Kinetics of Ethylene and Ethylene Oxide in Subcellular Fractions of Lungs and Livers of Male B6C3F1 Mice and Male Fischer 344 Rats and of Human Livers

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Ethylene (ET) is metabolized in mammals to the carcinogenic ethylene oxide (EO). Although both gases are of high industrial relevance, only limited data exist on the toxicokinetics of ET in mice and of EO in humans. Metabolism of ET is related to cytochrome P450-dependent mono-oxygenase (CYP) and of EO to epoxide hydrolase (EH) and glutathione S-transferase (GST). Kinetics of ET metabolism to EO and of elimination of EO were investigated in headspace vessels containing incubations of subcellular fractions of mouse, rat, or human liver or of mouse or rat lung. CYP-associated metabolism of ET and GST-related metabolism of EO were found in microsomes and cytosol, respectively, of each species. EH-related metabolism of EO was not detectable in hepatic microsomes of rats and mice but obeyed saturation kinetics in hepatic microsomes of humans. In ET-exposed liver microsomes, metabolism of ET to EO followed Michaelis-Menten-like kinetics. Mean values of \(V_{\text{max}} \text{[nmol/} (\text{min-mg protein})\) and of the apparent Michaelis constant (\(K_m\ [\text{mmol/} \text{l ET in microsomal suspension}]\) were 0.567 and 0.0093 (mouse), 0.401 and 0.031 (rat), and 0.219 and 0.013 (human). In lung microsomes, \(V_{\text{max}}\) values were 0.073 (mouse) and 0.055 (rat). During ET exposure, the rate of EO production decreased rapidly. By modeling a suicide inhibition mechanism, rate constants for CYP-mediated catalysis and CYP inactivation were estimated. In liver cytosol, mean GST activities to EO expressed as \(V_{\text{max}}/K_m\ [\mu l/([\text{min-mg protein}]\) were 27.90 (mouse), 5.30 (rat), and 1.14 (human). The parameters are most relevant for reducing uncertainties in the risk assessment of ET and EO.

*Key Words:* ethylene; ethylene oxide; subcellular fractions; suicide inhibition; mouse; rat; human.

The gaseous olefin ethylene (ET, CAS No.: 74-85-1) is the largest volume organic chemical produced worldwide. The estimated global production in 2005 was over 112.9 million tons. The gas is mainly used as feedstock in the production of polymers and industrial chemicals (Zimmermann and Walzl, 2007). It is ubiquitously present in the environment, arising predominantly as a natural product from vegetation, from burning of organic material, and from automotive engine exhausts (referenced, for example, in International Agency of Research on Cancer (IARC), 1994a). Plants produce ET as a ripening hormone as reviewed in Bleecker and Kende (2000). Mice (Lawrence and Cohen, 1985), rats (Csanády et al., 2000), and humans (Filser et al., 1992) exhale endogenously formed ET. In liver microsomes of rats pretreated with phenobarbital, it was first proved by Schmiedel et al. (1983) that cytochrome P450-dependent monooxygenases (CYP) epoxidize ET to ethylene oxide (EO, CAS No.: 75-21-8). EO is a directly protein- and DNA-alkylating agent in rodents and humans (for instance, see Boogaard et al., 1999; Huang et al., 2011; Walker et al., 2000; Wu et al., 2011). It is mutagenic in vitro and in vivo (reviewed, for example, in Dellarco et al., 1990; Kolman et al., 2002) and is carcinogenic in rat (Dunkelberg, 1982; Garman et al., 1985; Snellings et al., 1984) and mouse (Dunkelberg, 1981; National Toxicology Program, 1987). In EO-exposed humans, increased sister chromatid exchange and chromosomal aberrations were reported (reviewed in Kolman et al., 2002). Based on the results of several epidemiologic studies on EO-exposed workers and mechanistic evidences, IARC (1994b) evaluated EO as carcinogenic to humans.

In vivo, the formation of EO from ET was shown indirectly in rats, mice, and humans via characteristic adducts to macromolecules (Ehrenberg et al., 1977; Eide et al., 1995; Filser et al., 1992; Rusyn et al., 2005; Segerbäck, 1983; Törnqvist et al., 1989; Walker et al., 2000) and directly in ET-exposed rats determined as EO in exhaled air (Filser and Bolt, 1984) and as EO in blood (Fennell et al., 2004; Maples and Dahl, 1993). In spite of the formation of its carcinogenic metabolite EO, ET was found to be negative in mutagenicity
studies in vitro and in vivo in rodents (Victorin and Stählberg, 1988; Walker et al., 2000) and in a long-term carcinogenicity study in rats (Hamm et al., 1984). The negative outcome of the carcinogenicity study was expected from toxicokinetic studies with ET and EO in rats and from studies on hemoglobin adducts in rats and mice. The EO burden was predicted to be too low to lead to tumors in a long-term carcinogenicity study with ET (Bolt and Filsner, 1984; Osterman-Golkar and Ehrenberg, 1982; Walker et al., 2000).

Toxicokinetic data on EO were obtained in blood of mice during, and in blood, brain, muscles, and testes of mice and rats after exposing the animals up to 4 h to constant concentrations of atmospheric EO (Brown et al., 1996, 1998). Also, concentration-time courses of EO were monitored in the atmosphere of closed exposure chambers after the exposure of mice for 3 h to constant EO concentrations (Sega et al., 1991). In this species, no measured toxicokinetic data on ET are published neither in vivo nor in vitro. In humans, the kinetics of ET was investigated only at low ET concentrations (< 50 ppm) by means of gas uptake studies (Filser et al., 1992). Brugnone et al. (1985, 1986) reported EO concentrations in blood and ratios of alveolar to atmospheric EO concentrations in EO-exposed workers. Gas uptake experiments were also conducted to investigate in rats the kinetics of ET (Andersen et al., 1980; Bolt et al., 1984) and of EO (Filsner and Bolt, 1984; Krishnan et al., 1992). EO was rapidly eliminated following first-order kinetics at EO concentrations of up to at least 100 ppm. ET showed saturation kinetics that could be described according to Michaelis and Menten by \( V_{\text{max}} \) and an apparent Michaelis constant (\( K_m \)). The metabolic elimination of ET was quantitatively inhibited following pretreatment with sodium diethyldithiocarbamate; \( V_{\text{max}} \) was doubled after pretreatment with a mixture of polychlorinated biphenyls (Bolt et al., 1984). These results hinted to a CYP-mediated metabolism of ET, later confirmed by Fennell et al. (2004). In rats exposed to high ET concentrations (Filsner and Bolt, 1984; > 1000 ppm; Fennell et al., 2004 and Maples and Dahl, 1993: 600 ppm), metabolically formed EO increased rapidly in exhaled air and in blood shortly after starting the exposures. Thereafter, the EO concentrations declined, reaching plateaus (Filsner and Bolt, 1984; Maples and Dahl, 1993) that remained rather constant until the end of exposure. The decline of EO was explained by an inactivation of hepatic CYP down to 68% after 360 min of exposure (Maples and Dahl, 1993) and of hepatic 4-nitrophenol hydroxylase activity (predominantly CYP2E1) to 50% after 4 h (Fennell et al., 2004). The loss of CYP activity reflects the findings in liver microsomes of phenobarbital-pretreated rats that ET destroyed CYP by self-catalyzed prosthetic heme alklylation (Ortiz de Montellano and Correia, 1983; Ortiz de Montellano and Mico, 1980).

Toxicokinetic data of ET and EO in mice, rats, and humans were used for developing physiological toxicokinetic models of ET together with EO (Csánady et al., 2000) or solely of EO (Fennell and Brown, 2001; Krishnan et al., 1992). However, a toxicokinetic model that describes the initial EO blood peaks together with the concentration-time courses of the ET-metabolizing CYP species during exposure to ET is missing. Such a model is required for providing a quantitative basis for the assessment of the risk of ET to humans. The goal of the present work was to acquire the toxicokinetic parameters that are necessary for the model by using ET- and EO-exposed subcellular fractions of livers and lungs of mice and rats and of livers of humans.

**MATERIALS AND METHODS**

**Chemicals**

ET 3.5 (> 99.95%) and EO 3.0 (> 99.9%) were obtained from Linde, Unterschleissheim, Germany. Reduced glutathione (GSH; ≥ 99%), pig heart isocitrate dehydrogenase Type IV, buffered to pH 6.0 in 50% aqeous glycerol solution (13 or 15 U/mg protein), N,N,N-triisocitic acid trisodium salt (96%), \( \beta \)-nicotinamide adenine dinucleotide phosphate sodium salt (NADPH*; 97%), and \( \beta \)-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt (NADPH; 97%) were purchased from Sigma-Aldrich, Steinheim, Germany. Racemic propylene oxide (PO; 99.9%) was provided by Serva GmbH, Heidelberg, Germany. All other chemicals were from Linde; Merck, Darmstadt, Germany; and Sigma-Aldrich at the highest analytical grade available.

