Designing safer chemicals: Predicting the rates of metabolism of halogenated alkanes

(hydrochlorofluorocarbons/inhalation anesthetics/toxicity/computational model/cytochrome P450)

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ABSTRACT A computational model is presented that can be used as a tool in the design of safer chemicals. This model predicts the rate of hydrogen-atom abstraction by cytochrome P450 enzymes. Excellent correlations between biotransformation rates and the calculated activation energies (ΔH_{act}) of the cytochrome P450-mediated hydrogen-atom abstractions were obtained for the *in vitro* biotransformation of six halogenated alkanes (1-fluoro-1,1,2,2-tetrachloroethane, 1,1-difluoro-1,2,2-trichloroethane, 1,1,1-trifluoro-2,2-dichloroethane, 1,1,1,2-tetrafluoro-2-chloroethane, 1,1,1,2,2-pentafluoroethane, and 2-bromo-2-chloro-1,1,1-trifluoroethane) with both rat and human enzyme preparations: $\ln(\text{rate, rat liver microsomes}) = 44.99 - 1.79(\Delta H_{\text{act}})$, $r^2 = 0.86$; $\ln(\text{rate, human CYP2E1}) = 46.99 - 1.77(\Delta H_{\text{act}})$, $r^2 = 0.97$ (rates are in nmol of product per min per nmol of cytochrome P450 and energies are in kcal/mol). Correlations were also obtained for five inhalation anesthetics (enflurane, sevoflurane, desflurane, methoxyflurane, and isoflurane) for both *in vivo* and *in vitro* metabolism by humans: $\ln[F^-]_{\text{peak plasma}} = 42.87 - 1.57(\Delta H_{\text{act}})$, $r^2 = 0.86$. To our knowledge, these are the first *in vivo* human metabolic rates to be quantitatively predicted. Furthermore, this is one of the first examples where computational predictions and *in vivo* and *in vitro* data have been shown to agree in any species. The model presented herein provides an archetype for the methodology that may be used in the future design of safer chemicals, particularly hydrochlorofluorocarbons and inhalation anesthetics.

The design of chemicals with lowest possible toxicity would decrease the damage to the environment; decrease the costs of production, health care, and site remediation; and increase the safety in the workplace (1). Although many factors are involved in the design of safer chemicals, one significant aspect is the prediction of rates of bioactivation of protoxins and procarcinogens to toxic metabolites (2). Bioactivation plays a major role in the toxicity of many chemicals, and the most important enzymes involved are the cytochromes P450 (CYPs) (2). The CYP enzymes catalyze the activation of molecular oxygen to a reactive monooxygen species (3), which can oxidize a variety of compounds (4). The transition state for the hydrogen-atom abstraction reaction is not, however, stabilized by the enzyme (3). Furthermore, the energetics of the active oxygen species is conserved among the several CYP enzymes studied (5). These factors make the CYP enzymes ideal candidates for predictive computational methods.

We describe herein the application of a computational method for predicting the CYP-mediated oxidation rates of halogenated alkanes. Developing strategies for the design of safer halogenated alkanes is a particularly relevant objective, since hydro(chloro)fluorocarbons [H(C)FCs] are being devel-

oped as replacements for the ozone-depleting chlorofluorocarbons (6). The metabolic fate and toxicity of H(C)FCs have been reviewed.

The energetics of the active heme-iron oxygen species of CYP has been estimated to be similar to that of a primary oxy radical (5). In previous studies (8), we exploited this finding to refine the pioneering work of Pudzianowski and Loew (9) and established an isoenergetic surrogate, *p*-nitrosophenoxy radical (PNR) model, for the heme-iron oxygen species. The PNR model can be used to calculate activation energies from ground-state energies with the established free-energy relationship shown in Eq. 1 (8):

$$\Delta H_{\text{act}} = 2.60 + 0.22(\Delta H_{\text{reac}}) + 2.38(\text{I.P.}), \quad [1]$$

where ΔH_{act} is the activation energy, ΔH_{reac} is the heat of reaction, and I.P. is the ionization potential of the intermediate carbon-based radical. Thus, this model depends only on ground-state energies to calculate activation energies. Previous studies on the relationship of activation energies for hydrogen-atom abstraction, calculated with this model, to the LD₅₀ values of 26 nitriles (10) and to the amount of urinary trifluoroacetic acid formed from four H(C)FCs in rats (11) indicated that the PNR model may have the potential to predict rates of biotransformation by CYP. The present study was undertaken to test the capacity of the PNR model to predict rates of halogenated alkane biotransformation.

