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Applications of dosimetry modeling to assessment of neurotoxic risk☆

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Abstract

Risk assessment procedures can be improved through better understanding and use of tissue dose information and linking tissue dose level to adverse outcomes. For volatile organic compounds, such as toluene and trichloroethylene (TCE), blood and brain concentrations can be estimated with physiologically based pharmacokinetic (PBPK) models. Acute changes in the function of the nervous system can be linked to the concentration of test compounds in the blood or brain at the time of neurological assessment. This set of information enables application to a number of risk assessment situations. For example, we have used this approach to recommend duration adjustments for acute exposure guideline levels (AEGLs) for TCE such that the exposure limits for each exposure duration yield identical tissue concentrations at the end of the exposure period. We have also used information on tissue concentration at the time of assessment to compare sensitivity across species, adjusting for species-specific pharmacokinetic differences. Finally this approach has enabled us to compare the relative sensitivity of different compounds on a tissue dose basis, leading to expression of acute solvent effects as ethanol-dose equivalents for purposes of estimating cost–benefit relationships of various environmental control options.

Keywords: Haber's rule; Duration adjustments; PBPK model; Acute toxicity; Toluene; Trichloroethylene

1. Introduction

In many cases risk assessments could be improved by consideration of the dose delivered to the target tissue. Understanding the relationships between applied dose, target tissue dose and adverse outcomes can help in reducing uncertainties in risk assessment extrapolations such as those associated with species, ages, or exposure conditions including different levels or durations of exposure. In particular, we will consider how target-tissue dose can help in exposure duration adjustments and cross-species extrapolations. We shall also consider another topic that has become more visible lately, the issue of cost benefit analysis.

2. Exposure dose response models

The ability to understand tissue dosimetry as a link between exposure to toxic compounds and the development of adverse outcomes has many potential uses in risk assessment (Fig. 1). The ability to do so in a quantitative way incorporates both pharmacokinetic and pharmacodynamic information to describe the relationships between the dose applied to the organism, the amount of the dose absorbed, the amount of the active toxic compound that arrives at the target tissue, and the eventual toxic outcome (e.g., Andersen, 1995, 2003; Shuey et al., 1994). The scheme depicted in Fig. 1 focuses on a segment of the "exposure–dose response model" described in Bushnell et al. (in press), which also considers factors such as modeling the relationships between emissions of compounds from a source and the environmental fate and transport to the individual resulting in the external exposure conditions, or the "applied dose" of Fig. 1. For the case of inhaled volatile compounds, applied dose takes the minimal form of the concentration of the compound in inspired air and

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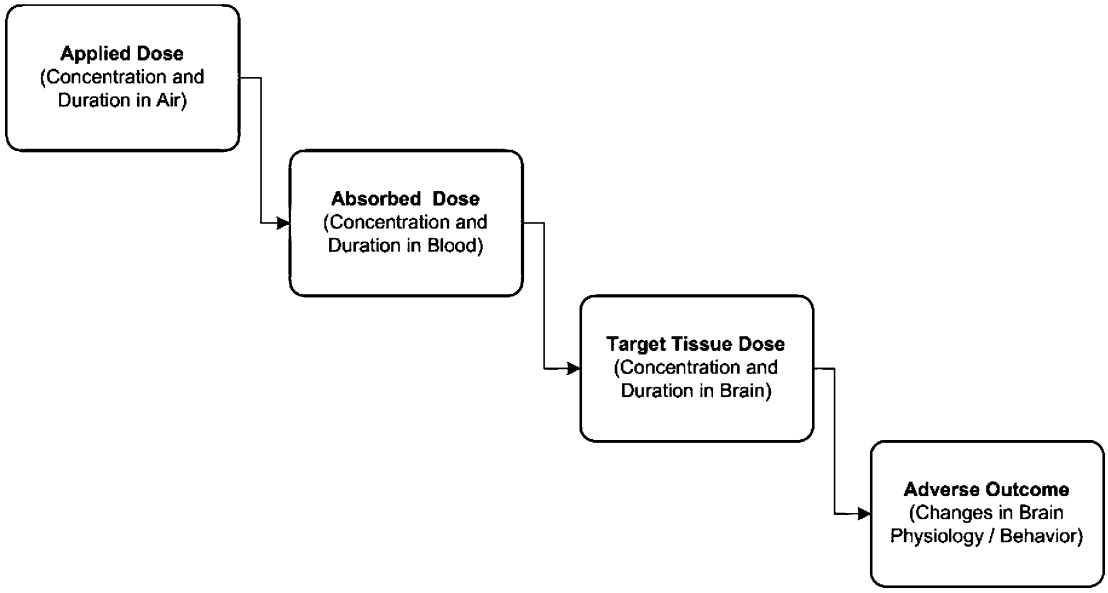

