HOST AND AGENT FACTORS MODIFYING METABOLISM OF TRICHLOROETHYLENE

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An apparent difference in trichloroethylene metabolism was observed between animals; human, rabbit and rat exposed to trichloroethylene. Rats metabolized the substance most rapidly, while metabolism in human proceeded at the slowest rate. The ratio of trichloroethanol to trichloroacetic acid, both the main metabolites in urine, was the largest in rabbit and the smallest in human.

There was not observed a significant sex difference in trichloroethylene metabolism in rat, while there seemed to exist the age difference in trichloroethylene metabolism in rat though not significant.

The metabolic pathway of trichloroethylene in rats did not alter with the size of trichloroethylene intraperitoneally injected at dose levels of 22-368 mg/kg. This might suggest to establish the maximum allowable concentration of trichloroethylene in the working environment from the viewpoints other than the altered metabolic pathway of trichloroethylene with the difference dose level. It was probably due to the physical mechanism that the ratio of total trichloro-compounds in urine to the dose of trichloroethylene decreased with the increase of dose.

Physiological factors have been reported to modify the metabolism of organic solvents. For example, females are more susceptible to benzene than males, because females produce much of the toxic intermediate, pyrocatechol, than do males¹⁾. Other factors such as pregnancy, nutrition and labour conditions also affect the individual susceptibility to benzene²⁾. The main metabolites of trichloroethylene have been reported to be trichloroethanol and trichloroacetic acid, both of which were expressed as total trichloro-compounds³⁾. Since Tanaka and Ikeda (1968)⁴⁾ reported the altered metabolism of trichloroethylene in human with the different dose level, there have been some discussions on factors modifying the metabolism of trichloroethylene⁵⁻⁷⁾, on which the present authors will discuss again based on the animal data. Furthermore, the meaning of these results on the maximum allowable concentration will be discussed.

MATERIALS AND METHODS

For the study of species difference, human being, rabbit and rat were employed. Data on human, rabbit and rat exposure were partly cited from Nomiya and Nomi-

yama^{5,7)}. Five male students were exposed to 250 to 380 (mean 315) ppm trichloroethylene in an exposure room (60 m³) for 160 minutes (equivalent to 25 mg/kg intake of trichloroethylene⁵⁾). Whole urine was collected every 12 hours for 6 days. Three male rabbits of Japanese white strain weighing 2.5 kg were injected intraperitoneally with 500 mg trichloroethylene (200 mg/kg). Whole urine was collected using a metabolic cage and a bladder catheter at 12 hr intervals for 4 days. Five male rats of Holtzman strain weighing 200 g were given intraperitoneally 10 mg trichloroethylene (50 mg/kg). Twenty four hr urines were collected for 3 days using a metabolic cage.

For the study on sex and age difference, 15 rats of Holtzman strain (5 each rats for 8 week old male, 8 week old female and 4 week old male groups) were given 10 mg trichloroethylene intraperitoneally; urine samples were collected in metabolic cage at 24 hr intervals for 3 days.

For study of different dose levels, 16 male rats of Holtzman strain weighing 200 g were divided into 4 groups of 4 each which were injected with 22, 74, 221 or 368 mg/kg trichloroethylene intraperitoneally. Urine samples were collected at 3, 6, 12, 36 and 48 hr after the injection.

Total trichloro-compounds, trichloroacetic acid and trichloroethanol in these urine samples were determined by a modification of the method of Seto-Schultze⁴⁾.

RESULTS

1. *Animal Species*

As shown in Fig. 1, urinary excretion of total trichloro-compounds decreased exponentially with observed period in human, rabbit and rat. Rates of decrease differed among animal species; in human it was very slow and a large amount was still detected 6 days after exposure. In rat the rate of decrease was fast and total trichloro-compounds were barely detected over 4 days. The decreasing rate of trichloroacetic acid was extremely slow.

Trichloroethanol decreased as fast as total trichloro-compounds except in rat, in which urine trichloroethanol was not detected over 2 days. The ratio of trichloroethanol to trichloroacetic acid was found to decrease rapidly with time in all 3 animal species.