**Animals**

Male B6C3Fl mice and male Fischer 344 rats were obtained from Charles River, Sulzfeld, Germany. Male 129/Sv-Cyp2e1tm1Gonz/J mice (CYP2E1 knockout mice) were bred in-house from three pairs of revitalized mice of this strain that were obtained from The Jackson Laboratory, Bar Harbor, ME. Male 129S1/SvImJ mice (wild-type mice) were also obtained from The Jackson Laboratory. CYP2E1 knockout and the corresponding wild-type mice were used only to investigate the relevance of CYP2E1 on the ET metabolism. All animals were housed in macronol type III cages in an IVC top-flow system (Tecniplast, Buguggiate, Italy). A light and dark cycle of 12 h was maintained in the animal room. The animals were kept at constant room temperature (23°C) and received standard diet 1324 (Altromin, Lage, Germany) and tap water ad libitum. Immediately before harvesting the livers and lungs for the preparation of microsomes and cytosol, the animals (mice about 25 g and rats about 250 g body weight) were asphyxiated with carbon dioxide.

**Human Liver Samples and Subcellular Human Liver Fractions**

A sample of a liver of a 75-year-old woman was obtained from the University Hospital “Rechts der Isar” of the “Technische Universität München”, Munich, Germany. The donor, suffering from metastasis of rectum cancer, was not treated with oncological drugs when the liver sample was collected on the occasion of a liver transplantation. The other liver samples were from five male and one female trauma victims who were between 16 and 58 years of age. All these liver samples were obtained at autopsies carried out in the forensic “Institut für Rechtsmedizin” of the “Ludwig-Maximilians-Universität München”, Munich, Germany. Death occurred between 6.5 and 29 h prior to autopsy. The liver samples were used to prepare cytosol in order to test the glutathione S-transferase (GST) activity to EO as substrate. In addition, microsomes were prepared from the liver samples of the 75-year-old woman and of a 22-year-old man who died 6.5 h before autopsy. Immediately after collection, the samples were stored at –20°C until transport (on ice) within 30 min to the Institute of Toxicology of the Helmholtz Center Munich, where they were stored at –80°C until preparation of the subcellular liver fractions.

Liver cytosol of 6 human individuals (1 male, 5 female; 42–78 years old) and microsomes pooled from the livers of 18 male and 7 female Caucasian and Hispanic donors, 21–64 years old, as well as cytosol pooled from the livers of
1 female Afro-American, 1 male Asian, 5 female and 3 male Caucasians, and 1 male Hispanic subject, between 1.9 and 70 years old, were obtained from BD Biosciences, San José, CA and delivered from Becton Dickinson, Heidelberg, Germany. Microsomes consisted of 20 mg protein/ml in a sucrose solution (250 mmol/l). Cytosol (protein content 20 mg/ml) was in a solution consisting of 150 mmol/l KCl, 50 mmol/l Tris-HCl buffer (pH 7.5), and 2 mmol/l EDTA.

Preparation of Microsomes and Cytosol

Microsomes and cytosol were prepared from tissue samples as described in Kreuzer et al. (1991). Both fractions were stored at −80°C until use. The protein content of the subcellular fractions was determined using a modified Biuret method (Gornall et al., 1949).

Incubation Procedure

All the experiments described in the following are “gas uptake experiments” with the gaseous compound administered only once into the atmosphere of closed all-glass exposure vessels. Microsomal and cytosolic incubation conditions were chosen as described in Kreuzer et al. (1991) and in Faller et al. (2001). In the latter publication, the quality of such preparations was verified by comparing specific activities of CYP, GST, and epoxide hydrolase (EH) for a series of standard substrates with literature data. The protein concentrations used in the present work were selected according to Faller et al. (2001). Generally, a subcellular fraction, either native or inactivated by heat (96°C for 10 min in a water bath), was incubated with ET or EO with or without coenzymes in a cone-shaped headspace vessel (~38 ml). The all-glass vessel contained two ports, one at the upper end, closed by a stopcock made out of glass, and one laterally, closed by a Teflon-coated rubber septum. It was located at 37°C in a continuously vigorously shaken water bath. Concentration-time courses of ET and EO were monitored by gas chromatography with flame ionization detection (GC/FID) using gas samples collected periodically from the headspace, immediately after shortly but vigorously shaking the vessel by hand in order to maintain the compound-specific partition coefficient incubation medium-to-air.

Microsomal Incubations

**Ethylene.** The headspace vessels contained microsomes, MgCl₂ (5 mmol/l in the final incubate volume) and potassium phosphate buffer (0.15 mol/l, pH 7.4). In those experiments in which only ET was monitored, either hepatic microsomal suspensions of 10 ml with protein concentrations of 10 mg/ml in the 38-ml vessels or hepatic microsomal suspensions of 4 ml with protein concentrations of 5 mg/ml in commercially available 8-ml headspace vials were used. When monitoring additionally the EO formation in ET incubations, the incubation volume was always 1 ml. After a preincubation period of 10 min, a defined volume of gas was removed from the vessel and replaced by an EO air mixture hereby starting the exposure. In order to investigate the GST-mediated EO elimination, initial EO concentrations of between 10 and 3000 ppm were used. Control experiments were done with heat-inactivated microsomes and cytosol in the absence of GST but in the presence of maleic acid diethyl ester (DEM, 3 mmol/l incubation medium) for the depletion of residual GSH (Boyland and Chasseaud, 1970). For determining the nonenzymatic conjugation of EO with GSH, incubations were done with heat-inactivated cytosol and GSH (15 mmol/l in the incubation medium). For investigating whether the presence of cytosolic proteins might have an influence on the rate of EO hydrolysis, some EO incubations were carried out in the presence of native cytosol and DEM (3 mmol/l incubation medium).

Gas Chromatographic Analysis

Atmospheric ET, EO, and PO (a proven EH substrate that was used for comparative purposes, see below) were analyzed according to Faller et al. (2001) using gas chromatographs GC-8A (Shimadzu, Duisburg, Germany) equipped with flame ionization detectors and the integrators CR5A and CR6A (from Shimadzu) for recording the chromatograms. Separation was carried out on 1/8” stainless-steel columns filled with Tenax TA from Chrompack, Frankfurt, Germany (60–80 mesh for ET and 80–100 mesh for EO and PO). The column used for ET was 1.5 m that for EO and PO 3.5 m long. Nitrogen with pressures of 200 (ET, PO) or 250 (EO) kPa was used as carrier gas. The oven temperatures were 60°C (ET), 150°C (EO), and 180°C (PO). The temperatures of the injectors and detectors were held at 130°C (ET), 200°C (EO), or 230°C (PO). The detectors were provided with hydrogen (60 kPa) and synthetic air (60 kPa). Gas samples of 50 μl (ET, PO, and EO > 10 ppm in the vessel atmosphere) and of 500 μl (EO < 10 ppm) were injected directly onto the column. The retention times of the gases were 0.5 min (ET), 2.5 min (EO), and 2.4 min (PO).

Calibration curves were constructed with the three substances in the ranges of between 200 and 50,000 ppm (ET), 5 and 5000 ppm (EO; injection volume 50 μl), 0.22 and 10.02 ppm (EO; injection volume 500 μl), and 10 and 1000 ppm (PO). Linear regression analysis through the origin revealed correlation coefficients of at least 0.999 between peak height and gas concentration. The detection limits, defined as three times the background noise, were 0.1 ppm (ET), 0.5 ppm (EO; injection volume 50 μl), 0.1 ppm (EO; injection volume 500 μl), and 0.3 ppm (PO).

For each exposure, a single calibration of each measured compound was performed.

Kinetic Analysis of Concentration-Time Courses

Atmospheric concentrations given in ppm at 37°C were transformed to mol/l by division with the molecular volume of an ideal gas at 37°C (25.43 l). Actual amounts of compounds in the vessel were obtained by multiplication of the actual concentration in air $y_{c0}$ with the compound-specific volume of distribution $V_d$:
Because the vessels were always vigorously shaken, $V_d$ is always the sum of the air volume $V_a$ in the vessel and the product of the volume of the liquid phase $V_l$ in the vessel with the compound- and medium-(cytosolic or microsomal incubation) specific thermodynamic partition coefficient medium-to-air $K_{eq}$.

$$V_d = V_a + V_l \cdot K_{eq}$$

(2)

The values of $K_{eq}$ ($K_{eq,ET}$ for ET and $K_{eq,EO}$ for EO) were determined at 37°C according to Csanady et al. (2000). They were $K_{eq,ET} = 0.2 \pm 0.06$ (n = 5) and $K_{eq,EO} = 68.4 \pm 0.6$ (n = 3) in microsomal incubations. In cytosolic suspensions, $K_{eq,EO}$ was 75.9 ± 3.1 (n = 5).