Fig. 1. Schematic of exposure, absorbed dose, target tissue dose and response relationship.

the duration of exposure. More complicated considerations of exposure pattern profiles with expressions for multiple exposures or the contributions of other exposure routes to target tissue dose are also possible. Absorbed dose refers in our case to the amount of the compound transferred from the air to the blood. This will be a function of the applied dose and duration of exposure as well as factors such as the ventilation rate, movement of the compound across the alveolar or other tissue barriers, the rate of blood flow and the partitioning of the compound into the blood. The transition from absorbed dose to dose of the active compound at the target tissue is a function of blood flow to the target organs, partitioning of the compound between the blood and the tissue, metabolism of the compound to active or inactive metabolites, the role of elimination and, perhaps, sequestering of the compound in other non-target tissues. Finally, the relationship between target tissue dose and neurotoxic outcome includes a host of factors associated with the ultimate neurotoxic mechanism, as well as measurement of the outcomes with a variety of procedures that vary in proximity to the actual toxic insult. These issues are discussed in more detail in Bushnell et al. (in press).

3. Physiologically based pharmacokinetic models

The process through which quantitative relationships can be established for the first three components of Fig. 1 involves use of a physiologically based pharmacokinetic (PBPK) model, or as they are being increasingly referred to, a physiologically based toxicokinetic model. In recent years the development and use of PBPK models has expanded considerably (Andersen, 2003). A simple conceptual framework of a PBPK model is presented in Fig. 2. The model contains parameters describing the concentrations and movement of the chemical or chemicals of interest through the tissue compartments depicted in the figure. It would include such elements as concentration of the test compound in inspired air, the ventilation rate, an air/blood partition coefficient, cardiac output, proportional blood flow to tissue compartments, blood/tissue partition coefficients for each tissue, and metabolic rate constants. More sophisticated model structures have been developed for a variety of applications including parameters for binding to tissue receptor proteins, dose-dependent processes, formation of metabolites, and suicide inhibition of further metabolism. One strength of PBPK models is that the parameters that are used are either known, measurable or can be estimated by the model. Physiological parameters can be adjusted to suit various applications such as comparing across species, ages or other conditions (e.g., Benignus et al., 1998; Evans et al., 2002). Another strength is that the models can be tested to determine whether they predict the observed absorption and distribution of compounds under a variety of conditions.

In this paper we discuss application of dose response models to specific risk assessment applications concerning the neurotoxic effects of acute exposure to trichloroethylene (TCE) and toluene. The PBPK model structure we have used was developed from previous models for volatile organic compounds (Ramsey and Andersen, 1984). Since most of our laboratory animal research for neurotoxicity has involved pigmented rat strains, the model was adapted to include parameters specific for Long-Evans rats and also expanded to include an explicit brain compartment (Simmons et al., 2002). It is important to evaluate the ability of the model to predict tissue concentrations of the compounds of interest. To this end, we quantitatively compared model predictions and experimental determinations of brain, liver,

