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Kinetic modeling of β-chloroprene metabolism: Probabilistic in vitro–in vivo extrapolation of metabolism in the lung, liver and kidneys of mice, rats and humans

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ABSTRACT

β-Chloroprene (chloroprene) is carcinogenic in inhalation bioassays with B6C3F1 mice and Fischer rats, but the potential effects in humans have not been adequately characterized. In order to provide a better basis for evaluating chloroprene exposures and potential effects in humans, we have explored species and tissue differences in chloroprene metabolism. This study implemented an in vitro–in vivo extrapolation (IVIVE) approach to parameterize a physiologically based pharmacokinetic (PBPK) model for chloroprene and evaluate the influence of species and gender differences in metabolism on target tissue dosimetry. Chloroprene metabolism was determined in vitro using liver, lung and kidney microsomes from male or female mice, rats, and humans. A two compartment PK model was used to estimate metabolism parameters for chloroprene in an in vitro closed vial system, which were then extrapolated to the whole body PBPK model. Two different strategies were used to estimate parameters for the oxidative metabolism of chloroprene: a deterministic point-estimation using the Nelder-Mead nonlinear optimization algorithm and probabilistic Bayesian analysis using the Markov Chain Monte Carlo technique. Target tissue dosimetry (average amount of chloroprene metabolized in lung per day) was simulated with the PBPK model using the in vitro-based metabolism parameters. The model-predicted target tissue dosimetry, as a surrogate for a risk estimate, was similar between the two approaches; however, the latter approach provided a measure of uncertainty in the metabolism parameters and the opportunity to evaluate the impact of that uncertainty on predicted risk estimates.

1. Introduction

β-Chloroprene (chloroprene, 2-chloro-1,3-butadiene, CAS 126–99-8) is a volatile colorless liquid used to manufacture polychloroprene, a synthetic rubber (Lynch, 2001a). Occupational exposure can occur during monomer synthesis, shipping, and polymerization processes, and inhalation is the only significant route of exposure (Lynch, 2001b). The health effects in humans have focused on the potential carcinogenicity of chloroprene in the liver, lung and lymphohematopoietic systems (reviewed by Bukowski, 2009). Although epidemiological findings do not support a substantial link between chloroprene exposure and increased cancer mortality (Marsh et al., 2007), it is still important to understand species differences.

Extensive animal studies have been performed to understand possible adverse health effects of chloroprene in humans including acute, sub-chronic, and chronic toxicity studies (Melnick and Sills, 2001; Valentine and Himmelstein, 2001; Pagan, 2007). The most toxicologically significant finding was chloroprene-induced tumorigenicity in F344/N rats and B6C3F1 mice exposed to ⩽80 ppm for 2 years (Melnick et al., 1996, 1999; NTP, 1998). Tumors in Fischer rats included the lung, oral cavity, thyroid gland, kidney, and mammary gland. Mouse tumors were in the lung, circulatory system, Harderian gland, forestomach, kidney, mammary gland, skin, mesentery, Zymbal gland, and liver. In contrast, no tumors occurred in Syrian hamsters and only a weak response in mammary tissue in female Wistar rats (Trochimowicz et al., 1998) indicating species and gender differences in tumorigenesis in rodents.

Chloroprene is oxidized by cytochrome P450 enzymes (Cottrell et al., 2001; Himmelstein et al., 2001b). One reactive intermediate formed is the epoxide (1-chloroethenyl) oxirane which was mutagenic in the Ames assay, but not clastogenic at cytotoxic concentrations in vitro (Himmelstein et al., 2001a). This epoxide also shows reactivity with DNA in vitro and is a potential cross-linking agent (Munter et al., 2002; Wadugu et al., 2010). The reactive metabolites of chloroprene are likely to contribute to the tumorigenicity of chloroprene seen in animal studies. Given the important role of metabolic activation for toxicity, it is important to understand chloroprene metabolism to assess its potential health effects. To this end, a physiologically based pharmacokinetic (PBPK) model

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was developed for chloroprene based on in vitro metabolism data. Previous PBPK models for chloroprene in male rodents and humans (Himmelstein et al., 2004a, b) suggested significant differences in chloroprene metabolism among liver and lung and among different species. Intrinsic clearance of chloroprene metabolism in hepatic microsomes was two fold higher in mouse compared to human, while clearance in lung microsomes was forty times higher in mouse than either rat or human. With the application of PBPK modeling, the species differences in metabolism (amount chloroprene metabolized per gram lung tissue) were shown to be the underlying mechanism for the difference in lung tumor incidence among different species (Himmelstein et al., 2004b).

Here we extend the chloroprene PBPK model using additional data for chloroprene metabolism from different species and genders. The models evaluated the role of metabolism differences in species- and sex-dependent tissue dose metrics (a potential marker for tumorigenesis). A key objective of this effort was to develop a probabilistic parameter estimation approach; so that the impact of uncertainty in the metabolic parameter estimates on risk predictions can be as illustrated in Fig. 1. While previous studies used deterministic approaches to estimate metabolism parameters, we estimated these parameter values by two different methods: deterministic point-estimation and a probabilistic (Bayesian) approach.

