A comparative study of the excretion of Fujiwara reaction-positive substances in urine of humans and rodents given trichloro- or tetrachloro-derivatives of ethane and ethylene

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Ikeda, M., and Ohtsui, H. (1972). *Brit. J. industr. Med.*, 29, 99-104. A comparative study on the excretion of Fujiwara reaction-positive substances in urine of humans and rodents given trichloro- or tetrachloro-derivatives of ethane and ethylene. 1,1,1-Trichloroethane, 1,1,2-trichloroethane, 1,1,1,2-tetrachloroethane, 1,1,2,2-tetrachloroethane, trichloroethylene, and tetrachloroethylene were administered to rats and mice as vapours at 200 p.p.m. for 8 hours and urine was collected for 48 hours. The urine was analysed by the Fujiwara reaction for total trichlorocompounds (TTC), trichloroacetic acid (TCA), and trichloroethanol (TCE). All compounds except 1,1,2-trichloroethane yielded substantial TCA and TCE but 1,1,1,2-tetrachloroethane and trichloroethylene much more than the rest. The results obtained during two periods of 48 hours after intraperitoneal injection were similar. The variations in the amounts of metabolites are shown to be consistent with the vapour pressures of the solvents (compounds with high vapour pressures are lost from the lungs before being metabolized) and with their known chemical properties, according to which 1,1,1-trichlorocompounds should yield TCE and TCA readily, whereas 1,1,2-chlorocompounds should not.

Excretion of metabolites from men exposed intermittently to vapours of tetrachloroethylene and trichloroethylene were also studied. Both gave enough TCE and TCA, but trichloroethylene gave considerably more, in accordance with its relative instability to oxidation.

In recent years increasing attention has been paid to the evaluation of urinary metabolites as a measure of exposure to organic solvents. The Fujiwara reaction enjoys a wide reputation as a method of analysis of urine for trichloroethylene metabolites (Browning, 1965a), but investigation of possible applications to the analysis of the metabolites of other chlorinated solvents is rather limited.

In the present study urine of humans and rodents exposed to several chlorinated hydrocarbons was analysed with a modification (Tanaka and Ikeda,

1968) of the Fujiwara reaction and the results are discussed in relation to the metabolism and alveolar excretion of the solvents.

Materials and methods

Animals

Wistar rats (about 70 g) of both sexes and female DD mice (about 25 g) were used. Preliminary experiments revealed no differences in metabolism between the two sexes under the conditions studied. They were fed on commercial laboratory chow and water *ad libitum*.

Chemicals

1,1,1,2-Tetrachloroethane was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A., while other sources were local. The solvents and their physical properties are listed in Table 1.

Analysis of urine

The urine from each group of animals (5 to 6 rats or 10 mice per group) was collected separately from faeces and pooled. Metabolites were determined colorimetrically by the Fujiwara reaction under the conditions described previously (Tanaka and Ikeda, 1968). The optical extinction obtained after oxidation of the urine sample was attributed to total trichloro compounds (TTC), that without oxidation to trichloroacetic acid (TCA), and the difference between the two to trichloroethanol (TCE). The spectral patterns of the urinary metabolites after the Fujiwara reaction were the same for all the solvents studied.

Vapour concentrations

The concentrations of trichloroethylene in an exposure chamber were determined with Kitagawa detection tubes (Kitagawa, 1960). For other solvents, equimolar amounts were introduced into the chamber.

Results**Urinary metabolites**

After exposure Groups of rats were exposed to the vapour of the solvents listed in Table 1 at a concentration of 200 p.p.m. for 8 hours. Urine was collected for 48 hours from the beginning of exposure. The results of analyses of urine are summarized in Table 2. Exposure to trichloroethylene led to excretion of both TCA and TCE, as reported in various papers (for a review, see Browning, 1965a). Both

TABLE 1
CHEMICAL AND PHYSICAL PROPERTIES OF THE SOLVENTS

Solvent	Structure	Vapour pressure ¹ (mmHg at 38°C)	Boiling point (°C)
1,1,1-Trichloroethane	$\begin{array}{c} \text{Cl} \quad \text{H} \\ \quad \\ \text{Cl}-\text{C}-\text{C}-\text{H} \\ \quad \\ \text{Cl} \quad \text{H} \end{array}$	220	74.0
1,1,2-Trichloroethane	$\begin{array}{c} \text{Cl} \quad \text{H} \\ \quad \\ \text{H}-\text{C}-\text{C}-\text{Cl} \\ \quad \\ \text{Cl} \quad \text{H} \end{array}$	42	113.5
1,1,1,2-Tetrachloroethane	$\begin{array}{c} \text{Cl} \quad \text{H} \\ \quad \\ \text{Cl}-\text{C}-\text{C}-\text{Cl} \\ \quad \\ \text{Cl} \quad \text{H} \end{array}$	26	129.2
1,1,2,2-Tetrachloroethane	$\begin{array}{c} \text{Cl} \quad \text{Cl} \\ \quad \\ \text{H}-\text{C}-\text{C}-\text{H} \\ \quad \\ \text{Cl} \quad \text{Cl} \end{array}$	14	146.3
Trichloroethylene	$\begin{array}{c} \text{Cl} \quad \quad \text{Cl} \\ \diagdown \quad \diagup \\ \text{C}=\text{C} \\ \diagup \quad \diagdown \\ \text{Cl} \quad \quad \text{H} \end{array}$	137	87.2
Tetrachloroethylene	$\begin{array}{c} \text{Cl} \quad \quad \text{Cl} \\ \diagdown \quad \diagup \\ \text{C}=\text{C} \\ \diagup \quad \diagdown \\ \text{Cl} \quad \quad \text{Cl} \end{array}$	38	121.2

