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Styrene, its metabolism and the evaluation of hazards in industry

by ZDENĚK BARDODĚJ[1]

BARDODĚJ, Z. Styrene, its metabolism and the evaluation of hazards in industry. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 95—103. The main products of styrene biotransformation excreted in human urine are mandelic and phenylglyoxylic acids. Phenylethylene oxide seems to be the first metabolite; this compound has been reported to be mutagenic and carcinogenic. The polarographic determination of mandelic acid has been used for about 20 years in Czechoslovakia as an exposure test for persons occupationally exposed to styrene. Only single cases of health damage have been reported to be due to styrene exposure during this time. Recently, however, elevated frequencies of chromosomal aberrations in peripheral lymphocytes have been observed in persons exposed to styrene in Czechoslovakia. The amount of phenylethylene oxide that can be formed in the human body during exposure to 50 ppm of styrene in air during a workshift is about 0.7 g. It would be hardly justifiable now to believe that styrene is not carcinogenic and mutagenic in man. The present Czechoslovakian maximum allowable concentration of styrene (200 mg/m^3) should be reduced, and the concentration of 50 mg/m^3 may be recommended as reasonable at this time. The corresponding biological limit value of mandelic acid might be 300 mg/l of urine from the last 2 h of the workshift.

Key words: exposure test, mandelic acid, phenylglyoxylic acid, styrene, styrene oxide.

Styrene toxicity was investigated during World War II by Spencer and coworkers (79) and by Carpenter and coworkers (18), who also detected acidic metabolites of styrene in urine. Valuable data on the biotransformation of styrene in rats were published in 1954 by Danishefsky and Willhite (23), who tried to investigate the mechanism of tumor formation by the polymer. In 1957 El Masri and coworkers (25) described hippuric acid to be the main metabolite of styrene in the rabbit; some mandelic acid and phenylethylene glycol were also present.

In the 1960s styrene biotransformation studies in man were started in Czechoslovakia (2, 3, 6, 7, 8, 9) and also in other countries (1, 24, 26, 29, 30, 35, 37 38, 40, 61, 62, 63, 84). Mainly mandelic acid, followed by phenylglyoxylic acid, was demonstrated in the urine of persons exposed to styrene (3, 8). About 20 metabolites seem to be formed, and species-specific differences in the quantitative balance of styrene metabolism have been stressed (7).

METABOLIC FATE OF STYRENE

The probable metabolic pathways of styrene in mammals are shown in fig. 1. The very important metabolite formed from styrene by oxidation of the vinyl group appears to be phenylethylene oxide,

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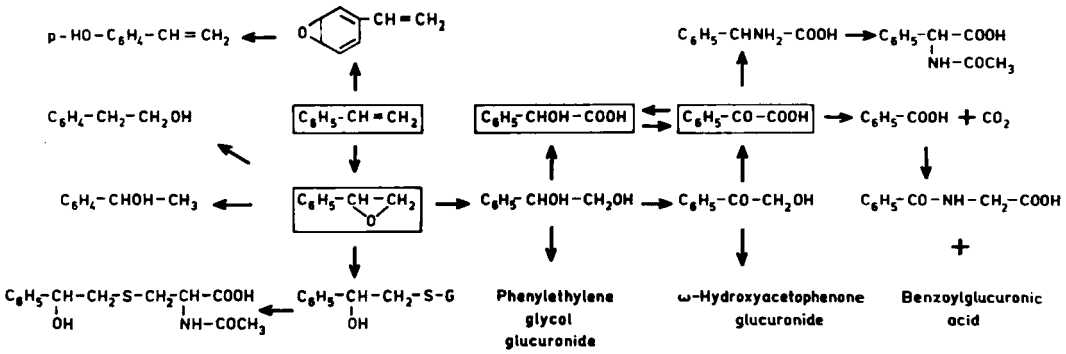

Fig. 1. Biotransformation of styrene.

Table 1. Half-time of mandelic acid excretion in urine in humans.

Author	Half-time (h)
Engström et al. (26)	6.4
	9.4
	16.6 [a]
Ikeda et al. (40)	7.8±1.9
Bardoděj (3)	7
	2 [b]

[a] At low concentration of mandelic acid.
[b] After administration of 2.05 g of mandelic acid per os.

Table 2. Toxicity of styrene and its metabolites (20, 49, 76, 77).