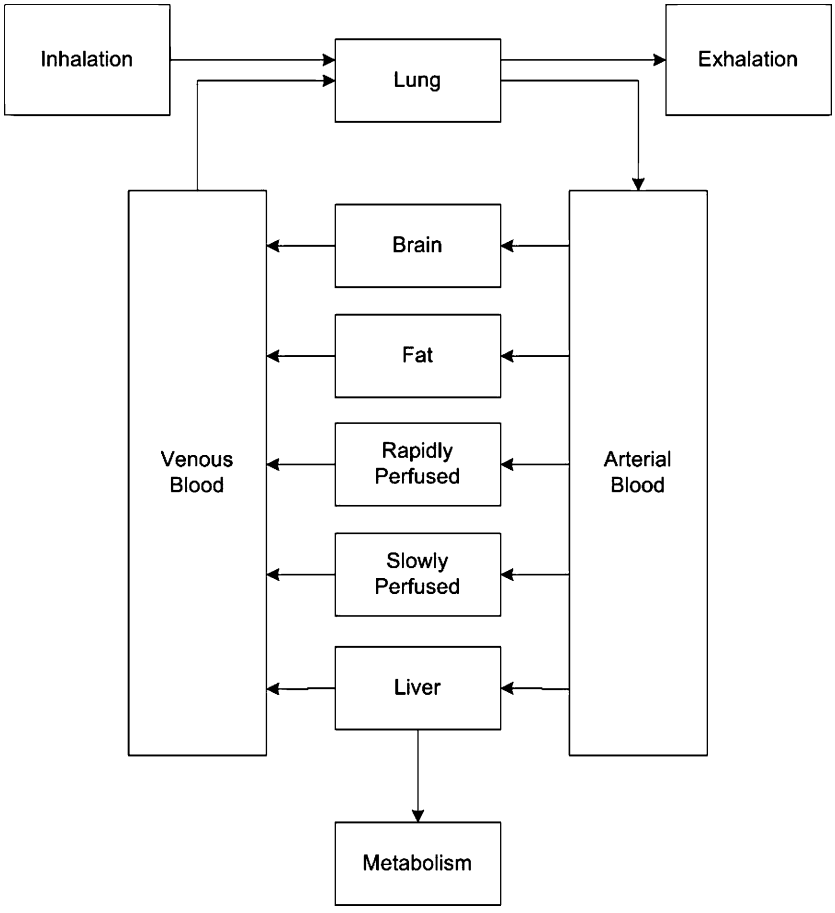

Fig. 2. Schematic of physiologically based pharmacokinetic (PBPK) model. This schematic represents the model structure used for TCE (Simmons et al., 2002) and is being used for toluene.

fat and blood concentrations of TCE (Simmons et al., 2002).

Our laboratory work has concentrated on two approaches to assess the acute neurological effects of treatment with the volatile organic compounds. One approach involves a behavioral assessment of sustained attention (Bushnell, 1997). Rats are trained to detect intermittent illumination of a visual signal light and to respond on one of two levers to reflect the presence or absence of a signal during specific time intervals. The training and testing apparatus is housed inside an inhalation chamber to allow testing during exposure. Exposure to TCE or toluene has impaired performance of this task, as measured with a number of dependent variables, including poor sensitivity for signal detection (Bushnell, 1997; Bushnell et al., in press).

An alternative procedure we have used to ascertain the acute neurological effects of exposure to volatile organic compounds involves recording visual evoked potentials (VEPs). We have measured VEPs elicited with visual patterned stimuli from rats restrained in a novel head-only exposure chamber with a glass face to allow viewing of the stimulus screen (Boyes et al., 2000, 2003). This apparatus also enables measuring neurotoxic outcomes during the period of exposure to the test compound. VEP waveforms are submitted to spectral analysis, and the amplitude of the primary response component occurring at twice the stimulus frequency (Boyes, 1994) is measured as the primary dependent variable. Exposure to volatile organic compounds reduces F2 amplitude (Boyes et al., 2000, 2003).

