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Family Approach for Estimating Reference Concentrations/Doses for Series of Related Organic Chemicals

H. A. Barton,*[,1,2] P. J. Deisinger,† J. C. English,† J. M. Gearhart,*[,2] W. D. Faber,† T. R. Tyler,‡ M. I. Banton,§ Justin Teeguarden,* and M. E. Andersen*[,3]

*K. S. Crump Group, ICF Kaiser, Research Triangle Park, North Carolina 27709; †Health and Environment Laboratories, Eastman Kodak Company, Rochester, New York 14652-6272; ‡Health, Safety, and Environmental Affairs Department, Union Carbide Corporation, Danbury, Connecticut 06817-0001; and §Toxicology, Health, Safety, and Environment, Shell Chemical Company, Houston, Texas 77002

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The family approach for related compounds can be used to evaluate hazard and estimate reference concentrations/doses using internal dose metrics for a group (family) of metabolically related compounds. This approach is based upon a simple four-step framework for organizing and evaluating toxicity data: 1) exposure, 2) tissue dosimetry, 3) mode of action, and 4) response. Expansion of the traditional exposure-response analysis has been increasingly incorporated into regulatory guidance for chemical risk assessment. The family approach represents an advancement in the planning and use of toxicity testing that is intended to facilitate the maximal use of toxicity data. The result is a methodology that makes toxicity testing and the development of acceptable exposure limits as efficient and effective as possible. An example is provided using butyl acetate and its metabolites (butanol, butyraldehyde, and butyrate), widely used chemicals produced synthetically by the industrial oxo process. A template pharmacokinetic model has been developed that comprises submodels for each compound linked in series. This preliminary model is being used to coordinately plan toxicity studies, pharmacokinetic studies, and analyses to obtain reference concentrations/doses. Implementation of the family approach using pharmacokinetic modeling to obtain tissue dose metrics is described and its applications are evaluated.

Key Words: **hazard identification; dose-response assessment; butyl acetate; tissue dosimetry; mixtures; risk assessment; pharmacokinetics; reference dose (RfD); reference concentration (RfC).**

Traditionally, risk assessment has relied upon correlations between No Observed Adverse Effect Levels (NOAELs) and exposure concentrations (or doses) for establishing acceptable exposure standards. Toxicity testing has provided qualitative hazard identification for increasingly wider ranges of responses including carcinogenesis, target organ toxicity, and neurologic, reproductive, and developmental toxicity. In general, regulatory agencies have considered it necessary to test individual chemicals for each of these toxicities using all relevant exposure routes. The two major routes have been oral and inhalation, whereas dermal studies have been carried out less frequently. This exposure–dose-response approach leads to a large three-dimensional (i.e., exposure routes, doses/concentrations, responses) matrix of studies required for evaluation of the chemicals of interest.

In support of hazard and dose-response assessments, efforts to establish the biologic foundation for observed toxicologic effects have focused upon two general groups of processes. The first are processes that affect the dose of chemical reaching relevant sites in the body, i.e., the pharmacokinetic processes of absorption, distribution, metabolism, and elimination. Those processes that produce sufficient biologic perturbations to result in toxic effects form the second group. General descriptions of these latter processes are referred to as the mode of action producing the toxicity. Thus, the traditional exposure-response framework has evolved into an exposure–tissue dosimetry–mode-of-action–response framework. This four-step framework facilitates organizing and utilizing scientific information in toxicology research and quantitative health risk assessment (Barton *et al.*, 1998; U.S. EPA, 1996).

Consideration of this four-part framework suggests that there are opportunities for greater efficiency and effectiveness in toxicity testing when chemicals of interest are metabolically related (i.e., parent and metabolites). For example, if people were exposed to chemicals A and B, then exposure limits would be needed for both chemicals. Traditionally, toxicity studies would be carried out with A and B and acceptable exposure limits would be derived independently based upon the results of each study. However, if B were a metabolite of A, the study in which animals were exposed to chemical A would result in internal exposures to both A and B, thus identifying hazards associated with both. By quantifying inter-

[1] To whom correspondence should be addressed at present address: Pharmacokinetics Branch, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, MD 74, 86 T. W. Alexander Dr., Research Triangle Park, NC 27711. E-mail: habarton@alum.mit.edu.

[2] Current address: Proctor & Gamble, Human Safety Department, Miami Valley Lab, P.O. Box 538707, Cincinnati, OH 45253-8707.

[3] Current address: Department of Environmental Health, Colorado State University, CETT-Foothills Campus, Fort Collins, CO 80523.

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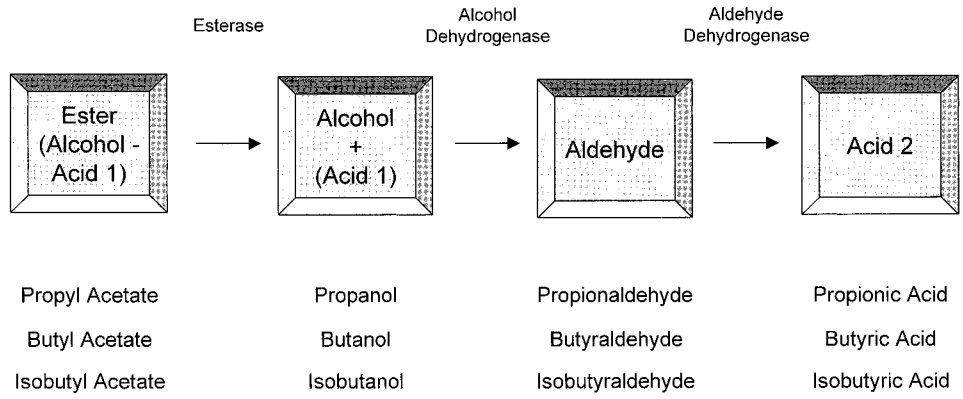

FIG. 1. Oxo process chemical families and metabolic pathways for acetate esters. Acetate esters are metabolized by mammals in a three-step process producing sequentially more oxidized metabolites of the alcohol component of the ester. A few examples of commercially important families are illustrated. Acid 1 is acetate in all these examples.

nal doses, the hazards identified from the study with external exposure to A can be used to estimate acceptable exposures to both chemicals A and B. This approach was named the family approach in reference to its application to a family of metabolically related chemicals. This paper describes this approach and its potential implementation for a specific family of compounds.

The examples to be used throughout this paper relate to a series of four compounds derived *in vivo* from sequential metabolism of the parent alkyl ester, butyl acetate (Fig. 1). The fifth compound, the initial acid formed by cleaving the ester, is acetate. Acetate is an important intermediate produced by normal metabolic processes in the body and generally is not associated with systemic toxicity. This family of compounds, coincidentally, is also related in terms of industrial production. The oxo process chemicals are produced first by the catalytic hydroformylation of short-chain alkene feed stocks, e.g., ethylene, propylene, butylene. The reaction (alkene plus carbon monoxide and hydrogen) is performed in closed reactors and the resulting aldehydes (e.g., propionaldehyde, butyraldehyde, isobutyraldehyde, and pentaldehydes) are refined and hydrogenated to form the respective alcohols. The alcohols may be further reacted with acetic acid to form the acetate esters. Examples of these families of related chemicals are also illustrated in Figure 1.

