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Evaluation of stable isotope-labeled probes in the study of solvent pharmacokinetics in human subjects

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Summary. The relationship between biomarkers of exposure (such as concentrations of toxicants in blood or breath, or metabolites in urine) and toxicant dose for individuals is influenced by many person- and episode-specific factors which contribute to overall variability in biomarker level for a given dose. This variability results in imprecise biological marker-based estimates of dose for individuals. We hypothesize that pharmacokinetic data from stable-isotope (deuterated) analogs can be used with a pharmacokinetic model to account for individual-related sources of variation, leading to more precise methods of dose estimation for individuals. To establish the degree of similarity in the pharmacokinetics of unlabeled (d_0-) and fully deuterated (d_8-) toluene, 21 men (ages 20-45) inhaled an equal molar mixture for 2 h. Washout kinetics for both compounds were followed for 4 d in alveolar air and blood. Both compounds exhibited three-phase elimination kinetics in both fluids. The third phase was not always definable for d_0-toluene because of concurrent uncontrolled environmental exposures. Considering data from only the first two phases, concentrations of d_0- and d_8-toluene in alveolar air and blood were well correlated for all subjects, even though pharmacokinetic parameters varied among individuals by 5-9 folds. Further experiments are needed to discern whether correlations between d_0- and d_8-toluene for the third phase are influenced by an isotope effect; present data support use of d_8-toluene as a suitable probe for d_0-kinetics.

Key Words: Deuterium – Metabolism – Models – Toluene

Introduction

Biological indicators of exposure are used in exposure assessment to address the combined influences of: 1.) environmental concentration of an agent; 2.) multiple routes of exposure; 3.) differential biological availability; 4.) personal characteristics including ventilatory rate, absorption, metabolism and excretion; and 5.) previously accumulated dose.

The existing rationale for regulating exposure to occupational hazards has relied on empirical relationships observed for groups of workers exposed by one route to single chemicals or simple mixtures. These relationships are used to relate a biological concentration to an environmental concentration and duration of exposure. For example, the ACGIH Biological Exposure Indices are the estimated biological concentrations expected after exposure for one shift at the Threshold Limit Value (ACGIH 1991). While this approach is useful as a qualitative means of assessing exposure, particularly for groups of workers, these indices cannot be generalized to populations with characteristics different from the worker of average physical make-up, age, or work rate (Lauwerys 1983). An additional limitation of current biological indices of exposure is the lack of a dose-response relationship, making quantitative application to low-level continuous or intermittent exposures infeasible.

It is also well recognized that large inter-individual variation exists in the distribution and fate of xenobiotics in humans (Wallen 1986). Factors that contribute are phenotype, nutrition, health status, body size and composition, toxicant interactions, and life style habits such as ethanol consumption. Empirical modeling is not able to account for these variations; therefore a biologically realistic model that is able to accommodate individual physiologic variations which are relevant to the absorption, distribution and elimination of a toxicant is needed. Such a model would serve as a framework for integrating personal characteristics into the dosimetric relationship between environmental exposure and biological markers of body burden.

We propose to use physiologically-based pharmacokinetic modeling (Andersen 1981) on individual subjects to relate exposure to biological indices, and to evaluate the model with pharmacokinetic data from tracer compounds (USEPA 1985). The tracer will be a probe of the individual's pharmacokinetics, which should account for sources of variation and lead to a more precise and powerful method of dose estimation.

The specific aims of this project are to: 1) determine the pharmacokinetic parameters that describe absorption, distribution, metabolism and excretion of toluene and its stable isotope-labeled analogs in human subjects given controlled acute inhalation exposure; 2) compare the

pharmacokinetic behavior of toluene and its analogs; and 3) assess the effect on pharmacokinetic parameters of person- and episode-specific factors including age, body composition, and repeated exposures.

A successful probe should exhibit the same pharmacokinetics as toluene through each phase of the elimination. To examine this, we conducted controlled simultaneous exposures to toluene and deuterated toluene in adult males, and monitored the washout kinetics through measurements of the two compounds and their metabolites in breath, blood and urine.