MATERIALS AND METHODS

Instrumental Analyses. Gas chromatography/mass spectrometry analyses were conducted with a Hewlett-Packard model 5880A GC equipped with a HP-1 capillary column (dimethyl silicone gum, 25 m × 0.2 mm × 0.5 μm film thickness) and coupled to a HP-5970 mass selective detector (70 eV, electron impact). Conditions for GC analyses were as follows: splitless injection; injector temperature, 250°C; initial column temperature, 30°C for 0.5 min; program rate, 10°C/min to 200°C; final column temperature, 200°C for 3 min; interface temperature, 250°C; carrier gas, He.

Chemicals. 1-Fluoro-1,1,2,2-tetrachloroethane (HCFC-121), 1,1-difluoro-1,2,2-trichloroethane (HCFC-122), 1,1,1-trifluoro-2,2-dichloroethane (HCFC-123), 1,1,1,2-tetrafluoro-2-chloroethane (HCFC-124), pentafluoroethane (HFC-125), dichlorofluoroacetic acid, and chlorodifluoroacetic acid were obtained from PCR Research Chemicals (Gainesville, FL) and were used as received. 2-Bromo-2-chloro-1,1,1-trifluoroeth-

Abbreviations: CYP, cytochrome P450 enzyme; PNR, *p*-nitrosophenoxy radical; H(C)FCs, hydro(chloro)fluorocarbons; HCFC-121, 1-fluoro-1,1,2,2-tetrachloroethane; HCFC-122, 1,1-difluoro-1,2,2-trichloroethane; HCFC-123, 1,1,1-trifluoro-2,2-dichloroethane; HCFC-124, 1,1,1,2-tetrafluoro-2-chloroethane; HFC-125, 1,1,1,2,2-pentafluoroethane; haloethane, 2-bromo-2-chloro-1,1,1-trifluoroethane.

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ane (halothane) was purchased from Halocarbon Products (North Augusta, SC). Sodium trifluoroacetate and pyridine were purchased from Aldrich. Hep G2 cells were obtained from American Type Culture Collection. TK⁻ cells and CYP2E1-recombinant vaccinia virus were obtained from Frank Gonzalez (National Cancer Institute, Bethesda, MD).

Preparation of Pyridine-Induced Rat Liver Microsomes. Male Fischer 344 rats (Charles River Breeding Laboratories; 250–270 g) were given pyridine at 100 mg/kg (in 0.2 ml of saline per rat) per day for 4 consecutive days; the rats were kept on a 12-h light-and-dark cycle and were starved for 12 h before sacrifice (12). Microsomes were prepared according to ref. 13. Protein concentrations were measured by the method of Bradford (14), and the CYP content was determined by CO difference spectra (15).

Expression of CYP2E1 Isozyme. Human CYP2E1 enzyme was expressed in Hep G2 cells, which have CYP reductase but lack CYP activity, with a vaccinia-virus expression system, according to the method of Gonzalez *et al.* (16).

Characterization and Quantification of Metabolites *In Vitro*. Trihaloacetic acid metabolites from H(C)FCs and halothane were characterized by gas chromatography/mass spectrometry after conversion to their benzyl esters (17): (i) benzyl trifluoroacetate: retention time, 10.8 min; m/z 204 (molecular ion, M), 91 (base peak, C₆H₅CH₂); (ii) benzyl chlorodifluoroacetate: retention time, 13.9 min; m/z 220 (M), 91 (base peak, C₆H₅CH₂), 85 (CClF₂), 135 (M – CClF₂), 77 (C₆H₅); (iii) benzyl dichlorofluoroacetate: retention time, 16.72 min; m/z 236 (M), 91 (base peak, C₆H₅CH₂), 101 (CCl₂F), 135 (M – CCl₂F), 201 (M – Cl), 77 (C₆H₅). The retention times and mass spectral fragmentation patterns were identical with esters prepared from authentic trihaloacetic acids.