¹Estimated from the observed values at adjacent temperatures

TABLE 2
EXCRETION OF URINARY METABOLITES FROM RATS AND MICE EXPOSED TO THE
VAPOUR OF THE SOLVENTS

Solvent	Species	No. of experiments	Urinary metabolites (mg/kg body weight)		
			TTC	TCA	TCE
Control (no exposure)	Rat	6	0.3 ± 0.1	0 ¹	0.3 ± 0.1
	Mouse	2 ²	0.05, 0.17	0.05, 0.17	0 ¹
1,1,1-Trichloroethane	Rat	8	3.6 ± 1.0	0.5 ± 0.2	3.1 ± 1.0
1,1,2-Trichloroethane	Rat	8	0.6 ± 0.3	0.3 ± 0.1	0.3 ± 0.1
1,1,1,2-Tetrachloroethane	Rat	8	199.0 ± 24.0	39.4 ± 5.0	159.6 ± 24.4
1,1,2,2-Tetrachloroethane	Rat	8	8.2 ± 3.0	1.7 ± 0.9	6.5 ± 2.7
Trichloroethylene	Rat	8	113.4 ± 32.0	21.2 ± 7.6	92.2 ± 28.1
Tetrachloroethylene	Rat	8	8.5 ± 3.3	5.3 ± 1.6	3.2 ± 1.2
	Mouse	2 ²	23.0, 26.9	18.9, 22.4	4.1, 4.5

Wistar rats (about 50 g, 6 animals per group) and DD mice (about 25 g, 10 animals per group) were exposed to the vapour of the solvent (200 p.p.m.) for 8 hours. Urine samples were collected (separately from faeces) for 48 hours from the beginning of exposure, and analysed with Fujiwara reaction as described under Materials and methods. Values in the table are means ± S.D.

¹Amount not measurable

²Single results

metabolites, although in much smaller amounts, were found in urine after exposure to tetrachloroethylene. The same was true for human subjects (Table 3) and mice (Table 2) exposed to tetrachloroethylene. It is noteworthy that 1,1,1,2-tetrachloroethane gave rise to large amounts of TCA and TCE whereas its isomer, 1,1,2,2-tetrachloroethane, yielded less than one-twentieth of these amounts. Both isomers of trichloroethane produced very small amounts of metabolites in urine, but 1,1,2-trichloroethane yielded less than its 1,1,1-isomer.

After intraperitoneal injection To eliminate effects of possible differences in the efficiency of pulmonary uptake among the vapours of the solvents tested, equimolar amounts of the solvents (2.78 mmol/kg body weight) were injected intraperitoneally into

the animals and their urine was collected for the following two 48-hour periods. Results from urine analyses (Table 4) are essentially the same as those obtained from inhalation experiments (Table 2), except that only very little, if any, TCE was excreted in the urine of rats and mice after an injection of tetrachloroethylene. Chloral hydrate, an intermediate in the conversion of trichloroethylene to TCE (Friedman and Cooper, 1960) and TCA (Cooper and Friedman, 1958), gave rise to TCE and TCA. The TCE/TCA ratio was quite close to that observed after the trichloroethylene injection.

Discussion

Organic solvents taken into the body may be eliminated mainly by excretion into the alveolae and

TABLE 3
EXCRETION OF METABOLITES IN URINE OF MALE WORKERS EXPOSED TO TETRACHLOROETHYLENE
AND TRICHLOROETHYLENE

Solvent	Concentration (p.p.m.)	No. of workers	Urinary metabolites (mg/l)		
			TTC	TCE	TCA
Tetrachloroethylene	20- 70	4	8- 56	4- 20	4- 35
	200-400 ¹	66	54-195	21-100	32- 97
Trichloroethylene	10- 50	6	38-376	11-281	18- 95
	120-250	14	55-487	33-347	22-177

The workers were subject to daily, intermittent exposure. The maximum vapour concentrations recorded are shown, as determined by Kitagawa detection tubes (1960). The ranges of urinary concentrations are shown.

¹This worker was also in direct skin contact with the liquid.