These oxo process chemicals typically do not have high toxic potency, often producing fairly nonspecific effects at high exposure levels (Bisesi, 1994; David *et al.*, 1998; Lington and Bevan, 1994). Several are found to occur naturally in food, are used as flavoring agents in food, or are normal components of intermediary metabolism. However, because they are produced in high volume and there is the potential for worker and consumer exposure during their manufacture and use, they are subject to increased regulatory scrutiny and are targets for test rules requiring additional toxicity testing. The large number of these compounds and the breadth and sophistication of required tests have placed a high demand on available resources, prompting a search for more efficient and effective ways to assess hazards posed by the compounds.

The family approach for risk assessment uses a dosimetry-based analysis to interpret a toxicity study with the parent compound and obtain toxicity information from it on other members of the family. Results of exposures with a single compound can be extrapolated to subsequent metabolites and from one dose route to another (e.g., oral to inhalation) (Gerrity and Henry, 1990). This approach is useful for internal or systemic toxicity; contact site effects (i.e., stomach, respiratory tract, or skin) would be evaluated by direct studies because they are influenced by the route of exposure and the physical/chemical properties of the material. In essence, the goal of the family approach is to use the toxicity data from hazard identification studies with the parent compound together with pharmacokinetic data to derive acceptable exposure levels for the metabolites, decreasing the amount of toxicity testing.

The remainder of this paper describes several aspects of the family approach, including: methods for developing exposure limits, approaches for necessary pharmacokinetic analyses, and issues for its implementation. Analysis for the n-butyl series of compounds will be used as an example to illustrate the family approach. The members of this series are n-butyl acetate (BuAc) and its subsequent metabolites, n-butanol (BuOH), n-butyraldehyde (BuCHO), and n-butanoic acid (BuCOOH). (Hereafter these compounds are referred to without noting that they are the straight chain isomer.) This example is in its preliminary stages; it is used to illustrate how the well-developed family approach to risk assessment is applied relatively early in the testing process to coordinately develop both pharmacokinetic and toxicity information.

Development of Exposure Limits Using the Family Approach

Chronic exposure limits for systemic effects, such as reference concentrations (RfC) or reference doses (RfD), are typically based upon the correlation between response and exposure (Barton *et al.*, 1998; Dourson, 1994; U.S. EPA, 1994). The RfC methodologies have explicitly incorporated tissue dosimetry into the process, an example of the transition away from analyses based on exposure dose. The RfDs and RfCs are derived from NOAELs, inhalation benchmark concentrations (BMC), or oral benchmark doses (BMD), which are determined for the compound to which the animals in the critical

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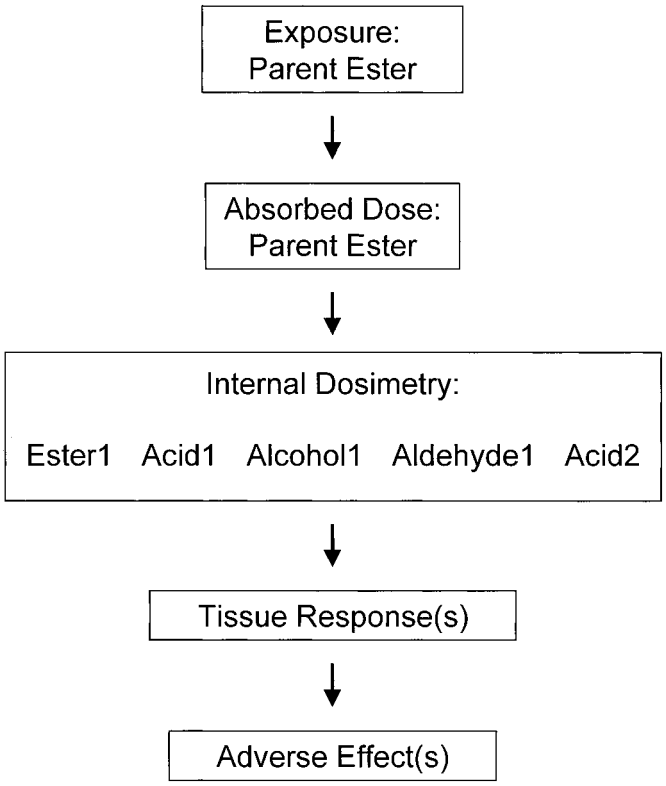

FIG. 2. Biologic processes leading from exposure to toxicity. The schematic illustrates major steps following exposure to acetate esters, notably their metabolism resulting in internal exposures to the five compounds comprising the series.

study were exposed. The BMC/D represent statistical estimates of exposure concentrations/doses that would produce a selected low level of response, usually 10% or less (Crump, 1984; Crump, 1995; U.S. EPA, 1995). In current risk assessment practice, the NOAEL and BMC/D are used essentially equivalently. Adjustments and uncertainty factors are then applied to obtain the RfC or RfD.

Implementation of the family approach relies upon internal dose metrics rather than the exposure concentration or dose (Fig. 2). Dose metrics could include peak blood concentration or area under the curve (AUC) of parent compound and metabolites in blood or in tissues. Dose metrics in blood are generally good surrogates for the dose to the target tissue, though mode of action information may indicate that specific tissue dose metrics are necessary (Collins, 1987; O'Flaherty, 1989; Voisin *et al.*, 1990). Toxic effects would be assumed to result from each circulating chemical individually, and a NOAEL or BMC/D would be established for each compound using the blood concentration time course. The pharmacokinetic analyses used to obtain values for internal dose metrics will vary depending upon the available pharmacokinetic database and the characteristics of the compound. In some cases, estimates obtained directly from data will be available, so little extrapolation is required. Pharmacokinetic models may include traditional compartmental analyses (Gibaldi and Perrier, 1982), physiologically based pharmacokinetic (PBPK) models (Andersen, 1991; Clewell and Andersen, 1994; Clewell *et al.*, 1995; Leung, 1991), and hybrid models that describe chemicals and their metabolites using combinations of physiologic and compartmental approaches (e.g., Gerlowski and Jain, 1983; Fisher *et al.*, 1989; Fisher *et al.*, 1990). This last approach can provide some of the advantages of physiologic modeling for extrapolation while limiting the data requirements for compounds for which simpler descriptions of tissue distribution or metabolism suffice.

