Rat to Human Extrapolation of HCFC-123 Kinetics Deduced from Halothane Kinetics: A Corollary Approach to Physiologically Based Pharmacokinetic Modeling

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The goal of this study was to develop a human physiologically based pharmacokinetic (PBPK) model for the chemical HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane) and its major metabolite, trifluoroacetic acid (TFA). No human kinetic data for HCFC-123 are available, thus a corollary approach was developed. HCFC-123 is a structural analog of the common anesthetic agent halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) and follows a common pathway of oxidative biotransformation, resulting in the formation of a specific metabolite, TFA. In this study, halothane models for rats and humans were used and validated. Then the corollary approach was used to develop a human HCFC-123 model from a rat HCFC-123 model. This strategy was implemented by using a previously validated PBPK model for HCFC-123/TFA in the Fischer 344 rat as a template model for halothane in rats. Model predictions were then compared to, and were in good agreement with, measured values for the concentration of halothane in rat blood and fat tissue. A human PBPK model for halothane was developed. The identical model structure (with the exception of the description for the fat compartment) that was used to describe halothane and TFA in the rat was used for describing halothane and TFA in the human. Human physiological parameters for tissue volumes and flows were taken from the literature, and human tissue partition coefficients for halothane were measured in the laboratory. Based on reported similarity in metabolism of halothane by humans and rats, metabolic constants for halothane in the rat were used in the human model, and specific parameters describing the kinetics of TFA were estimated by optimization. The model was validated against human exposure data for halothane from six published studies (expired breath concentrations of halothane and serum/urine data for TFA). A similar approach was then used to derive a human HCFC-123 model for humans from the HCFC-123 rat model. The corollary approach described here illustrates the innovative use of template model structures to aid in the development and validation of models for structural analogs with similar metabolism and activity in biologic systems. Furthermore, given that the PBPK model adequately describes the kinetics of halothane in rats and humans and of HCFC-123 in rats, use of the human PBPK model is proposed for deriving dose–response estimates of human health risks in the absence of human kinetic data. © 1996 Society of Toxicology

In response to the requirements of the Montreal Protocol of 1987, hydrochlorofluorocarbons (HCFCs) are currently being considered as alternatives to ozone-depleting halons (used widely as fire-fighting agents) and replacements to chlorofluorocarbons used as refrigerants, solvents, foaming blowing agents, and aerosol propellants. HCFCs possess physicochemical characteristics that contribute to low ozone-depleting potential when compared to existing compounds. The presence of C–H bonds allow HCFCs to be degraded in the troposphere, minimizing any migration to the stratosphere (Fisher et al., 1990). Coincidentally, the same useful properties that allow for the tropospheric destruction of HCFCs also allow for C–H bond oxidation and the formation of potentially toxic moieties in biologic systems (Harris et al., 1992, 1991; Huwyler et al., 1992).

Many HCFCs are structural analogs to the anesthetic halothane. In particular, the candidate HCFC substitute HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane) differs from halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) only by the replacement of bromine with chlorine. Both compounds possess geminal dihalomethyl groups (–CHX₂) and undergo similar pathways of metabolic degradation. The major pathway is via oxidation, where cytochrome-P450-dependent hydroxylation produces a dichlorogeminal halohydrin that is unstable and releases hydrochloric acid to form trifluoroacetylt chloride. This oxidative intermediate may react covalently with any nucleophile available at the site of its generation. Subsequent hydrolysis to yield trifluoroacetic acid (TFA) is a major pathway but minimal amounts of trifluoroacetyl–protein adducts or CF₃CO–proteins may also be formed (Harris et
tered the optimal approach to derive a dose-response esti-
of rat and human PBPK models for the structurally similar
cokinetic data for HCFC-123, a corollary approach was
for the dose-response assessment of HCFC-123 (Jarabek
1994). Without available human exposure and pharma-
et al.,
cally based pharmacokinetic (PBPK) model for HCFC-123
incorporated into the model as an index of oxidative metabo-
and halothane (Harris et al., 1991; Eckes and Büch, 1985;
Biermann et al., 1989). Furthermore, exposure of rats to a
single dose of HCFC-123 resulted in the formation of
CF$_3$CO–proteins that were immunochemically identical to	hose obtained with a single dose of halothane as a substrate
(Huwyler et al., 1992). CF$_3$CO–proteins from exposure to
halothane have been postulated to precipitate an immune-
mediated hepatitis in susceptible humans (Pohl et al., 1989;
Martin, 1992). Thus, the formation of a covalent adduct may
be of toxicological significance since the neoantigens formed
by exposure to HCFC-123 are immunologically identical to
neoantigens from halothane exposure (Harris et al., 1992;
Huwyler et al., 1992). Further support for this hypothesis
comes from studies of Marit et al. (1994) which demon-
strated identical morphologic lesions in the liver of guinea
pigs exposed to both compounds. These included centrolobu-
lar vacuolar (fatty) change, multifocal random degeneration
and necrosis, centrolobular degeneration and necrosis, and
subcapsular degeneration and necrosis. Additionally, glu-
thione depletion prior to exposure to HCFC-123 or halothane
potentiates hepatic injury in guinea pigs (Lind et al., 1995).

Because of the historical use of halothane as an anesthetic,
the kinetics of halothane have been studied extensively in
humans (Torri et al., 1972; Yasuda et al., 1991; Cahalan
et al., 1981; Carpenter et al., 1986; Bentley et al., 1982; Rehder
et al., 1967) and rodents (Stern et al., 1990; Eckes and Büch,
1985; Biermann et al., 1989; Gargas and Andersen, 1982;
Andersen et al., 1980). While recent laboratory studies on
the kinetics of HCFC-123 in rats have been published (Vine-
gar et al., 1994; Loizou et al., 1994), HCFC-123 is a com-
 pound that does not have a historical commercial use and
human exposure data are not available.

