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Kinetics of m-xylene in man

General features of absorption, distribution, biotransformation and excretion in repetitive inhalation exposure

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RIIHIMÄKI, V., PFÄFFLI, P., SAVOLAINEN, K. and PEKARI, K. Kinetics of m-xylene in man: General features of absorption, distribution, biotransformation and excretion in repetitive inhalation exposure. *Scand. j. work environ. & health* 5 (1979) 217—231. Sedentary volunteer subjects were exposed to an m-xylene concentration of about 3.9 mmol/m³ over five successive days, 6 h/d. It was noted that about 60 % of the inhaled xylene was retained in the lungs. The estimated daily uptake of xylene was recovered nearly quantitatively as methylhippuric acid in the urine. Other pathways of xylene excretion played a minor role. The rate of 2,4-xyleneol excretion shortly after a day's exposure was about 1—2 % of that for methylhippuric acid excretion, and pulmonary excretion of unchanged xylene amounted to about 4 % of the estimated uptake. In the blood, xylene was mainly associated with serum proteins, and only small amounts resided in the cells. Postexposure excretion of xylene in expired air and the urinary excretion of methylhippuric acid were initially rapid (elimination half-time about 1 h), and after an intermediate phase it attained, some 6—16 h after the exposure, a slow phase of elimination (half-time about 20 h). These observations are congruent with the concept that xylene is distributed to at least two main tissue compartments in the body. The slow elimination takes place from tissues with a high xylene solubility and a small perfusion, for example, adipose tissue; in this compartment cumulation of xylene occurs over repeated exposures. Under the conditions studied no signs of saturation of the metabolic pathways or renal excretion were noted.

Key words: human exposure, kinetics, m-xylene.

Xylene is a common industrial solvent, and it is frequently handled in ways that may significantly expose workers. For the assessment of exposure to xylene and the ensuing hazards, an understanding of the kinetics of xylene in man is important.

Kinetic studies of xylene isomers (ortho-, meta-, para-) with human volunteer subjects have been performed recently (1, 13, 31, 32). These studies have involved single inhalation exposures of 8-h or less duration. Many of the basic kinetic features of inhaled xylene have been elucidated.

It is known that a rather constant fraction, about 60 %, of inhaled xylene is retained in the body when the xylene concentration in the air is moderate (1.9—8.2 mmol/m³, 46—200 ppm v/v) (1, 4, 31). About 95 % of absorbed xylene is excreted

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as urinary metabolites, and only about 5 % is exhaled unchanged (1, 31). Xylene isomers are mainly biotransformed to methylbenzoic acids (toluic acids), which are conjugated with glycine to form methylhippuric acid (toluric acid). Only a few percent of absorbed xylene is metabolized to xylenols (31). These observations agree well with previous experience from metabolic studies of xylene in experimental animals (3, 6, 7, 9, 15, 21). The post-exposure elimination of xylene in the blood and exhaled air (1, 31), as well as the excretion of methylhippuric acid in the urine (24, 31, 32), displays an initial rapid phase followed by one or more slower phases, and this phenomenon indicates that xylene is distributed to tissue compartments with different elimination rates. Some xylene is distributed to adipose tissue, from which its elimination is slow (13).

We wanted to investigate the kinetics of m-xylene, the predominant isomer in technical xylene, in human volunteers under conditions which would simulate occupational exposure. Five successive daily exposures were employed in the study of the possible cumulation of the compound in the body. Kinetic events observed under stable conditions, i.e., the volunteers inhaled xylene at rest and at a constant concentration, are dealt with in this report. The modifying effects of physical exercise and varying inhaled xylene concentrations on kinetics will be reported in a subsequent publication.