An example illustrates extrapolation to the alcohol metabolite and across dose routes for a toxicity study in which animals were exposed to parent ester by inhalation (Fig. 3). For extrapolation to the alcohol, it might be assumed that the alcohol was the only metabolite responsible for the toxicity observed following ester exposure. Estimating the inhalation concentration of alcohol that would be equivalent to the ester BMC is a two-step process: 1) determine the internal dose of alcohol (e.g., blood AUC in Fig. 3A) at the ester BMC, and 2) estimate the alcohol exposure concentration that would produce the same internal dose. The exposure concentration of alcohol that would produce the same AUC would be estimated using a pharmacokinetic analysis describing the blood levels resulting from alcohol inhalation.

Dose-route extrapolation involves the same basic process except the second step would use pharmacokinetic analysis describing the alcohol blood levels resulting from oral alcohol dosing (Fig. 3B). Thus, the atmospheric exposure concentrations, skin exposures, or ingested amounts of the compounds expected to produce the given blood exposures would be calculated with pharmacokinetic models.

The family approach provides estimates of the BMC/D for a metabolite in animals exposed by inhalation or oral dosing. Derivation of the RfC/D for humans from the animal-based BMC/D may rely upon the default methods; the interspecies extrapolation may also be based upon internal dosimetry if appropriate data or pharmacokinetic models for humans are available (Barton *et al.*, 1998; Clewell and Andersen, 1987; Jarabek, 1995).

With this analysis, exposure limits can be defined for the parent and metabolites using the single toxicity study in which the animals received external exposure to the parent compound. Toxicity observed with exposure to the parent ester would be assumed to be due to each of the metabolites in turn (i.e., alcohol, aldehyde, acid) for obtaining acceptable exposure limits. Assuming the toxicity resulted only from a single metabolite may overestimate the potential for toxicity if a metabolite were not, in fact, responsible for the toxic effect. Thus, this approach should provide reasonable or conservative (i.e., health-protective) estimates of acceptable exposure levels.

MATERIALS AND METHODS

Pharmacokinetic Model for Butyl Acetate and Metabolites

A template pharmacokinetic model was developed for the oxo process series of compounds. This model was then parameterized for the butyl series based

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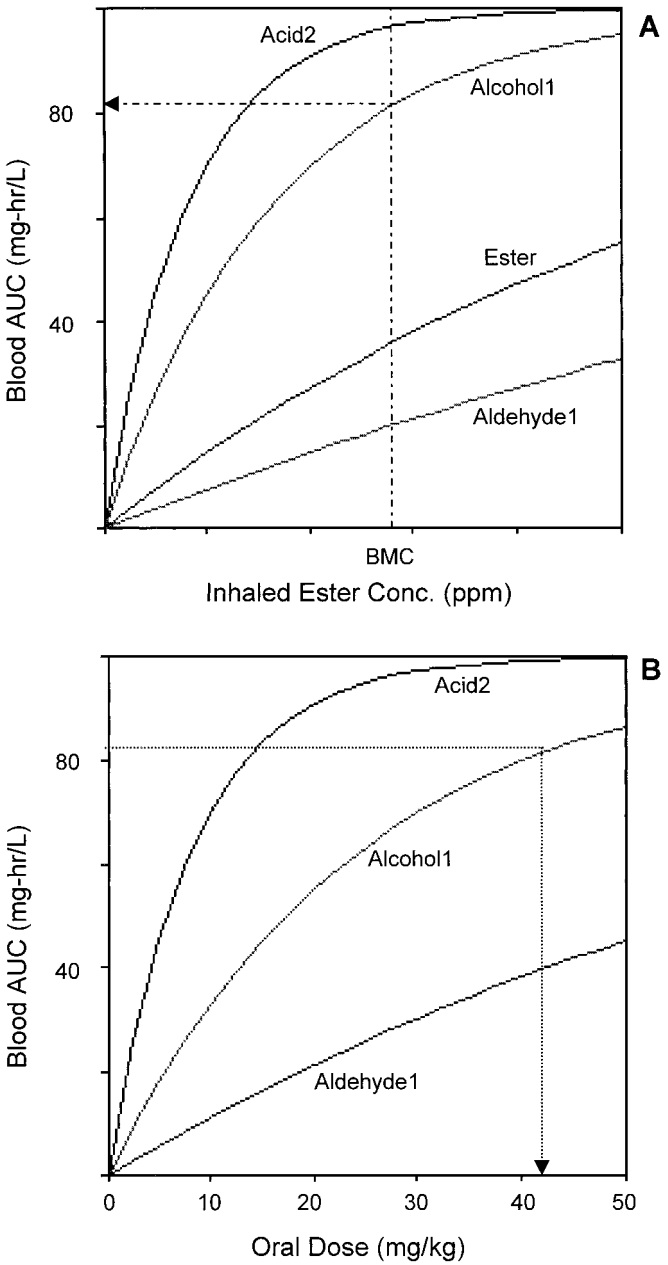

FIG. 3. Extrapolating from the benchmark concentration (BMC) for the parent ester to the BMC for the alcohol metabolite. Graphs illustrate hypothetical areas under the curve (AUC) for each metabolite in blood established in pharmacokinetic experiments using (A) ester inhalation and (B) oral dosing with the alcohol. The BMC would be obtained by analyzing a toxicity study with exposure to the parent ester. The exposure concentration in (A) results in a blood AUC of 82 mg*h/L for alcohol1. The results of oral pharmacokinetic studies (B) are used to estimate that an oral alcohol dose of 42 mg/kg would have the same blood AUC, so that would be the equivalent benchmark dose (BMD) for alcohol given by the oral route.

on limited literature data and experiments presented here. This provisional parameterization has been used to explore the family approach and to plan pharmacokinetic studies to improve the parameterization. The model does not track acetate formed in the initial ester cleavage because it is unlikely to contribute significantly to systemic toxicity, though it may contribute to nasal toxicity (i.e., a contact site effect). The model consists of submodels for each chemical that are linked by metabolism, forming the subsequent metabolite in the same tissue (Fig. 4). This approach has been used for a number of PBPK models, including 2-butanol and its metabolites (Dietz *et al.*, 1981), trichloroethylene (Abbas and Fisher, 1997; Clewell *et al.*, 1995; Fisher *et al.*, 1998), and several glycol ethers (Borghoff *et al.*, 1996; Corley *et al.*, 1994; Shyr *et al.*, 1993). The metabolic steps were assumed to be irreversible. Preliminary studies indicated low levels of formation of alcohol from the BuCHO, but no detectable reversibility with the other compounds (data not shown).

The pharmacokinetic model includes tissues that are important for systemic toxicity, storage, metabolism, and elimination. It uses units of μmoles, hours, and milliliters; amounts in moles were converted by the model to grams for purposes of comparison with available data. Three exposure routes are included: iv injection, inhalation, and oral dosing. The model was written using Advance Continuous Simulation Language (ACSL®) obtained from Mitchell & Gauthier Associates (MGA) Inc. (Concord, MA). Equivalent software is now available from Pharsight (Palo Alto, CA).