Accumulated urinary excretion of trichloro-compounds, trichloroacetic acid and trichloroethanol, and the ratio of trichloroethanol to trichloroacetic acid are listed in Table 1. The ratio of trichloroethanol to trichloroacetic acid indicated a significant difference in trichloroethylene metabolism between 3 animal species. In rabbits the major metabolite was trichloroethanol and the minor trichloroacetic acid, while in human [the reverse was true.

2. *Sex and Age*

A significant difference in trichloroethylene metabolism with time after trichloroethylene administration were not observed between sex or age as shown in Fig. 2.

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Fig. 1. Species difference in urinary excretion of trichloroethylene metabolites after exposure to trichloroethylene. Dots, circles and crosses represent human, rabbit and rat data, respectively. TTC, TCA, TCE and TCE/TCA represent total trichloro-compounds, trichloroacetic acid, trichloroethanol and the ratio of trichloroethanol to trichloroacetic acid, respectively.

Table 1. Animal species difference in accumulated urinary excretion of trichloroethylene metabolites in human, rabbit and rat.

Trichloroethylene		Species		
Metabolites		Human	Rabbit	Rat
Total Trichloro-Compounds	(mg)	597.3(212.0)	174.3(13.8)	2.65(2.13)
Trichloroacetic Acid	(mg)	221.4(111.0)	0.9(0.4)	0.21(0.05)
Trichloroethanol	(mg)	343.8(111.1)	157.0(14.7)	2.23(1.96)
Trichloroethanol Trichloroacetic Acid		1.90(0.97)	206.6(75.0)	12.45(13.58)

Numbers express average values and those in blanket standard deviation.

As to the accumulated urinary excretion of trichloroethylene metabolites in 3 days after trichloroethylene administration, 8-week-old female rat was found to excrete more metabolites in urine than 4-week-old male rat. The differences in the ratio of trichloroethanol to trichloroacetic acid were not significant, but 4-week-old group showed the highest ratio.

Fig. 2. Urinary excretion of trichloroethylene metabolites after exposure to trichloroethylene in rat. Dots, circles and crosses represent 8-week-old male rats, 8-week-old female rats and 4-week-old male rats, respectively.

Table 2. Accumulated excretion of trichloroethylene metabolites in 48 hours after trichloroethylene administration in rat in sex and age

Trichloroethylene Metabolites	Sex	Male	Female	Male
	Age	8 Weeks	8 Weeks	4 Weeks
Total Trichloro-Compounds	(mg)	2.65 (2.13)	4.18 (0.97)	1.19 (0.99)
Trichloroacetic Acid	(mg)	0.21 (0.05)	0.44 (0.23)	0.05 (0.04)
Trichloroethanol	(mg)	2.23 (1.96)	3.04 (0.95)	1.04 (0.88)
Trichloroethanol Trichloroacetic Acid		12.45 (13.58)	9.87 (16.41)*	27.37 (21.73)

Numbers in blanket express standard deviations. * An extraordinary high value in trichloroethanol/trichloroacetic acid ratio was observed with one rat, while the average for other rats was 5.54 (0.39).

3. Dose

No differences were observed in the profile of excretion curves of trichloroethylene metabolites, and also of the changes in the ratio of trichloroethanol to trichloroacetic acid with time after intraperitoneal administration of 22, 74, 221 or 368 mg/kg trichloroethylene.

Accumulated urinary excretion of trichloroethylene metabolites increased almost pro-

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Fig. 3. Dose difference in urinary excretion of trichloroethylene metabolites after exposure to trichloroethylene in rat. Dots, circles, crosses and triangles represent 367, 221, 74 and 22 mg/kg groups, respectively.

Fig. 4. Relationship between injected dose of trichloroethylene and accumulated urinary excretion of trichloroethylene metabolites in 48 hours after the injection in rat. Vertical bars represent one standard deviations.

Fig. 5. Relationship between injected dose of trichloroethylene and the ratio of trichloroethylene metabolites to the injected dose of trichloroethylene in rat.

portionally to the administered dose of trichloroethylene, and the ratio of trichloroethanol to trichloroacetic acid remained unchanged regardless the administered dose as shown in Fig. 4. The ratio of trichloroethylene metabolites to the administered dose of trichloroethylene decreased from 42% to 16% with the increase of dose of trichloroethylene as shown in Fig. 5.