MATERIAL AND METHODS

Subjects

The subjects of this study were six healthy male volunteers, 21–28 years of age. In a few details of this report reference is made to 12 additional volunteers (18–35 years of age) who took part in other series of exposures. The study was carried out with strict adherence to the principles of the Declaration of Helsinki adopted by the World Medical Association (38). No abnormal findings were disclosed in a routine

clinical examination (erythrocyte sedimentation rate, hematology, serum glutamate-oxalacetate transaminase, serum glutamate-pyruvate transaminase, serum creatinine, urinary albumin, glucose and sediment). Lung function (spirometry, capnography and transfer factor) and cardiac function (electrocardiography at rest and during submaximal ergometer exercise) were normal. Clinical chemical investigations were repeated after the exposure period, and no significant changes were noted. Some anthropometric and physiological characteristics of the subjects are presented in table 1.

Exposure

The exposures were carried out in a dynamic controlled-environment exposure chamber. The chamber had a volume of 15 m³, and the ventilation was adjusted to approximately ten air changes per hour. Meta-xylene (laboratory grade, Merck, Darmstadt, Federal Republic of Germany) vapor was generated in a saturator immersed in a thermostated water bath and diluted with carrier air to produce the desired environmental concentration. The xylene concentration in the chamber air was continuously monitored with an infrared analyzer (Miran 1 A, Wilks Scientific Corp., South Norwalk, U.S.A.) and graphically recorded. The temperature in the chamber was maintained at $23 \pm 2^\circ\text{C}$ and the relative humidity at $50 \pm 10\%$.

Six volunteers were exposed simultaneously to a constant xylene air concentration approximating the Finnish threshold limit value for xylene, 4.1 mmol/m³ (100 ppm), 6 h/d for five successive days. On the afternoon of the fifth day (Friday) the xylene concentration in the air was two times higher, 8.2 mmol/m³. The daily exposure was divided into two periods, i.e., 3 h in the morning and 3 h in the afternoon, with a 1-h lunch break in between. The exposure design is graphically presented in fig. 1.

The actual measured concentrations of m-xylene in the chamber air were somewhat lower than intended. Over the first 4 d and Friday morning the mean concentration was 3.9 mmol/m³ (range 3.6–4.1 mmol/m³); on Friday afternoon the mean

Table 1. Some anthropometric and physiological characteristics of the subjects.

Person	Age (years)	Skin surface area (m ²)	Weight (kg)	Ventilation of basal conditions, table values ^a (l/min)	Ventilation of 100-W exercise, in the laboratory (l/min)
E.I.	21	1.82	68	6.6	32
P.S.	24	1.78	65	6.4	37
H.M.	28	1.79	67	6.4	36
E.O.	22	1.95	68	7.0	30
K.L.	23	2.00	77	7.2	37
D.K.	22	1.94	74	7.0	32

^a From Comroe et al. (11).

concentration was 8.2 mmol/m³. The daily fluctuation of xylene concentration in the air was small and within the range of a few percent, with only a few accidental and brief exceptions. In a previous publication (14) this exposure was designated as exposure type III.

Reference will be made to two other series of exposures in connection with some of the kinetic observations. Exposure type I (8 volunteers) followed the previous-

ly presented exposure pattern, except that the subjects exercised on a bicycle ergometer for 10 min two times in the morning and two times in the afternoon with a 100-W work load (fig. 2). Exposure type II (8 volunteers) contained a more complex design involving varying xylene concentrations in the chamber air and the aforementioned periodic ergometer exercise. Since this particular exposure pattern is not likely to affect the fundamental find-

Fig. 1. Schematic presentation of the exposure conditions. Subjects were sedentary throughout the experiment.

Fig. 2. Exposure type I (reference exposure). Environmental conditions as in fig. 1, but the subjects exercised on a bicycle ergometer at 100 W for 10 min four times per day.

ings reported (distribution of m-xylene between the different components of blood), further details of exposure type II have not been included.

Collection of samples

Venous blood samples were obtained through a teflon catheter inserted into a large forearm vein at specified time intervals, as in fig. 3 and 4. The catheter was kept open by means of filling it with heparin (0.1 ml) after the blood sampling.

