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Novel and existing data for a future physiological toxicokinetic model of ethylene and its metabolite ethylene oxide in mouse, rat, and human

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ABSTRACT

The olefin ethylene is a ubiquitously found gas. It originates predominantly from plants, combustion processes and industrial sources. In mammals, inhaled ethylene is metabolized by cytochrome P450-dependent monooxygenases, particularly by cytochrome P450 2E1, to ethylene oxide, an epoxide that directly alkylates proteins and DNA. Ethylene oxide was mutagenic in vitro and in vivo in insects and mammals and carcinogenic in rats and mice. A physiological toxicokinetic model is a most useful tool for estimating the ethylene oxide burden in ethylene-exposed rodents and humans. The only published physiological toxicokinetic model for ethylene and metabolically produced ethylene oxide is discussed. Additionally, existing data required for the development of a future model and for testing its predictive accuracy are reviewed and extended by new gas uptake studies with ethylene and ethylene oxide in B6C3F1 mice and with ethylene in F344 rats.

1. Introduction

1.1. Sources of ethylene

The olefin ethylene (ET; CAS No.: 74-85-1) is a gas found ubiquitously in the environment. It is produced by microorganisms, fungi, and plants (see, e.g., [74,40,89]. Mammals exhale endogenously formed ET (mice, [56]; rats, [82]; humans, [75,15,82,34]. ET is the largest volume chemical produced globally [1]. It is primarily used in the production of polymers and industrial chemicals. About 74% of environmental atmospheric ET was related to natural and 26% to anthropogenic sources. Important environmental sources of ET are natural fires and man-made combustion of organic material as well as releases during its production and use (reviewed, e.g., in [45,2]. Concentrations of ET in ambient air are generally below 15 μg/m^3 (about 13 ppb) in rural areas and can amount to up to 805 μg/m^3 in cities [2]. In the air of a fruit store ET concentrations were in the range of 0.02–3.35 ppm [88]. A concentration of 46 ppm of ET was measured during firefighting [51]. ET concentrations of up to 47 mg/m^3 (about 40 ppm) were reported in a Swedish plastic producing company [41]. In a Canadian ET production facility, general workplace exposures to ET were below 15 ppm [67]. Field measurements performed at 14 petrochemical facilities in North America revealed a mean 8-h time weighted average atmospheric ET concentration of 2.6 ppm (range <0.05–2100 ppm). In two of the 146 samples collected during 4-h periods, average 4-h concentrations of atmospheric ET were 3200 and 4200 ppm [61].

1.2. Biological fate of ET

ET is only slightly soluble in water (Ostwald coefficient of 0.1198 at 101.325 kPa and 25 °C; [81]). Its blood/air partition coefficient,

Abbreviations: CYP, cytochrome P450-dependent monooxygenase(s); CYP2E1, cytochrome P-450 2E1; dithiocarbamate, sodium diethyldithiocarbamate trihydrate; EH, epoxide hydrolase; ET, ethylene; EO, ethylene oxide; GC/FID, gas chromatograph equipped with flame ionization detector; GSH, glutathione; GST, glutathione S-transferase; GSTT1, GST class Theta 1; K_{eq}, partition coefficient whole organism/air; k_{GSH}, pseudo first-order rate constant of the spontaneous conjugation of EO with GSH; k_{hydrol}, first-order rate constant of the non-enzymatic hydrolysis of EO; k_{inhib}, first-order rate constant of the ET-mediated suicide inhibition of CYP2E1; K_m, apparent Michaelis constant; K_{mCYP}, apparent Michaelis constant of CYP2E1-catalyzed EO formation; K_{mEH}, apparent Michaelis constant of epoxide hydrolase-catalyzed EO hydrolysis; K_{mGST}, apparent Michaelis constant of GST-catalyzed conjugation of EO with GSH; PT model, physiological toxicokinetic model; V_{max}, maximum rate of metabolism; V_{maxCYP}, maximum rate of CYP2E1-catalyzed EO formation from ET; V_{maxEH}, maximum rate of epoxide hydrolase-catalyzed EO hydrolysis; V_{maxGST}, maximum rate of GST-catalyzed conjugation of EO with GSH; V_1, volume of the air space in a closed exposure chamber; V_2, volume of the sum of the animals exposed in a closed chamber.

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determined at 37 °C, was low: 0.48 in rodents and 0.22 in humans [20]. As a consequence, the major part of ET inhaled by rats and humans was exhaled unchanged, only a minor part was metabolized. The pulmonary retention or the 1.5 times larger alveolar retention of ET at steady state (criteria for the metabolism of inhaled gases; see, e.g., [36]) were below 10% in humans and in rats [35,33]. In mammals, ET is biotransformed to ethylene oxide (EO, CAS No.: 75-21-8). The epoxide was detected in ET-exposed mice, rats, and humans [31,32,60,30,35]. The formation of EO from ET is catalyzed by cytochrome P450-dependent monooxygenase(s) (CYP) as was shown in liver microsomes of the same species [83,58]. Hydroxyethyl-adducts to hemoglobin and DNA, which are characteristic for EO, were quantified in ET-exposed rodents and hydroxyethyl-adducts to hemoglobin in ET-exposed humans (summarized in, e.g., [35]). Metabolic elimination of EO proceeds in subcellular liver fractions of mice and rats predominantly via conjugation with glutathione (GSH) mediated by hepatic cytosolic glutathione S-transferase (GST) as was shown by [11] and [58]. In human liver subcellular fractions, microsomal epoxide hydrolase (EH) plays a pivotal role for the metabolic elimination of EO, too [58].