The objective of this study was to develop a physiologi-
cally based pharmacokinetic (PBPK) model for HCFC-123
and its oxidative metabolite, TFA, in the human that can be
used in dose–response analysis for evaluation of potential
human health risks. The stoichiometric yield of TFA was
incorporated into the model as an index of oxidative metabo-
lism, a pathway considered key to the hepatotoxicity of inter-
est for the dose–response assessment of HCFC-123 (Jarabek
et al., 1994). Without available human exposure and pharma-
cokinetic data for HCFC-123, a corollary approach was
taken based on the successful development and validation
of rat and human PBPK models for the structurally similar
compound, halothane. The corollary working hypothesis is
that a human PBPK model for HCFC-123 can then be formu-
lated based on a validated PBPK model for HCFC-123 and
its metabolite, TFA, in the Fischer 344 (F-344) rat.

PBPK models, in lieu of default equations, are consid-
ered the optimal approach to derive a dose–response esti-
mate for potential human toxicity (Jarabek et al., 1994).
Validation of the rat model, combined with the incorpora-
tion of physiologic parameters from humans into a human
model, may ultimately prove useful for extrapolating ac-
ceptable exposure limits of HCFCs in the workplace. The
human HCFC-123 PBPK model will be used to aid in the
development of occupational and community exposure
guidelines for HCFC-123.

**MATERIALS AND METHODS**

**Test materials.** HCFC-123 (98.9% pure) was obtained from Allied–
Signal, Inc. (Morristown, NJ). Halothane (99.9% pure) was obtained from
Halocarbon Laboratorones, Inc. (Hackensack, NJ).

**Tissue collection.** Rat blood obtained from the constant concentration
halothane exposure was injected into preweighed vials capped with a Teflon/
rubber septum using a side-port needle. The vials were reweighed and
placed in a freezer at −40°C until analyzed. Fat tissue was harvested next
by clipping replicate 100- to 200-mg samples from the perrenal area and
stored in a manner similar to that of the blood samples. Tissue was taken
as rapidly as possible to minimize the potential loss of chemical by exposure
to the ambient air. The time from opening the abdomen of the animal to
capping of the last tissue vial did not exceed 40 sec.

**Analytical methods.** Analysis of halothane and HCFC-123 in (1) vial
headspace, for partition coefficient determinations; (2) closed-chamber at-
mospere (gas uptake), for estimation of metabolic constants; and (3) open-
chamber atmosphere (constant concentration exposure), for exposure mon-
toring; was performed by gas chromatography. Instrumentation was selected
based on availability and component configuration. Optimization of meth-
ods was performed on each system selected. Table 1 summarizes the specific
analytical conditions and instrumentation used for all experiments in this
study.

To determine the concentration of halothane in tissue of exposed animals,
headspace analysis of the chemical in blood and fat was performed by gas
chromatography (Table 1). Vials with fat tissue were removed from the
freezer, heated to 50°C, injected with 0.5 ml of 0.4 g NaOH/mL water and
vortex mixed for 1 hr to digest the tissue. It was not necessary to digest
the whole blood. All sample vials (blood and fat) were then heated to 70°C
for 15 min to drive the halothane into the headspace. Samples were diluted
as necessary to avoid overloading the gas chromatograph detector. In order
dilute the samples, they were heated in the capped headspace vial for
15 min at 70°C, the same temperature as in the headspace sampler before
injection. A sample was removed from the vial with a syringe at 70°C and
immediately injected into a headspace vial at room temperature. The amount
of hot sample injected was corrected for expansion due to temperature.

**Animals.** Male F-344 (200 to 300 g) rats (Rattus norvegicus) were
obtained from Charles River Breeding Laboratories (Kingston, NY). Ani-
mals received Purina Formulab 5008 and softened water ad libitum. They
were housed in plastic cages (2–3 cage) with hardwood chip bedding prior
to exposure and were maintained on a 12-hr light/12-hr dark light cycle at
constant temperature (22 ± 1°C) and humidity (40–60%). Cages were
changed twice per week. All animals were euthanized by CO$_2$ asphyxiation.

**Inhalation exposures.** To determine postexposure blood and fat hal-
othane kinetics, two groups of six rats were exposed to 1000 ppm of halothane
for 4 hr. Exposures were performed in a 22.5-liter glass bell jar. The
halothane was sparged at 150 ml/min using a gas washing bottle maintained
at 0°C and introduced counterflow to ensure mixing into the chamber air
supply giving a total chamber flow of halothane in air of about 7 liters/
min. The chemical concentration was analyzed using a loop-injected gas
chromatographic system (Table 1). Halothane concentrations were con-
trolled in response to analysis by varying the sparging flow rate.
## TABLE 1
Analytical Methods for Gas Chromatographic Conditions