Trihaloacetic acid metabolites were quantified by gas chromatography/mass spectrometry after conversion to benzyl esters (17). Pentafluoropropionic acid was used as the internal standard. The molecular ion of each ester was selectively monitored. The integration ratio was linear with the mole ratio of trihaloacetate to pentafluoropropionate in the range tested (0–100 nmol). Modification of the reference protocol improved the yield of esters by $\approx$ 2-fold. Briefly, 34 mg of tetrabutylammonium hydrogen sulfate, 0.7 ml of 100 mM potassium phosphate buffer (pH 7.0), 100 μ l of 0.2 mM pentafluoropropionic acid in water (20 nmol), 0–100 nmol of trihaloacetic acid, and water to a total volume of 0.9 ml were mixed in a crimp-top glass vial and then 1 ml of methylene chloride and 20 μ l of benzyl bromide were added. The vials were sealed with Teflon-lined septa and incubated at room temperature for 2 h on a reciprocal shaker. The organic layer was then separated, extracted once with water, dried over potassium carbonate, and analyzed by gas chromatography/mass spectrometry. Standard curves were prepared in triplicate.

Measurements of Biotransformation Rate. *In vitro* rates of biotransformation of H(C)FCs and halothane were determined by measuring end-point product formation. Incubations were carried out in Teflon-lined septum-sealed 10-ml glass vials that contained 100 mM potassium phosphate buffer (pH 7.0), liver microsomes from pyridine-induced rats at 6.2 mg of protein per ml (8.5 nmol of CYP per ml) or expressed human CYP2E1 at 366 pmol/ml, 2 mM freshly prepared NADPH, and 2 μ l of liquid substrate (HCFC-121, HCFC-122, HCFC-123, or halothane) or 5 ml of gaseous substrate (HCFC-124 or HFC-125), which was bubbled slowly into the solution through a needle while a separate needle provided pressure relief. Control incubations lacked substrate. Reactions were initiated by injecting the NADPH solution into the vials after a 5-min preincubation at 37°C and were terminated after 30 min by heating the samples at 70°C in a water bath for 5 min followed by cooling in ice for 10 min (18, 19). Preliminary experiments were conducted to ensure that all the substrates were present

at saturating concentrations and that the reaction progress was linear for at least 30 min, according to the method established by Kharasch and Thummel (20). The samples were transferred to 1.5-ml plastic centrifuge tubes and were centrifuged for 5 min at 3000 rpm on a Sorvall RT6000 bench-top centrifuge to remove precipitated proteins. Samples of the supernatant were collected for quantification of trihaloacetic acids by gas chromatography/mass spectrometry after conversion to benzyl esters, as described above.

Calculation of Activation Energies. Activation energies for CYP-dependent hydrogen-atom abstraction reactions were calculated on the basis of the PNR model (Eq. 1). Gas-phase energies were calculated by MOPAC 6.0 program with AM1 Hamiltonian and precise criteria on a Silicon Graphics (Mountain View, CA) workstation. Molecular structures were constructed with the SYBYL program (Tripos Associates, St. Louis).

RESULTS AND DISCUSSION

Metabolic Fate of Selected H(C)FCs and Halothane. The *in vitro* metabolic fate of HCFC-123, HCFC-124, HFC-125, and halothane was reported previously (11, 21–24) to be trifluoroacetic acid. The sole metabolites of HCFC-121 and HCFC-122 after incubation with liver microsomes from pyridine-induced rats or expressed human CYP2E1 in the presence of NADPH were dichlorofluoroacetic acid and chlorodifluoroacetic acid, respectively (see *Materials and Methods* for details).