TABLE 4
EXCRETION OF URINARY METABOLITES FROM RATS AND MICE AFTER INTRAPERITONEAL INJECTION
OF THE SOLVENT

Solvent	Species	No. of experiments	Urinary metabolites (mg/kg body weight)					
			TTC		TCA		TCE	
			A	B	A	B	A	B
Control (no injection)	Rat	6	0.3 ± 0.1		0 ¹		0.3 ± 0.1	
	Mouse ²	2	0.05, 0.17		0.05, 0.17		0, 0 ¹	
1,1,1-Trichloroethane	Rat	7	4.0 ± 1.5	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	3.5 ± 1.4	0 ¹
1,1,2-Trichloroethane	Rat	7	0.6 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0 ¹
1,1,1,2-Tetrachloroethane	Rat	7	114.2 ± 10.0	1.6 ± 0.8	16.9 ± 1.6	0.8 ± 0.1	97.3 ± 8.8	0.9 ± 0.3
1,1,2,2-Tetrachloroethane	Rat	8	2.1 ± 0.4	0.3 ± 0.03	1.3 ± 0.2	0.3 ± 0.03	0.8 ± 0.4	0 ¹
Trichloroethylene	Rat	8	99.5 ± 20.2	1.2 ± 0.3	13.3 ± 3.6	0.6 ± 0.1	86.2 ± 17.7	0.6 ± 0.2
Tetrachloroethylene	Rat	7	5.6 ± 1.1	0.4 ± 0.03	5.5 ± 1.1	0.3 ± 0.1	0.08 ± 0.04	0.03 ± 0.02
	Mouse ²	2	23.8, 23.3	0.6, 0.4	23.7, 22.9	0.5, 0.4	0.1, 0.4	0.1, 0.02
Chloral hydrate	Rat	8	232.2 ± 23.2	4.1 ± 2.6	28.1 ± 4.5	1.1 ± 0.6	195.1 ± 23.2	2.9 ± 1.0

Wistar rats (about 50 g, 5 animals per group) and DD mice (about 25 g, 10 animals per group) were given 2.78 mmol/kg body weight of the solvents intraperitoneally diluted with or suspended in soy-bean oil to a total volume of 1 ml/kg body weight. Urine samples collected (separately from faeces) for two successive 48-hour periods were analysed by the Fujiwara reaction as described under Materials and methods. Values in the tables are means ± S.D. for the first (A) and second (B) 48-hour period after injection.

¹Amount not measurable. ²Single results.

excretion of metabolites through the kidney into urine. Studies by Stewart (1968), Stewart *et al.* (1969; 1970a, b), and Hake, Waggoner, Robertson, and Rowe (1960) suggest that the proneness of solvents to be excreted into alveolar breath is closely related to such physical properties of the solvents as vapour pressure and boiling point (Table 1). 1,1,1-Trichloroethane, with the highest vapour pressure and the lowest boiling point among the solvents studied, was excreted almost completely in the expired air when its ¹⁴C-labelled preparation was administered intraperitoneally to rats (Hake *et al.*, 1960). The low metabolite levels in urine (Tables 2 and 4) are in accordance with this observation. Trichloroethylene, with a shorter alveolar excretion half-life than tetrachloroethylene (Stewart *et al.*, 1970a, b), has a higher vapour pressure and a lower boiling point than its tetrachlorinated homologue (Table 1).

Another factor which determines the rate of elimination is the tendency of the compound to undergo biotransformation. The high stability of 1,1,1-trichloroethane (Irish, 1963), coupled with rapid elimination through the lungs, as stated above, may account for the low levels of metabolites of this compound in urine (Tables 2 and 4). The relative levels of TCE and TCA, with TCE considerably the higher, may indicate that the TCA in this case is derived from TCE, which is itself the primary product from the oxidation of 1,1,1-trichloroethane. Although the metabolic pathways of the isomer 1,1,2-trichloroethane are still unknown (Browning, 1965b), the structure of this compound apparently

requires a shift of one chlorine atom from one carbon to the other to form TCA or TCE. Consequently, when this solvent is administered to rats, the levels of TCA and TCE in urine are very low as the occurrence of such a reaction is unlikely. One should also consider the possibility that these estimates by the Fujiwara reaction represent metabolites other than TCA and TCE, as those halogen compounds which have at least two halogen atoms bound to one carbon atom are reactive to the test (Feigl, 1960). The very high yields of TCA and TCE (Tables 2 and 4) from 1,1,1,2-tetrachloroethane can be explained by hydrolytic dehalogenation with the formation of an oxidized hydrocarbon (Williams, 1959). The first step may be the formation of TCE, which then is further oxidized to TCA. As the vapour pressure of 1,1,1,2-tetrachloroethane is lower than that of trichloroethylene or tetrachloroethylene and its boiling point is higher than that of the two chlorinated ethylenes, the rate of elimination of this solvent through the lungs is likely to be very low. Rates of conversion of its 1,1,2,2-tetrachloro isomer to TCA and TCE are much slower, in agreement with the observation that the yields of urinary metabolites from 1,1,2-trichloroethane are lower than those from its 1,1,1-isomer. It should be noted that the toxicities of 1,1,1-trichloroethane and 1,1,1,2-tetrachloroethane are lower than those of 1,1,2-trichloroethane and 1,1,2,2-tetrachloroethane (Table 5). Accordingly, from the rate of decrease in urinary metabolite concentration, the former two compounds have faster rates of elimination than the latter two (Table 4).

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