<table>
<thead>
<tr>
<th></th>
<th>Partition coefficients</th>
<th>Gas uptake</th>
<th>Tissue Blood/Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Halothane, rat</td>
<td>HCFC-123, human</td>
<td>Halothane, human</td>
</tr>
<tr>
<td>Gas chromatograph</td>
<td>Hewlett Packard 5890 or 5880</td>
<td>Hewlett Packard 5890</td>
<td>Hewlett Packard 5890</td>
</tr>
<tr>
<td>Auto sampler</td>
<td>Hewlett Packard 19395A</td>
<td>Hewlett Packard 19395A or Tekmar 7050</td>
<td>Hewlett Packard 19395A</td>
</tr>
<tr>
<td>Gas chromatographic column</td>
<td>OV-210, 6 in. 100/120 chromosorb W HP or Megabore capillary DB-5 30 Mx.53 mm 1D</td>
<td>10% SE-30 80/100 chromosorb W HP 12 ft x ⅛ in. SS or Vocel 30 Mx.53 mm 3.0 μm</td>
<td>10% SE-30 80/100 chromosorb W HP 12 ft x ⅛ in. SS or 10% SP-2100 80/100 Supelcoport 10 ft x ⅛ in. SS</td>
</tr>
<tr>
<td>Carrier gas (flow)</td>
<td>Nitrogen (18 ml/min) or helium (12 ml/min)</td>
<td>Nitrogen (29 ml/min) or helium (20–30 ml/min)</td>
<td>Helium (30 ml/min) or Nitrogen (33 ml/min)</td>
</tr>
<tr>
<td>Hydrogen gas flow</td>
<td>30 ml/min</td>
<td>20–40 ml/min</td>
<td>20–40 ml/min</td>
</tr>
<tr>
<td>Air flow</td>
<td>300 ml/min</td>
<td>200–400 ml/min</td>
<td>300–400 ml/min</td>
</tr>
<tr>
<td>Oven temperature</td>
<td>70°C</td>
<td>45–50 and 100°C</td>
<td>70–100°C</td>
</tr>
<tr>
<td>Injector temperature</td>
<td>125°C</td>
<td>125–130°C</td>
<td>125°C</td>
</tr>
<tr>
<td>Detector temperature (type)</td>
<td>250°C (FID)</td>
<td>250°C (FID)</td>
<td>250°C (FID)</td>
</tr>
</tbody>
</table>
Rats were euthanized serially immediately following the termination of halothane exposure and at 1, 2, 4, and 12 hr postexposure. The abdomen was opened and whole blood and fat tissues were harvested. Blood was collected from the posterior vena cava using a heparinized polyethylene syringe and 23-gauge needle. Triplicate 100-μl aliquots of blood were transferred to preseald/preweighed 20-ml glass vials as described above.

**Partition coefficient determination.** Partition coefficients for the chemical halothane were determined in the rat by using a modified version of the vial equilibration technique described by Gargas et al. (1989). Whole tissue was harvested and minced into a tissue slurry instead of homogenized in saline as reported by Gargas et al. (1989). This modification of the method eliminates the intermediate saline phase and the need for determining saline partitions. Rats were euthanized with CO₂ and whole blood was collected from the posterior vena cava using a heparinized syringe. Liver (L), quadriceps muscle (M), epididymal and perirenal fat (F), and stomach and small intestine from the gastrointestinal tract (G) were removed from the rat for vial equilibration studies. Blood samples (1.0 ml) were placed in 12.4-ml glass vials and incubated/mixed for 3 hr at 37°C with 800 ppm of chemical in the vial headspace. Whole tissue samples (1.0 g L and M; 0.50 g F and G) were minced and incubated/mixed under the same conditions as for blood, except that, which required further time, was equilibrated for 8 hr. The vial headspace containing halothane was analyzed by gas chromatography (Table 1).

Human tissue partition coefficients for HCFC-123 and halothane were determined by the saline method (Gargas et al., 1989) and the minced slurry method. Frozen liver, abdominal muscle, abdominal fat, and small intestine (human autopsy samples obtained from John L. McClellan Veteran's Administration Medical Center, Little Rock, AR) were thawed in plastic bags in a 37°C water bath. Liver and muscle were prepared as saline homogenates and as whole tissue slurries. Fat and small intestine were prepared only as tissue slurries. Whole blood was drawn from human volunteers into heparinized vacutainer tubes and processed immediately. All human tissues were then handled and prepared for analysis as described for the rat.

**Metabolic parameter estimation.** A closed-chamber gas uptake system (Gargas et al., 1986) with a volume of 8.0 liters was used for the determination of metabolic constants for rats. Six halothane exposures (N = 3 rats per exposure) were performed for 6 hr each at starting concentrations of 110, 300, 540, 1100, 2400, and 4500 ppm. Ascarite (sodium hydroxide on nonfibrous silicate carrier; Thomas Scientific, Swedesboro, NJ) (150 g) was used as the CO₂ absorber. Oxygen concentrations were maintained at 21 ± 1% during the exposures. The system flow was maintained at 2.1 liters/min with the flow to the sample loop of the gas chromatograph at 120 ml/min. The chemical concentrations in the chamber atmosphere were monitored by gas chromatography (Table 1) using a six-port gas sampling valve system. Samples were taken every 5 min for the first 30 min and every 15 min through the end of the exposure.

**Corollary modeling approach.** Without available human exposure and pharmacokinetic data for HCFC-123 and the considerable evidence that the metabolic pathways and toxicity of HCFC-123 are similar to those of halothane, a corollary approach for the dose-response analysis of HCFC-123 was used as shown in Fig. 1. Experimental kinetic studies were conducted to determine halothane kinetics in rats. These experimental studies, combined with other published kinetic studies for halothane in the rat (Stern et al., 1990; Eckes and Büch, 1985; Biermann et al., 1989; Gargas and Andersen, 1982; Andersen et al., 1980), and the validated HCFC-123 rat PBPK model served as the basis for the development and validation of a PBPK model for halothane in the rat. The human PBPK model for halothane was derived from the rat model by adjusting physiological parameters to correspond to human values. Several published human kinetic studies (Torr et al., 1972; Yasuda et al., 1991; Cahan et al., 1981; Carpenter et al., 1986; Bentley et al., 1982; Rehder et al., 1967) were used for the development or validation of the human PBPK model for halothane.

The development of a human HCFC-123 PBPK model is based, corollarily, on the development of the human halothane PBPK model. The validated rat HCFC-123 PBPK model was corrected to a human PBPK model by adjusting physiological parameters and using limited human experimental data (tissue solubility). Without human kinetic data for HCFC-123, this model cannot be directly validated. However, success using this approach with halothane gives confidence that the approach is reasonable.

