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Physiologically based pharmacokinetic modeling of ethyl acetate and ethanol in rodents and humans

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ABSTRACT

A physiologically based pharmacokinetic (PBPK) model was developed and applied to a metabolic series approach for the ethyl series (i.e., ethyl acetate, ethanol, acetaldehyde, and acetate). This approach bases toxicity information on dosimetry analyses for metabolically linked compounds using pharmacokinetic data for each compound and toxicity data for parent or individual compounds. *In vivo* pharmacokinetic studies of ethyl acetate and ethanol were conducted in rats following IV and inhalation exposure. Regardless of route, ethyl acetate was rapidly converted to ethanol. Blood concentrations of ethyl acetate and ethanol following both IV bolus and infusion suggested linear kinetics across blood concentrations from 0.1 to 10 mM ethyl acetate and 0.01–0.8 mM ethanol. Metabolic parameters were optimized and evaluated based on available pharmacokinetic data. The respiratory bioavailability of ethyl acetate and ethanol were estimated from closed chamber inhalation studies and measured ventilation rates. The resulting ethyl series model successfully reproduces blood ethyl acetate and ethanol kinetics following IV administration and inhalation exposure in rats, and blood ethanol kinetics following inhalation exposure to ethanol in humans. The extrapolated human model was used to derive human equivalent concentrations for the occupational setting of 257–2120 ppm ethyl acetate and 72–517 ppm ethyl acetate for continuous exposure, corresponding to rat LOAELs of 350 and 1500 ppm.

1. Introduction

The ethyl series, comprised of ethyl acetate and its metabolites ethanol, acetaldehyde, and acetate, is a family of metabolically related compounds with a variety of industrial applications as solvents, substrates, and intermediates. Acetaldehyde and acetate are additionally endogenous by-products of normal metabolism (Jones, 1995; Richards et al., 1976). Human exposure to the parent compound ethyl acetate leads to systemic exposure to its three sequential metabolites; exposure to ethanol leads to systemic exposure to its two sequential metabolites, and so forth.

The metabolic series approach (previously, the "family approach") is a methodology that leverages the metabolic relationships between related compounds or series members (Barton et al., 2000). This method can facilitate efficient toxicity testing and the development of acceptable exposure levels, and has been used to assess the butyl series (n-butyl acetate, n-butanol, n-butyraldehyde, and n-butyric acid) (Teeguarden et al., 2005). Dosimetry-based analyses can be used to develop toxicity information using pharmacokinetic data for each related compound and toxicity data for the parent compound, because this data necessarily includes exposure to the downstream metabolites (Barton et al., 2000).

Physiologically based pharmacokinetic (PBPK) models are mathematical descriptions of the physiology and biochemistry that determine chemical disposition within an exposed organism. Based on their robust, quantitative descriptions of the factors affecting pharmacokinetics, PBPK models enable extrapolation between different routes of exposure, dose levels, and organisms of interest. Because of this utility, such models are being used increasingly to develop human exposure limits and provide context to interpretation of laboratory studies on chemical toxicity. The family approach is one such application (Barton et al., 2000; Teeguarden et al., 2005).

For ethyl acetate, the primary human exposure route of concern for the chemical industrial setting is inhalation, while for ethanol, oral ingestion is a primary route of concern, as is the generation of ethanol secondary to ethyl acetate exposure. There are a number of

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PBPK models previously developed to describe the pharmacokinetics of ethanol in rodents and humans (Dumas-Campagna et al., 2014; Martin et al., 2012; Pastino and Conolly, 2000; Plawecki et al., 2008; Umulis et al., 2005), but currently no models describing ethyl acetate.

Here we present data describing IV and inhalation exposure to ethyl acetate and resulting pharmacokinetics of ethyl acetate and ethanol in rat blood, as well as a PBPK model developed for the two exogenous members of the ethyl series, ethyl acetate and ethanol. Further, we have used the extrapolated human model to estimate inhalation route ethyl acetate human equivalent concentrations (HECs) based on rat inhalation route lowest observed adverse effect levels (LOAELs) of 350 ppm for systemic toxicity, corresponding to reduced body weight, and 1500 ppm for neurotoxicity, corresponding to transient reduction in motor activity (Christoph et al., 2003).

2. Materials and methods

2.1. Pharmacokinetic studies

Because intravenous (IV) studies bypass absorption processes in the lung, gastrointestinal tract, and liver, they are generally considered preferable for validation of metabolic rate constants measured in *in vitro* studies, or for optimization of such parameters in lieu of measured values. Therefore, IV studies were conducted to facilitate initial model development. Dose rates or exposures for all studies were selected by balancing limitations imposed by endogenous ethanol blood concentrations and the goal of calibrating the model for the range of application: 500–1500 ppm ethyl acetate. Model performance was evaluated against pharmacokinetic data from inhalation exposures, as this is a primary exposure route of concern.

Data from three unpublished reports are used for model development and evaluation (Corley et al., 2000; Deisinger and English, 1998, 2003), alongside data collected by the authors. All studies involving animals were IACUC approved and were performed in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH, 2011). Experimental details not provided here may be found in the reports, which are available by request from the sponsor (American Chemistry Council, 1300 Wilson Blvd., Arlington, VA 22209).