In the ET or EO gas uptake studies, the atmospheric concentration-time courses of ET or EO were fitted using an e-function (Equation 3) or a differential equation describing saturation kinetics according to Michaelis and Menten (Equation 4).

$$c_a = c_{a0} \cdot e^{-k \cdot t} + A$$

(3)

The concentration in the air phase of the vessel is represented by $c_a$, and the elimination rate constant by $k$. "A" means a constant concentration of A below:

$$\frac{dc_a}{dt} = -(V^*_{max} \cdot c_a)/(K_{m} + c_a)$$

(4)

$V^*_{max}$ is the maximum concentration change in the atmosphere of the vessel. $K_m$ is the apparent Michaelis constant related to the atmospheric concentration. With the amount of protein (Pr) in the experimental onset, the expression $V_{max} = V^*_{max} \cdot V_d/\text{Pr}$ gives the maximum elimination rate $V_{max}$ (amount of compound per time unit per mg protein):

$$V_{max} = V^*_{max} \cdot V_d/\text{Pr}$$

(5)

The compound- and species-specific value of $K_{m}$ (the apparent Michaelis constant related to the incubation medium) is given by the product of $K_m$ with $K_{eq}$ (for definition of $K_{eq}$, see above):

$$K_m = K_{m} \cdot K_{eq}$$

(6)

In ET incubation experiments with quasi-constantly remaining ET concentrations, metabolically formed EO was detected in the air phase of the exposure systems. Because the EO formation took place only in the liquid phase, each measured air-phase concentration $c_{aEO}$ was used to calculate the corresponding amount of EO in the whole system and relating it to the volume of the liquid phase, solely:

$$c_{EO} = \frac{c_{aEO} \cdot (V_g + K_{eq,EO} \cdot V_l)}{V_l}$$

(7)

Here, $c_{EO}$ is the "theoretical" EO concentration in the liquid phase and $K_{eq,EO}$ is the thermodynamic partition coefficient of EO in microsomal incubations.

The following function was fitted to the time-dependent, experimentally obtained $c_{EO}$ data. This function was derived from theoretical considerations (see below):

$$c_{EO} = C_1 \cdot [1 - e^{-k \cdot t}]$$

(8)

$C_1$ is the fitted EO concentration for $t \to \infty$ and k is a rate constant.

Initial formation rates of EO ($v_{max}$), normalized to a protein content of 1 mg, were calculated from the derivative of Equation 8 at $t = 0$ by taking into account $V_l$ and the actual amount of protein (Pr):

$$v_{max} = \frac{k \cdot C_1 \cdot V_l}{\text{Pr}}$$

(9)

By means of the program Prism 5 for Macintosh (GraphPad Software, California) Michaelis-Menten-type kinetics were fitted to the data representing various $v_{max}$ values of EO against the corresponding ET substrate concentrations. The Berkeley Madonna 8.3.18 (University of California, Berkeley) program was used to fit Michaelis-Menten-type kinetics to the human microsomal EO concentration-time data.

Suicide Inhibition Model

The CYP-mediated metabolic production of EO from ET together with the irreversible inactivation of CYP (by N-hydroxyethylation of the pyrrole ring D in the porphyrin; reference, for e.g., Correia and Ortiz de Montellano, 2005) was described by the following reaction scheme (adapted from Collman et al., 1990), which incorporates the metabolite formation via the enzyme-substrate complex ES according to Briggs and Haldane (1925) together with simultaneous suicide inactivation of ES:

$$E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P \xrightarrow{k_4} EX$$

(10)

The concentration of the ET-metabolizing CYP species is given by $E$, that of the substrate ET by $S$, and that of the product EO by $P$. ES stands for irreversibly inactivated CYP. The rate constants of the four reactions shown in Equation 10 are represented by $k_1$, $k_2$, $k_3$, and $k_4$. In the literature, $k_1$ is often named "$k_{inact}\)" and $k_4$, the inactivation constant, "$k_{inact}\). The co-substrates O2 and NADPH are not considered because O2 was in excess and NADPH was assumed constant during the exposure period because of the NADPH regeneration system. According to Equation 10, the change of ES over time (t) is described by:

$$\frac{dES}{dt} = k_1 \cdot S \cdot E - (k_2 + k_3) \cdot ES - k_4 \cdot ES$$

(11)

When the rate of the suicide inhibition

$$\frac{dEX}{dt} = k_4 \cdot ES$$

(12)

is zero, Equation 11 reads

$$\frac{dES}{dt} = k_1 \cdot S \cdot E - (k_2 + k_3) \cdot ES$$

(13)

As discussed by Briggs and Haldane (1925), $\frac{dES}{dt}$ in Equation 13 can be considered negligibly small. In other words, the expression given at the right side of Equation 13 can be treated as zero. Taking this assumption of a very fast quasi-steady state into account and considering $\frac{dEX}{dt}$ (see Equation 12) being much slower than the time to reach the quasi-steady-state, Equation 11 can be reformulated to:

$$\frac{dES}{dt} = -k_4 \cdot ES$$

(14)

At the experimental onset used in the present microsomal studies, the ES complex was already formed in the preincubation phase before the reaction was started by the addition of the NADPH regenerating system. During this phase, both $k_1$ and $k_4$ are evidently not existent (compare Equation 10); the ES complex $ES_0$ at $t = 0$ (the end of the preincubation phase, when the reaction starts) is given by:

$$k_1 \cdot S \cdot E = k_2 \cdot ES_0$$

(15a)

or

$$ES_0 = \frac{k_1 \cdot S \cdot E}{k_2}$$

(15b)

At the end of the preincubation phase, the total enzyme concentration $E_t$ is given by:

$$E_t = ES_0 + E$$

(16)

Combining Equation 15b with Equation 16 and canceling $E$, one obtains $ES_0$ as a function of $E_t, S$, and the rate constants $k_1$ and $k_2$:...
\[ E_S^0 = \frac{E_t \cdot k_1 \cdot S}{k_2 + k_1 \cdot S} \]  

(17)

It can be derived from Equation 17 that \( E_S^0 \) becomes \( ES_0 \) at very high concentrations of \( S \) because \( k_2 \) can be neglected if compared with \( k_1 \cdot S \) for such exposure conditions.

The solution of the differential Equation 14 is:

\[ E_S = E_S^0 \cdot e^{-k_1 \cdot t} \]  

(18)

The exposure time is given by \( t \).

Equation 10 shows that the rate of the ET-derived formation of the EO concentration (\( \frac{dP}{dt} \)) is represented by:

\[ \frac{dP}{dt} = k_3 \cdot E_S \]  

(19)

or with Equation 18:

\[ \frac{dP}{dt} = k_3 \cdot ES_0 \cdot e^{-k_1 \cdot t} \]  

(20)

Because \( ES_0 \) becomes equal to \( E_t \) at very high concentrations of \( S \), the maximum rate of metabolite formation \( \frac{dP_{\text{max}}}{dt} \) (at the time point \( t_0 = 0 \), when the reaction is started) is given by:

\[ \frac{dP_{\text{max}}}{dt} = k_3 \cdot E_t \]  

(21)

The concentration-time course of \( P \) at a given constant concentration of \( S \) is obtained by integrating Equation 20 and considering that \( P = 0 \) at \( t_0 = 0 \):

\[ P = \frac{k_3}{k_4} \cdot ES_0 \cdot \left[ 1 - e^{-k_1 \cdot t} \right] \]  

(22)

The term (\( k_3/k_4 \)) is a partition ratio and represents the number of products formed per one inactivation event. The partition ratio is constant, independent of the substance concentration. Both reactions (\( k_3 \cdot ES \) and (\( k_4 ES \)) follow saturation kinetics.