The BuAc model includes compartments for the liver, lung, fat, other tissues, arterial blood, and venous blood. Fat was not included in the models of the metabolites due to their lower lipophilicity. All tissues were described as well-mixed compartments with rapid equilibration between the blood and the tissue, i.e., perfusion-limited uptake (see Abbas and Fisher, 1997; Barton *et al.*, 1999; Corley *et al.*, 1994 for examples of the relevant equations).

The rate of metabolism was described with a Michaelis-Menten equation in which metabolism is a function of the maximum metabolic rate for that tissue ($vmax_t$), the free concentration in tissue, and the concentration at which half-maximal activity occurs (Km_t). Values for $vmax_t$ were allometrically scaled by [body weight (g)]$^{0.75}$ to adjust for differing weights in experimental studies. The rate of metabolism was subtracted from the tissue in the submodel for the chemical being metabolized and then added in the analogous equation in the tissue in the submodel for the chemical being formed (see Fig. 4). Metabolism of BuAc occurred in the nose, venous and arterial blood, and liver, forming BuOH. Metabolism of BuOH occurred in the liver and the tissue compartments forming BuCHO. Oxidation of BuOH in the tissue compartment was included because metabolic clearance was too low to account for observed data if metabolism was assumed to occur only in the liver. Tissue metabolism of BuOH was described as a first-order process in which the rate constant, *koth*, is multiplied by that tissue's venous concentration to obtain the metabolic rate. Finally, metabolism of BuCHO to BuCOOH was assumed to occur in the liver.

Urinary excretion was included for BuAc, BuOH, and BuCOOH; BuCHO is too reactive for significant urinary elimination. Urinary filtration was described as a first-order process, removing compound from the venous blood.

Three routes of administration were included: iv injection, oral intubation, and inhalation. Intravenous dosing was described as a brief infusion into the arterial blood at a constant rate. Orally absorbed chemical was added directly to the liver compartment, simulating the first-pass portal flow from the gastrointestinal tract to the liver prior to distribution to the remainder of the body.

The respiratory tract for BuAc consists of the nasal compartments through which air passes and chemical is extracted prior to reaching the lung compartment. This description was used because nasal esterases affect the respiratory uptake of ethyl acetate (Morris *et al.* 1993). For the metabolites, only a lung compartment is included.

The modeling of the nose was based upon the models of Morris *et al.* (1993) and Plowchalk *et al.* (1997). These models described two airflows in the nose: the dorsal medial and lateral/ventral airstreams. The lateral/ventral airstream contacted respiratory tissue, whereas the dorsal medial contacted respiratory and olfactory tissues. These tissues were broken up into layers composed of several subcompartments, e.g., mucus or epithelium. Some of these subcompartments contain esterase activity (Plowchalk *et al.*, 1997). The model for butyl acetate simplified the description of the subcompartments in Plowchalk *et al.* (1997) to three tissue layers: mucus, epithelium, and blood exchange layers. Future modeling efforts will focus on nasal tissue as a site for local toxicity, but for this initial effort metabolism in these compartments served as presystemic clearance, decreasing the compound available to the lungs for systemic uptake.

Butanol is highly water soluble. Its alveolar absorption is reduced due to absorption of the chemical in the upper respiratory tract (Johanson 1991 and

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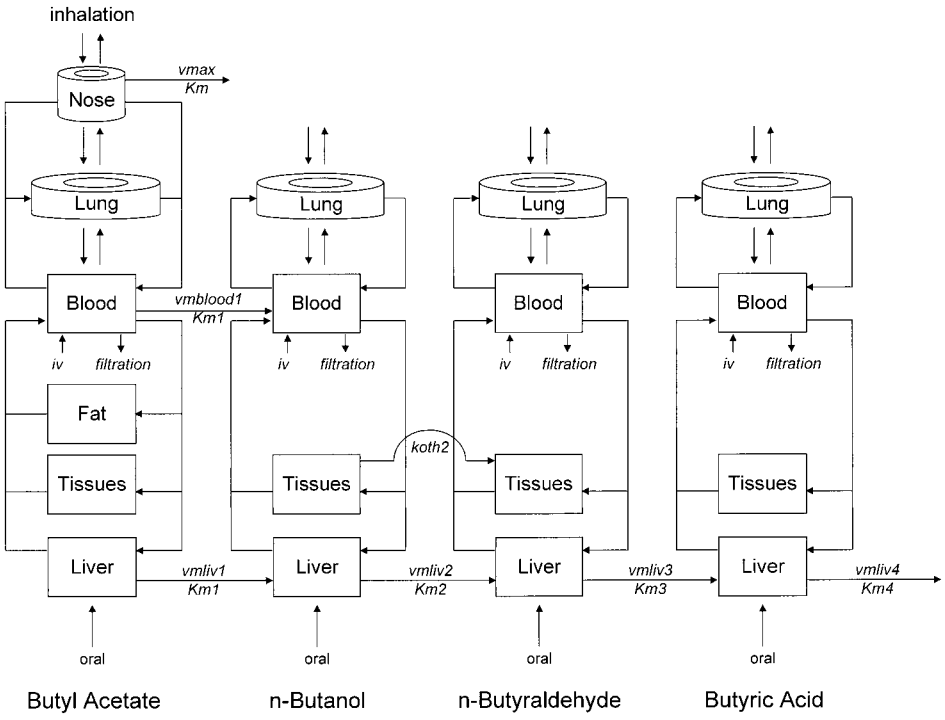

FIG. 4. Schematic of linked pharmacokinetic models for the BuAc series. Tissue compartments, blood flows, metabolic connections (i.e., vmax, Km, koth), excretion, and exposure pathways (i.e., iv, oral, inhalation) are illustrated.

references therein). This behavior has been described as a wash in-wash out phenomenon in which chemical equilibrates in the aqueous surface layer of the upper respiratory epithelium, i.e., mucus, during inhalation. It then diffuses back into exhaled air and is removed from the body. A simple approach used here for incorporating this phenomenon in a PBPK model is to limit the fraction of the incoming chemical available for absorption, as was done for 2-butoxyethanol and ethanol (Johanson, 1986; Pastino *et al.*, 1997). Butanol in humans is approximately 50% available at rest; this decreases to roughly 40% at higher levels of activity (Johanson, 1991). The 50% value was assumed to be similar for rats, for which there are no data. Thus, the incoming air concentration was multiplied by a factor representing the fraction of chemical that would be available for absorption. More complex models such as those described elsewhere could be attempted (Gerde and Dahl, 1991; Johanson, 1991).