4. Determination of the appropriate dose metric

To apply exposure–dose response models in a risk assessment situation, it is necessary to understand the appropriate dose metric for the active compound in the target tissue. This problem involves knowing whether the active compound is the parent compound or a metabolite, as well as determining the nature of the interaction between the active compound and the target tissue. In our evaluations of TCE neurotoxicity, there have been clear dose–response relationships for the neurotoxicity outcome measures at exposure levels in excess of those that the PBPK model showed to be associated with saturation of the metabolic pathways (Boyes et al., 2000). Above metabolic saturation, the rate of formation of metabolites remains constant. The fact that increasing deficits in signal detection behavior and VEP amplitude were observed above metabolic saturation suggests that the parent

Table 1
Data from Boyes et al. (2003)

Air [TCE] (ppm)	Exposure duration (h)	$C \times t$ product (ppm × h)	Brain [TCE] (mg/l) at test time	Brain [TCE] (mg/l)h AUC	F2 amplitude (μV)
0	4	0	0	0	4.4
1000	4	4000	45	130	3.1
2000	2	4000	75	107	3.1
3000	1.3	4000	98	98	2.3
4000	1	4000	120	81	1.9

compound and not one or more of the metabolites is the principal active moiety.

It is also important to understand what measure of target tissue dose is most predictive of the adverse outcome. This can be difficult to ascertain in many experimental designs where the different measures are correlated. For example, a typical toxicity study would incorporate a fixed exposure duration, and assess the effects of several air concentrations of the test compound. In this experimental design, tissue concentration at the time of assessment and total tissue load (area under the curve) are strictly correlated, and it is not possible to distinguish which is the better determinant of outcome. We have used an experimental design in which different exposure durations were used, enabling tissue concentration at the time of assessment to be distinguished from area under the curve because they are inversely-correlated (Table 1). Rats were exposed to TCE under conditions leading to a constant $C \times t$ product. The dose groups were exposed to either 1000 ppm for 4 h, 2000 ppm for 2 h, 3000 ppm for 1.3 h or 4000 ppm for 1 h. As can be seen in Table 1, the tissue concentration of TCE increased with concentration, while the area under the curve increased with exposure duration. VEP amplitudes were reduced in proportion to increasing tissue concentration at the time of assessment. These results demonstrate that for the acute neurotoxic effects of TCE exposure, tissue concentration at the time of assessment is an appropriate dose metric (Boyes et al., 2000, 2003).

5. Duration adjustments in acute solvent toxicity

In risk assessments, duration adjustments are often necessary because the duration of concern for protecting potentially exposed populations does not match the exposure duration of the available evidence. For example, there may be concern for people potentially exposed for 24 h/day, but the experimental evidence was obtained using a 6 h/day exposure protocol. In another example, acute exposure guideline levels (AEGLs) are set for 10, 30 min and 1, 4, and 8 h exposure durations, but experimental data are rarely available for each of those explicit times. Typically, the solution to this problem has involved invoking Haber's rule, stated as $C \times t = K$ where C refers to the concentration of the compound in air, t the duration of exposure and K a constant toxic effect. Alternatively, non-linear derivatives of Haber's rule have been used such as: $C^n \times t = K$, where the parameter n is fit to experimental data. Ideally, in setting regulatory standards or in providing other types of exposure guidance, the exposure concentrations selected as the standards would provide comparable levels of protection at each exposure duration.

In many cases however, Haber's rule does not describe very well the changing toxicity observed across different exposure conditions. The example in Table 1 illustrates that exposure conditions with different $C \times t$ products provided different degrees of toxic outcome. An alterative is to employ the conclusion described previously, that the tissue concentration at the time of testing is an appropriate dose metric that accurately predicts acute neurotoxic effects of TCE. We have done this by providing guidance to the AEGL Committee for duration adjustments for acute TCE exposures in their development of draft TCE AEGL values.

The AEGL values are intended to provide guidance on exposure levels below which risks of various degrees of severity can be avoided. The three levels of concern include: irritation or other reversible acute outcomes (AEGL-1); irreversible health effects or impaired ability to escape (AEGL-2); and lethal threat (AEGL-3) (NRC, 2001). In order to establish AEGL values for TCE, the committee responsible for establishing AEGL standards selected outcomes appropriate for AEGL-1, AEGL-2 and AEGL-3 levels of concern from the experimental literature. These values were from the human experimental literature for AEGL-1 and AEGL-2, representing the NOAEL (300 ppm for 2 h) and LOAEL (1000 ppm for 2 h), respectively, for performance of a visual-motor task in a human volunteer study (Vernon and Ferguson, 1969). The AEGL-3 value reflected the minimum lethal dose to mice of 4600 ppm for 4 h (Friberg et al., 1953). We used a PBPK model for TCE, equipped with human physiological parameters, to predict blood TCE concentrations of approximately 4.8 and 18.3 mg/l achieved during exposure conditions reflective of AEGL-1 and AEGL-2 levels of concern, respectively. The original source did not report TCE blood concentrations values at the time of testing, which would have enabled comparison to the PBPK model predictions; however, the parameters of the model were based on the published literature and reflect models that have been shown to be sufficiently acceptable in ability to predict tissue levels (Boyes et al., in press). We then used the model to predict the air concentrations that would be required to produce equivalent blood TCE levels following exposure durations of 10, 30 min or 1, 2, 4, or 8 h. The approach to AEGL-3 involved an additional initial step since the original starting point was derived from a study using mice. Therefore, we first developed a mouse PBPK model to predict the blood level associated with the exposure param-