**Linkage of the Suicide Inhibition Model with Experimental Parameters**

The amount of ET was much greater in the relatively large atmospheric volumes of the closed vessels than in the small volumes of the liquid phase, where the reaction took place because of the very low \( K_{\text{eq,ET}} \) of 0.2 (see above). Consequently and because of the relatively slow metabolic ET elimination, the substrate concentration ET remained almost constant during the exposure periods. The maximum EO production \( V_{\text{max,EO}} \) was obtained experimentally as described in the text to Equation 9. Considering ET to be metabolized exclusively by CYP2E1 (see Results), the mean microsomal CYP2E1 contents (nmol/mg microsomal protein) of 96.6 \( \pm \) 10\(^{-3}\) (SD = 12 \( \times \) 10\(^{-3}\); \( n = 3 \)), 35.8 \( \times \) 10\(^{-3}\) (SD = 8.3 \( \times \) 10\(^{-3}\); \( n = 3 \)), and 70.5 \( \times \) 10\(^{-3}\) (SD = 17 \( \times \) 10\(^{-3}\); \( n = 3 \)); weighted mean and SD of three times 10 determinations) in livers of mice, rats, and humans, respectively (Seaton et al., 1995), were taken as the species specific total enzyme concentrations \( E_t \). Replacing \( \frac{dP_{\text{max}}}{dt} \) in Equation 21 by \( V_{\text{max,EO}}V_{\text{CYP2E1}} \) and \( E_t \) by the CYP2E1 content/\( V_{\text{CYP2E1}} \), Equation 23 was used to calculate the value of \( k_3 \):

\[ k_3 = \frac{V_{\text{max,EO}}}{V_{\text{CYP2E1}}} \]  

(23)

Equation 22 has the same structure as Equation 8. Therefore, the value of the inactivation constant \( k_4 \) equals the experimentally obtained value of \( k \) in Equation 8. The concentration \( \frac{P_{\text{max}}}{ES_0} \) (Equation 22) stays for the experimental EO plateau concentration \( C_t \) which, when multiplied with \( k_4 \), equals the initial EO production rate at the corresponding ET exposure concentration and microsomal protein content (see Equations 9 and 20).

It has to be stressed that the \( K_m \) values and the rate constants \( k \), dealt with in the present work are apparent constants because the experiments were not performed with purified enzymes. The use of microsomal and cytosolic suspensions is, however, preferable to that of purified enzymes because the translation of \textit{in vitro} results to the \textit{in vivo} situation requires fewer assumptions when using microsomal and cytosolic data.

**Statistics**

Arithmetic means (\( \bar{x} \)) and SEs and two-way ANOVA were calculated using the software Prism 5. SE values were also obtained from SD calculated by means of Microsoft Excel 2004 for Mac (Microsoft, Unterschleissheim, Germany) using the following relation:

\[ SE = \frac{SD}{\sqrt{n}} \]  

(24)

with \( n \) being the sample size.

Error propagation of SE was calculated as described in Sachs (1973).

**RESULTS**

**Gas Uptake Studies with ET in Microsomal Incubations**

**Elimination of ET.** A series of ET gas uptake studies was performed using pooled rat liver microsomal incubations in order to monitor the disappearance of ET in the atmosphere of the vessels as a measure of ET metabolism. No enzyme-catalyzed concentration losses could be detected when using protein concentrations of 5 mg/ml incubate in 8-ml vials as the very flat slopes of the ET concentration-time courses of incubations containing native or heat-inactivated microsomes were almost identical (Fig. 1A). Only when using 38-ml vessels containing a microsomal suspension of 10 ml with a high protein concentration of 10 mg/ml, declines of atmospheric ET could be monitored (Figs. 1B and 1C). The slopes of the curves fitted to the data in native microsomes flatten with the exposure time and become, after about 20 min, parallel to the curves fitted to the data in heat-inactivated microsomes. The small decay of the latter curves results from the ET loss due to the sampling of the gas probes. The curves gained with native microsomes level off with time because of the suicide inactivation of the ET-metabolizing CYP species which results from the NADPH-dependent activation of ET (Ortiz de Montellano and Mico, 1980). The functional integrity of the hepatic microsomes of the three species was verified over a time span of 60 min using gaseous styrene as model substrate (data not shown). Styrene is known to have a very high partition ratio \( k_3/k_4 \) of 10,000 (determined using a synthetic iron porphyrin; Collman et al., 1986). Initial rates of ET elimination were calculated from the e-functions describing the curves in Figures 1B and 1C.

In mouse liver microsomes, the initial rate of metabolic ET elimination was 0.069 nmol/min/mg protein at an ET exposure concentration of 170 ppm in the vessel atmosphere. In rat liver microsomes, initial rates of 0.022 and 0.051 nmol/min/mg protein were obtained in two incubations with ET, each of
which with an initial concentration of 200 ppm. At an ET concentration of 30 ppm, the initial elimination rate was 0.005 nmol/min/mg protein. Figures 1D–F show the concentration-time courses of ET in those mouse, rat, and human liver microsomal incubation experiments that were carried out in order to study the formation kinetics of metabolically produced EO. The slopes of the ET concentration-time courses result predominantly from the repeated collection of air samples of 50 and 500 l each required for determining ET and EO, respectively, as is evidenced by the concentration-time curves that represent e-functions calculated by considering the experimental removal of atmospheric ET, solely. The extent of metabolic elimination of ET from the vessel atmospheres was too slight to become discernible.

**Formation of EO.** The results shown in Figure 2 were obtained when measuring the EO formation instead of the ET elimination in a series of ET incubation studies using pooled liver microsomes of mice (Fig. 2A), rats (Fig. 2B), or humans (Fig. 2C) and liver microsomes of individual human subjects (Figs. 2D and 2E) as well as pooled lung microsomes of mice or rats (Figs. 2F and 2G). In addition, the time courses of EO formation in ET-exposed pooled hepatic microsomal suspensions of 50 wild-type and 50 CYP2E1 knockout mice are shown in Figure 2H. The curves describing total EO in the liquid phase (cEOl, see Equation 7) flatten with the exposure time and approach plateaus. This finding corresponds to the flattening of the ET elimination curve in dependence of the exposure time (Figs. 1B and 1C).

The curves plotted to the concentration-time data in Figure 2 were fitted by the function given in Equation 8. The rate constant in Equation 8 equals the apparent suicide inactivation rate constant k₄ of the ET-metabolizing CYP species, which is predominantly CYP2E1 in the liver. This can be concluded from the findings that the maximum rate of EO formation (VmaxEO) was about 15 times higher in liver microsomes of wild-type mice (1.1 nmol/min/mg protein) than in the corresponding CYP2E1 knockout mice (0.074 nmol/min/mg protein).
protein). Even higher (340) was the ratio of $V_{\text{maxEO}}/K_m$ in wild-type mice (162 $\mu$L/min/mg protein) to $V_{\text{maxEO}}/K_m$ in CYP2E1 knockout mice (0.476 $\mu$L/min/mg protein).

Based on the fits to the EO concentration-time data, the initial EO formation rates $v_{\text{init}}$ were calculated and are presented for B6C3F1 mice and Fischer 344 rats versus the ET exposure concentrations in Figure 3. The data obtained in lung microsomes at high ET concentrations of 16,500 and 75,400 (mice) and of 18,600 and 57,800 ppm (rats) probably represent the maximum EO formation rates of $V_{\text{maxEO}} = 0.073 \text{ nmol/min/mg protein}$ (mean value, mice) and 0.055 nmol/min/mg protein (mean value, rats). The curves shown in Figure 3 are fitted to the data gained from mouse, rat, and human liver microsomes considering saturation kinetics according to Michaelis and Menten. The obtained $V_{\text{maxEO}}$ and $K_m$ values for mice and rats are given in Table 1. The $V_{\text{maxEO}}$ values in mice and rats are about double as high as in humans. The $K_m$ value in humans is lower than that in rats and higher than that in mice. When comparing the ratios $V_{\text{maxEO}}/K_m$, human and rat liver microsomes show almost the same values which are about four times less than in mouse microsomes.