**General model structure.** Three PBPK models were developed based on the model used to describe the kinetics of HCFC-123 and TFA in the rat (Vinegar et al., 1994). The identical model structure was used to describe the kinetics of halothane and TFA in the rat and human and of HCFC-123 in the human.

All models contain five anatomical compartments plus a mechanism for exchange of the parent compound between the lung blood and the end alveolar air (Fig. 2). In the rat model, a fat compartment was added that increases in size with aging, but not in direct proportion with body weight (Anderson et al., 1993). While in the human model, the fat compartment was described as a fixed proportion of body weight. All models include a gastrointestinal compartment, the blood flow from which enters the liver compartment. The kinetic behavior of the oxidative metabolite, TFA, is described by a classical single-compartment model. The kinetic disposition of TFA in the model is controlled by the rate of metabolism of the parent compound (halothane or HCFC-123) to TFA (V_{max}, K_m, K_s), the apparent volume of distribution (V_D) for TFA in the body, and the rate of renal elimination (K_{el}) for TFA. TFA formation is assumed to occur solely by metabolism in the liver and elimination by excretion from the kidney into the urine. The metabolic constants, V_{max}, K_m, and K_s, were obtained from the gas uptake data for halothane. As was the case with HCFC-123 (Vinegar et al., 1994), suppression of metabolism at high substrate concentration was observed with halothane, and gas uptake data alone were not sensitive enough to evaluate the suppression constant K_s. The suppression constant was estimated by fitting data for TFA blood concentrations using the high exposure concentrations of halothane, 15000 to 10000 ppm, from studies by Eckes and Büch (1985).

The differential and mass-balance equations describing the model are discussed in detail in Vinegar et al. (1994). All simulations were performed with SIMULEX (Mitchell & Gauthier Associates, Concord, MA), a Fortran-based continuous simulation language with optimization capabilities, on a VAX 8530 (Digital Equipment Corp., Maynard, MA).

**Metabolism.** The ability of a human to metabolize halothane was assumed to be quantitatively equivalent to the rat. This assumption was based on metabolic capacity calculations by Cahan et al. (1982) who determined that metabolic capacities, V_{max}, during vapor exposure to halothane were similar in humans, rats (Anderson et al., 1980), and miniature swine (Sawyer et al., 1971) (Table 2). Allometric scaling of metabolic capacity was used for intraspecies scaling in the rat and human and was assumed to be related by the allometric relationship, BW^{0.25}. Scaling of the elimination rate for TFA was proportional to BW^{0.25}. At high exposure concentrations of halothane (1000 ppm and above), a fitted first-order parameter, K_s, was used to account for decreased production of TFA in the rat and was assumed to occur in an equivalent manner in the human.
The physiological parameters, compartment volumes and flows, used in the rat model for halothane, the physiological parameters values of Arms and Travis (1988) for cardiac output and alveolar ventilation human model were taken from Davis and Mapleson (1981). The reference both the rat and the human model are shown in Tables 3 and 4, respectively.

Model parameters. Physiological and biochemical parameters used in both the rat and the human model are shown in Tables 3 and 4, respectively. The physiological parameters, compartment volumes and flows, used in the human model were taken from Davis and Mapleson (1981). The reference values of Arms and Travis (1988) for cardiac output and alveolar ventilation rate were used. In the rat model for halothane, the physiological parameters for compartment volumes and flows were taken from Vinegar et al. (1994). Cardiac output and alveolar ventilation rates were taken from Arms and Travis (1988). Both these parameters were scaled to body weight to the exponent 0.75 within rats and humans. Blood:air and tissue to air partition coefficients were experimentally obtained in humans and rats as described above (Analytical methods). In rats, metabolic constants ($V_{\text{MAX}}, K_{\text{M}}, K_{\text{S}}$) for halothane were fit from the closed-chamber gas uptake data (Fig. 3) and TFA blood concentrations (Fig. 4). All metabolic parameters were scaled to body weight using the allometric relationship described above (Andersen et al., 1987).

**RESULTS**

Partition coefficients. The tissue:air partition coefficients determined for HCFC-123 and halothane in the rat and the human are summarized in Table 4. Both compounds are only moderately soluble in all tissues, with rat and human fat having the largest partition coefficient values. Halothane was slightly more soluble than HCFC-123 in all rat and human tissues. Blood:air and fat:air partition coefficient values were lower in humans than in rats for both compounds. There were no differences in partition coefficient values between sources of fat tissue (epididymal and perirenal) or gastrointestinal tissue (stomach and small intestine) so the values from multiple sources were combined for each tissue, respectively.

Gas uptake studies. Chamber clearance of halothane, represented by the series of six gas uptake curves with initial concentrations ranging from 110 to 4500 ppm (Fig. 3), was adequately described as a single saturable process. The metabolic rate constant ($V_{\text{MAX}}$) and the Michaelis–Menten con-

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**TABLE 2**

Comparison of Kinetic Constants for the Metabolism of Halothane in Human, Rat, and Miniature Swine

<table>
<thead>
<tr>
<th>$V_{\text{MAX}}$ (ml/kg/min)</th>
<th>$K_{\text{M}}$ (%)</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>0.0072</td>
<td>Human</td>
<td>Cahalan et al., 1982</td>
</tr>
<tr>
<td>0.014</td>
<td>0.029</td>
<td>Rat</td>
<td>Andersen et al., 1980</td>
</tr>
<tr>
<td>0.015</td>
<td>0.04</td>
<td>Swine</td>
<td>Sawyer et al., 1971</td>
</tr>
</tbody>
</table>