eters selected. Then, as before, we used the human model to predict the exposure concentrations that would lead to that blood concentration at each of the specified durations. The exposure concentrations derived in this manner differed from those of the traditional duration adjustments based on $C^n \times t$ approach. At durations shorter than the point of departure, the PBPK model predicted that lower air TCE concentrations would be required to reach the same blood concentration than those derived from the traditional approach. On the other hand, for exposure durations longer than the point of departure, the PBPK approach predicted that higher air concentrations of TCE would be required than those predicted by the traditional approach. The magnitude of the difference between the two approaches increased with the magnitude of the time intervals across which predictions were made.

6. Cross-species extrapolations

Dosimetric evaluations can be used to help address the issue of cross species extrapolations. For example, Benignus et al. (1998) conducted a dosimetric analysis of behavioral effects of acute exposure to toluene in rats and humans. First, the scientific literature on behavioral effects of acute exposure to toluene was surveyed, and studies with rats or human subjects that provided sufficient data to conduct a dosimetric evaluation were selected. A PBPK model was used with parameters appropriate for rats or humans to estimate arterial blood concentrations of toluene at the time of behavioral assessment. Sufficient data for the meta-analysis were identified in the scientific literature for avoidance behavioral in rats and choice reaction time tasks in humans. Data from different manuscripts using similar behavioral procedures were pooled into a meta-analysis through conversions of the various dependent measures to a common outcome parameter based on proportion of unexposed control responding. Outcomes across the studies were then plotted as a function of arterial toluene concentration at the time of testing. Logistic dose-effect functions were fit to the resulting data. The probability of successful avoidance in rats was a uniformly decreasing function of blood toluene concentration. A 10% decrement in performance was obtained in rats with a blood toluene concentration of approximately 73 mg/l.

Human choice reaction performance also showed a uniformly decreasing function of blood toluene concentration. In contrast to rats, the human function showed a 10% decrement in performance at a blood toluene concentration of approximately 3 mg/l.

This analysis suggests a large difference in susceptibility of rats and humans to acute behavioral effects of toluene. It should be considered, however, that several other factors might also account for this difference. The rat and human experiments available in the literature employed very different behavioral tasks. It is possible that choice reaction time performance is more sensitive to disruption from toluene than is avoidance behavior. The informational and motivational aspects of these tasks are very different, and these factors are known to influence sensitivity to toxic interferences. This concern can be addressed through further studies in which the effects of toluene are evaluated in animal and human subjects using comparable behavioral procedures. Alternatively, it is possible that the PBPK model used is not consistently accurate in predicting rat and human arterial concentrations. This concern can also be addressed through more thorough evaluation of the toluene concentrations in tissues from rat and human exposure studies. A third possibility is that there is a systematic difference in the ability to mathematically describe the shape of the dose-effect curves from rat and human studies. The rat studies routinely employed higher dose levels, which enabled the shape of the dose response curve to be modeled with greater confidence than was the case for humans. The vast majority of the human data available originated from dose levels producing little or no significant behavioral deficits. The lack of high dose human studies, although ethically justifiable, serves to impair thorough animal to human comparisons. Finally, it is possible that human neurological function is more sensitive to disruption from toluene than is rat neurological function. This could be caused by species-specific differences in the sensitivity of target receptor proteins to toluene-induced disruption. This possibility can be evaluated through in vitro experiments such as those described in Bushnell et al. (in press). If this is found to be the case it could have important implications for risk assessments of toluene, or similarly acting compounds, if the primary data are from experimental studies of rats.