Table 2 shows the mean ± SE values of the rate constants of the EO formation ($k_3$), of the suicide inhibition ($k_4$), and the ratios $k_3/k_4$. The values of $k_3$ were calculated by dividing $V_{\text{maxEO}}$ (Table 1) by the CYP2E1 content (Equation 23), the values of $k_4$ were obtained from the above-described six, eight, and nine ET incubations of mouse, rat, and human liver microsomes, respectively, and from incubations (two each) of mouse and rat lung microsomes. The values of $k_4$, reflecting

![FIG. 2. Concentration-time courses of EO formed at various quasi-constant atmospheric concentrations of ET in closed exposure vessels (38 ml) containing hepatic or pulmonary microsomes. Produced EO is given as EO concentration in the liquid phase of the incubation vessels. (A–C and H) Incubations of pooled liver microsomes from (A) 50 B6C3F1 mice (2.5 mg protein/ml, incubation volume 4 ml), (B) 10 rats (5 mg protein/ml, incubation volume 4 ml), (C) 25 humans (10 mg protein/ml, incubation volume 2 ml), (H) 50 wild-type mice (2.5 mg protein/ml, incubation volume 4 ml), and 50 CYP2E1 knockout mice (10 mg protein/ml, incubation volume 2 ml). (D and E) Incubations of liver microsomes of 10 mg protein/ml (incubation volumes 2 ml each), from (D) a 75-year-old female human subject and (E) a 22-year-old male human subject. (F and G) Incubations (2 ml each) of pooled lung microsomes from (F) 150 mice (5 mg protein/ml) and (G) 50 rats (10 mg protein/ml). ET exposure concentrations and data symbols are given by (A) 200 ppm ( ), 720 ppm ( ), 2720 ppm ( ), 7040 ppm ( ), 36300 ppm ( ), 56700 ppm ( ); (B) 210 ppm ( ), 810 ppm ( ), 3010 ppm ( ), 7580 ppm ( ), 14200 ppm ( ), 3110 ppm ( ), 38600 ppm ( ); (C) 16500 ppm ( ), 75400 ppm ( ), 18600 ppm ( ), 57800 ppm ( ); (D) 66300 ppm ( ); (E) 270 ppm ( ), 3110 ppm ( ), 38600 ppm ( ); (F) 16500 ppm ( ), 75400 ppm ( ), 18600 ppm ( ), 57800 ppm ( ); (H) 1200 ppm ( ), 3420 ppm ( ), 8910 ppm ( ), 39800 ppm ( ), 10600 ppm ( ), 20100 ppm ( ), 30800 ppm ( ), 38300 ppm ( ). Lines are fits of Equation 8 to the data. The experiments represented by colors (Figs. 2A–C) are identical with those in Figures 1D–1F. The inset in Figure 2H enlarges the concentration-time courses obtained in ET exposed CYP2E1 knockout mice.](image-url)
a reaction occurring within the porphyrin ring, are rather similar between the species. Those of $k_3$ are highest in rat and lowest in human livers. These findings might be caused by species differences in active site amino acid residues of CYP2E1 (Lewis et al., 1997). As a result, the partition ratio $k_3/k_4$ is several times smaller in liver microsomes of humans than of rodents. This might be interpreted as a hint that humans could be less sensitive to eventual toxic effects from ET via the formation of EO. The values of the presently determined partition ratios are in agreement with the common observed values of less than 300 in unconjugated terminal olefins (Correia and Ortiz de Montellano, 2005).

The values of $k_4$ in lung microsomes of mice and rats are five and eight times larger than in liver microsomes of both species. The high $k_4$ values are unlikely to result from a bias in the interpretation of the EO concentration-times curves in lung microsomes because these microsomes were stable and metabolically active during the exposure time as was tested using the model substance styrene (data not shown). At present, we could only speculate why $k_4$ of the ET metabolism is organ-specific.

Gas Uptake Studies with EO in Microsomal Incubations

Figure 4 shows the results of EO uptake experiments in pooled hepatic microsomes of mice (Fig. 4A), rats (Fig. 4B), or humans (Fig. 4C), in hepatic microsomes of two adult human individuals (Fig. 4D) and in pooled lung microsomes of mice (Fig. 4E) or rats (Fig. 4F). In order to assess the microsomal functionality, uptake studies were carried out using the homologous PO and the same native pooled microsomes of mouse and rat livers as were prepared for the EO experiments. The slopes of the curves depicting the hepatic microsomal elimination of PO were about 10 times steeper than those of the corresponding EO curves (Fig. 4A and 4B), thereby proving the microsomal EH activity. With PO, about the same hepatic microsomal elimination clearances ($\mu\text{mol/min/mg protein}$) were obtained as had been found earlier (mouse 4.4 and rat 9.9; Faller et al., 2001). In spite of this, the slopes of the curves showing the EO elimination from the gas phase of the vessels containing native hepatic or pulmonary rodent microsomes were not distinguishable from those representing the spontaneous hydrolysis of EO, which was determined in 15 experiments with heat-inactivated microsomes of the three species (Figs. 4A–F). In other words, no EH-catalyzed EO elimination was found. Also, no CYP-mediated EO elimination was detected as was assayed for in a rat liver microsomal incubation containing an NADPH-regenerating system. In pooled native human liver microsomes, however, the EO elimination was clearly faster than in the heat-inactivated ones. The concentration-dependent flattening of the slopes hints to saturation kinetics of the EO elimination (Fig. 4C). From the best fits to the data by means of Berkeley Madonna, a $V_{\text{max}}$ value of 14.35 $\mu\text{mol/min/mg protein}$ was derived. The $K_m$ value in the microsomal environment was 12.74 $\mu\text{mol/l}$, and the ratio of $V_{\text{max}}$ to $K_m$ was 1.13 $\mu\text{mol/min/mg protein}$. The maximum EO production rates in microsomal incubations with ET (Table 1) were calculated without considering the microsomal elimination of EO and the loss of EO by spontaneous hydrolysis. The resulting error is negligible for rodents. For humans, it does not exceed 10% of the maximum production rate.

The slopes of the EO concentration-time curves of liver microsomes prepared from the two adults (Fig. 4D) were almost identical with the one obtained in pooled human microsomes at 100 ppm (see Fig. 4C).

Gas Uptake Studies with EO in Cytosolic Incubations

Figure 5 depicts time courses of atmospheric EO concentrations in incubations of GSH- or DEM-containing native or heat-inactivated pooled hepatic cytosol from mice, rats, and
humans (Figs. 5A–C) or GSH- or DEM-containing native or heat-inactivated pooled pulmonary cytosol from mice and rats (Figs. 5D and 5E). The slopes of the EO-concentration-time courses in the GSH-containing incubations with native cytosol are clearly different from those with GSH-depleted heat-inactivated cytosol. It is meaningful to compare the species-specific EO elimination by GSH conjugation directly from the figure because all incubations contained the same amount of cytosolic protein. Obviously, the conjugation rates are highest in mouse and lowest in human cytosol. In the semilogarithmic plots, the elimination curves are parallel over the whole exposure ranges up to about 3000 ppm and do not show any hint to saturation kinetics. In spite of this, the conjugation reaction was catalyzed by GST as has to be concluded from the evidently flatter slopes of the curves obtained in heat-inactivated GSH-containing cytosolic EO incubations. They represent the sum of both the spontaneous EO hydrolysis and the nonenzymatic conjugation of EO with GSH. The elimination rate constant resulting from the nonenzymatic conjugation reaction only was calculated to be $3.12 \times 10^{-3} \pm 3.0 \times 10^{-4} \text{min}^{-1}$ (means $\pm$ SE, $n = 8$; three measurements in heat-inactivated liver cytosol of human individuals included) corresponding to a mean half-life of 3.7 h. At the same GSH concentration of 15 mmol/l, Faller et al. (2001) reported a half-life of 1.7 h for the nonenzymatic GSH conjugation of the homologous PO.

The slopes of the curves obtained with heat-inactivated, DEM containing or with native, DEM-containing liver or lung cytosol did not differ significantly within and between each species. They were also not different from those obtained in Figure 4 with heat-inactivated microsomal EO incubations ($p < 0.05$; one-way ANOVA) representing the spontaneous hydrolysis. When calculating the parameters of enzyme-catalyzed reactions, nonenzymatic GSH conjugation and spontaneous hydrolysis were accounted for. The rate constant of the spontaneous EO hydrolysis was $9.3 \times 10^{-4} \pm 7 \times 10^{-5} \text{min}^{-1}$ (mean $\pm$ SE, $n = 36$). The corresponding mean half-life was 12.4 h. Faller et al. (2001) determined at pH 7.4 and 37°C a similar half-life of the spontaneous hydrolysis of PO (16 h) using also a MgCl$_2$ containing potassium phosphate buffer which has some catalytic activity due to its nucleophilic nature (Bundgaard and Hansen, 2001).
1981). Longer half-lives of between 77 and 99 h (EO) and of 88 h (PO) were estimated for the noncatalyzed aqueous hydrolysis at 37°C, pH 7 (reported in Faller et al., 2001).

The observed first-order decays in the EO concentrations signify that the rate of the GST-mediated EO elimination was directly proportional to the EO exposure concentration, the highest of which (3000 ppm) corresponds to an EO concentration of 9 mmol/l in the cytosolic suspension.

