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Glutathione pathway in ethylbenzene metabolism: Novel biomarkers of exposure in the rat

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ABSTRACT

Glutathione pathway was specifically studied in rats exposed by inhalation to a range of ethylbenzene vapours (5–2000 ppm). Urines were collected during exposure (6 h) and over the 18 h following the exposure. The potential metabolites coming from either side-chain or ring oxidation were synthesized: 1-, 2-phenylethylmercapturic acids (1-, and 2-PEMA) and 2-, 3- and 4-ethylphenylmercapturic acids (2-, 3-, and 4-EPMA). Their synthesis was fully described and the molecules characterized. Urine samples were analysed using a selective HPLC–fluorescence method. Among the five metabolites, 2-PEMA was never observed in any urine sample. By contrast, 1-PEMA was discovered in its two diastereomeric forms, and it was shown that one of them was mainly present. 2-EPMA, 3-EPMA and 4-EPMA (in the ratio 1:2:6) were also found, and their combined excretion levels were similar to that of 1-PEMA. The atmospheric concentrations and urinary excretions yielded very close correlations which allow us to consider these mercapturic acids as novel ethylbenzene exposure biomarkers.

1. Introduction

Ethylbenzene is among the highest production volume chemicals. It is commonly used as a raw material for manufacturing styrene (more than 99% of the production) and as an intermediate in synthetic organic chemistry. It is also present in refinery products such as mixed xylenes (at levels up to 25%) which are used as solvents for paints and lacquers, and in gasoline blending and aviation fuels as an antiknock agent. Like several other aromatic compounds, this substance has been shown to have various toxic effects. Classified as 2b by the IARC, ethylbenzene is also known for its effects on the nervous system. In particular, a recent study has shown that it causes damage to the inner ears in animals even at the lowest tested dose (200 ppm) (Gagnaire et al., 2007). For the aforementioned reasons, both threshold limit values, i.e. the time weighted average and short-term exposure limit, are currently 20 ppm (88.4 mg m^{-3}) and 100 ppm in France (100 ppm and 125 ppm respectively for ACGIH).

The metabolism of ethylbenzene has been studied in humans and other mammalian species (see lower Fig. 1) (McMahon and Sullivan, 1966; Kiese and Lenk, 1974; Chin et al., 1980; Engström, 1984, 1985; ATSDR, 2007; Gagnaire et al., 2007). Whatever the administration route, oral or inhalation, and whatever the species, the initial step in the metabolic pathway is oxidation of the side-chain to produce 1-phenylethanol and 2-phenylethanol. 1-phenylethanol, which results from the major pathway, is either excreted in glucuroconjugated form or converted by oxidation to acetophenone. Further oxidations of the side-chain lead to the sequential formation of 2-hydroxyacetophenone, 1-phenyl-1,2-ethanediol, and finally to mandelic acid and phenylglyoxylic acid (PGA). On the other hand, ring hydroxylation, which is considered a minor pathway, produces ethylphenols.

In humans exposed via inhalation, around 90% of the adsorbed dose is excreted as mandelic acid and PGA (with a ratio of approximately 3–1), the remaining 10% comprising 4-ethylphenol, p-hydroxyacetophenone, m-hydroxyacetophenone being excreted in their glucuronide and sulfate forms. In rats, the major metabolites identified are, in order of importance, hippuric (HA) and benzoic acids (38% of adsorbed dose), 1-phenylethanol (≈25%), mandelic acid (≈15–23%) and finally PGA (⩽10%). 2- and 4-ethylphenol have also been identified during assays on ethylbenzene with rat liver microsomes. Some other studies have been conducted with rabbits, but the results confirm that the rat appears to be a more appropriate model even if its metabolism is not completely consistent with that of humans (ATSDR, 2007).

In biomonitoring, ethylbenzene exposure is determined by measuring urinary mandelic acid and PGA or (possibly) by determining whole-blood ethylbenzene. However, mandelic acid and PGA are not specific to ethylbenzene exposure and can be found in the urine of workers exposed to styrene.

Therefore, the search for more suitable parameters to monitor the internal dose of ethylbenzene is thus still a major topic. In our quest for new ethylbenzene biomarkers, we wanted to ascertain if glutathione pathway could occur in the metabolism of ethylbenzene.