2.1.1. Rat IV bolus and IV infusion studies (Deisinger and English, 1998, 2003)

Male Sprague–Dawley rats (280–330 g, Charles River Laboratories, Wilmington, MA) were surgically prepared by the vendor with femoral and jugular vein cannulae. Cannulated animals were housed in polycarbonate “shoe box” rodent cages with wood chip bedding. Room lighting followed a 12 h light/dark cycle, and room temperatures and relative humidities were maintained in the range og 19.9–24.5 °C and 40–71.1%, respectively. Animals were allowed water and certified rodent diet (PMI, Inc. Rodent 5002 Pellet) *ad libitum*.

Pilot studies were conducted previously to identify acutely nontoxic IV dose levels for ethyl acetate and ethanol, verify the efficacy of the analytical methods, and establish sampling times for these studies. Pilot and definitive IV bolus studies were conducted using the same protocol. Groups of rats (4/group) were administered a bolus injection of ethyl acetate dissolved in a saline carrier (0.9% saline; plus or minus 1% Tween 20 vehicle) at 10 mg/kg and 100 mg/kg via the femoral vein cannula. In addition, ethyl acetate and ethanol were delivered by IV infusion at 10 and 50 mg/kg (ethyl acetate) or 15 and 50 mg/kg (ethanol) to 3 or 4 male SD rats in each group over a 15 min infusion using a syringe pump (Kd Scientific Model 100, Boston, MA) via the femoral vein cannula.

Sample collection and processing were the same for bolus and infusion experiments. Aliquots of blood (100 μL) were obtained by serial sampling from the jugular vein cannula. The blood was immediately de-proteinized with two volumes of acetonitrile containing a known amount of ethyl propionate internal standard. The precipitated protein was removed by centrifugation, and the supernatant was assayed for ethyl acetate and ethanol by gas chromatography-mass spectrometry with selected ion monitoring (GC–MS/SIM, Model 6890-5973N, Agilent Technologies, Santa Clara, CA). The injector was set to 160 °C, the transfer line to 150 °C. Injection volume was 1 μL (50:1 split ratio) using an Supelcowax 10 capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness, Supelco, Bellefonte, PA) with helium carrier gas at a constant pressure (5.9 psi). The gas chromatograph oven temperature program began at an initial 40 °C × 4 min, to 50 °C at 10 °C/min, to 120 °C at 40 °C/min, hold 1.5 min. Mass to charge ratios (m/z) for ethyl acetate, ethanol, and ethyl propionate were 43.1, 45.1, and 57.1, respectively.

2.1.2. Rat closed chamber exposure and blood kinetics

2.1.2.1. Animals and chamber. Male Sprague–Dawley rats (270–350 g, Hilltop Lab Animals, Scottsdale, PA) were surgically prepared by the vendor with jugular cannulae. Cannulated animals were housed in an American Association for the Accreditation of Laboratory Animal Care (AALAC) accredited facility with a 12 h light/dark cycle at 72 ± 3 °F and 50 ± 20% relative humidity. Deionized water and PMI 5002 Certified Rodent Diet (Animal Specialties, Inc., Hubbard, OR) were provided *ad libitum*.

A restrained, whole-body plethysmograph (designed and built at Battelle, Toxicology Northwest, Richland, WA) linked to a Buxco Biosystem XA Data Acquisition System (Buxco Electronics, Inc. Sharon, CT) was used for non-invasive ventilatory measurements on conscious rats. The animals were restrained in a constant-pressure plethysmograph with an attached pneumotachograph. A neck seal separated the head of the animal from the body chamber, and ventilation parameters were computed from flow measurements through the pneumotachograph from the body chamber (Fig. 1).

The tubular shaped plethysmograph chamber was modified to be positioned inside the 9.44 L gas uptake chamber so that respiratory parameters (tidal volume, respiratory rate, and minute

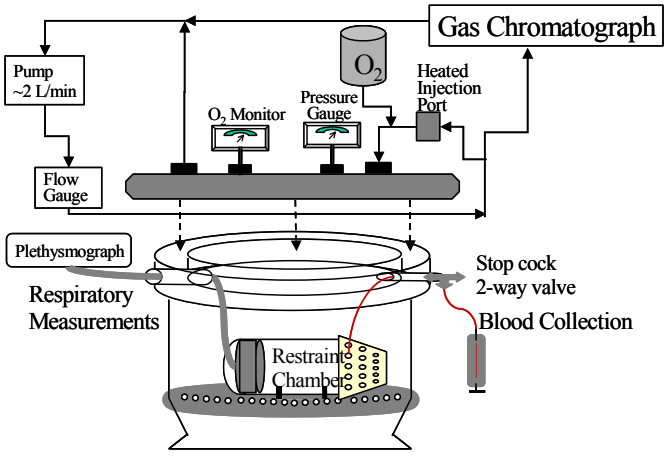

Fig. 1. Diagram of the gas uptake chamber described by Gargas et al. (1986) with modifications. The final chamber design utilized a heated injection port and inlet connecting tubing. A Plexiglas ring was added to place connections for the plethysmograph and to add a valve through which blood could be collected. For simplicity, the lid and recirculating tubing is shown disconnected from the chamber.