The EO-eliminating human GSTT1 (GST-theta; Föst et al., 1991, 1995) shows polymorphism, which may be genotoxically relevant (Hallier et al., 1993). Therefore, the GST-mediated conjugation of EO with GSH was additionally investigated in liver cytosol of six male and seven female individuals of between 16 and 78 years of age. The results are summarized in Table 3 together with those obtained in cytosol pooled from livers of 50 mice, 10 rats, or 11 humans or pooled from lungs of 150 mice or 50 rats. Only the ratio $V_{\text{max}}/K_m$ is given because of the first-order kinetics observed for the GST-catalyzed EO elimination. The ratio was obtained by dividing the product of the rate constant of the GST-dependent EO elimination with $V_{\text{d}}$ by the product of $K_{eqEO}$ with the actual amount of protein. The GST activity in pooled human liver cytosol is reasonably in the middle between the lowest individual GST activity (zero) and the highest one (2.87 μl/min/mg protein). The time span between death and autopsy did presumably not influence the GST activity because of the 13 hepatic cytosol samples obtained from non-Asian individuals, highest GST activities were found in the samples of a 22- and 42-year-old man, who died 6.5 and 29 h, respectively, prior to autopsy. Two samples (15%) did not show any GST activity to EO. The percentage agrees with that of the GSTT1*0 carriers reported for non-Asians (between 10 and 25%; Bolt and Thier, 2006).

In Johanson and Filser (1993), it was discussed that the GST-catalyzed conjugation of GSH with an epoxide can be described by saturation kinetics according to a sequentially ordered ping-pong mechanism. For EO, the $K_m$ concentration in livers or lungs of mice and rats and in livers of humans can be concluded to be at least 9 mmol/l (equivalent to the highest EO exposure concentration of 3000 ppm in cytosolic incubations). The GST-catalyzed EO elimination rate is almost independent of the GSH concentration at > 1 mmol/l because the $K_m$ of GSH is only about 0.1 mmol/l (Csánády and Filser, 2007; Johanson and Filser, 1993). Taking into account a $K_m$ value of 9 mmol/l tissue for the GST substrate EO, $V_{\text{max}}$ values (nmol/min/mg protein) of 251, 47.7, and 10.3 are obtained for the GST-mediated GSH conjugation with EO in mouse, rat,
and human pooled liver cytosol, respectively. Corresponding values for mouse and rat lung cytosol are 56.9 and 16.0.

**DISCUSSION**

**Toxicokinetics of ET**

If one assumes ET to be completely biotransformed to EO in the first metabolic step, EO formation rates should equal ET elimination rates (i.e., \( |V_{\text{maxEO}}| = |V_{\text{maxET}}| \) and \( K_m \) for EO formation = \( K_m \) of ET elimination). In Table 4, maximum rates of ET elimination and atmospheric \( K_m \) concentrations, calculated for *in vivo* conditions from the *in vitro* results given in Table 1, are compared with corresponding parameters obtained *in vivo* from gas uptake studies. On average, the predicted maximum ET elimination rates are around three times higher than those observed *in vivo*. At first glance, these results seem to be inconsistent. If, however, one bears in mind the values of the suicide inactivation constant \( k_4 \) of the ET-metabolizing CYP2E1 (Table 2), one has to expect that the amount of hepatic CYP2E1, which is available for the ET oxidation, decreases in between 11 min (humans) and 18 min (mice) to about one third of the initial status at very high ET concentrations. In gas uptake studies with ET in rats (Bolt et al., 1984) or mice (Artati et al., 2009), this effect could not be recognized from the concentration-time courses of ET in the atmosphere because it occurs within the initial phase during which the gas enriches in the organism. Predicted \( K_m \) concentrations in the atmosphere are 3.8- to 7.4-fold higher than actually determined *in vivo*. However, the predicted species-specific ratio \( V_{\text{maxET}}/K_m \) does not differ by more than a factor of two from the corresponding ratio obtained *in vivo* (Table 4). This ratio, which reflects the clearance of metabolism, is a direct measure of the metabolic elimination of ET at low atmospheric ET concentrations at which the ET elimination rate is proportional to the exposure concentration. The agreement between the predicted and the *in vivo* obtained \( V_{\text{maxET}}/K_m \) values is striking. Because the *in vivo*-derived values of \( V_{\text{maxET}}/K_m \) were obviously too low—not reflecting the suicide inhibition—the corresponding *in vivo* \( K_m \) values had also to be too low in order to describe correctly the metabolic elimination at low ET concentrations. For the same reason, an extremely small value of 0.0005 mmol/l was used for the apparent Michaelis constant of the ET oxidation in the liver compartment of the physiological toxicokinetic ET model of Csánády et al. (2000).

**Table 3**

Activity of GST (\( V_{\text{max}}/K_m \)) in Mouse and Rat Hepatic and Pulmonary Cytosol and in Human Hepatic Cytosol with EO as Substrate (Mean ± SE, \( n = 4 \))

| Tissue     | Species | Gender | Age (years) | \( V_{\text{max}}/K_m \) (μl/min/mg protein) | Time prior to autopsy (h) |
|------------|---------|--------|-------------|---------------------------------------------|--------------------------|
| Liver      | Mouse   | Male   | 0.25        | 27.90 ± 2.87                                | 0                        |
| Liver      | Rat     | Male   | 0.25        | 5.30 ± 0.10                                 | 0                        |
| Liver      | Human   | Both   | 1.9-70      | 1.14 ± 0.32                                 | n.a.                     |
| Liver      | Human   | Female | 51          | 0.00 ± 0.00                                 | n.a.                     |
| Liver      | Human   | Female | 54          | 0.00 ± 0.00                                 | 25                       |
| Liver      | Human   | Male   | 36          | 0.50 ± 0.25                                 | 23                       |
| Liver      | Human   | Male   | 63          | 0.54 ± 0.29                                 | n.a.                     |
| Liver      | Human   | Male   | 16          | 0.75 ± 0.26                                 | 24                       |
| Liver      | Human   | Female | 75          | 0.81 ± 0.34                                 | 0<sup>a</sup>            |
| Liver      | Human   | Female | 78          | 1.21 ± 0.16                                 | n.a.                     |
| Liver      | Human   | Female | 42          | 1.36 ± 0.18                                 | n.a.                     |
| Liver      | Human   | Male   | 58          | 1.49 ± 0.32                                 | 17                       |
| Liver      | Human   | Female | 53          | 1.62 ± 0.32                                 | n.a.                     |
| Liver      | Human   | Female | 48          | 1.76 ± 0.46                                 | n.a.                     |
| Liver      | Human   | Male   | 42          | 1.82 ± 0.51                                 | 29                       |
| Liver      | Human   | Male   | 22          | 2.87 ± 0.57                                 | 6.5                      |
| Lung       | Mouse   | Male   | 0.25        | 6.32 ± 0.52                                 | 0                        |
| Lung       | Rat     | Male   | 0.25        | 1.78 ± 0.33                                 | 0                        |

Note. n.a., not available.

<sup>a</sup>Pooled from livers of 50 mice or 10 rats.
<sup>b</sup>Pooled from livers of five male and six female humans.
<sup>c</sup>Pooled from lungs of 150 mice or 50 rats.
<sup>d</sup>Liver sample collected during transplantation.
concentrations of about 600 ppm. At exposure concentrations of about 1000 ppm, the concentration–time courses flattened with increasing exposure time (Csányi et al., 2000). Depletion of GSH was most probably the cause for this effect considering that McKelvey and Zemaitis (1986) observed a concentration-dependent decrease of GSH in Fischer rats exposed for 4 h to constant EO concentrations of 100, 600, or 1200 ppm. At the highest EO concentration, GSH levels in the livers decreased to less than 20% of the control value. In B6C3F1 mice exposed for 4 h to constant EO concentrations of up to 200 ppm, EO blood levels were linearly related to the exposure concentration (Brown et al., 1998). At exposure concentrations of 300 and 400 ppm, however, the EO blood levels increased overproportional when related to the exposure concentration. The authors linked these findings to a depletion of GSH. At EO concentrations of 300 and 400 ppm, GSH levels in the livers declined to less than 22% of the control value (Brown et al., 1998).

