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Model studies for evaluating the neurobehavioral effects of complex hydrocarbon solvents
III. PBPK modeling of white spirit constituents as a tool for integrating animal and human test data

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Abstract

As part of a project designed to develop a framework for extrapolating acute central nervous system (CNS) effects of hydrocarbon solvents in animals to humans, experimental studies were conducted in rats and human volunteers in which acute CNS effects were measured and toxicokinetic data were collected. A complex hydrocarbon solvent, white spirit (WS) was used as a model solvent and two marker compounds for WS, 1,2,4-trimethyl benzene (TMB) and *n*-decane (NDEC), were analyzed to characterize internal exposure after WS inhalation. Toxicokinetic data on blood and brain concentrations of the two marker compounds in the rat, together with in vitro partition coefficients were used to develop physiologically based pharmacokinetic (PBPK) models for TMB and NDEC. The rat models were then allometrically scaled to obtain models for inhalatory exposure for man. The human models were validated with blood and alveolar air kinetics of TMB and NDEC, measured in human volunteers. Using these models, it was predicted that external exposures to WS in the range of 344–771 mg/m^3 would produce brain concentrations similar to those in rats exposed to 600 mg/m^3 WS, the no effect level (NOEL) for acute CNS effects. Assuming similar brain concentration–effect relations for humans and rats, the NOEL for acute CNS effects in humans should be in this range. The prediction was consistent with data from a human volunteer study in which the only statistically significant finding was a small change in the simple reaction time test following 4 h exposure to approximately 570 mg/m^3 WS. Thus, the data indicated that the results of animal studies could be used to predict a no effect level for acute CNS depression in humans, consistent with the framework described above.

Keywords: Trimethyl benzene; *n*-Decane; White spirit; Toxicokinetics; PBPK model; Central nervous system effects; Occupational exposure limits; Cross-species extrapolation

1. Introduction

Physiologically based pharmacokinetic (PBPK) models have been found useful in risk assessments for a number of compounds (Leung, 1991; Clewell, 1995; Clewell and Andersen, 1996; Benignus et al., 1998; Gargas et al., 2000; Simmons et al., 2005). These models were used for different extrapolations (e.g. route to route extrapolations, intra- or interspecies extrapolations, dose-duration adjustments). In each case, the PBPK model provided a framework for estimating relationships between external (environmental) exposure and internal (biologically effective) target tissue concentration (Krishnan and Andersen, 1994).

The central nervous system (CNS) is a target for acute effects of organic solvents, characterized, both in animals and in humans, by reversible signs of CNS depression. It has been proposed that CNS effects from hydrocarbon solvents in general are proportional to the hydrocarbon concentrations in the brain (Shugaev, 1969; Baker et al., 1985). Occupational exposure

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limits (OELs) for organic solvents are often intended to avoid signs of acute intoxication, and recommended OELs are often based on acute CNS effects (Scheffers et al., 1985; Hau et al., 2001; McKee et al., 2005). Acute, non-lethal CNS effects typically include nausea, loss of coordination and signs of intoxication at levels that are high by comparison to occupational exposure recommendations. At lower levels of exposure, there may be subtle changes in behavioral function particularly those related to information processing and psychomotor function (Anger, 1986; Iregren and Gamberale, 1990; Kulig, 1990; Arlien-Soborg, 1992; Dick, 1995). OELs that are based on the relatively mild indicators of acute CNS depression usually provide an adequate measure of protection for other, more profound manifestations of CNS effects as well as other toxic effects.

The work described in this paper was part of a larger program with the overall objective of defining and then utilizing a group of tests of acute CNS effects in animals as a mean of predicting exposure levels associated with minimal levels of CNS depression in humans. In this program, joint neurobehavioral/toxicokinetic studies with ethanol, white spirit (WS) and cyclohexane, involving inhalation exposure of rats and humans, were performed in order to evaluate a working model for extrapolating animal test data to humans (McKee et al., 2006; Lammers et al., 2007). These studies demonstrated qualitative similarity in response between rats and humans, providing evidence that the rodent tests could be used to predict levels of response in humans and to assist in setting occupational exposure levels for hydrocarbon solvents.