The *exhaled air* of a few subjects was monitored over longer time periods on selected days with the apparatus described later, and samples of *forced end-expiratory air* were collected in 250-ml polyester-lined polyethylene bags at specified times immediately after the venous blood sampling and throughout the postexposure period (fig. 3 and 4).

Urine samples were mostly obtained at about 2-h intervals during the exposures, and the subjects were requested to collect urine voided throughout Monday night up until the beginning of the exposure period on Tuesday and from Thursday afternoon through the weekend (fig. 5 and 6).

Fig. 3. Concentration of m-xylene in blood ($\diamond$ - $\diamond$) and end-expired air ($\blacklozenge$ - $\blacklozenge$) during and after the first exposure day. Mean values of 4-6 subjects are presented; bars denote standard deviations. For comparison the corresponding postexposure values of m-xylene in blood ($\circ$ - $\circ$) and end-expired air ($\bullet$ - $\bullet$) for 4-8 subjects from exposure type I are shown.

Monitoring of m-xylene concentration in exhaled air

The subjects breathed with a half-face mask (dead space volume 70 ml) or with a mouthpiece (dead space volume 40 ml) through teflon tubing (diameter 2.5 cm,

Fig. 4. Concentration of m-xylene in blood ($\diamond$ - $\diamond$) and end-expired air ($\blacklozenge$ - $\blacklozenge$) during and after the fifth exposure day. Mean values of 4-6 subjects are presented (number of fewer subjects is given in parentheses); bars denote standard deviations. For comparison the corresponding postexposure values of m-xylene in blood ($\circ$ - $\circ$) and end-expired air ($\bullet$ - $\bullet$) for 4-8 subjects from exposure type I are shown.

length 1 m) into a three-necked 2-l flask situated outside of the chamber. One outlet of the flask was connected to a gas meter for the estimation of expired air volume. The sampling tube of a portable gas chromatograph (Analytical Instrument Development Inc., Avondale, U.S.A.) equipped with a flame-ionization detector was placed in the center of the flask, and a sample was drawn automatically (pumping time 3–4 s) every minute.

Analysis of samples

The analysis of *m*-xylene in *whole venous blood* was performed with gas chromatography as described previously (27). Differential analysis of the *m*-xylene concentration in *serum*, *protein-free serum*, and *red blood cells* was performed for a limited number of samples as follows. Twelve milliliters of venous blood was centrifuged in well-closed tubes immediately after sampling. About 2.5 ml of serum was pipetted into two tubes in an ice bath, and 1 ml of 30 % trichloroacetic acid was added to one of the tubes. The precipitate formed was centrifuged at 5,000 *g* for 10 min, and the supernatant and untreated serum (1 ml) were pipetted into bottles for headspace analysis. Standards were made in pooled human serum. The blood corpuscles sedimented in the original centrifugation were also pipetted (2 ml) into headspace bottles, and standards were made in a corresponding preparation of pooled human blood. Headspace assays were performed in duplicate in the same way as for whole blood.

The *m*-xylene in the *end-expired air samples* was analyzed with gas chromatography as described previously (27).

Urinary m-methylhippuric acid (more accurately, total conjugates of *m*-methylbenzoic acid) was estimated as described elsewhere (27). 2,4-Xylenol conjugates were assayed in the urine with gas chromatography after acid hydrolysis, a modification of the method of Bakke and Scheline (3). The hydrolysis was carried out in approximately 1.6 M sulfuric acid by refluxing 20 ml of urine and 3 ml of concentrated sulfuric acid in a boiling water bath for 1 h. An internal standard,

o-cresol, was added to the samples. The hydrolyzed urine samples were extracted three times with 25 ml of dichloromethane, and the combined extracts were shaken three times with 50 ml of 5 % sodium bi-

METHYLHIPPURIC ACID EXCRETION, mmol/h

Fig. 5. Rate of methylhippuric acid excretion in urine during and after repetitive inhalation exposures to *m*-xylene on the first (○-○) and the fifth (●-●) day. Mean values of 4–6 subjects are presented (number of fewer subjects in parentheses); bars denote standard deviations.