In contrast to mice and rats, EH-catalyzed hydrolysis of EO is expected to represent at least one fourth of the overall EO metabolism in humans. This ratio is obtained taking into account a protein content of 30 mg/g liver in microsomes and of 95 mg/g liver in cytosol (Kreuzer et al., 1991) as well as a \( V_{\text{max}} \) to \( K_m \) ratio of the EH-catalyzed elimination in microsomes of 1.13 \( \mu \text{l/min/mg protein} \) and of the GST-catalyzed conjugation reaction in pooled cytosol of 1.14 \( \mu \text{l/min/mg protein} \). Neglecting extrahepatic metabolism, one can therefore expect for EO-exposed humans of the GSTT1*0/0 genotype the EO tissue burden to be at most four times higher than in equally exposed humans carrying the active GSTT1 form. This conclusion of a quantitatively relevant role of EH on the elimination of EO in humans is supported by the results of a thoroughly conducted study on smokers. Hydroxyethyl adduct levels to the N-terminal valine of hemoglobin were on average 1.61 times higher in GSTT1-null individuals than in GSTT1 carriers (Fennell et al., 2000).
increase continuously until reaching a plateau that is simply characterized by the equilibrium between EO formation (from ET) and EO elimination.

Outlook

A presently favored procedure to evaluate human health risks from chemicals uses toxicokinetic and toxicodynamic chemical-specific adjustment factors (CSAFs) in dose-response assessment (see, for example, Meek et al., 2002). Usually, the CSAF for the toxicokinetic uncertainty between species and that for the toxicokinetic uncertainty between human individuals is given a maximum number of $10^{0.6}$ and of $10^{0.5}$, respectively. In our laboratory, we are just finishing studies on the EO burden in ET-exposed mice, rats, and human individuals. The results together with the robust toxicokinetic parameters obtained in the present work will be used to improve a physiological toxicokinetic model (Csánya et al., 2000) for ET and EO in these three species. Because of its strong interspecies and interindividual data basis, we expect model predictions to be reliable enough for reducing the toxicokinetic CSAFs to the minimum.

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REFERENCES

Andersen, M. E., Gargas, M. L., Jones, R. A., and Jenkins, L. J., Jr. (1980). Determination of the kinetic constants for metabolism of inhaled toxicants in vivo using gas uptake measurements. Toxicol. Appl. Pharmacol. 54, 100–116.

Artai, A., Kessler, W., Richter, N., Pütz, C., and Filser, J. G. (2009). Toxicokinetics of inhaled ethylene and ethylene oxide in mice. Naunyn-Schmiedeberg’s Arch. Pharmacol. 379(Suppl. 1), 64.

Bleecker, A. B., and Kende, H. (2000). Ethylene: a gaseous signal molecule in plants. Annu. Rev. Cell Dev. Biol. 16, 1–18.

Bolt, H. M., and Filser, J. G. (1984). Olefinic hydrocarbons: a first risk estimate for ethene. Toxicol. Pathol. 12, 101–105.

Bolt, H. M., Filser, J. G., and Störmer, F. (1984). Inhalation pharmacokinetics based on gas uptake studies V. Comparative pharmacokinetics of ethylene and 1,3-butadiene in rats. Arch. Toxicol. 55, 213–218.

Bolt, H. M., and Thier, R. (2006). Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. Curr. Drug Metab. 7, 613–628.

Boogaard, P. J., ROCchi, P. S. J., and van Sittart, N. J. (1999). Biomonitoring of exposure to ethylene oxide and propylene oxide by determination of hemoglobin adducts: correlations between airborne exposure and adduct levels. Int. Arch. Occup. Environ. Health 72, 142–150.

Boyland, E., and Chasseaud, L. F. (1970). The effect of some carbonyl compounds on rat liver glutathione levels. Biochem. Pharmacol. 19, 1526–1528.

Briggs, G. E., and Haldane, J. B. S. (1925). A note on the kinetics of enzyme action. Biochem. J. 19, 338–339.

Brown, C. D., Asgharian, B., Turner, M. J., and Fennell, T. R. (1998). Ethylene oxide dosimetry in the mouse. Toxicol. Appl. Pharmacol. 148, 215–221.

Brown, C. D., Wong, B. A., and Fennell, T. R. (1996). In vivo and in vitro kinetics of ethylene oxide metabolism in rats and mice. Toxicol. Appl. Pharmacol. 136, 8–19.

Brugnone, F., Perbellini, L., Faccini, G., and Pasini, F. (1985). Concentration of ethylene oxide in the alveolar air of occupationally exposed workers. Am. J. Ind. Med. 8, 67–72.

Brugnone, F., Perbellini, L., Faccini, G. B., Pasini, F., Bartolucci, G. B., and DeRosa, E. (1986). Ethylene oxide exposure. Biological monitoring by analysis of alveolar air and blood. Int. Arch. Occup. Environ. Health 58, 105–112.

Bundgaard, H., and Hansen, J. (1981). Nucleophilic phosphate-catalyzed degradation of penicillins: demonstration of a penicilloyl phosphate intermediate and transformation of ampicillin to a piperazinedione. Int. J. Pharm. 9, 273–283.

Collman, J. P., Hampton, P. D., and Brauman, J. I. (1986). Stereochemical and mechanistic studies of the “suicide” event in biomimetic P-450 olefin epoxidation. J. Am. Chem. Soc. 108, 7862–7864.

Collman, J. P., Hampton, P. D., and Brauman, J. I. (1990). Suicide inactivation of cytochrome P-450 model compounds by terminal olefins. 1. A mechanistic study of heme N-alkylation and epoxidation. J. Am. Chem. Soc. 112, 2977–2986.

Correia, M. A., and Ortiz de Montellano, P. R. (2005). Inhibition of cytochrome P450 enzymes. In Cytochrome P450: Structure, Mechanism, and Biochemistry, 3rd ed. (P. R. Ortiz de Montellano, Ed.), pp. 247–322. Kluwer Academic/Plenum Publishers, New York, NY.

Csánya, G. A., Denk, B., Pütz, C., Kreuzer, P. E., Kessler, W., Baur, C., Gargas, M. L., and Filser, J. G. (2000). A physiological toxicokinetic model for exogenous and endogenous ethylene and ethylene oxide in rat, mouse, and human: formation of 2-hydroxyethyl adducts with hemoglobin and DNA. Toxicol. Appl. Pharmacol. 165, 1–26.

Csánya, G. A., and Filser, J. G. (2007). A physiological toxicokinetic model for inhaled propylene oxide in rat and human with special emphasis on the nose. Toxicol. Sci. 95, 37–62.

Dellarco, V. L., Generoso, W. M., Sega, G. A., Fowle, J. R., III, and Jacobson-Kram, D. (1990). Review of the mutagenicity of ethylene oxide. Environ. Mol. Mutagen. 16, 85–103.

Dunkelberg, H. (1981). Carcinogenic activity of ethylene oxide and its reaction products 2-chloroethanol, 2-bromoethanol, ethylene glycol and diethylene glycol. I. Carcinogenicity of ethylene oxide in comparison with 1,2-propylene oxide after subcutaneous administration in mice. Zentralbl. Bakteriol. Mikrobiol. Hyg. B. 174, 383–404.

Dunkelberg, H. (1982). Carcinogenicity of ethylene oxide and 1,2-propylene oxide upon intragastric administration to rats. Br. J. Cancer 46, 924–933.

Ehrenberg, L., Osterman-Golkar, S., Segerbäck, D., Svensson, K., and Calleman, C. J. (1977). Evaluation of genetic risks of alkylating agents. III. Alkylation of haemoglobin after metabolic conversion of ethene to ethene oxide in vivo. Mutat. Res. 45, 175–184.

Eide, I., Hagemann, R., Zahlen, K., Tareke, E., Törnqvist, M., Kumar, R., Vodicka, P., and Hemminki, K. (1995). Uptake, distribution, and formation
of hemoglobin and DNA adducts after inhalation of C2–C8 1-alkenes (olefins) in the rat. Carcinogenesis 16, 1603–1609.

Faller, T. H., Csanády, G. A., Kreuzer, P. E., Baur, C. M., and Filser, J. G. (2001). Kinetics of propylene oxide metabolism in microsomes and cytosol of different organs from mouse, rat, and humans. Toxicol. Appl. Pharmacol. 172, 62–74.

Fennell, T. R., and Brown, C. D. (2001). A physiologically based pharmacokinetic model for ethylene oxide in mouse, rat, and human. Toxicol. Appl. Pharmacol. 173, 161–175.

Filser, J. G., and Bolt, H. M. (1984). Inhalation pharmacokinetics based on gas uptake studies. VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats. Arch. Toxicol. 55, 219–223.

Filser, J. G., Denk, B., Törnqvist, M., Kessler, W., and Ehrenberg, L. (1992). Pharmacokinetics of ethylene in man; body burden with ethylene oxide and hydroxyethylolation of hemoglobin due to endogenous and environmental ethylene. Arch. Toxicol. 66, 157–163.