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**TABLE 3**

Physiological Parameters for Rats and Humans

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat* (%) of Free Fat Body Weight</th>
<th>Human* (%) of Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue volumes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slowly perfused</td>
<td>70.6</td>
<td>57.5</td>
</tr>
<tr>
<td>Rapidly perfused</td>
<td>5.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Liver</td>
<td>4.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Gut</td>
<td>3.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Fat</td>
<td>9.1–12.6′</td>
<td>21.5</td>
</tr>
<tr>
<td>Tissue blood flows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% of cardiac output)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slowly perfused</td>
<td>35.1</td>
<td>17.7</td>
</tr>
<tr>
<td>Rapidly perfused</td>
<td>43.4</td>
<td>53.0</td>
</tr>
<tr>
<td>Liver</td>
<td>13.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Gut</td>
<td>6.1</td>
<td>17.1</td>
</tr>
<tr>
<td>Fat</td>
<td>6.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Scaled parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(body weight$^\text{1/3}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar ventilation rate</td>
<td>14.0 $^\text{a}$</td>
<td>24.0 $^\text{a}$</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>2.7</td>
<td>16.5 $^\text{a}$</td>
</tr>
</tbody>
</table>

* Parameter values taken from Vinegar et al., 1994.  
$^\text{a}$ Parameter values taken from Davis and Mapleson, 1981.  
$^\text{b}$ Range for percentage of body weight.  
$^\text{c}$ Arms and Travis, 1988.
TABLE 4  
Biochemical Parameters Used in the PBPK Model for HCFC-123/Halothane and TFA

<table>
<thead>
<tr>
<th>Metabolic constants for parent chemical and metabolite</th>
<th>HCFC-123</th>
<th>Halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat*</td>
<td>Human</td>
</tr>
<tr>
<td>$V_{max}$ (mg/hr/kg), maximum metabolic rate</td>
<td>8.8</td>
<td>8.8*</td>
</tr>
<tr>
<td>$K_m$ (mg/liter), Michaelis-Menten constant</td>
<td>0.7</td>
<td>0.7*</td>
</tr>
<tr>
<td>$K_s$ (mg/liter), suppression constant</td>
<td>65.0</td>
<td>65.0*</td>
</tr>
<tr>
<td>$V_d$ (liters/kg), volume of distribution for TFA</td>
<td>0.35</td>
<td>0.34*</td>
</tr>
<tr>
<td>$K_{eke}$ (hr⁻¹ kg⁻¹), elimination rate constant for TFA</td>
<td>0.01</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Partition coefficients for parent chemical</th>
<th>HCFC-123</th>
<th>Halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat*</td>
<td>Human</td>
</tr>
<tr>
<td>PB, blood to air</td>
<td>3.2 ± 0.4</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>PLA, liver to air</td>
<td>3.3 ± 0.3</td>
<td>3.0 ± 1.6</td>
</tr>
<tr>
<td>PRA, rapidly perfused to air*</td>
<td>3.3 ± 0.3</td>
<td>3.0 ± 1.6</td>
</tr>
<tr>
<td>PGA, gut to air</td>
<td>3.1 ± 1.1</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>PFA, fat to air</td>
<td>7.0 ± 8.0</td>
<td>49.0 ± 18</td>
</tr>
<tr>
<td>PSA, slowly perfused to air</td>
<td>2.1 ± 0.3</td>
<td>3.0 ± 2.0</td>
</tr>
</tbody>
</table>

* Parameter values taken from Vinegar et al., 1994.
# Human constants equal rat constants.
TFA constants from human halothane studies.
Values are means ± SD.
Same value as liver tissue.

stant ($K_m$) of 7.4 mg/kg/hr and 0.1 mg/liter, respectively, were obtained using Simusolv to optimize these parameter values using the gas uptake data sets. Uptake of halothane vapor showed two discernable kinetic phases, a rapid equilibration phase that lasted approximately 30 min followed by a slow linear uptake and distribution phase for the duration of the exposure (Fig. 3).

Postexposure blood and fat data. Blood and fat concentrations in rats exposed to 1000 ppm halothane for 4 hr were in good agreement with model predictions for an 8-hr postexposure period (Fig. 4). Venous concentration decreased rapidly from 15.5 mg/liter immediately postexposure to 3.5 mg/liter at 1 hr postexposure. From 1 to 8 hr postexposure, the rate of decrease in venous concentration was more gradual and represented a 95% reduction in concentration. Fat concentrations decreased gradually from a concentration of 1233.6 mg/liter at 1 hr to 116.8 mg/liter at 8 hr postexposure, with no rapid decrease in the immediate postexposure hour as with blood concentrations. This represented a 91% reduction in tissue concentration over the same time interval. Halothane levels in the fat were at least 100 times greater than in the blood. Evidence for the fat tissue as the source of halothane in the postexposure kinetics is supported by the parallel decrease in fat and blood concentrations from postexposure Hours 1 to 8. This is also consistent with the much higher fat:air partition values compared to other tissue:air values (Table 4).

Simulation of published data in rats. The TFA suppression term, $K_s$, was obtained by simulating the TFA blood concentration profile in rats exposed to halothane at 15,000 ppm for 10 min and then 10,000 ppm for 50 min (Fig. 5) using the data of Eckes and Büch (1985). The values for the maximum metabolic rate constant ($V_{max}$) and Michaelis-Menten constant ($K_m$), determined from the gas uptake studies, were held constant to solve for the value of $K_s$. The optimized (using Simusolv) fitted value of $K_s$ for halothane was 18.1 mg/liter. This fitted value for $K_s$ was used to resimulate the gas uptake data. The final values for $V_{max}$ and $K_m$ were unchanged. The TFA concentrations in blood could not be adequately simulated without incorporating this term for suppression.

