

Styrene, its experimental and clinical toxicology

A review

by HANNU HÄRKÖNEN M.D.¹

HÄRKÖNEN, H. Styrene, its experimental and clinical toxicology: A review. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 104—113.

Key words: acute effects, chronic effects, metabolism, neurophysiological findings, psychological functions, styrene, symptoms.

Styrene is a clear, colorless liquid with a characteristic pungent odor. The odor threshold of styrene vapors is about 0.05 ppm (69). In an aqueous solution the mean of the odor threshold concentration is 0.73 ppm (range 0.02—2.6 ppm) (4). Styrene has the following physical properties (27):

Molecular weight	104.14
Specific gravity	0.90 g/cm ³
Melting point	—30.6°C
Boiling point	145.2°C
Vapor pressure (25°C)	858.93 Pa (6.45 mm Hg)
Vapor density (air = 1)	3.6
Conversion factors:	1 ppm = 4.260 mg/m ³ 1 mg/m ³ = 0.236 ppm
Solubility	little in water, miscible with ethanol and ether

Styrene monomer was discovered in 1831, but technical production did not begin until 1930 in Germany (27). During the Second World War the styrene industry developed rapidly, because styrene

monomer was in great demand for the production of synthetic rubber.

Styrene is produced commercially by the dehydrogenation of ethylbenzene. Because of its vinyl group, styrene is very reactive, and it polymerizes easily at room temperature. Before styrene can be stored, inhibitors are added to prevent polymerization.

Styrene is used in the production of plastics and resins [polystyrene resins, acrylonitrile-butadiene-styrene polymer (ABS), styrene-acrylonitrile copolymer (SAN) resins, styrene-butadiene copolymer resins, styrene butadiene rubber (SBR)]. It is also used as a cross-linking agent and a solvent for unsaturated polyester resins (72).

Especially workers manufacturing reinforced plastic products are exposed to styrene as it evaporates during the laminating process. The exposure level greatly depends on the work conditions. For example in four Swedish factories the time-weighted average of styrene in the ambient air ranged between 3 and 171 ppm during different work phases, i.e., 10—139 ppm during boat hull laminating, 18—171 ppm during boat deck laminating, 68—144 ppm during spraying and 3—91 ppm dur-

¹ Department of Occupational Medicine, Institute of Occupational Health, Helsinki, Finland

ing the assembling of small parts (8). The median value of the arithmetic means of styrene concentrations measured in the breathing zones of 68 Finnish laminating workers (8 1-h measurements/worker) was 66 ppm, range 20–299 ppm. The exposure level was dependent on the volume of the workroom. For example, the median styrene concentration of the large workrooms (over 5,000 m³, N = 6) was 53 ppm, while in the middle-sized (1,000–4,999 m³, N = 11) and small (below 1,000 m³, N = 5) sites it was 87 and 117 ppm, respectively (40). When laminates were manufactured in the laboratory from different commercial polyester resins, 6.3–12.8 % (mean 9.2 ± 2.2 %) of the styrene used evaporated into the air (40).

METABOLISM AND TOXICOLOGY

Absorption

Under work conditions styrene enters the body primarily through absorption in the lungs. Earlier studies indicated that about 59–66 % of inhaled styrene is absorbed (1, 7, 25, 56). However, in 1977 Fernandez and Caperos (24) exposed volunteers to 70–206 ppm of styrene in an exposure chamber and found that 88.7 ± 3.4 % of the inspired styrene was absorbed. The same proportion was absorbed throughout an 8-h exposure period.

Bodily absorption depends on alveolar ventilation. As alveolar ventilation increases, the amount of styrene absorbed increases. Åstrand and her coworkers (2) demonstrated this phenomenon when they exposed subjects to 50 ppm of styrene, both at rest and during 50-W exercise. They measured the following styrene concentrations at rest: 8.8 ± 0.7 ppm in alveolar air, 0.5 ± 0.0 ppm in arterial blood, and 0.3 ± 0.0 ppm in venous blood. During 50-W exercise the concentrations rose to 9.5 ± 1.3 ppm in alveolar air, 1.9 ± 0.1 ppm in arterial blood and 1.4 ± 0.1 ppm in venous blood. When the subjects were exposed to 50 and 150 ppm of styrene while at rest and during 50-, 100- and 150-W

exercise, the difference between the arterial and venous blood concentrations, which describes the amount of styrene absorbed, increased slightly as the alveolar ventilation increased. Also the concentration in arterial blood increased. However, no linear relationship between arterial and venous blood concentrations was observed. At the end of exposure during 50-W exercise the styrene concentration was 20–30 ppm in alveolar air and 5–7 ppm in arterial blood. One hour after exposure the styrene concentration in alveolar air had decreased to 1–5 ppm (2). In comparison with other industrial solvents styrene absorbs into the body easily, and the tissues become saturated slowly (1).

In liquid form styrene can be absorbed through the skin at a rate of about 9–15 mg per square meter per hour (21). The amount of styrene vapor absorbed through the skin is about 2 % of the amount absorbed via the lungs, and thus skin absorption of styrene vapors is of minor importance in the work environment (60).

