

Short Review

VINYL CHLORIDE AND VINYL BENZENE (STYRENE) — METABOLISM,
MUTAGENICITY AND CARCINOGENICITY

HARRI VAINIO

Department of Industrial Hygiene and Toxicology, Institute of Occupational Health,
Haartmaninkatu 1, SF-00290 Helsinki 29 (Finland)

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SUMMARY

Vinyl chloride and vinyl benzene (styrene) are mutagenic in microbial tests, in *Drosophila*, in yeast, and in mammalian cells. Reports from various countries have shown an excess of chromosomal aberrations in the lymphocytes of workers exposed to vinyl chloride monomer when the workers were compared with controls. Workers occupationally exposed to styrene also revealed a clear increase in the rate of chromosome aberrations in their lymphocytes. Both chloroethylene oxide and styrene oxide, the primary biotransformation products of vinyl chloride and styrene respectively, bind covalently to cellular macromolecules. Vinyl chloride is a carcinogen in both animals and man. Styrene is currently being tested in animals. These findings, the demonstration of mutagenic response via microbial and other test systems and with observations of significant excesses of chromosomal aberrations among workers exposed to these agents, raise scientific and health oriented concern about the possible genetic risks of vinyl chloride and styrene to man.

VINYL CHLORIDE

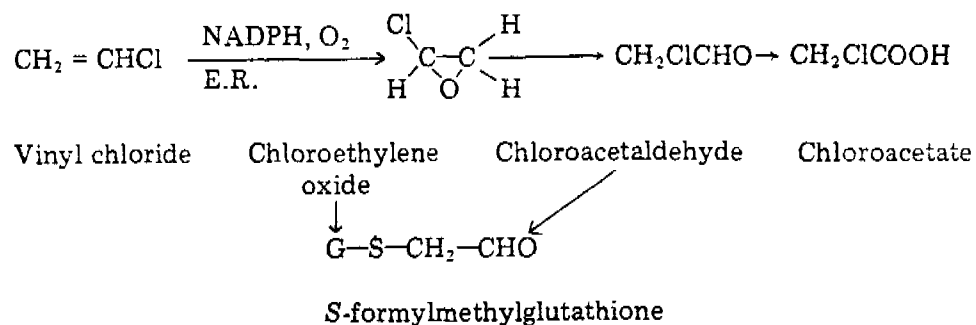
Metabolism

Vinyl chloride is readily metabolized, at least in small concentrations and it is partially converted into polar excretable metabolites [1]. Monochloroacetic acid has been reported to be excreted in the urine of workers exposed to vinyl chloride monomer [2]. The fact that exposure to vinyl chloride monomer reduces the non-protein sulfhydryl concentration in rat liver [1] suggests that the metabolites excreted in the urine are conjugated with glutathione [3].

Strong evidence has been obtained that the biotransformation of vinyl

chloride involves microsomal mixed-function oxidase, i.e. the metabolic activation of vinyl chloride by a liver microsomal system from rats, mice or humans depends on the presence of necessary cofactors for a NADPH generating system and oxygen [4-7]. Also the pretreatment of rats with phenobarbitone, which is known to increase the P-450 content of microsomal membranes, increases the mutagenic action of vinyl chloride.

Thus the metabolism of vinyl chloride can be described as follows:



Chloroethylene oxide rearranges to 2-chloroacetaldehyde spontaneously. In aqueous solution at pH 7.4 and 37°C the epoxy compound has a half-life of 1.6 min [8]. Chloroethylene oxide is capable of reacting with glutathione and with nucleophilic groups of macromolecules [9]. *N*-acetyl-*S*-(2-hydroxyethyl)cysteine (a major metabolite), *S*-(carboxymethyl)cysteine and *N*-acetyl-*S*-vinylcysteine are metabolites of vinyl chloride after inhalation or oral administration in rats [10]. The identification of S-containing metabolites from vinyl chloride-treated animals gives further support to the hypothesis that chloroacetaldehyde is formed and that chloroethylene oxide or chloroacetaldehyde reacts with glutathione [10]. Chloroethylene oxide and chloroacetaldehyde alkylate 4-(4-nitrobenzyl)pyridine and adenosine. Rat liver microsomes catalyze the covalent binding of [¹⁴C]vinyl chloride to proteins and nucleic acids [5].