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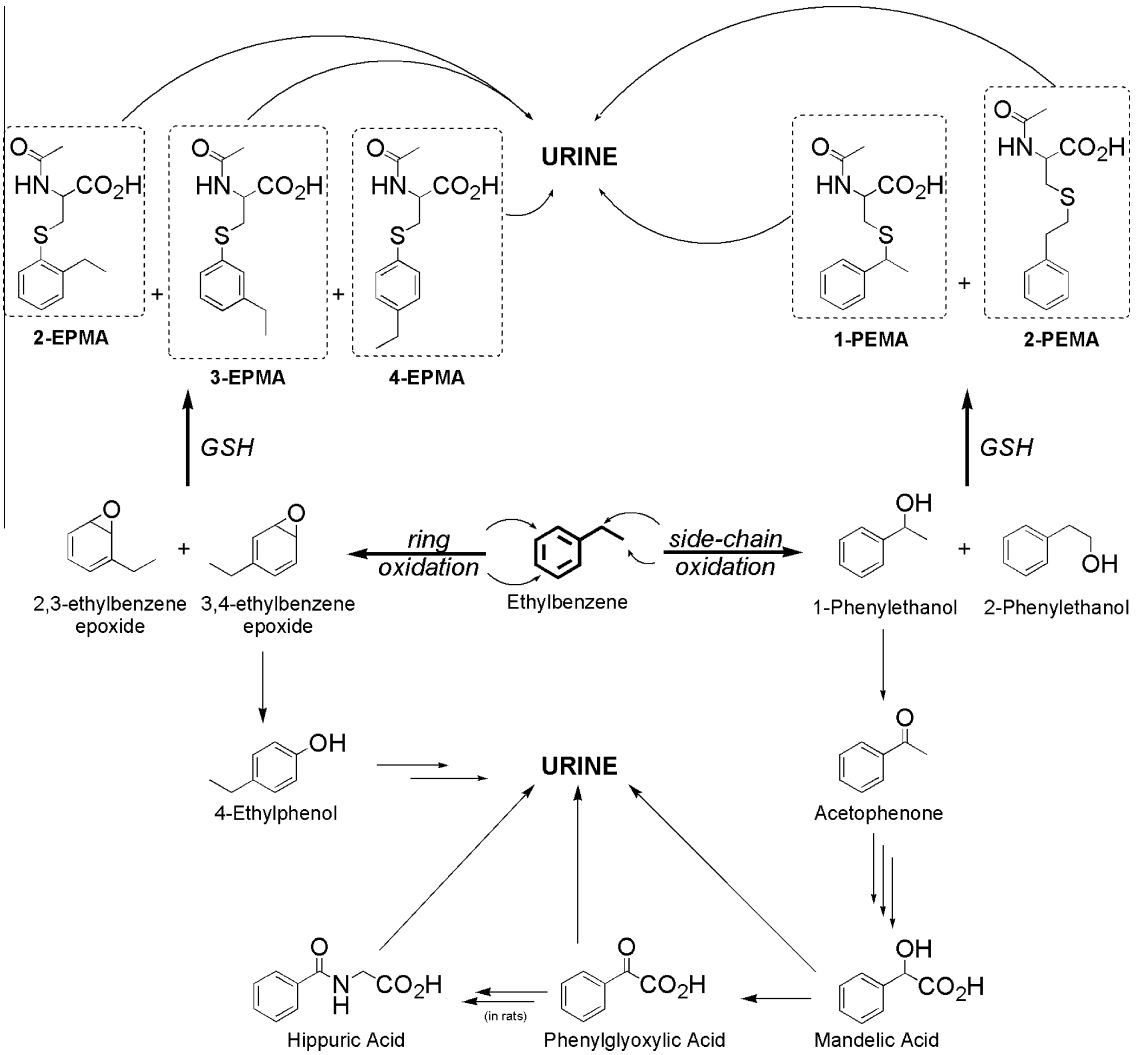

Fig. 1. Simplified metabolisation scheme of ethylbenzene oxidation pathway completed with glutathione (GSH) pathway (in rat).

The mercapturic acids (MAs), which are N-acetyl-L-cysteine-S-conjugates, are end products of the detoxification glutathione pathway (GSH). They may be potential candidates as biomarkers (ACGIH, 1998; DFG, 1998; Perbellini et al., 2002; Inoue et al., 2004; Haufroid and Lison, 2005). In recent decades, several MAs coming from aromatic hydrocarbons (benzene, toluene, xylenes, trimethylbenzenes, diethenylbenzenes) have been identified. These MAs may come from two metabolic pathways depending on the first metabolisation step, which may occur either via the side-chain or the aromatic ring oxidation.

In this respect, specific MAs stemming from the side-chain oxidation of toluene (benzylmercapturic acid), xylenes (methylbenzylmercapturic acids), 1,2,3- and 1,2,4-trimethylbenzene (dimethylbenzylmercapturic acids), diethenylbenzenes ((ethenylphenyl)-hydroxyethyl mercapturic acids) and styrene (phenylhydroxyethylmercapturic acids) have been identified by experimental exposures in rats (van Doorn et al., 1980; Linhart et al., 1989, 1992, 1996; Tanaka et al., 1990; Truchon et al., 1990; Maestri et al., 1997b; Tsujimoto et al., 1998, 1999, 2000). For these MAs, occupational exposure data are also available (Takahashi et al., 1993, 1994; Maestri et al., 1996, 1997b; Ghittori et al., 1997; Moriwaki et al., 2002; Sabatini et al., 2008).

MAs coming from aromatic ring oxidation such as *p*-toluyl mercapturic or dimethylphenyl mercapturic acids for toluene and xylenes respectively, have been identified both in the urine of rats and workers (Angerer et al., 1998; Gonzalez-Reche et al., 2003). Very recently 2-, 3- and 4-vinylphenyl mercapturic acids which are the three ring-oxidized metabolites of styrene have been found in the urine of mice exposed to styrene vapours (Linhart et al., 2010).

The aim of our investigation was to examine whether a comparable catabolism exists in the case of ethylbenzene. For this, five potential metabolites, the 1- and 2-phenylethylmercapturic acids (1- and 2-PEMA) and the 2-, 3- and 4-ethylphenyl mercapturic acids (2-, 3- and 4-EPMA) (see upper part of Fig. 1) were synthesized and characterized. These reference compounds and urine samples of rats exposed by inhalation to ethylbenzene vapours (ranging from 5 to 2000 ppm) were analysed separately using a specific HPLC–fluorescence method. The obtained results were compared. Finally, this glutathione pathway was put in context with other metabolic pathways, and the proportion of mercapturic acids relative to major urinary metabolites was estimated.

2. Material and methods

2.1. Chemicals

Ethylbenzene, 2-acetamidoacrylic acid, N-acetyl-L-cysteine, ammonium chloride, (1-bromoethyl)benzene, (2-bromoethyl)benzene, cesium carbonate, 2-ethyl-thiophenol, cyclopentyl methyl