Distribution

When rats were given β -¹⁴C-labeled styrene subcutaneously, 1 h after the injection the highest concentration was found in the liver (4.62 % of the dose) and kidney (1.82 % of the dose). Blood (1.02 %), adrenal tissue (0.91 %), and brain (0.01 %) showed the next highest concentrations (17). [See also the article of Withey (77) in this proceedings]. When rats were exposed by inhalation to 2,800 ppm of styrene for 4 h, the highest styrene concen-

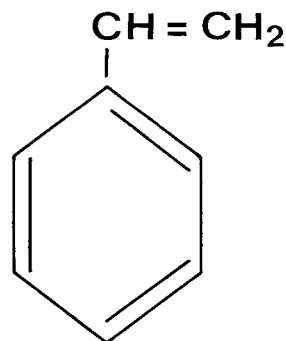


Fig. 1. Styrene (vinylbenzene, phenylethylene).

tration after the exposure was found in perirenal fat, 133 mg⁰%. In brain tissue the concentration was 25 mg⁰%, and in liver 20 mg⁰%. One hour after exposure the styrene concentration in the brain tissue was 8.6 mg/100 cm³ (68).

The styrene concentration in the adipose tissue of exposed workers was found to be 0.2–1.0 ppm for more than 48 h after exposure. The styrene concentration was roughly related to the exposure level and to the length of time since the cessation of exposure (79).

The duration of styrene exposure has been found to influence the concentration in brain tissue. When rats were exposed to 300 ppm of styrene for 11 weeks, the highest concentration (47 nmol/g) in the brain was observed at four weeks, but when the exposure continued, the concentration decreased, being only 10 nmol/g 10 weeks after the beginning of the exposure (65).

Metabolism

Leibman (42) has reviewed the metabolism of styrene in detail. Styrene is metabolized mainly in the liver. First it is oxidized to styrene oxide, an epoxide, by the microsomal monooxygenase system (43). Styrene oxide is biologically reactive and can bind covalently to the cellular macromolecules (48). It is hydrated to phenylethylglycol by microsomal epoxide hydratase (53) and can be conjugated with glutathione; the reaction is catalyzed by glutathione S-transferase (10). The glutathione conjugate is excreted mainly via the bile (63).

Styrene is metabolized also in extrahepatic tissues, e.g., the lungs and kidneys (38, 63). Phenylethylglycol can be conjugated with glucuronic acid to form water soluble glucuronide, which is mainly excreted in the urine (22). Phenylethylglycol is oxidized to mandelic acid and further to phenylglyoxylic acid, the two main urinary metabolites of styrene in man (7). Phenylethylglycol and mandelic acids can be further oxidized to benzoic acid, which, after glycine conjugation, is excreted in the urine as hippuric acid. A small fraction of styrene is metabolized to vinyl phenol and phenyl ethanol (5). Recently the existence of other metabolites has been proposed. The articles by Watabe et al. in

this proceedings (75) and Pantarotto et al. (57) offer more information in this respect.

Other foreign compounds can affect the metabolism of styrene. Phenobarbitone enhances the biotransformation of styrene (55), whereas the coadministration of toluene suppresses its metabolism (37). In addition, styrene metabolism can be induced by styrene exposure. When animals were given a styrene dose of 500 mg per kilogram per day intraperitoneally for 3–6 d, the activity of the epoxide hydratase increased (59). And when the animals were exposed to 450 ppm of styrene for 7 d, the activities of the enzymes catalyzing the metabolism of styrene and other xenobiotics were increased (64).

Excretion

When rats were given β -¹⁴C-labeled styrene intraperitoneally, 3 % of the activity was excreted in feces during the first 24 h, 12 % was exhaled as carbon dioxide, and 71 % was excreted in the urine (17). In rabbits about 40 % of the styrene absorbed is excreted in the urine as hippuric acid, 6 % as glucuronide-conjugate (22), about 30 % as mandelic acid, and 5 % as hydroxymercapturic acid (39).

In man 2.6 % of the styrene absorbed is excreted in exhaled air (24). About 85 % is excreted as mandelic acid, and 10 % as phenylglyoxylic acid in urine (7). According to the results of Åstrand (2) only 50 % is excreted in the urine as mandelic acid. The half-time of the mandelic acid excreted in the urine is 7.8 ± 1.9 h (36). The half-times of mandelic and phenylglyoxylic acids do not differ statistically significantly (36). However, the excretion seems to be biphasic. The half-time of the first phase is inversely related to the level of exposure (23). When the median exposure level was 248 ppm, the half-time of the first phase was 6.4 h, and, in a group with a median exposure level of 23 ppm, the half-time was 9.4 h. The half-time of the urinary mandelic acid concentration in the second phase was about 16 h (23).

The ratio of the urinary concentrations of phenylglyoxylic acid to mandelic acid varies as a function of the inhaled styrene concentration. As the exposure level increases, the relative amount of mandelic acid increases (54). It has been reported

that also in man some of the styrene absorbed could be metabolized to hippuric acid (36). However, human capacity is considered low in this respect (7, 54, 71). The metabolites of styrene, mandelic and phenylglyoxylic acids, have been used as biological exposure tests. For details, see the article of Bardodej in this proceedings (6).