Materials and Methods

Exposures and sampling. After providing informed consent, twenty one adult males between 20 and 45 years inhaled, via mouthpiece, 100 ppm of an equimolar mixture of toluene (d_0) and fully substituted deutero-toluene (d_8) for two hours. Inhaled and exhaled air were segregated and analyzed continuously for total organics by two photoionization detectors; exhaled air was also monitored continuously for flow rate (capillary flow meter) and CO_2 (fast infrared analyzer). Together these measurements permitted calculation of the rate of absorption and the accumulated absorbed dose, and determination of end-tidal concentrations.

At selected times after exposure, breath was collected by several normal exhalations into a Tedlar bag containing 10 L dry nitrogen added to prevent condensation. The concentration of CO_2 in the diluted breath was measured, and the organic vapors present were concentrated by drawing the entire sample through a standard charcoal tube using a calibrated personal pump. At the same time a 5 ml sample of venous blood was taken from the subject's arm.

During the first 24 hours after exposure total voided urine was collected; full void spot samples were collected at scheduled times during the subsequent 72 hours.

Chemical analysis. Breath analytes were desorbed using a fixed volume of CS_2, and d_0- and d_8-toluene were quantitated by gas chromatography with flame ionization detection. Results were corrected for measured recovery efficiency (85%). Adequate resolution of the two toluene forms was accomplished with a DB-5 capillary column (30 m, 0.25 mm ID, 1.0 μm film thickness, splitless injection). The concentration of each analyte was corrected to equivalent alveolar level using the measured sample CO_2 concentration and assumed alveolar CO_2 of 5.2%.

Blood toluenes were determined by automated headspace sampling and injection into a similar gas chromatograph with flame ionization detection (Dills 1991). Blood standards were prepared by addition of toluene compounds to toluene-free donor blood. Urinary hippuric acids, benzoic acids and o-, m-, and p-cresols were determined by extraction, derivatization (Andersson 1983) and analysis by gas chromatograph equipped with mass spectrometer or electron capture detectors.

Additional measurements. Subjects' body fat was determined by measurement of skin fold thickness with calipers, using published nomograms (Dumin 1974).

Pharmacokinetic analysis. For both d_0 and d_8 toluene, the concentration-time data from breath and blood were fit to three compartment exponential decay models of the form:

$$C_{breath}(t) = A_1\exp(-\alpha_1 t) + A_2\exp(-\alpha_2 t) + A_3\exp(-\alpha_3 t)$$

$$C_{blood}(t) = \beta_1\exp(-\beta_1 t) + \beta_2\exp(-\beta_2 t) + B_3\exp(-\beta_3 t)$$

Because of hypothesized extraneous uncontrolled exposure to d_0-toluene during the washout period, it was not possible to estimate reliably the third phase coefficients for d_0-toluene in four individuals. In those cases a constant term replaced the third phase exponential decay term. In every case data were fit using weighted non-linear least squares regression.

Results

D_0-toluene and d_8-toluene washout curves in breath are shown for two subjects in Figs. 1 and 2. For each subject the pre-exposure levels of breath d_0-toluene are also shown (d_8-toluene was never detected in pre-exposure samples). Data in Figure 1 are typical of subjects with low pre-exposure toluene levels, and with close agreement between the d_0-toluene and d_8-toluene levels at all times.

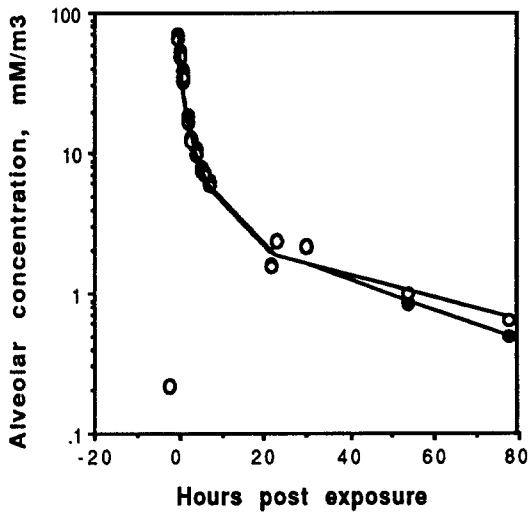

Fig. 1. Toluene washout in breath for a subject with low pre-exposure level. Open symbol: d_0-toluene; closed symbol: d_8-toluene; lines indicate calculations from three-compartment model.