1.3. Mutagenicity and carcinogenicity of EO

EO, a colorless gas at room temperature (boiling point: 10.8 °C at 101.3 kPa), is a high production volume chemical used primarily as an intermediate in the synthesis of various chemicals, especially ethylene glycol. A very minor part is used as fumigant and insecticide and as sterilizing agent, e.g., for food and medical devices (see, e.g., [46,47,48]). EO is a directly protein- and DNA-alkylating agent (reviewed in, e.g., [97]). It was mutagenic in vitro in bacteria and cells of animals and humans and in vivo in mice, rats, monkeys (reviewed, e.g., in [47]), and Drosophila [66]. Increased frequencies of micronucleated cells were found in EO-exposed mice, rats, and humans. In mice and rats, EO was carcinogenic (summarized in, e.g., [47,48]). IARC [48] evaluated EO as carcinogenic to humans (Group 1) by taking into account the "sufficient evidence for the carcinogenicity of ethylene oxide in experimental animals" and relying "heavily on the compelling data in support of the genotoxic mechanism" of EO.

1.4. Mutagenicity and carcinogenicity of ET

Despite its metabolism to EO, ET was neither mutagenic in Salmonella Typhimurium [91] nor mutagenic/genotoxic in rodents [90,94] nor carcinogenic in F344 rats that were long-term exposed to ET concentrations of up to 3000 ppm [43]. The negative results of the latter study agree with the suggestion that the tissue burdens of metabolically formed EO were too low to produce significant effects in a standard carcinogenicity study with ET [8,71,94].

1.5. Rationale for developing a physiological toxicokinetic model for ET and its metabolite EO in rodents and humans

A physiological toxicokinetic (PT) model for ET and its metabolite EO in mice, rats, and humans should reproduce quantitative differences in the tissue burdens by ET and EO between the three species. It should assess internal exposures to EO in relation to external EO concentrations for which dose–responses of carcinogenic effects were reported in both rodent species. In addition, it should "reflect interindividual differences in activation and clearance of the reactive epoxides" as has been asked by Melnick [63] when he was dealing with cancer risks from several olefins including ET. This statement is most relevant with respect to the kinetics of EO which is metabolically eliminated by GST class Theta 1 (GSTT1). The enzyme shows genetic polymorphism in humans, which may result in sub-population-specific kinetics of EO [87]. However, such a PT model has to be validated on experimental in vivo data in order to be considered as accurate and reliable: over a range of doses the predicted fate of the chemical (ET) and its toxicologically relevant metabolite (EO) in the body (blood, plasma, or other tissues) should agree with experimental data in laboratory animals and humans [17].

1.6. Aim of the present work

It was the aim of the present work to review the only published PT model for ET and its metabolite EO and to discuss newer data that will be most useful for the development of a revised PT model and for testing its predictive accuracy in the EO burden of ET-exposed B6C3F1 mice, F344 rats, and GSTT1 positive and negative humans. In addition, some novel gas uptake studies with ET and EO in B6C3F1 mice and with ET in F344 rats are to be presented. They will serve to validate model-predicted elimination kinetics of ET in both strains and of EO in B6C3F1 mice. A gas uptake study with EO in F344 rats was not carried out because such a study has already been published [55]. F344 rats were used in the carcinogenicity study with inhaled ET [43], B6C3F1 mice and F344 rats in carcinogenicity studies with inhaled EO (F344 rats: [37,38,59,84]; B6C3F1 mice: [68]).

2. Materials and methods

2.1. Chemicals

Synthetic air 5.5, helium 5.0, hydrogen 5.0, oxygen 4.5, nitrogen 5.0, ET 3.5, and EO 3.0 were obtained from Linde, Unterschleissheim, Germany. Soda lime "Drägersorb 800Plus" was from Drägerwerk, Lübeck, Germany, and sodium diethyldithiocarbamate trihydrate (dithiocarbamate) from Sigma–Aldrich, Taufkirchen, Germany.

2.2. Animals

Male F344 rats (in vivo studies, body weights: 280–320 g; control experiments with carcasses, body weights: 232 and 258 g) and male B6C3F1 mice (body weights: 25–30 g) were from Charles River Wiga Deutschland, Sulzfeld, Germany. Animal husbandry and experimental procedures were performed in conformity with the "Guide for the Care and Use of Laboratory Animals" [65]. In order to acclimate the animals, groups of 2 rats or 5 mice, respectively, were housed for at least 5 days before use in a Makrolon type III cage which was placed in an IVC top flow system (Tecniplast, Buguggiate, Italy). This system provided the animals with HEPA-filtered air of 22–25 °C and 50–60% humidity. A constant 12-h light/dark cycle was maintained in the chamber room. Animals had free access to standard chow (Nr. 1324 from Altromin, Lage, Germany) and tap water.

2.3. Inhalation studies in mice and rats

Before carrying out gas-uptake studies with ET or EO in closed all-glass exposure chambers, it was tested whether the presence of soda lime—required for adsorbing exhaled CO_2—leads to a loss of the concentration of ET or EO in chamber air. Closed chambers (about 2.8 L for ET and about 6.4 L for EO) contained 25 g of soda lime per chamber. The soda lime had been humidified with urine and exhaled water by exposing 5 mice per chamber to pure air for 5 h. After removing the animals from the chambers, initial concentrations of ET and EO were adjusted to 1090 ppm and 94 ppm, respectively, and concentration–time courses were monitored for about 7 h (both gases). In order to check whether skin and body of the animals influence the gas concentration of ET or EO in the