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## Abstract

Differences in urinary excretion of monochlorobenzene between rats and humans were studied. Monochlorobenzene was administered to rats and humans intraperitoneally, orally or by inhalation. Urinary p-chlorophenylmercapturic acid and 4-chlorocatechol, after hydrolysis of its conjugate, were measured. The excretion of p-chlorophenylmercapturic acid was somewhat more than that of 4-chlorocatechol in rats which were administered monochlorobenzene orally or intraperitoneally. The excretion of p-chlorophenylmercapturic acid was markedly less than that of 4-chlorocatechol in humans who received monochlorobenzene orally or by inhalation. The results indicate that the 4-chlorocatechol conjugate is a suitable index of metabolites in the urine of workers exposed to monochlorobenzene.

**KEYWORDS:** monochlorobenzene, species-difference, mercapturic acid, 4-chlorocatedchol, urinary metabolites

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## —— BRIEF NOTE ——

# DIFFERENCES IN URINARY MONOCHLOROBENZENE METABOLITES BETWEEN RATS AND HUMANS

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*Abstract.* Differences in urinary excretion of monochlorobenzene between rats and humans were studied. Monochlorobenzene was administered to rats and humans intraperitoneally, orally or by inhalation. Urinary p-chlorophenylmercapturic acid and 4-chlorocatechol, after hydrolysis of its conjugate, were measured. The excretion of p-chlorophenylmercapturic acid was somewhat more than that of 4-chlorocatechol in rats which were administered monochlorobenzene orally or intraperitoneally. The excretion of p-chlorophenylmercapturic acid was markedly less than that of 4-chlorocatechol in humans who received monochlorobenzene orally or by inhalation. The results indicate that the 4-chlorocatechol conjugate is a suitable index of metabolites in the urine of workers exposed to monochlorobenzene.

*Key words :* monochlorobenzene, species-difference, mercapturic acid, 4-chlorocatechol, urinary metabolites.

Monochlorobenzene is used as a solvent and chemical intermediate and has been used extensively in industry for many years but little has been published on its toxicity (1).

It is difficult to estimate the average concentration of monochlorobenzene in the air in places where it is used, as the concentration varies considerably within a working day. It is more practicable, therefore, to calculate the amount inhaled by workers from the metabolites excreted in their urine (2).

Parke and Williams (3) have shown that the major metabolites of monochlorobenzene found in the urine of rabbits administered chlorobenzene are p-chlorophenylmercapturic acid and the monoglucuronide and ethereal sulphate conjugate of 4-chlorocatechol. Differences in urinary excretion of trichloroethylene in rabbits, rats and mice have been studied (4) and differences in the urinary metabolite of o-xylene in rats and humans have been reported (5). The purpose of this report is to indicate the differences of metabolites between rats and humans administered monochlorobenzene, and to determine suitable index of the metabolites in the urine of workers exposed to monochlorobenzene.

*Materials and Methods*

*Reagents.* p-Chlorobenzenemercapturic acid (MA) and 4-chlorocatechol (4CC), both of reagent grade, were purchased from Kanko Shikiso Co. and

Tokyo Kasei Co.

*Urine specimens.* Four femal Wister rats weighing about 180 g were used. Rats were given a laboratory diet and supplied water liberally. Monochlorobenzene (MoCl-Bz) was diluted with polyethylene glycol and injected intraperitoneally into rats, or administered to rats and humans orally. MoCl-Bz was injected into rats at the dose of 0.5 m moles/kg, 1.0 m moles/kg and 2.0 m moles/kg. MoCl-Bz was also administered orally at a dose of 0.3 m moles/kg. Urine was collected periodically. MoCl-Bz was administered orally to a 57 year old male volunteer 3 times at a dose of 0.3 m moles/kg. Urine sample were taken during work hours from two workers (average of 30 years of age) working around the distillation appratus of a factory using MoCl-Bz.

