DISTRIBUTION, ELIMINATION AND RETENTION OF STYRENE IN RATS

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Abstract.....In rats exposed to styrene for 4 hours, the rate constant of elimination and biological half life of styrene were 0.11 and about 6 hours for adipose tissue, and 0.3 to 0.4 and about 2 hours for the other tissues, respectively. The relative ratio of apparent distribution of styrene decreased in the order adipose tissue >> liver > brain > kidney > blood $\div$ spleen > muscle. Almost the same results were obtained in the experiment by intraperitoneal injection of styrene.

Repeated 4-hour exposures at about 700 ppm daily for five days caused the results similar to those in a single exposure. A trend of increase in concentration of styrene was observed in adipose tissue by 5 successive intraperitoneal injections every 6 hours at a dose of 350 mg/kg.

Key words: distribution, elimination, retention, styrene, rats

INTRODUCTION

Styrene has been widely used in the plastics industries in the world. Since 1942 when Spencer et al. reported the response of animals to styrene, several studies have been made on styrene poisoning as reviewed by Oltamare et al. (1974). In Japan, styrene was categorized as one of the second sort of organic solvents under the revised regulation of the Ministry of Labor in 1972. We have reported a series of studies on styrene poisoning which include surveys of the actual conditions in the factories and fundamental experiments in both humans and animals (Horiguchi et al., 1971; Teramoto, 1978; Teramoto and Horiguchi, 1979).

The present paper describes a study on distribution, elimination and retention of styrene administered once or repeatedly by inhalation or by intraperitoneal injection in rats.

MATERIALS AND METHODS

1. Animals

Male rats, JCL: SD breeding of Nippon Clea Co. of 4 weeks old, were grown under conventional ambient condition at room temperature. Water and chow, CE-2 (Nippon

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Clea Co. Ltd., Tokyo), were furnished ad libitum. When the rats became 10 weeks old, they were used for each experiment.

2. Administration of styrene

1) Single administration

a) By inhalation : Rats were divided into 6 groups, each group consisting of 5 to 7 rats. They were exposed to styrene at 500 to 1000 ppm in exposure chambers for 4 hours. Considering the similarity of rate constants, the above difference of styrene concentration seems to be practically satisfactory. At 0, 1, 2, 5, 8, 24 and 48 hours after cease of exposure, styrene concentration in each tissue was determined.

Fig. 1 shows an apparatus for exposing animals to styrene. Styrene was vaporized and sent with air into the exposure chamber at a flow rate of 2 L/min. The concentration of styrene in the exposure chamber was adjusted by the amount of styrene in an impinger, being checked by the gaschromatography as required.

Fig. 1 Generator of styrene and exposure chamber

b) By intraperitoneal injection : Rats were divided into 5 groups, each group consisting of 4 to 5 rats. They were given styrene dissolved in olive oil (styrene : olive oil = 1 : 4) at a single dose of 350 mg/kg. At 1, 2, 4, 6 and 24 hours after administration, styrene concentration in each tissue was determined.

2) Repeated administration

a) By inhalation : Twenty five rats were divided into 5 groups, each group consisting of 5 rats. They were exposed to styrene at about 700 ppm in exposure chamber 4 hours daily for 5 days. Styrene concentration in each tissue was determined at 0 to 20 hours after cease of each exposure.

b) By intraperitoneal injection : Twenty rats were divided into 5 groups, each group consisting of 4 rats. They were given styrene dissolved in olive oil (styrene : olive oil = 1 : 4) at 5 successive doses of 350 mg/kg, every 6 hours. At 1, 2, 4 and 6 hours after administration, styrene concentration in each tissue was determined.

3. Analysis for styrene in tissue

The blood, liver, kidney, spleen, brain, muscle, and adipose tissue were excised from the ether-anesthetized rats. The excised tissues were washed with 0.85% saline and wiped with filter paper. Then a 0.5 g specimen of each tissue was taken into a tube with

5 ml n-hexane, homogenized by Polytron and analyzed for styrene by gas chromatography. The details of analytical method was previously reported (Teramoto and Horiguchi, 1980).

4. The instrumental conditions

Gas chromatograph : Shimadzu GC-6A (Shimadzu Seisakusho, Ltd., Kyoto)

Column : glass, 2m × 3mm, temp., 110°C

Packing : 10% PEG-20M on Chromosorb W, 60-80 mesh

Carrier gas : N₂, 50 ml/min.

H₂ : 0.7 kg/cm²

Air : 1 kg/cm²

Detector : FID temp., 150°C

Injection : temp., 150°C

RESULTS AND DISCUSSION

After a 4 hour single exposure to styrene at 500 to 1000 ppm in rats, the elimination of styrene from the blood, liver, kidney, spleen, brain and muscle was expressed by the following equation :

$$C = A \exp(-\alpha t) \dots \dots \dots (1)$$

where C is the concentration of styrene in each tissue at time t, α the rate constant for elimination, and A the intercept. Fig. 2 shows the elimination of styrene from the blood as a representative of those tissues. As shown in Fig. 3, the elimination from adipose tissue was expressed by the following equation :

$$C = P \exp(-\alpha_1 t) - Q \exp(-\alpha_2 t) \dots \dots \dots (2)$$

Fig. 2 Concentration of styrene in the blood of rats after a single exposure to about 700 ppm styrene

Fig. 3 Concentration of styrene in the adipose tissue of rats after a single exposure to about 700 ppm styrene

where C is the concentration of styrene in adipose tissue, α_1 the rate constant for elimination, and α_2 the rate constant for absorption, while P and Q are the intercepts.