Biotransformation Rates of Six Halogenated Alkanes. Previous studies indicated that the pyridine-inducible CYP2E1 is the predominant isoform responsible for the oxidation of small halogenated alkanes (13, 20). Therefore, pyridine-induced rat liver microsomes were used to increase the CYP enzyme content and to facilitate the detection of acid metabolites. The biotransformation rates of five H(C)FCs and halothane were measured (Table 1). The turnover rates by CYP decreased as fluorine substitution increased from HCFC-121 (0.27 nmol/min per nmol of CYP) through HFC-125 (0.0034 nmol/min per nmol of CYP). The rates for the different substrates span a nearly 100-fold range.

Further experiments on the biotransformation rates of these compounds were conducted with expressed human CYP2E1 (Table 1). As with the microsomal preparations, the substrate with the fewest fluorines, HCFC-121, had the highest turnover rate (5.13 nmol/min per nmol of CYP) and that with the most fluorine-substituted substrate, HFC-125, had the lowest reactivity (0.067 nmol/min per nmol of CYP). The rates also span a nearly 100-fold range.

Calculation of the Activation Energies for CYP-Mediated Hydrogen-Atom Abstraction from Substrates. The heats of reaction and ionization potentials of the product radicals for a series of halogenated alkanes were calculated (Table 2). The activation energies for CYP-mediated hydrogen-atom abstractions from different substrates calculated from Eq. 1 are also given in Table 2. The activation energies ranged from 25.48 (HCFC-121) to 28.27 kcal/mol (HFC-125) and increased as fluorine substitution was increased.

Correlation of Experimentally Determined $\ln(\text{Rate})$ Values with Calculated Activation Energies. To determine if the rates of CYP-mediated biotransformation of halogenated alkanes can be predicted with the PNR model, we compared the natural logarithm of the rates of biotransformation with the activation energies calculated by the PNR model. For the rat-liver microsomal enzyme preparations, a linear correlation ($r^2 = 0.86$) was found and is shown in Eq. 2.

$$\ln(\text{rate, microsomes}) = 44.99 - 1.79(\Delta H_{\text{act}}) \quad [2]$$

Table 1. *In vitro* experimental and predicted rates of trihaloacetic acid formation

Substrate*	Microsomal rate [†]	ln(microsomal rate)		CYP2E1 rate [§]	ln(CYP2E1 rate)	
		Experimental	Predicted [‡]		Experimental	Predicted
HCFC-121	0.271 ± 0.021	-1.268 ± 0.114	-1.170	5.129 ± 0.480	1.612 ± 0.106	1.959
HCFC-122	0.287 ± 0.020	-1.255 ± 0.066	-1.319	4.514 ± 0.433	1.489 ± 0.094	0.990
HCFC-123	0.247 ± 0.042	-1.442 ± 0.173	-2.673	1.338 ± 0.108	0.277 ± 0.084	-0.082
Halothane	0.134 ± 0.023	-2.054 ± 0.171	-2.763	0.822 ± 0.189	-0.337 ± 0.282	-0.171
HCFC-124	0.025 ± 0.010	-3.981 ± 0.465	-2.999	0.529 ± 0.188	-1.181 ± 0.615	-0.629
HFC-125	0.003 ± 0.002	-5.833 ± 0.582	-4.956	0.067 ± 0.026	-3.019 ± 0.795	-3.541

Rates are given in nmol of product per min per nmol of CYP and are reported as the mean ± SEM (*n* = 4). The experimental ln values are given as the mean of the ln of each rate determination ± the SEM.

*See Table 2 for chemical formulas.

[†]The rate of biotransformation of each compound was determined with liver microsomal mixtures from pyridine-induced rats.

[‡]The predicted rate from a cross-validated model that does not use the predicted compound in the model. The partial least squares cross-validation analysis was carried out with the SYBYL program.

[§]The rate of biotransformation of each compound in expressed CYP2E1 incubations (*n* = 4).