Fat and venous blood concentrations in Sprague-Dawley rats resulting from a 1-hr exposure to 15,000 ppm halothane (Stern et al., 1990) were simulated using the maximum metabolic rate constant and Michaelis-Menten constant derived from the gas uptake experiments (present study) and the
RAT TO HUMAN EXTRAPOLATION OF HCFC-123 KINETICS

FIG. 3. Gas uptake data for halothane in rats collected for 6-hr with starting chamber concentrations of 4500, 2400, 1100, 540, 300, and 110 ppm. (Symbols represent actual measurements, continuous lines represent the simulations used to estimate metabolic constants.)

fitted value for the suppression constant derived from the TFA blood concentration data (Eckes and Buch, 1985). The model did an adequate job of qualitatively simulating these data (both blood and fat) that span over 3 orders of magnitude (Fig. 6). Simulated results were in close agreement (within an average of a factor of 2) with the observed measurements (Table 5). When partition coefficient values measured in the same study for fat:air (112) and blood:air (2.79) were used in the model, the data were well simulated for both tissues (Fig. 6).

Simulation of published data in humans. Human studies used to validate the human PBPK model are summarized in Table 6. For humans exposed to 1100 to 4300 ppm halothane for 0.5 to 2.0 hr, the simulations of exhaled breath concentrations of halothane were in general agreement with experimental data (Fig. 7). Other human halothane exposures reported serum and urine TFA concentrations resulting from halothane exposure (Table 6). Good agreement was obtained

FIG. 4. Concentrations of halothane in rat fat and venous blood after a 4-hr exposure to 1000 ppm. (Symbols represent actual measurements for different individual animals at each time, continuous lines represent the simulations.)

FIG. 5. Concentrations of TFA in rat venous blood during and after a 1-hr exposure of halothane, 15,000 ppm for the first 10 min and 10,000 ppm for the last 50 min (Eckes and Buch, 1985). (Symbols represent mean measurements of four to six animals; the top and bottom lines represent, respectively, the simulation without and with a term describing the suppression of halothane metabolism to TFA.)

FIG. 6. Concentrations of halothane in Sprague–Dawley rat venous blood and fat after a 1-hr exposure to 15,000 ppm (Stern et al., 1990). (Symbols represent mean measurements of three animals; the top and bottom continuous lines represent, respectively, the simulation with current study values for blood:air and fat:air partition values; the top and bottom dotted lines represent the corresponding simulation with values determined by Stern et al. (1990).)
between these serum TFA concentrations and model predictions (Fig. 8). The model slightly overpredicted the amount of TFA excreted in the urine (Fig. 9). Data presented are cumulative excretion of TFA in urine for up to 13 days post inhalation exposure (Rehder et al., 1967).

**Simulation of human exposure to HCFC-123.** A simulation of human exposure to 1000 ppm HCFC-123 is shown in Fig. 10. This simulation is of exhaled breath during 2 hr of exposure. The simulation parallels the one shown for halothane in Fig. 7 but of course no human exposure data are available for HCFC-123.

**DISCUSSION**

Without available human exposure and toxicokinetic data for HCFC-123, a "corollary" working hypothesis was developed using human data for the structural analog halothane. This corollary approach relies on interspecies extrapolation from rats to humans and parallel assumptions of metabolism for HCFC-123 and halothane in both species. Based on structural similarities, evidence that HCFC-123 toxicity is likely to be mechanistically similar to halothane (Marit et al., 1994; Harris et al., 1992, 1991; Huwyler et al., 1992), and parallel assumptions for metabolism, it is proposed that this model structure provides a rational basis for describing the general kinetic behavior of HCFC-123 and the production and systemic clearance of its metabolite, TFA, in the human. Additionally, given that the oxidative metabolic pathway is responsible for the hepatotoxicity of HCFC-123.

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**TABLE 5**

Simulated and Measured Venous Blood and Fat Concentrations in Rats after Inhalation Exposure to 15,000 ppm Halothane for 1 hr

<table>
<thead>
<tr>
<th>Minutes postexposure</th>
<th>Measured* blood concentration (mg/liter)</th>
<th>Simulated* blood concentration (mg/liter)</th>
<th>Measured* fat concentration (mg/liter)</th>
<th>Simulated* fat concentration (mg/liter)</th>
<th>Simulated fat concentration (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>296</td>
<td>284</td>
<td>579</td>
<td>2506</td>
<td>4747</td>
</tr>
<tr>
<td>1</td>
<td>189</td>
<td>246</td>
<td>499</td>
<td>ND</td>
<td>2391</td>
</tr>
<tr>
<td>3</td>
<td>112</td>
<td>199</td>
<td>405</td>
<td>ND</td>
<td>2421</td>
</tr>
<tr>
<td>10</td>
<td>81</td>
<td>123</td>
<td>233</td>
<td>2151</td>
<td>2472</td>
</tr>
<tr>
<td>30</td>
<td>43</td>
<td>45</td>
<td>77</td>
<td>ND</td>
<td>2437</td>
</tr>
<tr>
<td>60</td>
<td>20</td>
<td>15</td>
<td>39</td>
<td>2052</td>
<td>2232</td>
</tr>
<tr>
<td>120</td>
<td>ND</td>
<td>6</td>
<td>27</td>
<td>1500</td>
<td>1789</td>
</tr>
<tr>
<td>240</td>
<td>ND</td>
<td>4</td>
<td>15</td>
<td>789</td>
<td>1131</td>
</tr>
<tr>
<td>480</td>
<td>ND</td>
<td>2</td>
<td>4</td>
<td>493</td>
<td>451</td>
</tr>
</tbody>
</table>

* Measured values are digitized from figures appearing in Stem et al. (1990).
* Simulated values using Stern et al. (1990) partition coefficients for blood and fat.
* ND, not determined.