Butyl Acetate Pharmacokinetic Studies

Pharmacokinetic studies used male Sprague-Dawley rats [Crl:CD(SD)BR VAF/PLUS] (Charles River, Stone Ridge, NY). The rats were dosed with radiolabeled BuAc by iv injection and analyzed for BuAc, BuOH, and Bu-COOH in blood and brain. Only the results from blood are reported in this paper. Prior to studies, animals were housed in wire-mesh, stainless steel cages in an environmentally controlled room with 12-hr light/dark cycles. Animals were fed certified rodent diet *ad libitum*; domestic tap water was supplied *ad libitum*. Rats were 9–12 weeks of age and weighed between 300 and 400 g at the start of the study. Animals were randomly selected from the study animal pool. Chemicals used for dosing or analytical standards were n-butyl acetate purchased from Eastman Chemical Co. (Kingsport, TN), n-butyric acid obtained from Sigma Chemical Co. (St. Louis, MO), and 1-[^{14}C]n-butyl acetate obtained from Wizard Laboratories, Inc. (West Sacramento, CA). [^{14}C]n-butanol was prepared by basic hydrolysis of [^{14}C]n-BuAc. Chemicals were assayed for HPLC and/or gas chromatography with mass selective detection to verify chemical structure and purity.

Preliminary studies were carried out to determine a high dose of BuAc that did not affect the health status of the rats (Deisinger and English, 1997). Each rat was administered 30 mg/kg (mean ± SD: 30.2 ± 0.1 for 32 rats or 16.8 ± 0.6 μCi) via a tail vein. Four animals were euthanized by exsanguination under CO_2 anesthesia at each of the following time points: 1.5–2, 2.5, 4, 7, 10, 15, 20, and 60 min following dose administration. Due to the several minutes required for obtaining the blood and stopping metabolism by deproteinizing it, for modeling purposes the measured blood concentrations were assumed to reflect *in vivo* concentrations 1 min earlier than the time of deproteinizing the sample. A sample of blood from each animal was deproteinized (Smith, 1984) using sodium tungstate and cupric sulfate. Samples were sedimented by centrifugation 16,000 × *g* for 4 min. at 5^{0}C. Clear supernatant (250 μl) was injected on a Hewlett Packard 1090 HPLC using a reverse-phase column (Whatman Partisil 10 ODS, 4.6 × 250 mm). The isocratic mobile phase consisted of 25 mM sodium formate buffer (pH 4.0) with 20% acetonitrile at 1 ml/min. The column effluent was analyzed with a radiochemical flow-through detector (Bechman 171) fitted with a 1000-μl scintillant mix flow cell. Column effluent (1 ml/min) was mixed with scintillant (4 ml/min; Mini-Scint, Radiomatic Instruments).

RESULTS AND DISCUSSION

The family approach integrates information on tissue dosimetry and toxicity in order to estimate acceptable exposure levels for all the chemicals (i.e., parent and metabolites) present in the animal's tissues following dosing with the parent compound.

Butyl Acetate Toxicity

The systemic toxicity of n-butyl acetate following inhalation exposure has been investigated in a 90-day subchronic study (Bernard and David, 1996) and a subchronic neurotoxicity study (David *et al.*, 1998). Reduced activity during exposure to relatively high levels of n-butyl acetate was noted in the neurotoxicity study; however, no cumulative neurotoxic effects were noted. Signs of systemic toxicity noted in the 90-day subchronic inhalation study were limited to reduced body

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TABLE 1
Partition Coefficient Values

Partition	Butyl acetate	Butanol
Rat blood/air	89.4 (5.5)[a]	1160 (396)
Liver/air	281 (19)	1250 (44)
Muscle/air	157 (15)	900 (130)
Fat/air	1520 (59)	900 (49)
Liver/blood[b]	3.14	1.08
Lung/blood[b,c]	1.76	0.78
Upper respiratory/blood[b,c]	1.76	0.78
Other tissues/blood[b,c]	1.76	0.78
Fat/blood[b]	17.0	0.78

Note. Data from Kaneko *et al.* (1994)
[a] The values in parentheses are standard deviations of the mean.
[b] Tissue/blood values were derived from the ratio of the tissue/air to the blood/air partition coefficient.
[c] The values for lung, upper respiratory, and other tissues were derived from the experimental muscle/air and blood/air values.

TABLE 3
Kinetic Constants for Metabolism Used in Fitting the Intravenous and Inhalation Pharmacokinetic Studies

Parameter	Inhalation study	Intravenous study
vmliv[a,b]	75	75
vmblood1[a,c]	20	45
vmliv2[a,b]	150	150
koth2[d,e]	40	40
vmliv3[a,b]	100	100
vmliv4[a,b]	4	4
Km [f]	0.1	0.1

[a] The metabolic rate constants are allometrically scaled values. The body weight (BW) specific values used in the model were generated by multiplying the allometrically scaled values by $(BW)^{0.75}$.
[b] *vmliv#*: maximal rate of metabolism in liver.
[c] *vmblood1*: maximal rate of metabolism in blood.
[d] This allometrically scaled constant was divided by body $weight^{0.25}$ to obtain the body weight specific value.
[e] *koth2*: first-order rate constant for metabolism in other tissues
[f] *Km#*: Michaelis constant (μmole/ml or mM). (All *Km* values were 0.1.)

weights and reduced weight gain in the groups exposed to relatively high levels of n-butyl acetate. Reproductive and developmental toxicity of n-butyl acetate has been investigated in both rats and rabbits (Hackett *et al.*, 1982) and suggests that the material is not a reproductive or developmental toxicant. A NOAEL of 500 ppm for 6 h/day is obtained based upon transient sedation and reductions in body weight gain at 1500 ppm.

Modeling Butyl Series Pharmacokinetics

The model was implemented for adult rats exposed to BuAc. Chemical-specific parameters were obtained from the literature or estimated by fitting previously published data or data presented here. Tissue:blood partition coefficients for BuAc and BuOH (Table 1) reflect their differing chemical properties (Kaneko *et al.*, 1994). The greater lipid solubility of BuAc compared to its metabolites is evident in the moderate fat:blood value, whereas the high water solubility of BuOH is evident from the tissue partition coefficient values near 1.0 and the large blood:air value. No data were available for the aldehyde and acid; the values for BuOH were used for both. The limited available pharmacokinetic data were used to estimate values for the metabolism (Aarstad *et al.*, 1985; Groth and Freundt, 1991; and data presented here) and clearance parameters. No data were available for BuCHO, so the modeling of this compound is highly uncertain, being bounded only by the observed production of BuCOOH. These data allow development of an initial set of values for the chemical-specific parameters (Tables 2, 3). These values are adequate for designing further pharmacokinetic studies and exploring issues of how to implement a family approach for testing and risk assessment. Subsequent studies will be used to modify these chemical-specific parameter values.

