adsorbent, in which unchanged 3,4,3',4'-TCB was first eluted with *n*-hexane and metabolites were then obtained in the subsequent fractions with methanol. Fractions containing metabolites were combined and purified by preparative TLC on the same conditions as above.<sup>5)</sup>

- 1) F.J. Biros, A.C. Walker and A. Medbery, *Bull. Environ. Contam. Toxicol.*, **5**, 317 (1970).
- 2) Study Group for "Yusho," Kyushu University, *Fukuoka Acta Med.*, **60**, 403 (1969).
- 3) a) H. Yoshimura and M. Oshima, *Fukuoka Acta Med.*, **62**, 5 (1971); b) H. Yoshimura, H. Yamamoto, J. Nagai, Y. Yae, H. Uzawa, Y. Ito, A. Notomi, S. Minakami, A. Ito, K. Kato and H. Tsuji, *ibid.*, **62**, 12 (1971).
- 4) S. Saeki, A. Tsutsui, K. Oguri, H. Yoshimura and M. Hamana, *Fukuoka Acta Med.*, **62**, 20 (1971).
- 5) TLC was carried out using silica gel plates (Wako gel B-5UA containing fluorescent indicators, 0.25 mm thick, activated at 105° for 30 min). The chromatograms were visualized by ultraviolet lamp (Manaslu Light, short wavelength). Phenolic metabolites were also revealed as blue spots or red spots by spraying with Folin-Ciocalteu reagent or diazotized benzidine reagent, respectively. *R<sub>f</sub>* values of the parent compound and its metabolites (M-1, M-2 and M-3) were 0.70, 0.53, 0.45 and 0.40, respectively, in the solvent system of *n*-hexane-AcOEt-AcOH (40:10:1). Retention times (min) were 10, 24.5, 23.5, 15.2, respectively, for the parent compound and TMS derivatives of three metabolites in the conditions as follows; Shimadzu GC-3AE gas chromatograph (ECD), 4 mm × 2.5 m glass column packed with 1.5% SE-30 on Chromosorb W (60—80 mesh), column temperature 200°.

A major metabolite (M-2), mp 167—168°, showing *R<sub>f</sub>* 0.45 in TLC described above, was isolated as colorless needles together with unchanged 3,4,3',4'-TCB, mp 172—173°, *R<sub>f</sub>* 0.70. This metabolite seemed to be major one in feces judging from the gas chromatogram (Fig. 1). The mass spectrum of M-2 showed M<sup>+</sup> and M+2 ion peak at *m/e* 306 and 308, respectively, suggesting the addition of a single oxygen atom to the parent 3,4,3',4'-TCB molecule [*m/e* 290 (M<sup>+</sup>), 292 (M+2)] to produce monohydroxylated TCB. This phenolic structure was further evidenced by the existence of a sharp band around 3500 cm<sup>-1</sup> due to hydroxyl group in infrared (IR) absorption spectrum and also by occurrence of bathochromic shift with a change from neutral to basic condition in ultraviolet (UV) absorption spectra ( $\lambda_{\text{max}}^{\text{EtOH}}$  262, 290 m $\mu$ ,  $\lambda_{\text{max}}^{0.1N \text{ NaOH}}$  255, 325 m $\mu$ ). In order to determine the location of hydroxyl group, the reaction of diazotized 3,4-dichloroaniline with 2,3-dichlorophenol was carried out utilizing the method of Colbert and Lacy.<sup>6)</sup> This reaction should afford three isomeric products, 2- and 5-hydroxy-3,4,3',4'-TCB and 4-hydroxy-2,3,3',4'-TCB. Therefore, if one of above three products is identical with M-2 (monohydroxylated 3,4,3',4'-TCB), it should be either 2- or 5-hydroxy-3,4,3',4'-TCB. In fact, colorless needles, mp 167—168°, which were isolated from the CHCl<sub>3</sub> extract of above reaction mixture after purification through preparative TLC with a solvent system of *n*-hexane–AcOEt–AcOH (40:10:1) and recrystallization from AcOH–H<sub>2</sub>O (1:1), showed complete identity with M-2 by chromatographic (TLC and GLC) and spectral (IR, UV, nuclear magnetic resonance (NMR) and mass) examinations. By these evidences, the structure of M-2 must be either 2- or 5-hydroxy-3,4,3',4'-TCB. The HCl-hydrolysates of both urine and feces after extraction of free metabolites were carefully examined by GLC, but no evidences were obtained concerning the excretion of conjugated metabolite.

Excretion rate of unchanged 3,4,3',4'-TCB and M-2 in the rat feces was determined during 14 days after oral administration of 3,4,3',4'-TCB at a single dose of 25 mg/body by GLC. It was found that about 64% of the dose was excreted as unchanged, most of which were considered to be unabsorbed material from the gastrointestinal tract. On the other hand, the excretion of M-2 in the feces was accounted for only 3.3% of the dose during 14 days.

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Received November 24, 1972

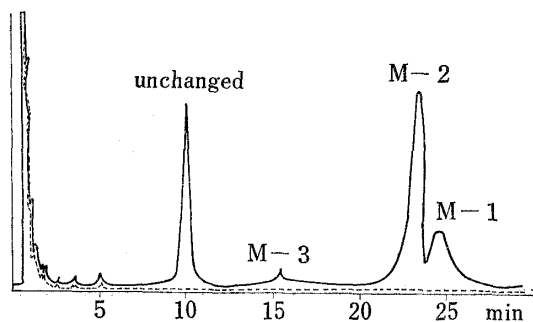


Fig. 1. A Typical Gas Chromatogram of Trimethylsilylated Extract of Fecal Sample of the Rat Excreted on Third Day after Administration of 3,4,3',4'-TCB

—: test, ----: control

6) J.C. Colbert and R.M. Lacy, *J. Amer. Chem. Soc.*, **68**, 270 (1946).