As shown in Table 1, the rate constants of elimination were similar (0.3 to 0.4) in all the tissues studied, except that in adipose tissue (0.11). The biological half lives (BHLs) were about 6 hours for adipose tissue and about 2 hours for the others. There were no significant differences in the rate constants of elimination and BHLs for all the tissues, except adipose tissue.

Table 1 Pharmacokinetic data of styrene in tissues following a single exposure to styrene at 500 to 1000 ppm in rats.

		blood	liver	kidney	spleen	muscle	brain	adipose tissue
Rate constant of elimination	Mean	0.30	0.33	0.34	0.33	0.37	0.35	0.11
	S.D.	0.057	0.092	0.097	0.096	0.13	0.11	0.065
Biological half life (h)	Mean	2.4	2.1	2.2	2.2	2.0	2.1	6.3
	S.D.	0.56	0.51	0.49	0.53	0.58	0.55	4.5
Relative ratio of apparent distribution	Mean	1	2.8	1.5	0.93	0.68	2.1	35
	S.D.		0.71	0.19	0.13	0.18	0.36	22

From the equations (1) and (2) at $t=0$, apparent concentration of styrene in each tissue was calculated. The relative ratio of this concentration in each tissue comparing with that in the blood decreased in the order adipose tissue > liver > brain > kidney > blood $\div$ spleen > muscle (see Table 1). As the rate constants are similar in the tissues except adipose tissue, the above order seems to be about the same in any time during 5 hours after cease of exposure. Almost the same results were obtained in an experiment with intraperitoneal injection of styrene as shown in Table 2.

Table 2 Pharmacokinetic data of styrene in tissues following a single intraperitoneal dose of 350 mg/kg in rats.

		blood	liver	kidney	spleen	muscle	brain	adipose tissue
Rate constant of elimination	Mean	0.29	0.37	0.43	0.37	0.33	0.29	0.15
	S.D.	0.11	0.13	0.29	0.049	0.070	0.11	0.055
Biological half life (h)	Mean	2.7	2.1	2.3	1.9	2.1	2.7	5.0
	S.D.	1.1	0.70	0.52	0.22	0.37	0.99	1.7
Relative ratio of apparent distribution	Mean	1	4.8	2.3	2.4	0.71	2.4	91
	S.D.		0.49	1.2	0.61	0.30	0.057	18

Styrene concentrations in the blood, liver, brain, kidney, spleen, muscle and adipose tissue were also determined after repeated administrations by inhalation and intraperitoneal injection. Fig. 4 shows styrene concentration in blood and adipose tissue after repeated 4-hour exposures at about 700 ppm daily for five days. Distribution and elimination of styrene in tissues were similar to those in a single exposure. No signifi-

cant accumulation of styrene in the body was observed.

Fig. 5 shows styrene concentration in blood and adipose tissue after repeated intra-peritoneal injections. Styrene concentration in the blood as well as in the liver, kidney, brain, spleen and muscle, was highest 1 hour after administration, followed by gradual decrease. Six hours after administration, styrene was still detected in the above tissues, but no increasing tendency of the concentration was observed. In adipose tissue, however, tendency of accumulation of styrene was observed.

Fig. 4 Variation of styrene concentration in blood and adipose tissue following repeated inhalation exposure to about 700 ppm of styrene: $\times$ — $\times$ blood, $\bullet$ — $\bullet$ adipose tissue, $\blacksquare$ styrene exposure (4 hours)

Fig. 5 Variation of styrene concentration in blood and adipose tissue following repeated intraperitoneal injection of 350 mg/kg of styrene: $\times$ — $\times$ blood, $\bullet$ — $\bullet$ adipose tissue, $\downarrow$ injection, No data at 2 hours after the first administration

From the standpoint of occupational health, intake of styrene through the respiratory organs is most important. In our experiments, animals were given styrene by inhalation and intraperitoneal injection respectively. As our exposure chambers were limited in an increase of styrene concentration more than 1500 ppm, we additionally made an experiment of intraperitoneal injection of styrene to observe its accumulation. It was reported that styrene was found in samples of subcutaneous fat from workers who had been heavily exposed to styrene and other solvents (Wolff et al., 1977). Determination of styrene in adipose tissue, accordingly, may serve as an index of styrene exposure in workmen exposed to high concentrations of styrene. For the maximum allowable concentration expressed as time-weighted average, 50 ppm in Japan (Japan Association of Industrial Health) and 100 ppm in U. S. A. (American Conference of Governmental Industrial Hygienists) are currently recommended. It seems that no accumulation of styrene occurs as far as the workers are under the above maximum allowable concentration of styrene.

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