On the other hand there were many subjects who showed washout curves as in Fig. 2, with a pre-exposure level four times higher, and with notable divergence of the d_0-toluene and d_8-toluene levels in breath which became marked at fifteen hours or more after exposure.

Summary statistics for the model parameters for each solvent are presented in Table 1. The overall agreement among the d_0-toluene and d_8-toluene model parameters is shown in Table 2, as a matrix of correlation coefficients for the breath data. Agreement of coefficients between the two solvents was excellent for the most rapid washout phase, whose median half-time was 0.5 h. However, neither the pre-exponential A_3 nor the time constant α_3 for the slowest phase (median half-time 38 h for d_8-toluene and 69 h for d_0-toluene) showed good agreement between the solvents.

Figs. 3 and 4 illustrate the extent of correlation for the

breath pre-exponentials A_1 and A_3, having the best and worst between-solvent agreement, respectively.

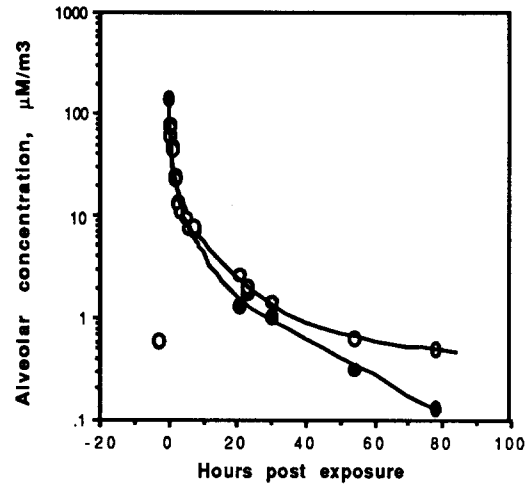

Fig. 2. Toluene washout in breach for a subject with higher pre-exposure level, and probable extraneous exposure. Symbols are as in Fig. 1.

Table 1. Coefficients of the three compartment PK model, determined by regression of breath data

d_8-Toluene	A_1	α_1	A_2	α_2	A_3	α_3
Mean	98	1.52	16.8	0.17	2.28	0.0197
Std. Dev.	51	0.68	7.63	0.07	1.38	0.0126
Median	94	1.35	16.0	0.14	2.01	0.0181
Maximum	238	3.92	38.7	0.34	5.93	0.0424
Minimum	27	0.80	6.86	0.09	0.50	0.00008
d_0-Toluene	A_1	α_1	A_2	α_2	A_3	α_3
Mean	94	1.51	15.6	0.16	2.09	0.0124
Std. Dev.	49	0.74	8.46	0.08	1.59	0.00085
Median	92	1.28	14.8	0.14	1.49	0.0101
Maximum	231	3.96	38.7	0.35	7.36	0.028
Minimum	28	0.67	1.4	0.04	0.57	0.00018

Units: A_i, µmmol/m^3; α_1, 1/hr

Table 2. Correlation coefficient (r) matrix for breath data, three compartment model parameters

d_8-Toluene	A_1	α_1	A_2	α_2	A_3	α_3
Toluene						
A_1	0.998					
α_1		0.945				
A_2			0.782			
α_2				0.576		
A_3					0.477	
α_3						0.583

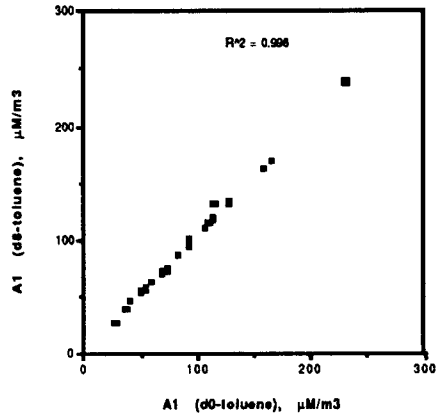

Fig. 3. Correlation of pre-exponential factors for the fastest compartment between d_0- and d_8-toluene in breath.