Eq. 3 is the equation for the line obtained for the correlation of the expressed human CYP2E1 enzyme with the activation energies calculated by the PNR model ($r^2 = 0.97$; Fig. 1).

$$\ln(\text{rate, CYP2E1}) = 46.99 - 1.77(\Delta H_{\text{act}}) \quad [3]$$

Leave-one-out cross-validation was carried out to validate the predictive ability of the PNR model (Table 1). The cross-validated predicted rates of reaction reflect the ability of the model to predict the rate of reaction of a given compound that is not included in the model. The cross-validated r^2 values were 0.71 and 0.91 for the microsomal and expressed systems, respectively, indicating that the PNR model has excellent predictive capabilities. The slopes for both enzyme systems are similar (1.79 and 1.77, respectively), pointing to similar kinetic mechanisms and energetics of the biotransformation mediated by the rat and human CYP enzymes.

Qualitative structure-activity relationships for the halogenated alkanes can be obtained from the combined experimental and computational results (Table 2). Substitution at the carbon (geminal) bearing the hydrogen atom that is abstracted influences the rate of biotransformation in the order Cl > Br

> F > H, whereas substitution at the adjacent position (vicinal) affects the rate in the order H > Br > Cl > F. The former reflects the combination of inductive destabilization and resonance stabilization of the forming carbon-centered radical by substituents, whereas the latter reflects inductive destabilization.

In addition to the data for halogenated alkanes obtained from *in vitro* experiments, data are available for halogenated anesthetic metabolism *in vivo* in humans. This data set incorporates observed values for peak plasma fluoride concentrations after exposure to volatile anesthetic agents and includes methoxyflurane ($[F^-] = 61 \mu\text{M}$) (25, 26), sevoflurane ($[F^-] = 26 \mu\text{M}$) (27, 28), enflurane ($[F^-] = 17.5 \mu\text{M}$) (29), isoflurane ($[F^-] = 4.2 \mu\text{M}$) (25, 27, 28), and desflurane ($[F^-] = 0.9 \mu\text{M}$) (30). The activation energies were calculated as described above and are shown in Table 2. Correlation of activation energies of the preferred oxidation site in each molecule with peak plasma fluoride concentrations gave the line shown in Eq. 4 ($r^2 = 0.86$).

$$\ln[F^-]_{\text{peak plasma}} = 42.87 - 1.57(\Delta H_{\text{act}}) \quad [4]$$

Table 2. Calculated heats of formation, ionization potentials, and activation energies of selected halogenated alkanes

Substrate	Formula*	$H_{\text{fsub}}^{\dagger}$ kcal/mol	$H_{\text{frad}}^{\ddagger}$ kcal/mol	I.P. [§] eV	$\Delta H_{\text{reac}}^{\parallel}$ kcal/mol	$\Delta H_{\text{act}}^{\parallel}$ kcal/mol
Ethane	CH ₃ CH ₃	-17.41	15.49	9.21	4.18	25.44
HCFC-121	CCl ₂ FCHCl ₂	-71.77	-48.55	10.12	-5.46	25.48
Methoxyflurane	CH ₃ OCF ₂ CHCl ₂	-171.00	-138.65	9.31	3.64	25.55
	CH ₃ OCF ₂ CHCl ₂	-171.00	-145.75	9.99	-3.45	25.63
HCFC-122	CClF ₂ CHCl ₂	-119.06	-95.15	10.22	-4.77	25.86
HCFC-123	CF ₃ CHCl ₂	-175.42	-150.42	10.39	-3.72	26.51
Halothane	CF ₃ CHClBr	-161.36	-137.53	10.54	-4.85	26.61
HCFC-132b	CClF ₂ CH ₂ Cl	-118.51	-89.03	10.05	0.76	26.68
Enflurane	CHF ₂ OCF ₂ CHFCI	-319.70	-287.90	10.67	3.08	28.68
	CHF ₂ OCF ₂ CHFCI	-319.70	-293.89	10.46	-2.91	26.86
HCFC-124	CF ₃ CHFCI	-214.04	-188.55	10.51	-3.19	26.91
Sevoflurane	CH ₂ FOCH(CF ₃)CF ₃	-414.22	-384.67	10.16	0.82	26.97
	CH ₂ FOCH(CF ₃)CF ₃	-414.22	-381.07	10.86	4.44	29.42
Isoflurane	CHF ₂ OCHClCF ₃	-320.10	-289.80	10.83	1.58	28.71
	CHF ₂ OCHClCF ₃	-320.10	-291.73	10.44	-0.35	27.37
1,1,1-Tribromoethane	CBr ₃ CH ₃	4.70	36.96	10.26	3.54	27.80
HFC-125	CF ₃ CHF ₂	-264.90	-237.06	10.87	-0.84	28.27
Desflurane	CHF ₂ OCHFCF ₃	-369.15	-338.96	10.82	1.47	28.68
	CHF ₂ OCHFCF ₃	-369.15	-339.11	10.68	1.31	28.31
HFC-134a	CF ₃ CH ₂ F	-217.08	-187.69	10.75	0.67	28.34