Föst, U., Hallier, E., Ottenwaler, H., Haller, H. M., and Peter, H. (1991). Distribution of ethylene oxide in human blood and its implications for biomonitoring. Hum. Exp. Toxicol. 10, 25–31.

Föst, U., Törnqvist, M., Leutbecher, M., Granath, F., Hallier, E., and Ehrenberg, L. (1995). Effects of variation in detoxification rate on dose monitoring through adducts. Hum. Exp. Toxicol. 14, 201–203.

Garman, R. H., Snellings, W. M., and Maronpot, R. R. (1984). Brain tumors in F344 rats associated with chronic inhalation exposure to ethylene oxide. Neurotoxicology 6, 117–137.

Gornall, A. G., Bardawill, C. J., and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177, 751–766.

Hallier, E., Langhof, T., Dannappel, D., Leutbecher, M., Schröder, K., Goergens, H. W., Müller, A., and Bolt, H. M. (1993). Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. Arch. Toxicol. 67, 173–178.

Hamm, T. E., Guest, D., and Dent, J. G. (1984). Chronic toxicity and oncogenicity bioassay of inhaled ethylene in Fischer-344 rats. Fundam. Appl. Toxicol. 4, 473–478.

Huang, C. C. J., Wu, C. F., Shih, W. C., Chen, M. F., Chen, C. Y., Chien, Y. C., Liou, S. H., Chiang, S. Y., and Wu, K. Y. (2011). Comparative analysis of urinary N7-(2-hydroxyethyl)guanine for ethylene oxide- and non-exposed workers. Toxicol. Lett. 202, 237–243.

IARC. (1994a). Ethylene. Some Industrial Chemicals, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 60, pp. 45–71. WHO, IARC Press, Lyon, France.

IARC. (1994b). Ethylene oxide. Some Industrial Chemicals, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 60, pp. 73–159. WHO, IARC Press, Lyon, France.

IARC. (1994c). Ethylene oxide. Some Industrial Chemicals, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 60, pp. 118–119. WHO, IARC Press, Lyon, France.

Johanson, G., and Filser, J. G. (1993). A physiologically based pharmacokinetic model for butadiene and its metabolite butadiene monoxide in rat and mouse and its significance for risk extrapolation. Arch. Toxicol. 67, 151–163.

Kolman, A., Chovanec, M., and Osterman-Golkar, S. (2002). Genotoxic effects of ethylene oxide, propylene oxide and epichlorohydrin in humans: update review (1990-2001). Mutat. Res. 512, 173–194.

Kreuzer, P. E., Kessler, W., Welter, H. F., Baur, C., and Filser, J. G. (1991). Enzyme specific kinetics of 1,2-epoxybutene-3 in microsomes and cytosol from livers of mouse, rat, and man. Arch. Toxicol. 65, 59–67.

Krishnan, K., Gargas, M. L., Fennell, T. R., and Andersen, M. E. (1992). A physiologically based description of ethylene oxide dosimetry in the rat. Toxicol. Ind. Health 8, 121–140.

Lawrence, G. D., and Cohen, G. (1985). In vivo production of ethylene from 2-keto-4-methylthiobutyrate in mice. Biochem. Pharmacol. 34, 3231–3236.

Lewis, D. F. V., Bird, M. G., and Parke, D. V. (1997). Molecular modeling of CYP2E1 enzymes from rat, mouse and man: an explanation for species differences in butadine metabolism and potential carcinogenicity, and rationalization of CYP2E2 substrate specificity. Toxicology 118, 93–113.

Li, Q., Csanády, G. A., Artati, A., Khan, M. D., Rietber, M. B., and Filser, J. G. (2008). Ethylene inhibits its own metabolism in liver and lung microsomes from male Fischer 344 rats and B6C3F1 mice. Naunyn-Schmiedeberg’s Arch. Pharmacol. 377(Suppl. 1), 70.

Li, Q., Csanády, G. A., Klein, D., and Filser, J. G. (2009). Metabolism of ethylene oxide in microsomes and cytosol from livers and lungs of B6C3F1 mice, Fischer 344 rats, and humans. Naunyn-Schmiedeberg’s Arch. Pharmacol. 379(Suppl. 1), 64.

Maples, K. R., and Dahl, A. R. (1993). Levels of epoxides in blood during inhalation of alkenes and alkenes oxides. Inhal. Toxicol. 5, 43–54.

McKelvey, J. A., and Zemaitis, M. A. (1986). The effects of ethylene oxide (EO) exposure on tissue glutathione levels in rats and mice. Drug Chem. Toxicol. 9, 51–66.

Meek, M. E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. D., and Vu, V. (2002). Guidelines for application of chemical-specific adjustment factors in dose/concentration–response assessment. Toxicology 181–182, 115–120.

National Toxicology Program. (1987). Toxicology and carcinogenesis studies of ethylene oxide (CAS No. 75-21-8) in B6C3F1 mice (inhalation studies). Natl. Toxicol. Program Tech. Rep. Ser. 326, 1–114.

Ortiz de Montellano, P. R., and Correa, M. A. (1983). Suicidal destruction of cytochrome P-450 during oxidative drug metabolism. Ann. Rev. Pharmacol. Toxicol. 23, 481–503.

Ortiz de Montellano, P. R., and Mico, B. A. (1980). Destruction of cytochrome P-450 by ethylene and other olefins. Mol. Pharmacol. 18, 128–135.

Osterman-Golkar, S., and Ehrenberg, L. (1982). Covalent binding of reactive intermediates to hemoglobin as an approach for determining the metabolic activation of chemicals—ethylene. Drug Metab. Rev. 13, 647–660.

Rusyn, I., Asakura, S., Li, Y., Kosyk, O., Koc, H., Nakanura, J., Upton, P. B., and Swenberg, J. A. (2005). Effects of ethylene oxide and ethylene inhalation on DNA adducts, apurinic/apyrimidinic sites and expression of base excision DNA repair genes in rat brain, spleen, and liver. DNA Repair (Amst.) 4, 1099–1110.

Sachs, L. (1973). Angewandte Statistik—Planung und Auswertung, Methoden und Modelle, 4. Aufl. Springer-Verlag, Berlin.

Schmiedel, G., Filser, J. G., and Bolt, H. M. (1983). Rat liver microsomal transformation of ethene to oxirane in vitro. Toxicol. Lett. 19, 293–297.

Seaton, M. J., Follansbee, M. H., and Bond, J. A. (1995). Oxidation of 1,2-epoxy-3-butene to 1,2,3,4-diepoxybutane by cDNA-expressed human cytochromes P450 2E1 and 3A4 and human, mouse and rat liver microsomes. Carcinogenesis 16, 2287–2293.

Sega, G. A., Brimer, P. A., and Generoso, E. E. (1991). Ethylene oxide inhalation at different exposure-rates affects binding levels in mouse germ cells and hemoglobin. Possible explanation for the effect. Mutat. Res. 249, 339–349.

Segerbäck, D. (1983). Alkylation of DNA and hemoglobin in the mouse following exposure to ethene and ethene oxide. Chem. Biol. Interact. 45, 139–151.

Snellings, W. M., Weil, C. S., and Maronpot, R. R. (1984). A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. Toxicol. Appl. Pharmacol. 75, 105–117.
Törnqvist, M. Å., Almberg, J. G., Bergmark, E. N., Nilsson, S., and Osterman-Golkar, S. M. (1989). Ethylene oxide doses in ethene-exposed fruit store workers. *Scand. J. Work Environ. Health* **15**, 436–438.

Victorin, K., and Ståhlberg, M. (1988). A method for studying the mutagenicity of some gaseous compounds in Salmonella typhimurium. *Environ. Mol. Mutagen.* **11**, 65–77.

Walker, V. E., Wu, K. Y., Upton, P. B., Ranasinghe, A., Scheller, N., Cho, M. H., Vergnes, J. S., Skopek, T. R., and Swenberg, J. A. (2000). Biomarkers of exposure and effect as indicators of potential carcinogenic risk arising from in vivo metabolism of ethylene to ethylene oxide. *Carcinogenesis* **21**, 1661–1669.

Wu, K. Y., Chiang, S. Y., Shih, W. C., Huang, C. C. J., Chen, M. F., and Swenberg, J. A. (2011). The application of mass spectrometry in molecular dosimetry: ethylene oxide as an example. *Mass Spectrom. Rev.* Doi: 10.1002/mas.20299.

Zimmermann, H., and Walzl, R. (2007). In *Ethylene. Ullman’s Encyclopedia of Industrial Chemistry*. Wiley-VCH Verlag GmbH, Weinheim, Germany.