Acute toxicity

Exposure to 1,300–10,000 ppm of styrene caused eye and nose irritation, lachrymation and salivation in animals (70). During an exposure level of 5,000 ppm animals lost their equilibrium and often showed tremors and clonic convulsion. Marked pulmonary lesions were produced at concentrations of 1,300–10,000 ppm, when the exposure was so long that some of the exposed animals died as a result. Pulmonary changes were characterized by congestion, hemorrhage, edema, exudation, and leucocytic infiltration (70).

In addition liver and kidneys were affected. However, only some animals had changes in these organs, and the changes found were not severe or persistent (70). A styrene dose of 3 g/kg, injected intraperitoneally to hamsters, caused periportal necrosis in liver tissue (58). Styrene also lowered the glutathione concentration of liver tissue and increased the level of alanine aminotransferase in serum. The gamma glutamyltransferase level was not altered (58).

Acute toxicity of styrene, expressed as the LD_{50} ² value for rats, is about 5.0 g/kg for oral administration (78) and 2.4 g/kg intraperitoneally (55). The corresponding intraperitoneal value for styrene oxide is 0.55 g/kg (55). The LD_{50} value for intravenous administration to mice is 139 mg/l for styrene, 415 mg/l for mandelic acid, and 465 mg/kg for phenylglyoxylic acid (80). The lethal concentration (LC_{50}) for 2-h exposure is 5,000 ppm for mice, while the corresponding value for 4-h exposure of rats is 2,800 ppm (68).

Long-term effects

Spencer et al. (70) exposed animals to 1,300 ppm of styrene 7–8 h, 5 d a week,

over a period of at least 6 months. A microscopic examination of various tissues revealed no essential changes. When results of repeated blood examinations of the exposed and control groups were compared, neither changes in the total erythrocyte count, total leucocyte count, nor in the differential count were found. Later Wolf and coworkers (78) exposed rats through the daily administration of peroral doses of 400 mg/kg for 185 days (oral doses 132) and found a slight increase in the weight of liver and kidney. A dose of 133 mg/kg did not, however, cause such changes. One year of styrene vapor inhalation at a level of 1,300 ppm did not cause changes in rhesus monkeys or rabbits (78).

Mutagenic and other toxic effects

The metabolite of styrene, styrene oxide, has been found to be mutagenic in microbiological mutagenicity tests (46, 52, 74). In addition styrene itself is mutagenic to *Salmonella* cultures in the presence of liver homogenate (S-9 mix) (74), and this phenomenon suggests that styrene must be metabolically activated before it is mutagenic.

When animals were exposed to 30 ppm of styrene vapor for 2–11 weeks, the chromosomal aberrations were increased statistically significantly after 9 weeks of exposure. Thereafter the aberrations did not increase. The amount of aberrant cells was 8–12 % in the exposed group and 1–6 % in the control group (50). Styrene also has toxic effects on chicken embryos; styrene (0.5–50 μ mol) injected into the air space of fertilized chicken eggs caused malformations in up to 20 % of the treated embryos (73).

CLINICAL FINDINGS

Symptoms

Carpenter and his colleagues (11) exposed humans in an exposure chamber to 800 ppm of styrene vapor and found that styrene immediately caused eye and nose

² LD_{50} = mean lethal dose.

irritation, a persistent metallic taste, drowsiness and impairment of equilibrium; in addition after the termination of exposure slight muscular weakness and depression occurred. Stewart et al. (71) exposed human volunteers and found that 7 h of exposure to 99 ppm or more caused slight eye and throat irritation. After 45 min at a level of 375 ppm one of the five subjects became nauseated, two complained of being slightly inebriated, and two developed a headache.

Wilson found (76) styrene exposed workers to have eye, throat and respiratory symptoms and a feeling of lassitude and fatigue. Styrene exposed workers themselves described "styrene sickness," with such symptoms as nausea, vomiting, loss of appetite, and general weakness. The duration of symptoms was only a few hours (61). Other reports have also described skin, eye and nose irritation, tiredness, headache, and disturbances of sleep, as well as changes in weight (32, 41, 56).

In a symptom survey of 98 male laminating workers occupationally exposed to styrene, the workers statistically significantly expressed symptoms of irritation, fatigue, and difficulties in concentration more often than a nonexposed group. Also during the workday irritation and "pre-narcotic" symptoms, e.g., nausea, dizziness and a drunken feeling, were reported significantly more often. However, none of the symptoms correlated statistically with the level of exposure, as indicated by urinary mandelic acid concentration, or with abnormal findings in the psychological or electroencephalographic examination (34).

Liquid styrene degreases the skin and can cause toxic eczema when contact is frequent (9, 76). The allergic effect of styrene is slight, however (26), and positive reactions to styrene are seldom found in epicutaneous tests (9, 49). In a cross-sectional study, which consisted of 70 styrene-exposed workers, no eczema was observed that could be attributed to contact with polyester or styrene (30).

If styrene is accidentally splashed into the eyes, moderate hyperemia of the conjunctiva and slight injury of corneal epithelium can occur. Such phenomena were found to return to normal within 48 h (31). The ophthalmological examination

of 300 styrene-exposed workers did not reveal any sign of optic neuritis of fundal abnormality (47).