Styrene or metabolite	Toxicity [a]	Dose
Styrene		
oral-rat	LD_{50}	4,920 mg/kg
oral-rat	LD_{50}	5,840 mg/kg
Phenylethylene oxide		
oral-rat	LD_{50}	4,290 mg/kg
oral-rat	LD_{50}	2,830 ml/kg
intraperitoneal-rat	LD_{50}	460 mg/kg
inspiratory-rat	LC	500 ppm/4 h
1-Phenylethanol		
oral-rat	LD_{50}	400 mg/kg
2-Phenylethanol		
oral-rat	LD	1,790 mg/kg
Mandelic acid		
oral-rat	LD	3,000 mg/kg
NH_4 mandelate		
oral-rat	LD_{50}	5,000 mg/kg
Benzoic acid		
oral-rat	LD_{50}	1,700 mg/kg

[a] LD = lethal dose, LD_{50} = mean lethal dose, LC = lethal concentration.

which is an electrophilic alkylating agent. It was detected in in vitro experiments with rat and rabbit liver preparations by Leibman and Ortiz (46). Also the corresponding glycol and S-/2-hydroxy-2-phenylethyl/mercapturic acid were demonstrated (25, 42, 46). Mixed function oxidase, epoxide hydratase and glutathione transferase are involved in these transformations. Phenylethylene oxide has not been detected in urine by the method used (3).

In man most of the styrene absorbed is eliminated as mandelic and phenylglyoxylic acids, the ratio of the acids being 8.5—3 : 1. Mandelic acid may be formed by the oxidation of phenylethylene glycol or by the hydrogenation of phenylglyoxylic acid. The latter of these two is also a metabolite of mandelic acid and has been found in urine after administration of ω-hydroxyacetophenone (5), together with its glucuronide and mandelic acid (5, 25). The attempt to discover ω-hydroxyacetophenone after styrene inhalation in urine was not successful. The fate of mandelic acid in the body was studied by Schultzen and Gräbe in 1867, and many times since then (34, 44, 54, 55, 56, 57, 60, 65, 66, 71, 72, 88). The half-times of its excretion in humans after styrene inhalation are presented in table 1 (3, 26, 40). Phenylglyoxylic acid is eliminated more slowly.

An increase in hippuric acid excretion has not been demonstrated in styrene workers (9). Benzoic acid seems to be formed from phenylglyoxylic acid. According to Montenbruck (54) mandelic acid and hippuric acid are metabolites of phenylglyoxylic acid in man and dog.

Some of the metabolites in fig. 1 have not been detected in experiments with styrene directly, but their formation can be expected because they are metabolites of styrene metabolites, e.g., phenylglycine and N-acetylphenylglycine (54, 56, 57).

BIOLOGICAL EFFECTS

The acute toxicity of styrene and its metabolites (20, 49), including phenylethylene oxide (76, 77), seems to be low (table 2). Some of the nonlisted metabolites, e.g., phenylethylene glycol, phenylglyoxylic acid and phenylglycine, are also only slightly toxic, for they were used in high doses without adverse effect in animals. Some were even given to man (25, 54). Styrene is an irritant of the eyes, nose and throat and a depressant of the central nervous system (18, 21, 80). However there is no information showing chronic toxicity of styrene or its metabolites, with the exception of styrene and benzoic acid.

In long-term studies irritation of mucous membranes and neurological symptoms have been observed in animals (32, 33, 79, 89) and in exposed persons (9, 10, 16, 18, 21, 39, 44). No toxic damage to liver, kidney or bone marrow tissue has been reported by most authors (16, 21, 33, 34, 43, 48, 89). For decades, styrene was handled as a relatively safe substance. With the exception of Rogers and Hooper (67), who reported styrene sickness in cases of extremely high styrene concentrations in air, it was mainly Soviet authors who described liver damage, blood count changes and neurological symptomatology in persons exposed to low styrene concentrations (43, 64, 69, 81, 86, 92).

In the last few years the possible mutagenicity (19, 47, 50, 51, 53, 82), carcinogenicity (83, 87), and teratogenicity (36) of styrene and phenylethylene oxide have been investigated. Mutagenic activity of styrene was demonstrated in microorganisms after metabolic activation by liver microsomes (47, 50, 53, 55) in vitro and in host-mediated assay (19); chromosomal aberrations in peripheral lymphocytes have been observed by a Finnish group (51) and by Zudová and coworkers (unpublished results of investigations carried out in Czechoslovakia).

DETERMINATION OF STYRENE EXPOSURE

Styrene exposure measurements are based on workplace air analyses and/or on the analysis of styrene metabolites in urine. Maximum allowable concentrations for styrene in air have been established and accepted in most industrially developed countries (table 3); however, the differences among individual limit values fall in the range of two orders of magnitude.

Table 3. Maximum allowable concentration or threshold limit value of styrene in various countries (41).