METHYLHIPPURIC ACID EXCRETION IN URINE, mmol/8h

Fig. 6. Methylhippuric acid excretion in urine in 8-h periods in the context of five successive daily inhalation exposures to *m*-xylene. Mean values of 4–6 subjects are given; bars denote standard deviations. For two subjects individual values are shown.

carbonate solution to remove acids. The dichloromethane solution was dried with sodium sulfate, evaporated carefully to dryness in a rotavapor, and then dissolved without delay in 1 ml of dichloromethane. The dichloromethane solutions were analyzed with gas chromatography.

The xyleneol was identified in a comparison with reference substances in two separate columns, i.e., in a packed XE60 (2 % on Chromosorb W AW, length 1 m, inner diameter 2 mm, temperature 90°C) and in an OV 225/151 glass capillary column. The occurrence of 2,4-xyleneol in the urine was also verified with gas chromatography/mass spectrometry.²

The procedure for capillary gas chromatography was as follows. A Perkin Elmer Sigma 3 equipped with a flame ionization detector was used. The column was a glass capillary, length 29 m, inner diameter 0.273 mm with liquid phase OV 225/151. The column temperature was programmed after a 1-min isothermal operation from 40 to 135 °C with a rate of 10 °C/min, then 15 min isothermal. The carrier gas was nitrogen with a flow of 1 ml/min. The injection volume was 1 µl.

The recovery of added 2,4-xyleneol (30 µg in 1 ml of urine) was about 99 %, and the coefficient of variation in 10 parallel assays was about 1 %.

Estimation of m-xylene uptake

A theoretical estimate was made of the total uptake of m-xylene in the lungs (uptake = percentage of retention × inhaled xylene concentration × pulmonary ventilation × time) over a typical exposure day. The current experiment required the co-operation of the subjects in a number of repeated tasks, and it was not feasible to measure pulmonary ventilation and xylene retention over the full length of a day or for several subjects at the same time. Therefore, it was assumed that for the whole group of volunteers the mean pulmonary ventilation was 9.0 l/min. This was the actual approximate value for a few subjects whose pulmonary ventilation was monitored on several occasions during

1 d. For interindividual comparison among the group members, pulmonary ventilation values under basal conditions taken from standard tables (11) are given in table 1.

The retention (R) of m-xylene in the lungs was calculated from the m-xylene concentration in exhaled air (C_E) and chamber air (C_I) as $R = (C_I - C_E)/C_I$ for a few sedentary subjects at different points in time during the day's exposure, and a correction was made for the dead space volume of the mask and the mouthpiece.

Estimation of m-xylene excretion via the lungs

The postexposure expiration of m-xylene after the first exposure day was calculated from the elimination curve of xylene based on end-expired air samples (fig. 3). The elimination half-times were obtained graphically from the curve over three consecutive time periods (0–3 h, 4–16 h and ≥ 17 h postexposure), and the corresponding elimination rate constants (k) were calculated ($k = \ln 2/T_{1/2}$). Because, after more than 5 h into the postexposure period, the concentration of xylene in the end-expired air samples was so low that it could not be accurately measured, a corresponding excretion after another type of exposure (exposure type I) served as the model for the elimination rate constant estimation (fig. 3). Excretion during the last time period starting 17 h after the exposure can best be visualized in the samples collected over the weekend in relation to exposure type I (fig. 4), and the corresponding elimination rate constant was obtained from these data. The total amount of xylene expired (Ω) in the different phases of elimination was calculated according to Sherwood (33) as follows:

$$\Omega = \frac{K_1 - K_2}{k_1} + \frac{K_2 - K_3}{k_2} + \frac{K_3}{k_3},$$

where K_1 is the initial elimination rate of xylene (µmol/h), K_2 is the rate at the end of the first phase (3 h) and at the beginning of the second, K_3 is the rate at the end of the second phase (16 h) and at the beginning of the third, and k_1 , k_2 and k_3 are elimination rate constants (h⁻¹) for the corresponding phases. The following rate