7. Cost–benefit assessment for acute solvent exposure

Recently, there has been an increasing emphasis on conducting cost–benefit analyses as a component of making risk management decisions (e.g., OMB, 2002). Part of the goal of this movement is to apportion limited public resources to remedy those situations offering the greatest cost–benefit gains. Providing information sufficient to support the implementation of this public policy presents a great challenge to neurotoxicologists. It is currently difficult, if not impossible, to estimate the public costs associated with many of the outcome measures typically evaluated in neurotoxicity studies. For example, how much does it cost if someone experiences a 10 or 20% decrement in choice reaction time? It is important to attempt to answer such difficult questions so that potential neurotoxic risks from exposure can be considered in the cost–benefit portions of risk management decisions.

We have been developing a framework in which some potential costs of acute exposure to volatile organic compounds could be expressed in a manner that enables monetization of the risks of exposure (Benignus et al., 2002, in press). This was begun in response to EPA's assessment of emissions from off-road recreational vehicles, which in some configurations emit substantial quantities of unburned volatile hydrocarbons. In the final assessment, predicted exposure levels

in this particular application were low enough that the neurotoxicity costs appeared to be negligible. The framework for conducting the analysis, however, could be employed in other situations involving potential acute exposures to similar compounds.

Our analysis considered the problem of estimating costs of behavioral deficits caused by acute exposure to toluene. The basis of the analysis was the meta-analysis of choice reaction time behavioral deficits in human subjects as a function of blood toluene concentration from Benignus et al. (1998), as discussed above. We reasoned that there was a longstanding observation that toluene and other organic solvents produced behavioral deficits that were in many ways similar to acute ethanol exposure (e.g., Balster, 1998; Evans and Balster, 1991; Rees et al., 1987). In addition there is a growing body of evidence that volatile organic compounds, including toluene, have many similar pharmacological actions to ethanol in cellular target sites within the central nervous system (see Bushnell et al., in press). Therefore, we conducted a meta-analysis of the published scientific literature on the acute effects of ethanol exposure in volunteer human subjects on choice reaction time behavior. Deficits in choice reaction time were modeled as a function of blood ethanol concentration. In possession of comparable functions for the effects of toluene and ethanol on choice reaction time, we were able to generate a function relating blood toluene concentration to blood ethanol concentration on the basis of equal magnitude effects on choice reaction time. Thus, we could now express blood toluene concentrations as blood ethanol equivalents. While it is recognized that choice reaction time is only one aspect of a complex suite of behaviors that together allow people to conduct complex tasks such as driving, we contended that choice reaction time deficits could be considered as a marker for those other unmeasured outcomes of exposure because of the similarities of toluene and ethanol effects at both the cellular and systemic levels.

The functions expressing toluene as ethanol equivalent dose levels expressed behavioral deficits in a context that economists could use to express the monetary costs of toluene exposure. There is a substantial amount of data on probability of highway traffic accidents as a function of blood ethanol concentration. There are also statistics on the severity of these accidents and their associated direct and indirect monetary costs. Although the risk of highway traffic accidents following toluene exposure is only one aspect of the potential total costs associated with such acute changes in behavior, it was the outcome for which potential costs could be estimated most readily. In the future it will be important to develop additional means to estimate costs or benefits associated with controlling exposure to neurotoxic substances.

In summary, being able to establish quantitative relationships between exposure, tissue dose and adverse outcome led to practical applications in several risk assessment situations. Key elements in developing the models included formulating a PBPK model and evaluating its ability to predict tissue concentrations, determining whether parent compound and/or metabolites were active, and establishing tissue dose metrics that predict adverse outcomes. Several applications that followed from this exposure–dose response model were illustrated, including duration adjustments in setting acute exposure guidelines, cross species extrapolations, and an approach to evaluate cost–benefit relationships through establishing dose-equivalent functions with ethanol. Many additional applications of these models could also be developed.

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