Mutagenicity

The mutagenicity of vinyl chloride in a bacterial test system depends, at least partially, on metabolic activation by microsomal enzymes [6,11,18]. Chloroethylene oxide, which is formed as a primary metabolite of vinyl chloride monomer [1,7] by microsomal monooxygenases, is a potent mutagenic and alkylating agent. Chloroethylene oxide was the most effective vinyl chloride metabolite among those studied in inducing forward mutations in *Schizosaccharomyces pombe* and gene conversions in *Saccharomyces cerevisiae* [12]. 2-Chloroacetaldehyde, a spontaneous rearrangement product of chloroethylene oxide [13], was found to induce 8-azaguanine-resistant mutants but, in contrast to chloroethylene oxide, it did not induce ouabain-resistant mutants in Chinese V79 hamster cells [14]. Vinyl chloride is also mutagenic in the recessive lethal test both after short-term and long-term exposures of *Drosophila* males [15].

Chromosome aberrations in workers exposed to vinyl chloride monomer

Ducatman et al. [16] examined cultured lymphocytes from 11 workers in a factory which polymerizes vinyl chloride monomer. There was a significantly higher incidence of chromosomal aberrations in the exposed group as compared with a control group. Similar findings were reported by Funes-Cravioto et al. [17], Purchase et al. [18] and Hansteen et al. [19]. These data indicate that chronic high-level exposure to vinyl chloride monomer is associated with an increase in all breaking events that occur in human chromosomes.

A study of pregnancy outcome among wives of workers exposed to vinyl chloride monomer has indicated that, in comparison with controls, there is apparently an excess fetal loss among women whose husbands are considerably exposed to vinyl chloride [20]. These findings, in conjunction with significant excess of chromosomal aberrations among workers exposed to vinyl chloride, raise public health concern about the possible genetic risks of vinyl chloride to man. They indicate a potential danger of mutagenic effects on the fetus via sperm.

Carcinogenicity

In 1971, Viola et al. [21] reported that tumors of the skin, lung and bone developed in rats exposed to an atmosphere containing 30 000 ppm vinyl chloride for 4 h/day, 5 days/week for 12 months. More recently, Maltoni and Lefemine [22] reported angiosarcomas of the liver in rats exposed to concentrations of vinyl chloride ranging from 50 to 10 000 ppm 4 h/day, 5 days/week for 12 months and subsequently maintained and observed until death. Later, Maltoni showed that even a 25-ppm concentration of vinyl chloride has a demonstrable carcinogenic effect. In 1974, 3 years after the first carcinogenicity data was obtained in rats, Creech and Johnson reported an association of vinyl chloride exposure with liver angiosarcoma in man [23]. Thereafter, other independent studies have shown that exposure to vinyl chloride results in an increased carcinogenic risk in man, involving liver, brain, lung and lymphatic tissue [24-30].

VINYL BENZENE (STYRENE)

Metabolism

A variety of information has been accumulated concerning the toxicity and metabolism of vinyl benzene (styrene), an important source of plastics, in animals as well as man [31-33]. Industrial workers exposed to styrene vapor excrete large quantities of mandelic acid and small quantities of phenylglyoxylic acid as major metabolites in their urine [34-36]. In addition the following urinary metabolites, all of which are now recognized as deriving from the intermediate epoxide styrene oxide, are known: styrene glycol, its glucuronide, S(1-phenyl-2-hydroxy-ethyl)glutathione, benzoic acid, and hippuric acid (Fig. 1) [31]. The epoxy intermediate is so reactive that it has never been detected in urine.