2. Materials and methods

2.1. In vitro microsomal experiments

2.1.1. Chemicals

β-Chloroprene (>99%) containing phenothiazine and *N*-nitrosodiphenylamine inhibitors was supplied by DuPont Performance Elastomers,LLC (LaPlace, LA). The inhibitors were removed as previously described (Himmelstein et al., 2001b). The purified chloroprene was stable at <−70 °C under nitrogen headspace atmosphere. For metabolism experiments, vapor concentrations were prepared by adding the liquid test substance to Tedlar® bags (SKC Inc., Eighty Four, Pennsylvania, USA) containing a known volume of room air. Further gas phase dilutions were made for calibration or exposure purposes. Gas tight syringes were used for the gas transfers.

2.1.2. Source of microsomes and cytosol

Fischer rat (F344/DuCrl) and mice (B6C3F1/Crl) were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The species and strains were selected to match those used for inhalation toxicity testing by the National Toxicology Program (NTP, 1998). The animals were acclimated for at least 7 days prior to use. A total of 15 female rats and 50 female mice were used for preparation of the liver and lung microsomes. A total of 15 rats/sex and 30 female mice/sex were used for preparation of kidney microsomes. Human kidney microsomes were purchased from Xenotech (H0610.R, Lot No. 0810236, Lenexa, Kansas, USA). Microsomes were prepared by differential centrifugation and pooled as described by Himmelstein et al. (2004a). The use of pooled tissue microsomes mitigates issues of inter-animal biological variability, yet supports the analysis on species and gender differences. Further details on the microsomal preparation are given in the Supplement data A.1

2.1.3. Microsomal oxidation of chloroprene

The time course of total chloroprene disappearance was measured in three tissues: liver and lung microsomes for female rodent; kidney microsomes of rodents for both genders and human kidney microsomes. Data on the (1-chloroethenyl) oxirane formation was not collected in the current experiments because of the focus on total chloroprene metabolism as a dosimetric for dose–response modeling (Himmelstein et al., 2004b). After pre-incubation (37 °C for 5 min), an equal volume of vial headspace was removed from the vial and replaced with known concentrations of chloroprene vapor. The vial was equilibrated for approximately 10 min and reactions were started by the addition of microsomal protein and $NADP^+$ (0.53 mM). Microsomal protein concentrations were established from previous work (liver and lung) or experimentally for kidney microsomes. Definitive experiments used protein concentrations that ranged from 1–3 mg/mL. Control incubations were performed without $NADP^+$ or with $NADP^+$ and heat-inactivated microsomes. Samples (200 μL) were injected on the GC using a robotic x-y-z programmable multipurpose sampler (MPS2, Gerstel US, Baltimore, Maryland, USA) and were analyzed at 12 min intervals for up to 1 h.

2.2. In Vitro Kinetic Model Description

A 2-compartment PK model modified from Himmelstein et al. (2004a) was used to describe the time-concentration measurements of chloroprene in the headspace in the closed vial system. The microsomal oxidation of chloroprene in tissues (liver, lung and kidney) was by saturable kinetics, with the exception of rat and human lung where a first-order process was used. In addition to microsomal metabolism, the current model included the loss of chloroprene from the headspace to describe the decline of headspace concentration of chloroprene observed in the control dataset. The background loss rates were modeled as a first order process. Estimates of the first order background loss rates were based on eight sets of control data (the complete female data set plus the male kidney dataset). The in vitro experimental background loss rate was assumed to be independent of gender, tissue, and dose. The same PK model was used to estimate the background loss rate by setting the parameter values for the microsomal process to zero. To estimate the gender-specific variability of the kinetic parameters, male tissue

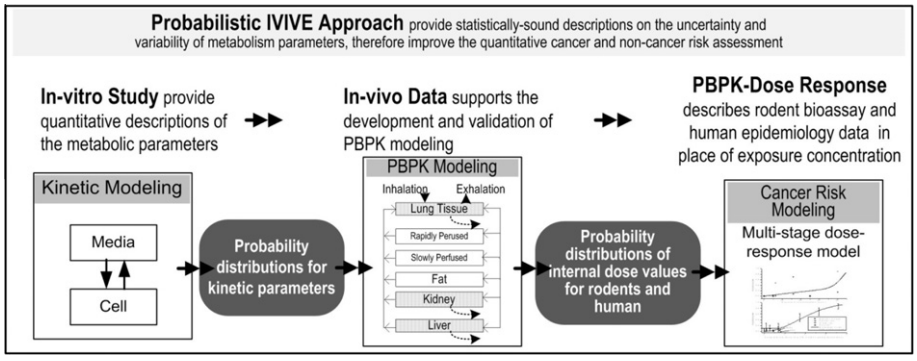

Fig. 1. Illustration of probabilistic approach.