DISCUSSION

There may be some critiques addressed to the present paper that comparison between human and animals is illegal on account of different routes of trichloroethylene uptake which may decisively influence the ratio of trichloroethanol to trichloroacetic acid. However, Ikeda and Ohtsuji (1972)⁹⁾ already demonstrated that the metabolism of inhaled and intraperitoneally administered trichloroethylene was the same in rat, that is, the administration route of trichloroethylene did not modify the metabolism of trichloroethylene in rat. Therefore, the present authors believed it not illegal to compare the metabolism of trichloroethylene in 3 animal species regardless the different routes of administration. The present authors gave trichloroethylene to 3 animal species in relatively a small dose levels of 25 to 200 mg/kg. And as already mentioned above, the significant difference in trichloroethylene metabolism was found among human, rabbit and rat. However, this seemed to be modified by the life span of the animals. The biological half time of urinary excretion of total trichloro-compounds ($\tau=1/2$), which was calculated from Fig. 1, varied directly with the life span of the animals; the longer the life span, the slower the fall in the excretion curve (Table 3).

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Table 3. Relationship between life span and biological half time of total trichloro-compounds in urine (τ 1/2) of human, rabbit and rat.

Species	τ 1/2 of Total Trichloro-Compounds in Days	Life Span of Animal in Years	τ 1/2
			Life Span in Days/Years
Human	1.33	70	0.019
Rabbit	0.36	6	0.060
Rat	0.22	3	0.074

Although a significant difference in trichloroethylene metabolism was observed in human⁵⁾, no sex difference was found in rat. That fact quite accorded with the report of Ikeda and Imamura (1973)⁸⁾ in rats exposed to 100 ppm trichloroethylene vapour.

Young animals seemed to excrete rather small trichloroethylene metabolites into urine although the same amount of trichloroethylene was intraperitoneally administered. The mechanism is still unclarified.

Ikeda *et al.* (1972)⁸⁾ claimed that urinary excretion of trichloroacetic acid both in human and in rat did not increase any more at the exposure level of over 50 ppm trichloroethylene, that is, the ratio of trichloroethanol to trichloroacetic acid increased over the level of 50 ppm. They therefore proposed 50 ppm for the maximum allowable concentration of trichloroethylene partly because trichloroethylene metabolism altered at this level. The present authors could not confirm such an alteration in trichloroethylene metabolism at the level of 50 ppm as shown above. Our experimental data on human also failed to support the finding of Ikeda *et al.* (1972)⁸⁾ as shown previously⁷⁾. It is possible that the discrepancy between our results and those of Tanaka and Ikeda (1968)⁴⁾ might arise from the fact that Tanaka and Ikeda determined the trichloroethylene metabolites in urine only for 24 hr after trichloroethylene exposure, although the ratio of trichloroethanol to trichloroacetic acid in urine decreased with time after 24 hr as shown in Fig. 3.

Based on the above data on the metabolism of trichloroethylene in rat and on our previous data on human⁷⁾, the maximum allowable concentration of trichloroethylene in the working environment should be discussed from the viewpoints other than the altered metabolic pathway of trichloroethylene in human and/or animals.

The ratio of total trichloro-compounds in urine to the dose of trichloroethylene decreased with the increased dose level, and remained the same level at over 331 mg/kg dose level, that is, equivalent to 120 ppm trichloroethylene exposure level, which was calculated on the report of Ikeda and Ohtsuji (1972)⁹⁾. The mechanism of the decrease in the ratio of total trichloro-compounds to the dose of trichloroethylene is not elucidated. It might be caused by the increased respiratory excretion of trichloroethylene with the dose of trichloroethylene, but not by the increased retention of trichloroethylene in fat tissue because the metabolism of trichloroethylene did not prolong with the dose of

trichloroethylene as shown in Fig. 3. It is still too early, however, to take the decreased metabolism of trichloroethylene with the dose into consideration in establishing the maximum allowable concentration of trichloroethylene in the working environment.

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