The intratracheal inhalation experiment of Groth and Freundt (1991) provided estimates of the uptake of BuAc by the lungs, its metabolism to BuOH, and the rate of clearance of BuOH. Anesthetized female Sprague-Dawley rats (290–40 g) were exposed to air containing an average concentration of 970 ppm BuAc for 5 h. Arterial blood samples were taken periodically and analyzed for BuAc and BuOH; data were digitized from graphs in this paper. Because the exposure was by tracheostomy tube, no presystemic nasal metabolic clearance would occur and metabolism rates for BuAc in the nose were set to zero. The concentration presented to the lungs was equal to the measured air concentration. These air concentrations were included in a TABLE function available in ACSL®, and interpolated values were used as the instantaneous inhalation concentrations for simulating the 5-h exposure. Metabolic rates (see Table 3) were estimated by simulating the BuAc and BuOH blood concentrations (Fig. 5).

TABLE 2
Chemical-Specific Uptake and Clearance Parameters

Parameter	Value (units)	Reference
Urinary filtration of BuAc (*kfilt1*)	0.1 (hr^{-1})	Fitted as described in text
Urinary filtration of BuOH (*kfilt2*)	30 (hr^{-1})	Based on Dietz *et al.*, 1981
Urinary filtration of BuCOOH (*kfilt4*)	0.1 (hr^{-1})	Based on DiVincenzo and Hamilton, 1979
Respiratory absorption of BuOH (*fa*)	0.5 (fractionofinhaled)	Aarstad *et al.*, 1985

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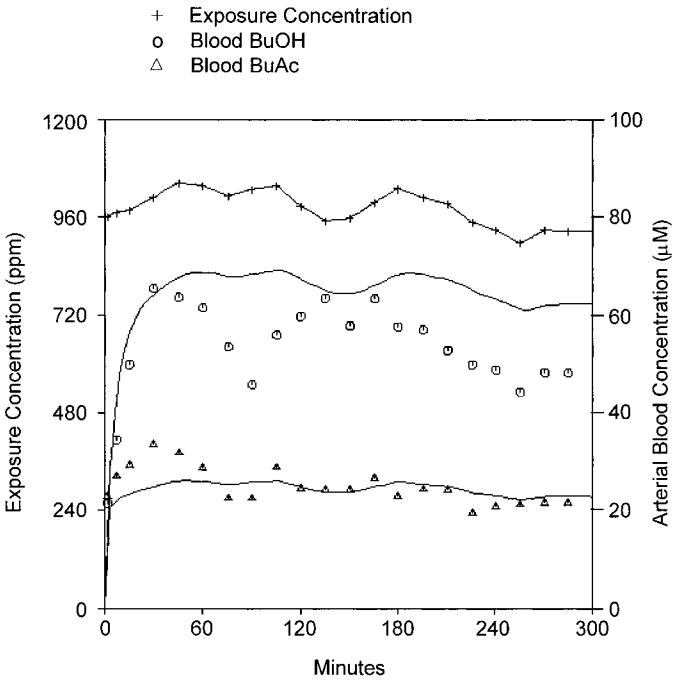

FIG. 5. Model simulation and data for inhalation exposure to BuAc (Groth and Freundt, 1991). The measured exposure concentrations (+) averaged 970 ppm. Blood concentrations of BuAc (Δ) were consistently lower than those of BuOH (○) except at the very earliest times. The solid lines are the model simulation while the symbols represent the measured data.

Blood concentrations of BuAc, BuOH, and BuCOOH were measured following iv dosing with 30 mg/kg radiolabeled BuAc as described in *MATERIALS AND METHODS*. Clearance of BuAc was rapid following iv dosing, resulting in the earliest measurements occurring in the tail of the peak (Fig. 6A). The maximum metabolic rate estimated from Groth and Freundt (1991) resulted in a peak concentration of BuOH that was too low. Therefore, higher rates of metabolism in blood or liver would be estimated from these iv data. Absent other information, the arbitrary choice was made to double the rate of metabolism in the blood (Table 3), resulting in a better simulation of the data (Fig. 6B). The rate and extent of filtration of BuOH is unknown. Studies with 2-butanol found urinary clearance up to 14% in different species, so a value of 30 h^{-1} was used for *kfilt2*, resulting in about 10% urinary excretion (Dietz *et al.*, 1981). The metabolism by tissues other than liver speeds the clearance of BuOH at later times. Simulations of BuCOOH concentrations (Fig. 6C) provided estimates for metabolism of BuCHO and BuCOOH. Again, no urinary excretion data were available for butyric acid, so it was assumed to be only a few percent, as observed for isobutyric acid in the rat (DiVincenzo and Hamilton, 1979).

Modeling Exposure Routes

An important element of the family approach is extrapolation of internal doses to multiple exposure routes. This process requires describing for each compound the characteristics of their absorption by the different routes. The model currently accommodates iv injection, an experimentally important route, and oral or inhalation exposures, routes that are important for human exposures. Dermal pathways can readily be incorporated in the future.

The inhalation model for BuAc incorporates compartments for the nose because it is a site of metabolism and toxicity. Currently, metabolism in these compartments results in approximately 10% of an inhalation dose being metabolized in the nose at 1000 ppm. This approximates the nasal metabolism observed with ethyl acetate and methyl methacrylate (Andersen *et al.*, 1999; Morris, 1990). The nasal compartment will play a more significant role when nasal tissue dosimetry is simulated for analyzing contact site toxicity.

The decreased respiratory tract availability of highly soluble alcohols such as BuOH was modeled using a factor for fractional absorption (Johanson, 1986). Blood BuOH concentrations of 0.09 mM were measured in Sprague-Dawley rats after 6-h exposure to BuOH (Aarstad *et al.*, 1985). Using the metabolic parameters previously established, a fractional absorption (*fa*) of 0.5 fitted the data.

No pharmacokinetic data were available for inhalation exposures to BuCHO or BuCOOH. The current model was structured assuming complete absorption of these compounds into the lung. No data were available for setting uptake parameters for oral absorption from the stomach; a first-order uptake rate of 1.0 h^{-1} was assumed for oral dosing simulations, based upon values used with a range of other compounds (Abbas and Fisher, 1997; Barton *et al.*, 1999; Corley *et al.*, 1994).

Estimating External Exposures Based upon Internal Dose Metrics

The fundamental principle underlying the family approach is that by estimating exposures of a compound and its metabolites that produce equivalent internal doses, the results of a toxicity study with that compound can be used to estimate acceptable exposure limits for its metabolites. Thus, if a NOAEL or BMC/D were established in a study using BuAc exposure, there also would have been internal systemic exposures to BuOH, BuCHO, and BuCOOH. By determining external exposures for these compounds resulting in the same systemic exposure, NOAELs or BMC/Ds can be estimated for each compound. This process can be done by modeling a specific NOAEL or BMC/D for BuAc. Alternatively, the models can be run iteratively for a range of doses to obtain the equivalent exposures at all those doses for the other compounds. From these graphs, equivalent external exposures can be determined and the relationships among compounds are more apparent.