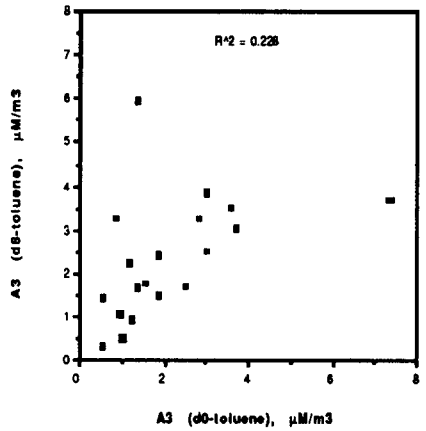

Fig. 4. Correlation of pre-exponential factors for the slowest compartment between d_0- and d_8-toluene in breath.

Discussion

Despite the large variation in model coefficients among individuals, the labeled solvent followed a washout time course that agreed closely with that of d_0-toluene for the first twelve h. The discrepancies in washout which became increasingly apparent after 24 h post-exposure could have at least two causes. First, uncontrolled extraneous toluene exposures from multiple environmental sources (Lonneman 1968; Wallace 1986) probably occurred in many subjects, during the post-exposure period. Toluene levels in subjects prior to exposure varied over a wide range, consistent with background exposures that differ among individuals, and that probably differ over time in the same person. A few subjects demonstrated highly variable d_0-toluene levels, sometimes even rising, during the last 24 hours of sampling. However, all subjects showed continuous declines in d_8-toluene levels in breath and blood during the entire sampling period. Excretion of labeled and unlabeled metabolites showed the same contrasting patterns.

The second important cause of disagreement between the two solvents is the possibility of a moderate isotope effect. Studies on rodent liver cells have shown that methyl substitution of deuterium for hydrogen in toluene causes a shift in the metabolic pathway in favor of the cresol isomers, and an increase in total metabolite production (Ling 1989). If d_8-toluene is metabolized faster than the unlabeled compound in humans at these levels of exposure, washout curves would be expected to show the pattern found in most subjects. Because of the additional confounding by probable concurrent environmental exposure to d_0-toluene, the magnitude of the putative isotope effect cannot be estimated. However, based upon our data neither effect appears to influence pharmacokinetics during the first 12 to 24 h of washout.

As an additional test of d_8-toluene as an individual tracer, the derived value of the half time for the slowest compartment was compared to the estimated body fat compartment size in each subject. Fig. 5 indicates considerable scatter, but a discernible increasing trend in the half time as body fat increases. These results are consistent with the physiological model prediction that the half time should be proportional to the compartment volume, divided by the compartment perfusion rate.

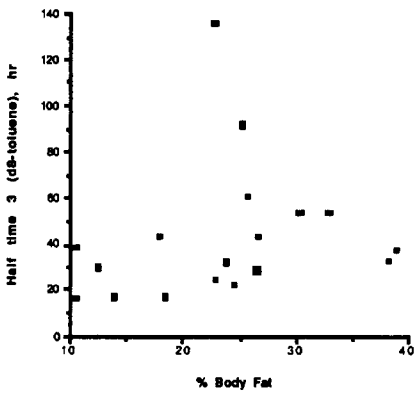

Fig. 5. Influence of fat compartment size on the half-time of the slowest compartment for d_8-toluene in breath.

In summary, the three-compartment pharmacokinetic model described the washout data well for both breath and blood measurements. There was wide variation in the model parameters among individuals, but in each case a satisfactory fit of model to data was obtained. Presumably, a multicompartment physiologically-based model will also describe the data well.

For the fastest two model compartments, model coefficients for d_0-toluene and d_8-toluene showed close agreement; the apparent disagreement for the slowest compartment was apparently caused by uncontrolled exposure to d_0-toluene from extraneous sources, and by a moderate isotope effect on metabolism, and perhaps overall pharmacokinetics, of d_8-toluene. Current experiments are directed at quantifying the isotope effects on metabolism. The consistent occurrence of a third washout phase for d_8-toluene provides strong encouragement for further use of this tracer compound in the study of individual human pharmacokinetics at low exposures consistent with occupational and environmental settings.

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