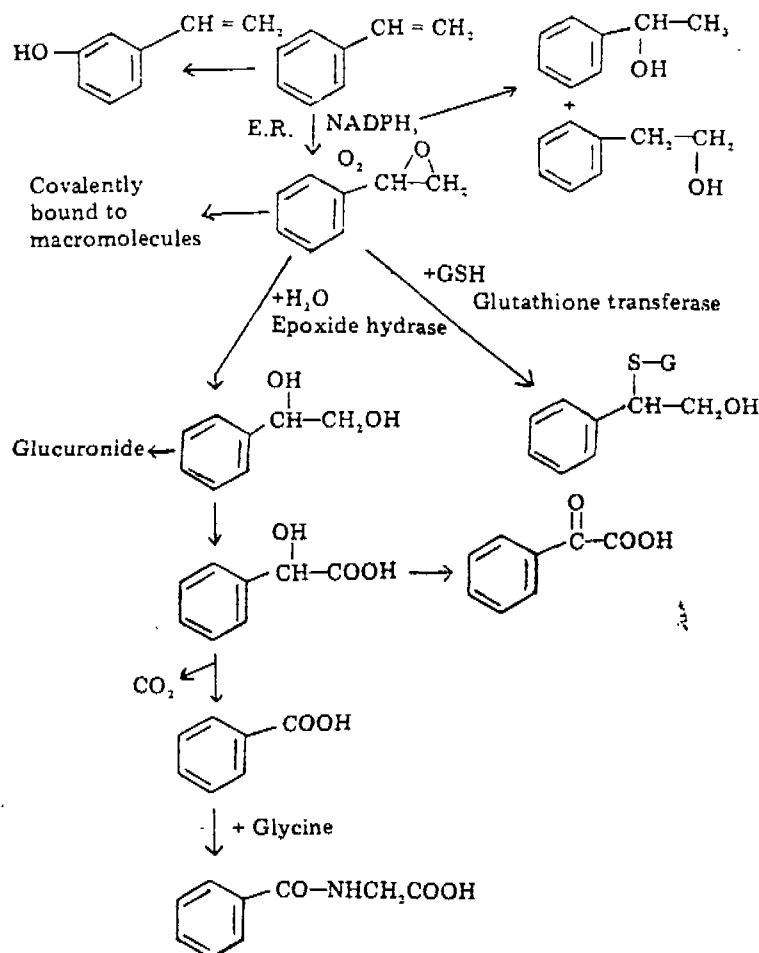


Fig. 1. Main pathways of the biotransformation of styrene.

The biotransformation of styrene to styrene oxide, as well as styrene oxide to styrene glycol, is stimulated by phenobarbital pretreatment of rats [37,38]. Styrene oxide binds covalently to liver homogenate, microsomes and to protein and nucleic acid fractions of rat liver [39]. Both styrene and styrene oxide have also been found to cause a depression of hepatic non-protein sulfhydryl content, probably through styrene oxide binding to glutathione [40].

Mutagenicity

Styrene oxide is mutagenic, without metabolic activation, to *Salmonella typhimurium* strains TA 1535 and TA 100, which have been devised to detect mutagens causing base-pair substitutions [41-43]. Styrene oxide also produces forward mutations in *Sch. pombe*, mitotic gene conversions in

strain D 4 of *S. cerevisiae* and azaguanine-resistant mutants in V79 hamster cells [44].

Styrene exhibits some mutagenic activity in *S. typhimurium*, but only after metabolic activation [42,43]. The mutagenicity after metabolic activation was enhanced in the presence of 3,3,3-trichloropropene oxide and the glutathione scavenger, diethylmaleate [42,43]. This suggests an increase in the metabolically formed epoxide in the microbial assay system.

The major factors determining the cellular level of the mutagen styrene oxide, formed from the promutagen styrene, could be the relative activities of monooxygenase (cytochrome P-450) to epoxide hydratase, localized in the endoplasmic reticulum of the cell and glutathione transferase activity, localized in the cytosol. In prolonged incubations of cell homogenates or microsomal cell fractions, the activities linked to cytochrome P-450 are more prone to inhibition by lipid peroxidation in the microsomal membrane than epoxide hydratase [45-47]. This phenomenon might affect the level of epoxide, as well as its biological half-life, for example, when added to microbial mutagenicity tests fortified with liver homogenate, thus making the testing more difficult.

Styrene has also been tested in *Drosophila melanogaster* by the sex-linked recessive lethal test [48]. A significant increase in recessive lethals was observed especially in the last brood after exposure to atmospheric styrene (300 ppm, 5 days, 6 h/day).

Chromosome aberrations in workers exposed to styrene

A significantly higher incidence of chromosome aberrations has been observed in styrene-exposed men from plants manufacturing polyester plastic products [49]. The aberrations were mainly chromosome-type breaks. Both decondensation of chromatin and disturbances similar to premature chromosome condensation were also seen [49].

Carcinogenicity

Styrene oxide has been tested by skin application in limited studies on mice [50-52]. According to van Duuren, styrene oxide is a weak carcinogen when applied to mouse skin [53,54]. On the other hand, an IARC monograph [55] states that no significant increase in skin tumors has been observed in animals. Styrene is currently being tested for animal carcinogenicity by several groups in the world.

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