In a Polish study styrene-exposed workers had symptoms of the respiratory tract, suggesting that long-term exposure to styrene could cause obstructive changes (15). In another study with a group of 472 styrene-exposed workers, the prevalence of chronic bronchitis was 19%. In a group of styrene-exposed nonsmokers the same prevalence was 6%. The authors reported that they had seen such findings in other industrial populations, and they considered the etiological importance of styrene exposure as questionable (47).

Effects on blood, liver function and glucose metabolism

In the previously mentioned Polish study 21 workers had been exposed for more than 10 years and 101 workers had been employed in styrene exposure for about 1 year. The authors found no statistically significant difference between the groups with respect to red or white cell count or differential count. The blood platelet count was statistically significantly lower, however, in the group with the longer exposure when it was compared with a non-exposed reference group. The reference group was not described in detail (16). In contrast, Lorimer et al. (47) found no correlation between the duration of exposure of workers to styrene and their blood hemoglobin, white cell count, or platelet count.

In the Polish study liver function tests [aspartate aminotransferase (S-ASAT), alanine aminotransferase (S-ALAT), lactate dehydrogenase (S-LD), cholinesterase, and bilirubin] did not suggest liver lesion (14). Also in the material of Lorimer et al. (47), the abnormal values of bilirubin, alkaline phosphatase (S-AFOS), alanine aminotransferase, and aspartate aminotransferase scattered randomly. Abnormal gamma glutamyl transferase (S- γ GT) values were found in 4.9 % of all the exposed workers, the corresponding value being 6.7 % for the exposed group with the high level of exposure and 2.8 % for the one with the low exposure level. The difference was statistically significant, and it was increased when those with an alcohol con-

sumption of more than about 60 g of pure ethanol per week were excluded.

In another Polish study Chmielewski et al. (13) found that the group with exposure of more than 10 years had higher alpha and beta lipoprotein levels, as well as higher cholesterol concentrations in serum. The less exposed group (approximately 1 year) had an increased glucose tolerance (13). The possible effect of age was not accounted for in these studies.

The effect of styrene exposure on chromosomes of cultured blood lymphocytes from occupationally exposed workers have also been studied. The exposed workers had more (11–26 %) chromosomal aberrations than a reference group (3 %) (51); this finding was in accordance with the results of a study of persons exposed to vinyl chloride (20).

Effects on the central and peripheral nervous systems

In an experimental study in which six volunteers were exposed to 99 ppm of styrene for 7 h, three of the six had difficulties on the Romberg test (71). The results of the Crawford manual dexterity test and Flanagan's coordination test did not differ from preexposure values. However, when the level of exposure was increased to 375 ppm for 50 min, two of five volunteers had 20 and 35 % lower results, respectively, on a test, and three of the five had coordination test results that were 10 % lower than preexposure results (71). Gamberale and Hultengren (28) found a statistically significant deterioration in reaction speed after 30 min of exposure at a level of 350 ppm; they suggested that perceptual speed was also affected by the exposure. However manual dexterity was normal. Oltramare et al. (56) found a slowing of reaction time already at an exposure level of 50 ppm, and the slowing was even clearer at 100 and 200 ppm.

When occupationally exposed workers were studied, Götell et al. (32) found that those exposed to 150 ppm of styrene had slower reaction times than an age-matched comparison group. Gamberale et al. (29) found that styrene-exposed workers had longer reaction times than the workers in mechanical industries who served as the control group. In fact the workers exposed

to styrene had a longer reaction time, a greater deterioration in reaction time, and a greater irregularity in performance on the test than nonexposed workers.

Lindström et al. (45) examined 98 laminating workers occupationally exposed to styrene with a psychological test battery. They used the duration of exposure in years as the measure of exposure and the urinary mandelic acid concentration as the measure of the current intensity of exposure. Only slight differences between the exposed and reference groups were found. The two groups differed only in visuomotor accuracy. In a multiple regression analysis, disturbances in visuomotor accuracy, psychomotor performance, and lowered vigilance proved to have some connection to a high mandelic acid concentration.

In a styrene-exposed group, a statistically significant difference was found in visuomotor accuracy when the urinary mandelic acid concentration exceeded 1,200 mg/l, and above this exposure level an almost statistically significant decline occurred in psychomotor performance (35). For a better understanding of the exposure-response relationship, the psychological function was classified as abnormal when the disturbance score was greater than the mean of the reference group + 1 SD. The percentage of workers with abnormally classified visuomotor accuracy (Symmetry Drawing) was about 17 when mandelic acid concentration was below 1,600 mg/l; above this concentration the corresponding percentage was 60. Visuomotor accuracy, as measured by the Bourdon-Wiersma test, was classified as abnormal in about 12 % of the workers with a mandelic acid concentration below 1,999 mg/l and in 50 % of those with a higher mandelic acid concentration. Psychomotor function (Mira Test) was found to be abnormal in 19 % of the workers with a mandelic acid concentration below 1,600 mg/l and in 50 % of those with a higher mandelic acid level (35). A mandelic acid concentration of 1,600 mg/l corresponds to an 8-h time-weighted average of about 75 ppm (35).