**TABLE 6**

Human Halothane Exposures with Exhaled Breath Measurements, a TFA in Serum, a and TFA in Urine a

<table>
<thead>
<tr>
<th>Concentration or inhaled dose</th>
<th>Exposure duration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000 ppm</td>
<td>1.0 hr</td>
<td>Tomer et al., 1972</td>
</tr>
<tr>
<td>2000 ppm</td>
<td>0.5 hr</td>
<td>Yassuda et al., 1991</td>
</tr>
<tr>
<td>1100 ppm (Grp I)</td>
<td>2.0 hr</td>
<td>Cahalan et al., 1981</td>
</tr>
<tr>
<td>1100 ppm (Grp II)</td>
<td>2.0 hr</td>
<td>Cahalan et al., 1981</td>
</tr>
<tr>
<td>4300 ppm (Grp III)</td>
<td>2.0 hr</td>
<td>Cahalan et al., 1981</td>
</tr>
<tr>
<td>2260 ppm</td>
<td>2.0 hr</td>
<td>Carpenter et al., 1986</td>
</tr>
<tr>
<td>9000–30000 ppm</td>
<td>2.0 hr</td>
<td>Bentley et al., 1982</td>
</tr>
<tr>
<td>166.26 mg/kg</td>
<td>1.25 hr</td>
<td>Rehder et al., 1967</td>
</tr>
</tbody>
</table>

**FIG. 7.** Ratio of end alveolar to inhaled concentration during exposure of humans to halothane. Data are a composite of six different exposure scenarios simulated; reference Table 6. (Symbols represent actual measurements; continuous lines represent the simulations.)
in both rats and humans, it is proposed that this approach to modeling be used for the dose–response analysis of HCFC-123.

The essential step toward developing a model to predict the toxicokinetics of HCFC-123 in humans was to indirectly validate the model against human exposure data for halothane. To do this, a previously validated PBPK model in the F-344 rat, for HCFC-123 and the oxidative metabolite, TFA, was used as a template model to simulate the pharmacokinetics of halothane in the rat. Empirical data on tissue solubilities (partition coefficients) for halothane were measured in the laboratory and incorporated directly into the model structure. All tissue:air partition coefficients for halothane in the rat show that it is more soluble in tissue than the analogous compound HCFC-123. Partition values agree with those reported in other studies (Table 7) where similar vial equilibration techniques were used (Gargas et al., 1989; Loizou et al., 1994).

With the exception of liver tissue, human partition coefficient values for HCFC-123 and halothane did not compare tissue-for-tissue to those measured in the rat. Tissue solubilities for blood, gastrointestinal, and fat were lower in humans than in rats for both HCFC-123 and halothane, whereas solubilities for muscle tissue were higher in humans for both chemicals. As noted under Materials and Methods, liver and muscle tissue were prepared as saline homogenates and as a minced tissue slurry. This dual analysis was performed because an earlier database for human tissue had been initiated using the saline homogenate technique. For uniformity and comparison purposes, the slurry technique was also used and the two methods were compared. There was less vari-

<table>
<thead>
<tr>
<th>Study</th>
<th>Blood:Air</th>
<th>Liver:Air</th>
<th>Muscle:Air</th>
<th>Fat:Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>6.1</td>
<td>7.0</td>
<td>4.7</td>
<td>178</td>
</tr>
<tr>
<td>Loizou et al., 1994</td>
<td>5.7</td>
<td>7.6</td>
<td>ND*</td>
<td>147</td>
</tr>
<tr>
<td>Gargas et al., 1989</td>
<td>5.3</td>
<td>7.6</td>
<td>4.5</td>
<td>182</td>
</tr>
</tbody>
</table>

* Data are shown as mean values
* ND, not determined.
ability in the slurry technique when compared to the saline homogenate technique for the same tissue. This is assumed to be related to the more homogenous presentation and greater surface area of tissue being exposed to chemical in the vial headspace. Further studies are indicated to support this finding.

Metabolic parameters were optimized from experimental data for halothane (gas uptake studies) and published data for TFA blood concentrations (Eckes and Büch, 1985). The PBPK model predictions were simultaneously optimized against data for all exposure concentrations by adjusting the maximum rate of metabolism constant ($V_{\text{max}}$) and the Michaelis–Menten constant ($K_m$). The model did a good job of simulating the data with greater than 98% of the variation accounted for with optimized values of 7.4 mg/kg/hr and 0.1 mg/liter for $V_{\text{max}}$ and $K_m$, respectively. This value for $V_{\text{max}}$ did not differ greatly from reported values of 9.7 to 10.0 mg/kg/hr (Gargas and Andersen, 1982). The value for $K_m$ in the two studies is not comparable due to the fact that Gargas and Andersen (1982) originally described $K_m$ in terms of the external exposure concentration, whereas $K_m$ in this study is defined in terms of free chemical in the liver.

Vinegar et al. (1994) demonstrated the lack of sensitivity of gas uptake data to detect the suppression of HCFC-123 metabolism at high concentrations. However, data for TFA in the blood could be simulated adequately by including a term for substrate inhibition ($K_s$, 65 mg/liter). The suppression constant decreased the loss of HCFC-123 from the chamber by 4.6%, but decreased the TFA blood concentrations by 73%. Suppression of TFA production during exposure to halothane has been demonstrated in rats (Eckes and Büch, 1985) and in guinea pigs (Lind and Gandolfi, 1992, 1993). Further discussion of substrate inhibition and the justification for its use in the PBPK model have been reviewed (Vinegar et al., 1994). The same approach for deriving the suppression constant as described by Vinegar et al. (1994) was used in this study. The value for $K_s$ was optimized by iteratively fitting TFA blood concentrations from exposures to high concentrations of halothane (10,000 to 15,000 ppm) with fixed values for $V_{\text{max}}$ and $K_m$. The need to include suppression for halothane ($K_s$, 18.1 mg/liter) is analogous to HCFC-123 ($K_s$, 65 mg/liter). The derived value of $K_s$ for halothane is 3.5 times smaller (showing greater suppression than HCFC-123); without this term, data for TFA could not be adequately simulated.