In rats, exposure to WS for 9 h on three consecutive days produced small but statistically significant effects in the domains of neuromuscular function, sensorimotor reactivity, motor activity and performance of learned behavior (Lammers et al., 2007). These effects were most evident at 4800 mg/m^3, but statistically significant differences were also observed at 2400 mg/m^3. There were no statistically significant differences at 600 mg/m^3. In humans, one small but statistically significant CNS effect was associated with a single 4 h exposure to 570 mg/m^3 WS. Due to the nature and magnitude of this effect and the number of statistical comparisons performed, it was unclear as to whether this effect was due to treatment or chance. During both studies concentrations of two WS constituents, 1,2,4 trimethyl benzene (TMB) and *n*-decane (NDEC), were measured in blood and brains of rats or in blood and exhaled alveolair air of humans, respectively. TMB and NDEC were used as biomarkers for aromatic and aliphatic fractions of WS to determine the internal exposure to the respective hydrocarbon solvent. Kinetic data on TMB and NDEC derived from the rat study together with experimentally determined partition coefficients were used to develop PBPK models for the kinetics of both compounds in the rat. The rat models were allometrically scaled to obtain human models, which were used to predict blood, brain and alveolar air concentrations. The measured brain concentrations of NDEC and TMB in rats at no effect and effect levels were used as input values for the human PBPK models to predict the external exposure levels of WS which would be associated with equivalent brain levels in humans. Data from acute CNS studies in volunteers were then compared to model predictions to determine if it was reasonable to assume similar concentration–response relationships for acute CNS effects in humans and rats.

2. Materials and methods

2.1. Chemicals

White Spirit (CAS no. 64742-82-1) was supplied by Chempropack, the Netherlands. White spirit is a generic name for complex hydrocarbon solvents that contain straight and branched chain paraffins (C9–C12), naphthenes and aromatic hydrocarbons. Other generic terms that are used for this type of hydrocarbon solvent are Stoddard solvent and mineral spirit. More specifically, the test sample met the general requirements for a type I mineral spirit (ASTM, 2002). The initial boiling point was 160 °C, the aromatic content of the supplied sample was 21.3%, and the density was 0.785 kg/L (at 288 K). The value used for average molecular weight was 140 Da, and the relationship between mg/m^3 and ppm was 1 mg/m^3 = 0.175 ppm.

1,2,4-Trimethyl benzene (CAS no. 95-63-6) and *n*-decane (CAS no. 124-18-5), the aromatic and paraffinic molecules present in white spirit in the highest concentrations, were used as analytical markers. To increase analytical sensitivity, the concentration of TMB was increased from 2.6 to 7.8%, the maximum amount that could be added while remaining consistent with the CAS description, i.e. the overall aromatic content was increased from 21.3 to 25.6% aromatics by the addition of TMB. NDEC concentration in the fortified WS was 10.0%. NDEC was purchased from Boom (Meppel, Netherlands) and had a purity >99%. TMB was purchased from Merck (Amsterdam, the Netherlands) and had a purity >98%. All other chemicals used for analyses were obtained from Merck (Darmstadt, Germany) and were of analytical grade.

2.2. Animal study

2.2.1. Animals and housing

Adult male Wistar-derived WAG/RijCR/BR rats were purchased from Charles River Wiga (Sulzfeld, Germany). The animals at time of testing were approximately 15 weeks old with body weights of 242 to 296 g. Animals were housed in suspended wire mesh steel cages under normal housing conditions in temperature-controlled (20–25 °C) animal rooms with a relative humidity between 40 and 70%. Animals were maintained on a 12-h light:dark cycle. Feed and drinking water were available *ad libitum* from the arrival of the rats until the end of the study.

2.2.2. Exposure conditions and collection of samples

Groups of rats were exposed to WS at target concentrations of 0 mg/m^3 (control group), 600 mg/m^3 (~102 ppm), 2400 mg/m^3 (~410 ppm) and 4800 mg/m^3 (~820 ppm) in modified H1000 inhalation chambers (Hazleton Systems, Inc., USA). Each chamber had a pyramidal top and bottom and was constructed of stainless steel with glass doors on two sides. The