The connection between occupational styrene exposure and electroencephalographic (EEG) abnormalities has also been studied. Klimková-Deutschová (41) found

that 7 of 17 exposed persons had pathological EEGs and only 7 had normal EEGs. Later 14 styrene-exposed workers were studied, and 9 had an abnormal EEG finding (62). In a Polish study 57—68 % of the styrene-exposed group had a pathological finding in the EEG examination (18, 19). Seppäläinen et al. (67) studied 98 male laminating workers neurophysiologically. The urinary mandelic acid concentration (mean of five determinations) was used as the measure of exposure intensity. Mandelic acid concentration varied from 7 to 4,715 mg/l, median 808 mg/l. The overall prevalence of abnormal EEGs was 24 % (23 out of 96).

Abnormal EEGs were found in one-third of the subjects with a urinary mandelic acid concentration of more than 700 mg/l, while a low level of styrene exposure (urinary mandelic acid below 700 mg/l) did not increase the prevalence of abnormal EEGs above that found in a normal population (67).

Lilis et al. (44) found the radial nerve conduction velocity of 88 styrene-exposed workers to be less than 55 m/s in 20 %, and peroneal nerve conduction velocity was lower than 40 m/s in 21 % of 91 examinees. No major difference was found between the groups with "high" and "low" exposure. However, in a Finnish study (66) nerve conduction velocity measurements performed on 40 subjects did not reveal any definite relationship between styrene exposure and neuropathy.

Clinical picture of styrene effects

The clinical picture of styrene poisoning has been dealt with in the literature. Slight neurological disturbances connected with vegetative symptoms have been described (19, 33, 47). In a case report a relationship between styrene exposure and depressive psychic status and diffuse brain damage in primary psychasthenic and neurotic workers was suggested (3). A Swiss study concluded that neurological disturbances in styrene-exposed workers are rare and that the chronic toxicity is slight at concentrations of 100—400 ppm (12).

Symptoms of styrene exposure have been listed as skin, eye and nose irritation, tiredness, loss of appetite, difficulties in

concentration and, in higher exposure, dizziness. Reliable reports on the toxicity to the liver have not yet been published.

So far, direct evidence of carcinogenic effects caused by styrene is lacking, but, if such effects become established, the setting of standards for safe exposure must be regarded from a completely new angle.

REFERENCES

1. ÅSTRAND, I. *Upptag av lösningsmedel i blod och vävnader hos människa* (Arbete och hälsa no. 6). Arbetarskyddsverket, Stockholm 1975, pp. 5—30.
2. ÅSTRAND, I., KILBOM, Å., ÖVRUM, P., WAHLBERG, I. and VESTERBERG, O. Exposure to styrene: I. Concentration in alveolar air and blood at rest and during exercise and metabolism. *Work environ. health* 11 (1974) 69—85.
3. AXELSON, O., FRÖBÄRJ, G. and WEDEFELT, U. Kan styrenexposition orsaka cerebralläsionella tillstånd? *Läkartidningen* 71 (1974) 137—138.
4. BAKER, R. A. Threshold odors of organic chemicals. *J. am. water works assoc.* 55 (1963) 913—916.
5. BAKKE, O. M. and SCHELIN, R. R. Hydroxylation of aromatic hydrocarbons in the rat. *Toxicol. appl. pharmacol.* 16 (1970) 691—700.
6. BARDODĚJ, Z. Styrene, its metabolism and the evaluation of hazards in industry. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 95—103.
7. BARDODĚJ, Z. and BARDODĚJOVÁ, E. The metabolism of ethylbenzene, styrene and alpha-methylstyrene. *15th international congress on occupational health, Vienna, 19—24 Sept. 1966* (vol. II—1). Wiener Medizinischen Akademie, Wien 1966, pp. 457—460.
8. BERGMAN, K. Styrenexposition i plastbåtsindustri: I. Teknisk-hygienisk studie (Arbete och hälsa no. 3) Arbetarskyddsverket, Stockholm 1977, pp. 1—9.
9. BOURNE, L. B. and MILNER, F. J. M. Polyester resin hazards. *Br. j. ind. med.* 20 (1963) 100—109.
10. BOYLAND, E. and WILLIAMS, K. An enzyme catalysing the conjugation of epoxides with glutathione. *Biochem. j.* 94 (1965) 190—197.
11. CARPENTER, C. P., SHAFFER, C. B., WEIL, C. S. and SMYTH, H. F. Studies of the inhalation of 1:3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. *J. ind. hyg.* 26 (1944) 69—78.