Validation of the rat model for halothane was then performed by comparing model predictions against other experimental data not used for model development. Fat and venous blood concentrations resulting from exposures to 1000 ppm halothane were reasonably simulated by the model. Sensitivity analysis of the model parameters that have the greatest influence on uptake and elimination of halothane, indicated that the blood:air (PB) and fat:air (PFA) partition values greatly influenced the goodness of fit for this data set.

As a further evaluation of the soundness of the model, an additional set of data from the literature (Stern et al., 1990) was simulated. Overall, the model predictions were within a factor of 1.1 to 3.6 to those obtained experimentally. When partition coefficient values measured in their study for fat:air and blood:air were used in the PBPK model, the data were well simulated for both tissues. As discussed above, PB and PFA have a significant impact on the uptake and elimination kinetics. The partition values reported by Stern et al. (1990) were 47–54% and 23–37% lower, for PB and PFA, respectively, than those reported in more recent studies (Table 7). It should be noted that the values measured by Stern et al. (1990) were performed with different analytical methods from those referenced in Table 7.

Stern et al. (1990) alluded to analytical factors that could have contributed to lower than expected tissue concentrations. They noted that concentrations in adipose tissue were consistently 20% higher when measured by chromatography compared to $^{19}$F nuclear magnetic resonance, but never clarified which technique was used for the data reported. Chemical concentrations in the homogenates used for calculating tissue partitioning were analyzed as described for tissues from the postexposure studies. In both instances, extraction efficiencies of less than 100% could also result in loss of quantifiable chemical. Other factors that could have contributed to analytical error (loss of volatile chemical) were the ability of polyethylene tubing to pick up chemical and atmospheric off-gassing of volatile chemicals during homogenizing in an open system. Both techniques, the injecting of tissue through PE-240 tubing and the extracting, homogenizing, and centrifuging of tissue with a system not designed to minimize exposure to the ambient atmosphere, were used by Stern et al. (1990) to prepare tissues for analysis. Nonetheless, it appears that the model did a reasonable job of simulating halothane data generated independent of model development in the rat. The next step in this corollary approach to modeling was to construct a human PBPK model for halothane.

A human PBPK model for halothane was derived from the rat model and validated against human exposure data for halothane taken from the literature. The human PBPK model structure for halothane and the metabolite, TFA, was similar to that used to describe the kinetics of halothane and TFA in the rat, differing only by the description of the fat compartment which was a fixed proportion of body weight in the human as opposed to an increasing proportion with age in the rat (Anderson et al., 1993). Using relevant human data from six published studies (expired breath concentrations of halothane and serum and urine data for TFA), specific parameters describing metabolism of halothane and the kinetics of TFA were estimated by optimization.

Once constructed, the human PBPK model for halothane was validated against published studies. Though it is recog-
nized that there is an effect of anesthesia on the circulatory and ventilatory functions in this model, no attempt was made to accommodate such physiologic perturbations. Parameters such as alveolar ventilation rate, cardiac output to tissues, and respective tissue flows are expected to change during anesthesia. Regardless, in the absence of such adjustments, the model simulations agree closely with the experimental data.

Other human halothane exposures reported serum and urine TFA concentrations resulting from halothane exposures. Good agreement was also obtained between serum TFA concentrations and model predictions. The model slightly overpredicted the amount of TFA excreted in the urine. As reported by Vinegar et al. (1994), simulations for accumulated TFA in the urine of rats exposed to HCFC-123 at three different exposure concentrations were also higher than the experimental measurements. This issue was not specifically addressed. It is likely that the one-compartment classical description is inadequate to describe the excretion of TFA in the urine. The compartmental model structure used in this model has been used successfully to model trichloroethylene and its major metabolite, trichloroacetic acid (Fisher et al., 1989), in addition to HCFC-123 and its oxidative metabolite, TFA, in serum (Vinegar et al., 1994). Again, the model was useful in providing acceptable simulations of several datasets generated by multiple authors (cited above) spanning years of research (1967–1991).

Validation of the rat and human models for halothane were performed by comparing model predictions against experimental data and data collected from the literature for both species. In both instances, the model predictions were adequate in describing the kinetics of halothane, including TFA, the proposed marker for oxidative metabolism which has been hypothesized to be involved in the mechanism of toxicity. By structural and metabolic analogy, the PBPK model structure developed for halothane in the human is also likely to satisfactorily simulate human HCFC-123 kinetics. A simulation showing exhaled breath during inhalation exposure to 1000 ppm of HCFC-123 shows that the ratio of end alveolar to inhaled concentration during exposure is higher than when being exposed to halothane. This is a consequence of the lower blood:air partition coefficient for HCFC-123. Given the unavailability of human data, the modeling of halothane (a structurally similar chemical, differing by a bromine for chlorine substitution, having virtually identical metabolic pathways, having virtually identical pathologic manifestations, and having human pharmacokinetic data available) is a reasonable approach to substantiate the use of the described model for human exposure to HCFC-123.

Ultimately, this corollary approach to modeling may be an appropriate way to make estimates on the kinetic behavior of compounds with similar structure and activity in biologic systems. Given that the PBPK model structures discussed adequately describe the kinetics of halothane and HCFC-123 in both rats and humans, use of the PBPK models is proposed as a more reasonable approach to dosimetrically adjust observed effect levels in rats to a human equivalent concentration than the use of default equations (Jarabek et al., 1994). The corollary approach described here illustrates how the innovative use of template model structures may aid in the development and validation of models for structural analogs that are similar in mechanism of action.

ACKNOWLEDGMENTS

The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHS. National Institute of Health Publication No. 86-23, 1985, and the Animal Welfare Act of 1966, as amended. The authors thank H. Leahy and J. Creech for inhalation support; R. Black, S. Neurath, J. Lipscomb, and B. Schmidt for determination of partition coefficients; D. Pollard and M. Ketcha for analytical chemistry support; and J. Frazier for critical discussion of the manuscript.

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