Country	mg/m³		ppm	
Australia	420		100	
Belgium	420		100	
Bulgaria	5		1	
Czechoslovakia	200	1,000 [a]	47	235 [a]
Finland	420		100	
Federal Republic of Germany	420		100	94 [a]
German Democratic Republic	200	400 [a]	47	
Great Britain	420		100	
Hungary	50		12	
Italy	200	300 [b]	47	70 [b]
Japan	210		50	
Poland	50	100 [b]	12	24 [b]
Romania	200	250 [b]	47	60 [b]
Soviet Union	5		1	
Sweden	210		50	
Switzerland	420		100	
The Netherlands	420		100	
United States	420	525 [c]	100	125 [c]
Yugoslavia	42	420 [b]	10	100 [b]

[a] Ceiling value.
[b] Both values found in the literature.
[c] Short-term exposure limit.

Styrene in the atmosphere can be determined spectrophotometrically (8, 14, 68, 91), polarographically (73), and gas chromatographically (1, 26, 29, 70), and its concentration can also be recorded continuously (3). In Czechoslovakia, a polarographic method has already been standardized (73), and a gas chromatographic method is to be standardized in the near future.

Styrene analysis of alveolar air (1, 80, 90) has found only a very limited use because the amount of styrene eliminated from the body through the lungs is generally below 2 % of the amount retained (3, 28); consequently this method is far from being representative.

An exposure test based on the determination of mandelic acid has been used in Czechoslovakia since 1960 (8). Biological limit values for mandelic acid determination are given in table 4. In contrast to the maximum allowable concentrations of styrene in the air, the biological limits for mandelic acid proposed by various authors are similar. Methods used for determining mandelic acid in urine are given in table 5. In Czechoslovakia, the polarographic method (3) has become standard. It is simple and fast, and there are no background interferences in the urine of nonexposed persons. In 1 h three to six determinations can be made by a laboratory technician.

Phenylglyoxylic acid has been determined spectrophotometrically (3, 59), polarographically (5), and gas chromatographically (11, 15, 17, 31, 35, 76), the polarographic method being the fastest. But the mandelic acid exposure test is preferred to the determination of phenylglyoxylic acid in Czechoslovakia.

Hippuric acid determination cannot be used as an exposure test because of the high background interference in normal urine (9).

Table 4. Biological limits of mandelic and phenylglyoxylic acids in urine according to various authors.

Author	Styrene (ppm)	Biological limit [a]
Engström et al. (26)	100	2,300 mg MA/g cr.
Götell et al. (29)	50	1,000 mg MA/l
	100	2,000 mg MA/l
Härkönen et al. (35)	50	1,660 mg MA/l [b]
	100	3,000 mg MA/l [b]
Institute of Occupational Health Helsinki (1974)		1,500 mg MA/l
Philippe et al. (63)	100	350 mg PGA/g cr.
Ohtsuji and Ikeda (59)	10—30	875 mg MA/l
	10—30	381 mg PGA/l
Schaller and Valentin (71)		350 mg MA/l
Bardoděj (3, 5)	50	1,500 mg MA/l [c]
	50	1,000 mg MA/g cr.
	100	3,000 mg MA/l [c]

[a] MA = mandelic acid, PGA = phenylglyoxylic acid, cr. = creatinine.
[b] Specific gravity of urine = 1.018.
[c] Specific gravity of urine = 1.024.

OCCUPATIONAL HAZARDS

The extent of styrene air contamination depends on the amount of styrene used, the surface of the objects from which evaporation occurs, technology, ventilation, and also on the low vapor pressure. The irritating odor of styrene is a limiting property. The concentration of styrene in the general workplace atmosphere is usually relatively stable, but it fluctuates in the breathing zone of a worker according to the operations performed. Styrene concentrations in plants producing the same goods are probably similar in all countries, because technology, as well as work conditions, is generally similar. My coworkers and I have measured occupational exposure to styrene in Czechoslovakian industries. The highest exposure of workers was observed in the production of glass laminates, where styrene was used as a solvent and a cross-linking agent for polyester resins. The styrene concentrations were generally at 50 ppm, with peaks of up to 120 ppm; the concentrations of mandelic acid in urine were 300—7,000 mg/l, most often between 500 and 2,000 mg/l. Occupational exposures in furniture producing plants, where polyester resins in a styrene solution are used for varnishing, were a little lower than in the first case — styrene 25—75

Table 5. Methods for determining mandelic acid (MA) in biological material.