*High performance liquid chromatography (HPLC) (6).* For the assay, urine (1 ml) was placed in a stoppered tube. Its pH was adjusted to 2.0 with HCl and nearly saturated with NaCl (0.3 g). MoCl-Bz was extracted with benzene 3 times. For the separation of MA by HPLC, 1 $\mu$l of urine was introduced into the column in which the mobile phase was a mixture of ethanol water and phosphoric acid (50/50/0.26). The flow rate was 1 $\mu$l/min, and the pressure was 80 kg/cm$^2$.

For 4CC measurement, HCl was added to urine to a final concentration of 3M and the mixture was heated for 3 h in a water bath. The hydrolysate was nearly saturated with 0.3 g/ml of NaCl. After drying under reduced pressure, the residue was dissolved with a volume of methanol equal to that of the original urine sample. For the separation of 4CC by HPLC, the molibe phase was methanol and water (50/50), the flow rate was 0.7 ml/min, and the pressure was 100 kg/cm$^2$.

*Results and Discussion*

The results are summarized in Table 1. The urinary excretion ratio of

TABLE 1. RATIO OF URINARY EXCRETION OF MERCAPTURIC ACID (MA) TO 4-CHLOROCATECHOL (4CC) CONJUGATES IN RATS AND HUMANS ADMINISTERED MONOCHLOROBENZENE

| Administration | Intraperitoneal | | | Oral | | Inhalation | |
|---|---|---|---|---|---|---|---|
| Species | Rat | | | Human | Rat | Human | |
| Dose (m moles/kg) | 0.5 | 1.0 | 2.0 | 0.3 | 0.3 | 0.88 ppm for 415 min | 0.50 ppm for 225 min[a] |
| Urinary metabolites | m moles/kg | | | | | | |
| MA/4CC mean | 9.10 | 7.71 | 6.22 | 0.04 | 2.85 | 0.02 | |
| MA + 4CC mean | 0.13 | 0.29 | 0.50 | 0.09 | 0.05 | 25.6 | 8.9 |
| USD[b] | 0.02 | 0.04 | 0.06 | 0.01 | 0.01 | 0.43 | 0.46 |
| Excretion ratio[c] | 0.26 | 0.29 | 0.25 | 0.31 | 0.18 | | |

*a* based on a personal communication from Prof. I. Hara. *b* unbiased standard deviation.
*c* MA + 4CC (m moles/kg)/Dose of MoCl-Bz (m moles/kg).

MA to 4CC was 9.1, 7.7 and 6.2 in rats injected intrapertoneally with MoCl-Bz at the doses of 0.5, 1.0 and 2.0 m moles/kg, respectively. Excretion of urinary metabolites was 25.6, 29.0 and 25.2% of each dose, respectively. The ratio of MA to 4CC showed a decreasing tendency, reciprocal to the increase in the amounts injected, but there were no significant differences among these ratios.

The amount of total metabolites (MA + 4CC conjugates) excreted was nearly proportional to the doses of MoCl-Bz injected into rats. The human volunteer and rats received MoCl-Bz orally, after which urinary MA and 4CC were determined by HPLC. The mole-ratio of MA to 4CC was 0.04 in the human volunteer and 2.84 in rats. The ratio of MA to 4CC in the human volunteer was low because of the extremely small amounts of MA excreted.

The mole-ratio of MA to 4CC in the urine of the volunteer who received MoCl-Bz orally was comparable to that of the workers who inhaled MoCl-Bz, namely 0.04 and 0.02, respectively.

Lindsay (7) reported that the major metabolites of monochlorobenzene are p-chlorophenylmercapturic acid and ethereal sulfate and monoglucuronide conjugates of 4-chlorocatechol. In humans, Wainer (8) reported that there is no detectable mercapturic acid in the urine following the administration of bromobenzene. However, Boyland (9) reported that small amounts of MA have been detected chromatographically in human urine following the administration of naphthalene. Our results using monochlorobenzene agree with previous reports (8, 9). Furthermore, the 4CC conjugate was shown to be the main metabolite of inhaled monochlorobenzene and accordingly is an excellent index of metabolites in the urine of workers exposed to monochlorobenzene.

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