By way of example, we'll assume a NOAEL of 500 ppm for 6 h/day for butyl acetate inhalation. A 13-week subchronic inhalation toxicity study (Bernard and David, 1996) and a neurotoxicity study of BuAc observed no effects at this exposure level (David *et al.*, 1998). Transient sedation and reductions in body weight gain in some animals were observed at 1500 ppm, with more significant effects at 3000 ppm.

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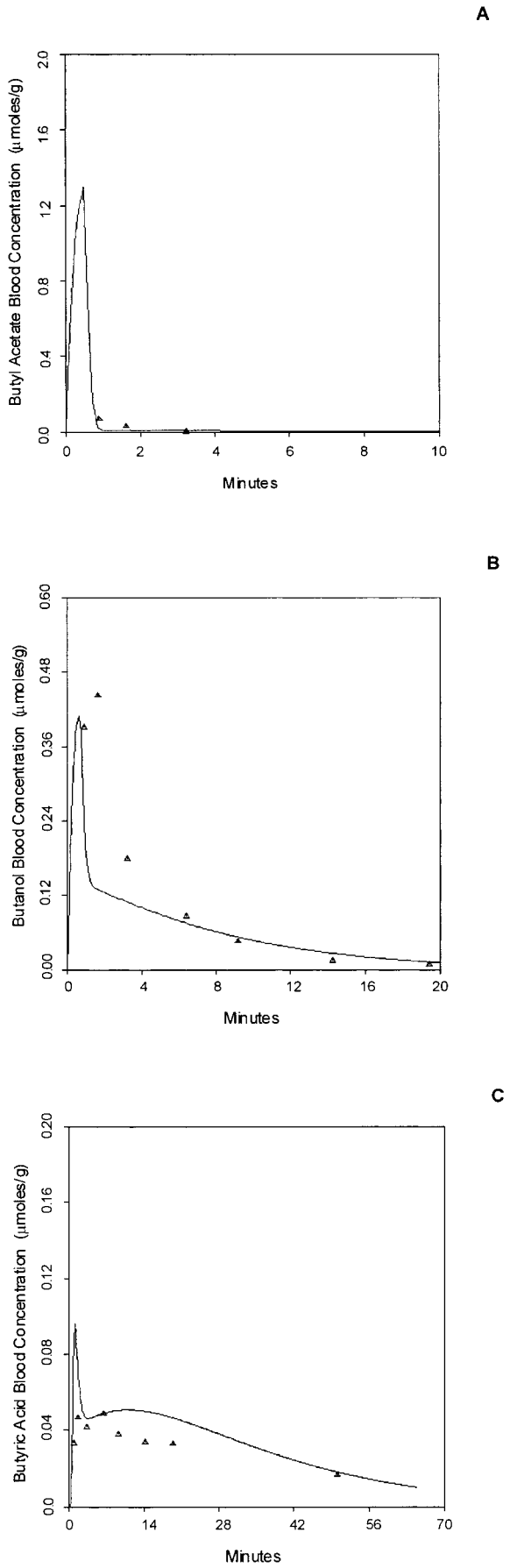

FIG. 6. Blood concentrations of BuAc, BuOH, and BuCOOH following iv injection of BuAc. The symbols represent the measured data; the solid line is the model simulation of the BuAc blood concentration. (A) BuAc (B) BuOH (C) BuCOOH.

In the simulations of exposure to 500 ppm BuAc, blood concentrations rapidly came to steady-state levels estimated at 6 μM and 26 μM for BuAc and BuOH respectively. Exposure to 820 ppm of BuOH for 6 h/day also rapidly achieves a steady-state blood concentration of 26 μM BuOH. The blood AUCs for BuOH are the same (0.16 μmol*h/ml) arising from these simulated BuAc or BuOH exposures. These exposures also result in similar steady-state concentrations and blood AUCs for BuCOOH (41 or 42 μM and 0.24 or 0.25 μmol*h/ml from BuAc or BuOH, respectively). [The focus will be on these three compounds (BuAc, BuOH, and BuCOOH) to simplify the discussion and because there was some data supporting the parameterization for these compounds.] Thus, exposure to 500 ppm BuAc or 820 ppm BuOH would be equivalent NOAELs when effects are proportional to blood concentrations of BuOH or its metabolites. If the effects arose in full or part from BuAc, this would underestimate the NOAEL for BuOH, providing a conservative health-protective point of departure for developing an acceptable exposure limit.

The apparently counterintuitive finding that a higher exposure concentration of BuOH achieves similar blood levels to those resulting from exposure to a lower concentration of BuAc simply reflects the differences in their respiratory tract absorption. Butyl acetate is essentially fully absorbed and its systemic availability is likely reduced only 10–20% by nasal metabolism (Morris, 1990). In contrast, BuOH is a highly water-soluble compound and its systemic availability may only be about 40–50% by inhalation.

The PBPK model simulates internal concentrations arising from external exposures. Estimating equivalent concentrations requires repeated model runs to identify the appropriate exposures. Therefore, we modified the model to run repeatedly, incrementing the exposure concentration each time, to obtain the dose-response relationship for the different internal dose metrics. Equivalent exposures then could be estimated from the graphs of these dose-response relationships. This is illustrated with the blood AUCs for BuCOOH arising from inhalation (Fig. 7) or oral dosing (Fig. 8) of BuAc or BuOH. Equivalent exposures producing the same internal value for the dose metric can be read off these graphs.

Under steady-state conditions achieved during extended inhalation exposure, matching the blood concentration of BuAc and BuOH results in very similar values for the AUCs in blood. Some differences exist due to metabolism in different tissues (e.g., liver and other tissue for BuOH). For transient exposures, such as single oral doses, the relationships between concentrations and AUCs are more complex. Both BuAc and BuOH are subject to very extensive first-pass metabolism by the liver following oral doses. Thus, equivalent molar amounts (e.g., 500 mg/kg BuAc and 320 mg/kg BuOH) are predicted to

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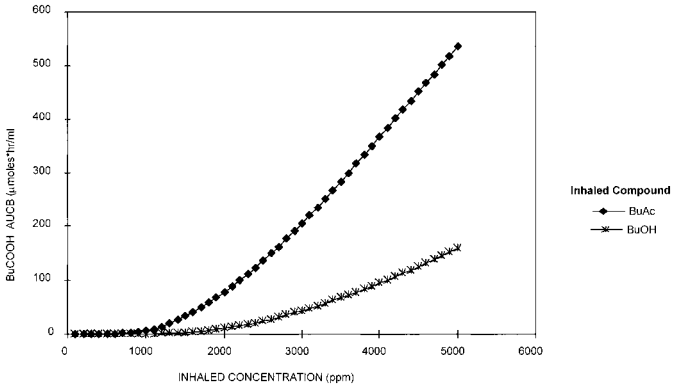

FIG. 7. Dose response for BuCOOH in blood following inhalation of BuAc or BuOH. The areas under the curve in blood for BuCOOH formed from BuAc or BuOH were estimated using the model. The curves are displaced due to the differences in the absorption of the two compounds in the respiratory tract.

produce higher peak concentrations of BuOH when dosing with BuAc than with BuOH (12 vs. 4 μM for BuAc or BuOH dosing, respectively). However, for BuCOOH the peak concentrations and AUC in blood and liver are predicted to be nearly equivalent regardless of whether BuAc or BuOH is dosed, because both are rapidly and relatively completely metabolized to BuCOOH.