12. CARRARD, D. *Etude sur le styrene: Toxicité, métabolites urinaires selon une enquête effectuée dans quatre entreprises.* Université de Lausanne — Faculté de médecine. Institut universitaire de médecine sociale et préventive 1975. 47 p.
13. CHMIELEWSKI, J. Clinical and experimental research into the pathogenesis of toxic effect of styrene: V. Impact of styrene on carbohydrate balance in people in the course of their work. *Bull. inst. mar. trop. med. gdynia* 27 (1976) 177—184.
14. CHMIELEWSKI, J. and HAC, E. Clinical and experimental research into the pathogenesis of toxic effects of styrene: IV. Estimation of liver functions in persons exposed to the action of styrene during their work. *Bull. inst. mar. trop. med. gdynia* 27 (1976) 69—74.
15. CHMIELEWSKI, J. and RENKE, W. Clinical and experimental studies on the pathogenesis of toxic effects of styrene: II. The effect of styrene on the respiratory system. *Bull. inst. mar. trop. med. gdynia* 26 (1975) 299—302.
16. CHMIELEWSKI, J. and RENKE, W. Clinical and experimental research into the pathogenesis of toxic effect of styrene: III. Morphology, coagulation and fibrinolysis systems of the blood in persons exposed to the action of styrene during their work. *Bull. inst. mar. trop. med. gdynia* 27 (1976) 63—68.
17. DANISHEFSKY, I. and WILLHITE, M. The metabolism of styrene in the rat. *J. biol. chem.* 211 (1954) 549—553.
18. DOLMIERSKI, R., KWIATKOWSKI, S. R. and NITKA, J. A preliminary evaluation of the studies on central nervous system in workers exposed to styrene. *Bull. inst. mar. med. gdansk* 25 (1974) 399—406.
19. DOLMIERSKI, R., KWIATKOWSKI, S. R. and NITKA, J. Clinical and experimental research into the pathogenesis of toxic effects of styrene: VII. Appraisal of the nervous system in the workers exposed to styrene. *Bull. inst. mar. trop. med. gdynia* 27 (1976) 193—196.
20. DUCATMAN, A., HIRSCHHORN, K. and SELIKOFF, I. J. Vinyl chloride exposure and human chromosome aberrations. *Mutat. res.* 31 (1975) 163—168.
21. DUTKIEWICZ, T. and TYRAS, H. A study of the skin absorption of ethylbenzene in man. *Br. j. ind. med.* 24 (1967) 330—332.
22. EL MASRI, A. M., SMITH, J. N. and WILLIAMS, R. T. Studies in detoxication. *Biochem. j.* 68 (1958) 199—204.
23. ENGSTRÖM, K., HÄRKÖNEN, H., KALLIOKOSKI, P. and RANTANEN, J. Urinary mandelic acid concentration after occupational exposure to styrene and its use as a biological exposure test. *Scand. j. work environ. & health* 2 (1976) 21—26.
24. FERNANDEZ, J. G. and CAPEROS, J. R. Exposition au styrène. I. Etude expérimentale de l'absorption et l'excrétion pulmonaires sur des sujets humains. *Int. arch. occup. environ. health* 40 (1977) 1—12.
25. FISEROVA-BERGEROVA, V. and TEISINGER, J. Pulmonary styrene vapor retention. *Ind. med. surg.* 34 (1965) 620—622.
26. FISHER, A. *Contact dermatitis* (2nd ed). Lea & Febiger, Philadelphia, Pa. 1973, p. 182.
27. FOERST, W. Styrol. In: *Ullmans Encyklopädie der technischen Chemie* (16 Band). Urban et Schwarzenberg, München 1965, pp. 460—476.
28. GAMBERALE, F. and HULTEGREN, M. *Exposition för styren.* Psykofysiologiska funktioner (Arbete och hälsa no. 3). Arbetskyddsverket, Stockholm 1973, pp. 43—61.
29. GAMBERALE, F., LISPER, H. O. and ANSHELM-OLSON, B. *Styrenegasers effekt på reaktionsförmåga hos arbetare i plastbåtsindustri* (Arbete och hälsa no. 8). Arbetskyddsverket, Stockholm 1975. 23 p.
30. GOLEBIEWSKA-PODGORCZYK, I. I. Evaluation of the role of styrene in the occurrence of occupational dermatoses. *Bull. inst. mar. trop. med. gdynia* 26 (1975) 289—297.
31. GRANT, W. M. *Toxicology of the eye* (2nd ed). Charles C. Thomas Publisher, Springfield, Ill. 1974, pp. 946—948.
32. GÖTELL, P., AXELSON, O. and LINDELÖF, B. Field studies on human styrene exposure. *Work environ. health* 9 (1972) 76—83.
33. HRUBÁ, E., SALCMANOVÁ, Z. and SCHWARTZOVÁ, K. Dlouhodobé sledování pracujících v riziku styrenu [Long-term follow-up of subjects exposed to possible styrene intoxication]. *ČS. neurol. neurochir.* 38 (1975) 116—122.
34. HÄRKÖNEN, H. Relationship of symptoms to occupational styrene exposure and to the findings of electroencephalographic and psychological examinations. *Int. arch. occup. environ. health* 40 (1977) 231—239.
35. HÄRKÖNEN, H., LINDSTRÖM, K., SEPÄLÄINEN, A. M., ASP, S. and HERNBERG, S. Exposure-response relationship between styrene exposure and central nervous functions. *Scand. j. work environ. & health* 4 (1978) 53—59.
36. IKEDA, M., IMAMURA, T., HAYASHI, M., TABUSHI, T. and HARA, I. Evaluation of hippuric, phenylglyoxylic and mandelic acids in urine as indices of styrene exposure. *Int. Arch. Arbeitsmed.* 32 (1974) 93—101.
37. IKEDA, M., OHTSUJI, H. and IMAMURA, P. In vivo suppression of benzene and styrene oxidation by co-administered toluene in rats and effects of phenobarbital. *Xenobiotica* 2 (1972) 101—106.
38. JAMES, M. O., FOUTS, J. R. and BEND, J. Hepatic and extrahepatic metabolism in vitro, of an epoxide (8-¹⁴C-styrene oxide) in the rabbit. *Biochem. pharmacol.* 25 (1976) 187—193.
39. JAMES, S. P. and WHITE, D. A. The metabolism of phenethyl bromide, styrene and styrene oxide in the rabbit and rat. *Biochem. j.* 104 (1967) 914—921.