² Courtesy of J. Taskinen, Ph.D., Orion Pharmaceuticals Co., Espoo, Finland.

constants were observed and applied in the calculations: $k_1 = 0.90 \text{ h}^{-1}$, $k_2 = 0.09 \text{ h}^{-1}$, and $k_3 = 0.04 \text{ h}^{-1}$.

End-expired air contains, in the elimination phase, a higher concentration of xylene than tidal air due to the diluting effect of uncontaminated air in the pulmonary dead space. In our end-expired air samples the xylene concentration was 1.1–1.5 times higher than the mean xylene concentration in expired air (Riihimäki et al., unpublished data). The latter figure is in agreement with Sherwood's findings with benzene (33). In the present calculations we multiplied the xylene concentrations in end-expired air by 0.7 to obtain the mean tidal air xylene concentrations, and an average pulmonary ventilation of 10 l/min was assumed for all subjects over the whole postexposure period.

RESULTS

Uptake and distribution of m-xylene

Absorption of m-xylene in the lungs. After the first 5–10 min of exposure the retention of xylene in the lungs was relatively constant throughout the exposure day, approximating 60 % with a range of 54 to 68 % (table 2). The determinations were limited to two individuals only of this study, supplemented with corresponding values obtained during the rest periods of exposure type I. For each individual monitored, a slight increase in the morning level of retention was noted after lunch in the afternoon.

Xylene in venous blood. There was an initial rapid rise in the venous blood xylene concentration during the first hour of exposure and a tendency to level-off towards the end of the 3-h periods of the morning and the afternoon (fig. 3 and 4). The somewhat high xylene concentration in the first blood sample taken during exposure of the fifth day is an artifact due to an accidental peak in the air xylene level. In the afternoon of the first day, after an hour-long break, the blood xylene values were consistently somewhat higher

than the corresponding morning values. During the fifth day of exposure the xylene concentrations of the morning blood samples were about 20 % higher than corresponding concentrations of the first day, a phenomenon suggesting cumulation of the compound in the body. Another unequivocal indicator of cumulation over four exposure days is the significant blood xylene level in the pre-exposure morning sample of the fifth day (fig. 4). In the afternoon of this same day the environmental xylene concentration was doubled, and a similar doubling of the blood xylene concentrations, in comparison to the morning values, occurred. The course of events with respect to the venous blood xylene concentration clearly illustrates that equilibrium between the blood and most tissues was about to be reached within the 6-h of exposure, but the cumulation of xylene took place from day to day in some tissues.

Transport of m-xylene in blood. In a limited number of venous blood samples of exposure type II, the m-xylene content was analyzed separately in serum, protein-free serum, and cells. With a reservation for the fact that the headspace assay applied may be influenced by the presence of trichloroacetic acid, it seemed that approximately 90 % of the blood xylene occurred in the serum fraction, and little was left in serum (about 10–15 % of the original content) after protein precipitation with trichloroacetic acid (table 3). The relationship between xylene in serum and whole blood was fairly constant in all the samples taken at different times of the day, the lowest mean of 79 % occurring in the morning preexposure sample (only two specimens) and the highest of 93 % ap-

Table 2. Percentage of retention of m-xylene in the lungs at rest. Mean value of successive determinations at 1-min intervals over a time period of 10–30 min.

	Morning	Afternoon
This study	63 (N = 1)	56–68 (N = 2)
Exposure type I	54–58 (N = 2)	62–63 (N = 2)