The mode of action resulting in the toxicity of concern is the key determinant for selecting the appropriate dose metric (Barton *et al.*, 1998; U.S. EPA, 1996). Many systemic effects of butyl acetate and similar oxo process compounds are transient, of low adversity/severity, or apparently nonspecific, such as decreases in body weight gain. These effects appear to be concentration dependent and unlikely to depend on interactions with specific biologic receptors. For diffuse effects, there is actually no way to define a specific target tissue. Thus, average blood concentrations of the compounds should suffice as measures of overall exposure of internal tissues during toxicity testing for many of the systemic effects associated with this series of compounds.

For some effects, such as neurotoxicity, the literature on related alcohols or esters may provide a sufficient basis for selecting the appropriate dose metric (e.g., average daily concentration of alcohol) or limiting the choices of reasonable dose metrics (e.g., excluding the acid as unlikely to cause the effects). In other cases, such as alterations in body weight gain, it may simply be necessary to assume that each metabolite alone might cause the effect (and the others were inactive) and then select the lowest equivalent exposure, to be protective in the absence of better information.

Applicability of the Family Approach

The family approach offers the opportunity for efficiently obtaining and utilizing toxicity data. The example illustrated here, the oxo process compounds, may have particularly good qualities for this approach. The metabolic pathway is a series of consecutive steps that are fairly efficient, i.e., near 100% conversion in all species. Thus, the body is exposed to similar molar amounts and concentrations of each compound. The compounds are generally not highly toxic and are often associated with relatively nonspecific systemic effects, making the blood AUC a very reasonable choice for the appropriate internal dose metric. This approach would also be applicable for other dose metrics such as peak concentration in blood. Dose metrics in tissues could also be obtained by modeling, although the supporting experimental work can be much more extensive.

For other compounds such as trichloroethylene, metabolism involves complex branched metabolic pathways, making the pharmacokinetic modeling more complex (Abbas and Fisher, 1997). Branched pathways or low yield of a specific metabolite may limit the utility of the toxicity data within the family approach. The family approach will generally provide health-protective exposure limits for a minor metabolite. However, there may be little information about the toxicities that such a metabolite would produce if exposures occurred at higher concentrations. If direct human exposures to the metabolite would be at higher concentrations, additional hazard identification and dose-response information would be needed, requiring toxicity studies for that particular compound.

The family approach uses toxicity data obtained from testing with a naturally produced mixture, i.e., the parent and metabolites. The composition of the mixture changes as exposure and metabolism proceed, although steady-state conditions can be achieved such as occur for several members of the butyl series with inhalation exposure. Generally, the resulting exposure limits will be similar to those derived from testing individual chemicals or even more conservative. When there is no interaction among the compounds and the effect arises from a downstream metabolite, the results should be the same as for

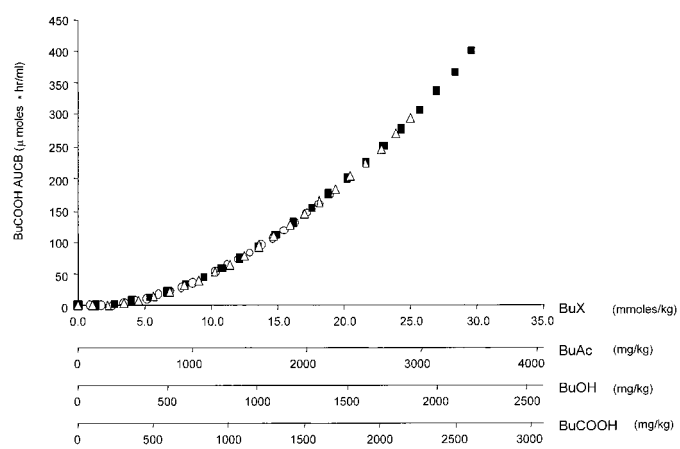

FIG. 8. Dose response for BuCOOH in blood following oral dosing with BuAc, BuOH and BuCOOH. BuX represents the dose of each compound expressed on a molar basis. The AUCs in blood for BuCOOH are essentially identical on a molar basis because virtually all of the dosed compound is metabolized to BuCOOH. Multiple x-axes illustrate the differences in exposure dose when expressed on a milligram per kilogram basis due to the different molecular weights of the dosed compounds.

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testing individual compounds. For example, if toxicity arose only from butyrate, then RfC/Ds obtained using a BuAc toxicity study with tissue dosimetry should be similar to those estimated from toxicity studies with each individual compound. If the toxicity results from some combination of upstream and downstream compounds, the limits will be conservative for the downstream compounds as compared to those obtained in the absence of the upstream compounds (e.g., the example with BuOH versus BuAc discussed above). This can be inferred from the fact that if toxicity results from more than one member of the family, assigning the total response (toxicity) to any single compound will overestimate the toxicity of that compound. Under some conditions of antagonistic interactions, the limits potentially could be less health protective than those derived from studies with each compound. Conversely, equal or more health-protective limits will generally be derived if the presence of multiple compounds increases the toxicity more than additively (i.e., synergism or potentiation).

Contact site toxicities are an issue that must also be addressed in a separate but coordinated manner. The approach outlined is based upon systemic exposures and assumes limited impact of contact site toxicities upon the pharmacokinetics. This is appropriate because toxicity studies for systemic effects would generally be done at concentrations or doses lower than those causing severe contact site toxicity. Contact site toxicities often would require direct studies, such as for the effects of esters in the nose (Plowchalk *et al.*, 1997).

There are also some specific pharmacokinetic limitations on the applicability of dose-route and intercompound extrapolations. For example, if a compound causes liver toxicity and is subject to significant first-pass clearance in the liver, it is not appropriate to use AUC in blood for dose-route extrapolation. The liver essentially acts as presystemic clearance for the oral dose route, altering the relationship between blood and liver concentrations as compared to other exposure routes. For other systemic tissues, the relationship between blood and tissue concentrations would be unaffected.

Finally, when applicable, the family approach brings another significant benefit to dose-response assessment because it inherently considers the consistency of the acceptable exposure limits for the chemicals involved. Dose-response assessment has generally been done for each chemical individually with no consideration of the results for other compounds. Yet many uses of RfC/Ds involve making decisions among chemicals. For example, choosing the chemical to use for some purpose by minimizing the risk assumes that the derivation of the RfC/Ds is consistent for the chemicals one is choosing amongst and therefore reflects their relative ranking. The family approach offers a method that can increase the use of toxicity data in a consistent manner.

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