40. KALLIOKOSKI, P. *Lujitemuoviteollisuus — ongelmallinen työympäristö* [The reinforced plastic industry — a problem work environment] (Työterveyslaitoksen tutkimuksia 122). Institute of Occupational Health, Helsinki 1976. 130 p.
41. KLIMKOVÁ-DEUTSCHOVÁ, E. Neurologische Befunde in der Plastikindustrie bei Styrol Arbeitern. *Int. Arch. Gewerbepath. Gewerbehyg.* 19 (1962) 35—50.
42. LEIBMAN, K. C. Metabolism and toxicity of styrene. *Environ. health perspect.* 11 (1975) 115—119.
43. LEIBMAN, K. C. and ORTIZ, E. Oxidation of styrene in liver microsomes. *Biochem. pharmacol.* 18 (1969) 552—554.
44. LILIS, R., LORIMER, W. V., DIAMOND, S. and SELIKOFF, I. J. Neurotoxicity of styrene in production and polymerization workers. In: *Proceedings of 4th international conference, Haifa, Israel, 7—10 Sept. 1976*. Medichem.
45. LINDSTRÖM, K., HÄRKÖNEN, H. and HERNBERG, S. Disturbances in psychological functions of workers occupationally exposed to styrene. *Scand. j. work environ. & health* 2 (1976) 129—139.
46. LOPRIENO, N., ABBONDANDOLO, A., BARALF, R., BARONCELLI, S., BONATTI, S., BRONZETTI, G., GAMMELLINI, A., CORSI, C., CORTI, G., FREZZA, D., LEPORINI, C., MAZZACCARO, A., NIERI, R., ROSELLINI, D. and ROSSI, A. M. Mutagenicity of industrial compounds: Styrene and its possible metabolite styrene oxide. *Mutat. res.* 40 (1976) 317—324.
47. LORIMER, W. V., LILIS, R., NICHOLSON, W. J., ANDERSON, H., FISCHBEIN, A., DAUM, S., ROM, W., RICE, C. and SELIKOFF, I. J. Clinical studies of styrene workers: Initial findings. *Environ. health perspect.* 17 (1976) 171—181.
48. MARNIEMI, J., SUOLINNA, E.-M., KAARTINEN, N. and VAINIO, H. Covalent binding of styrene oxide to rat liver macromolecules in vivo and in vitro. In: V. ULLRICH, I. ROOTS, A. HILDEBRAND, R.W. ESTABROOK and A. CONNEY (eds.), *Microsomes and drug oxidations: Proceedings of the third international symposium, Berlin, July 1976*. Pergamon Press, Oxford 1977, pp. 698—701.
49. MENEGHINI, C. L., RANTUCCIO, F. and RIBOLDI, A. Klinisch-allergologische Beobachtungen bei beruflichen ekzematösen Kontakt-Dermatosen. *Berufsdermatosen* 11 (1963) 181—202.
50. MERETOJA, T., VAINIO, H. and JÄRVEN-TAUS, H. Clastogenic effects of styrene exposure on bone marrow cells of rat. *Toxicol. lett.* 1 (1978) 315—318.
51. MERETOJA, T., VAINIO, H., SORSA, M. and HÄRKÖNEN, H. Occupational styrene exposure and chromosomal aberrations. *Mutat. res.* 56 (1977) 193—197.
52. MILVY, P. and GARRO, A. J. Mutagenic activity of styrene oxide (1,2-epoxyethylbenzene), a presumed styrene metabolite. *Mutat. res.* 40 (1976) 15—18.
53. OESCH, F., JERINA, D. M. and DALY, J. A radiometric assay for hepatic epoxide hydrolase activity with (7-³H) styrene oxide. *Biochim. biophys. acta* 227 (1971) 685—691.
54. OHTSUJI, H. and IKEDA, M. A rapid colorimetric method for the determination of phenylglyoxylic and mandelic acids: Its application to the urinalysis of workers exposed to styrene vapour. *Br. j. ind. med.* 27 (1970) 150—154.
55. OHTSUJI, H. and IKEDA, M. The metabolism of styrene in the rat and the stimulatory effect of phenobarbital. *Toxicol. appl. pharmacol.* 18 (1971) 321—328.
56. OLTRAMARE, M., DESBAUMES, E., IMHOFF, C. and MICHIELS, W. *Toxicologie du styrene monomere. Recherches experimentales et cliniques chez l'homme*. Editions Medecine et hygiene, Geneve 1974. 100 p.
57. PANTAROTTO, C., FANELLI, R., BIDOLI, F., MORAZZONI, P., SALMONA, M. and SZCZAWINSKA, K. Arene oxides in styrene metabolism, a new perspective in styrene toxicity? *Scand. j. work environ. & health* 4 (1978): suppl. 2, 67—77.
58. PARKKI, M. G., MARNIEMI, J., EKFORS, T. and LOUHIVUORI, A. Hepatotoxic changes in hamster by styrene. In: *Abstracts of international conference on in vivo aspects of biotransformation and toxicity of industrial and environmental xenobiotics, September 13—15, 1977, Prague, Czechoslovakia*. p. 74.
59. PARKKI, M. G., MARNIEMI, J. and VAINIO, H. Action of styrene and its metabolites styrene oxide and styrene glycol on activities of xenobiotic biotransformation enzymes in rat liver in vivo. *Toxicol. appl. pharmacol.* 38 (1976) 59—70.
60. RIIHIMÄKI, V. and PFÄFFLI, P. Percutaneous absorption of solvent vapors in man. *Scand. j. work environ. health.* 4 (1978) 73—85.
61. ROGERS, J. C. Industrial hygiene problems in the field of plastics. *Arch. ind. health* 12 (1955) 470—471.
62. ROTH, B. and KLIMKOVÁ-DEUTSCHOVÁ, E. The effect of the chronic action of industrial poisons on the electroencephalogram of man. *Rev. czech. med.* 9 (1963) 217—227.
63. RYAN, A. J., JAMES, M. O., BEN-ZVI, Z., LAW, F. C. P. and BEND, J. R. Hepatic and extrahepatic metabolism of ¹⁴C-styrene oxide. *Environ. health perspect.* 17 (1976) 135—144.
64. SANDELL, J., MARNIEMI, J., PARKKI, M. G. and VAAHTORANTA, K. The effects of styrene inhalation on the drug metabolizing enzymes in rat. In: *Abstracts of international conference on in vivo aspects of biotransformation and toxicity of industrial and environmental xenobiotics. September 13—15, 1977 Prague, Czechoslovakia*. p. 81.
65. SAVOLAINEN, H. and PFÄFFLI, P. Effects of chronic styrene inhalation on rat brain protein metabolism. *Acta neuropath. (Berl.)* 40 (1977) 237—241.