Method		Reference
IODOMETRIC TITRATION	of benzadehyde after oxidation of MA and distillation with water vapor	Bardoděj and Bardodějová (4) and Bister and Wolff (12)
SPECTROPHOTOMETRY	of benzaldehyde after oxidation of MA and distillation with water vapor	Bardoděj and Bardodějová (4)
	of MA after extraction into ether and reaction with ferric chloride	Bardoděj (3) and Bardoděj and Bardodějová (5)
	of MA after extraction into ether and reaction with formaldehyde in sulfuric acid	Ohtsuji and Ikeda (59)
GAS CHROMATOGRAPHY	of benzaldehyde after oxidation of MA and distillation with water vapor	Nicholson (58) and Šedivec and Flek (74)
	of MA after extraction and methylation	Buchet at al. (15), Caperes and Fernandez (17), Dagliesh et al. (22) and James and White (42)
	of MA after extraction and silylation	Engström and Rantanen (27), Guillemin and Bauer (31), Meszka and Jakubowski (52), Schaller et al. (70), Slob (75), Vivoli and Vecchi (84) and Völmin et al. (85)
ISOTACHOFORESIS	of MA after extraction	Sollenberg and Baldestein (78)
POLAROGRAPHY	of benzaldehyde after oxidation of MA and distillation with water vapor	Bardoděj (3), Bardoděj and Bardodějová (4) and Bister and Wolff (13)

ppm; mandelic acid 250—3,500 mg/l, mostly below 1,000 mg/l. Similar values of mandelic acid were also found in the production of artificial mother of pearl. Lower concentrations were observed in the production of styrene-butadiene rubber (with mandelic acid in the urine mostly below 400 mg/l), in the production of compact and porous polystyrene (where mandelic acid concentrations only seldom reached levels of 250 mg/l), and in the thermal destruction of styrene-butadiene rubber (mandelic acid below 50 mg/l urine). In 1959—1962 the average mandelic acid concentrations were slightly above 1,000 mg/l; in Prague styrene levels reached their maximum between 1962 and 1966. Now the average concentrations are below this level, although most urine samples analyzed in our laboratory come from persons who do glass laminating manually.

Over the 20 years of its industrial use styrene has been reported to produce only single cases (2) of occupational disease in Czechoslovakia.

MAXIMUM ALLOWABLE CONCENTRATIONS AND BIOLOGICAL LIMIT VALUES

In recent years the mutagenic activity of styrene and phenylethylene oxide has been described not only in animals, but also in man, and it has been shown that the latter compound is carcinogenic in mouse skin (83).

On one hand, depression of bone marrow function, which is often seen after the administration of cytostatics with mutagenic and carcinogenic side-effects, has not been observed even after very high exposures to styrene; on the other, there are indisputable signs of mutagenic activity which cannot be neglected.

As has already been mentioned, the maximum allowable concentration for styrene in Czechoslovakia is 200 mg/m^3 (21). The volume of inhaled air during a workshift is about 5 m^3. Sixty percent of inhaled styrene vapors have been reported to be retained in the human

respiratory system (2, 3, 28). Under these assumptions the retained amount of styrene is approximately 600 mg. If it is assumed that almost all of the vapors are oxidized to phenylethylene oxide, the dose of phenylethylene oxide becomes 700 mg during a workshift.

I think that the present Czechoslovakian maximum allowable concentration for styrene should be reduced to 50 mg/m^3 (= 12 ppm) — at least temporarily until the mutagenicity, carcinogenicity and teratogenicity of styrene have been further clarified. The corresponding biological limit value of mandelic acid might be 300 mg/l or 2 mmol/l of urine sampled at the end of the workshift.

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QUESTIONS AND ANSWERS

Questions to to Dr. BARDODĚJ

Prof. IKEDA: What were the main symptoms in the cases identified as styrene poisoning in your report?

Dr. BARDODĚJ: In the few cases of occupational styrene poisoning mainly neurological symptoms were observed that were similar to those observed in poisoning with other solvents (CNS symptomatology).

Dr. GUILLEMIN: To what extent can mandelic acid interfere with the polarographic analysis of phenylglyoxylic acid and to what extent can phenylglyoxylic acid interfere with the analysis of mandelic acid?

Dr. BARDODĚJ: 1. There is no interference in phenylglyoxylic determination caused by mandelic acid once human urine has been diluted with a sodium tetraborate solution because this acid is not polarographically active itself.
2. The determination of mandelic acid is based on the oxidation to benzaldehyde by periodic acid; benzaldehyde is isolated by steam distillation. Phenylglyoxylic acid is not oxidized to benzalhyde and cannot interfere. Phenylethylene glycol could interfere in this procedure, but it is not present in measurable concentrations in urine of persons exposed to styrene.

Prof. LOPRIENO: You have mentioned a chromosome aberration analysis done on exposed workers. Could you, please, tell more about it?

Dr. BARDODĚJ: Chromosomal aberration study on lymphocytes — a cytogenetic analysis according to Hungerford — was performed by Z. Zudová and coworkers on 10 women exposed to styrene in one plant. The frequency of aberrations averaged 8 % (4—10.5 %) in this group. Gaps and diploid cells were not included. If the percentage was 5 or higher, 200 mitoses were evaluated. Mandelic acid in the urine was 1,100 mg/l in the sample from the end of the workshift.

No elevated frequency (> 3 %) was found for 200 mg of mandelic acid per liter of urine. The frequency of aberrations in a control group was 2.4 %.