66. SAVOLAINEN, H. and VAINIO, H. Organ distribution and nervous system binding of styrene and styrene oxide. *Toxicology* 8 (1977) 135—141.
67. SEPPÄLÄINEN, A. M. and HÄRKÖNEN, H. Neurophysiological findings among workers occupationally exposed to styrene. *Scand. j. work. environ. health* 2 (1976) 140—146.
68. SHUGAEV, B. B. Concentrations of hydrocarbons in tissues as a measure of toxicity. *Arch. environ. health* 18 (1969) 878—882.
69. SMITH, H. O. and HOCHSTETTLER, A. D. Determination of odor thresholds in air using C^{14} -labeled compounds to monitor concentrations. *Environ. sci. techn.* 3 (1969) 169—170.
70. SPENCER, H. C., IRISH, D. D., ADAMS, E. M. and ROWE, V. K. The response of laboratory animals to monomeric styrene. *J. ind. hyg.* 24 (1942) 295—301.
71. STEWART, R. D., DODD, H. C., BARETTA, E. D. and SCHAFFER, A. W. Human exposure to styrene vapor. *Arch. environ. health* 16 (1968) 656—662.
72. TOSSAVAINEN, A. Styrene use and occupational exposure in the plastics industry. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 7—13.
73. VAINIO, H., HEMMINKI, K. and ELOVAARA, E. Toxicity of styrene and styrene oxide on chick embryos. *Toxicology* 8 (1977) 319—325.
74. VAINIO, H., PÄÄKKÖNEN, R., RÖNNHOLM, K., RAUNIO, V. and PELKONEN, O. A study on the mutagenic activity of styrene and styrene oxide. *Scand. j. work environ. & health* 2 (1976) 147—151.
75. WATABE, T., ISOBE, M., SAWAHATA, T., YOSHIKAWA, K., YAMADA, S. and TAKABATAKE, E. Metabolism and mutagenicity of styrene. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 142—155.
76. WILSON, R. H. Health hazards encountered in the manufacture of synthetic rubber. *J. am. med. assoc.* 124 (1944) 701—703.
77. WITHEY, J. R. The toxicology of styrene monomer and its pharmacokinetics and distribution in the rat. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 31—40.
78. WOLF, M. A., ROWE, V. K., MC COLLISTER, D. D., HOLLINGSWORTH, R. L. and OYEN, F. Toxicological studies of certain alkylated benzenes and benzene. *Arch. ind. health* 14 (1956) 387—398.
79. WOLFF, M. S. Evidence for existence in human tissues of monomers for plastics and rubber manufacture. *Environ. health perspect.* 17 (1976) 183—187.
80. VRBA, J. and MÁDLO, Z. Some new aspects of the metabolism of styrene. In: *15th international congress of occupational health, Vienna 19—24 Sept. 1966* (vol. II—Akademie, Wien 1966, pp. 461—464.