*For compounds with multiple positions of metabolism the energies for the oxidation site in boldface type are given.

[†]AM1 heat of formation of the substrate (1 kcal = 4.18 kJ).

[‡]Heat of formation of the radical.

[§]Ionization potential, calculated as described in ref. 8.

^{||}Heat of reaction calculated from $\Delta H_{\text{reac}} = H_{\text{frad}} + H_{\text{f}} \text{ of } p\text{-nitrosophenol} (-14.65) - H_{\text{fsub}} + H_{\text{f}} \text{ of PNR} (14.0737)$.

^{||}Calculated from $\Delta H_{\text{act}} = 2.60 + 0.22(\Delta H_{\text{reac}}) + 2.38(\text{I.P.})$, as in ref. 8.

FIG. 1. Correlation between the measured biotransformation rates of six halogenated alkanes in human expressed CYP2E1 and the activation energies calculated with the PNR model.

A poorer correlation ($r^2 = 0.77$) is obtained when the percent oxidation at each site and the number of fluoride ions released from each site are taken into account.¹¹ Since peak plasma fluoride concentrations correlate well with the amount of fluoride released in incubations *in vitro* with human liver microsomes (20), the computational model also predicts successfully the *in vitro* metabolic rates of these five anesthetics. Such correlations are important because these inhalation anesthetics are initially biotransformed by CYP to give fluoride (20), and an excessive amount of fluoride causes kidney damage (31).

To our knowledge, these are the first *in vivo* human metabolic rates to be quantitatively predicted. Furthermore, this is one of the first examples where computational predictions and *in vivo* and *in vitro* data have been shown to agree in any species.

The two data sets presented herein validate the PNR model as a predictive method for the CYP-mediated biotransformation of halogenated alkanes and provide an excellent quantitative structure-metabolism relationship. This relationship can be exploited in the design of safer halogenated alkanes, based on the predicted amount of metabolism to potentially toxic intermediates. For example, if two structures are under consideration for development, the compound with the lower predicted metabolic rate may be a reasonable candidate for further development, assuming that toxicity and metabolism are correlated. The relationship between metabolic rates and toxicity is discussed below.

Although these results indicate that the electronic PNR model is sufficient for predicting the metabolic rates for the halogenated alkanes, the relationship between the predicted rates of biotransformation and toxicity is less certain. For compounds that are directly biotransformed to toxic products—e.g., nitriles being converted to cyanide—biotransformation and toxicity are well correlated (10). Similarly, the conversion of H(C)FCs to acyl halides is correlated with

neoantigen formation (23). For the inhalation anesthetics presented in this study, a correlation between rate of metabolism to give fluoride and renal toxicity also exists (20). Furthermore, 1,2-dichloro-1,1-difluoroethane (HCFC-132b) (Table 2) is predicted to have relatively high biotransformation rate and has proven to be toxic (32), whereas 1,1,1,2-tetrafluoroethane (HCFC-134a) (Table 2) is predicted to have low biotransformation rate and is now in commercial production and use. If, however, toxicity is associated with further biotransformation of initial products, predicted rates of biotransformation may not be correlated with toxicity. For example, 1,2-difluoroethane is metabolized to fluoroacetic acid, which is highly toxic (33). In this instance the relative rate of biotransformation can be predicted and compared with that of a compound that does not produce fluoroacetic acid, but it would not be expected to be related to the toxicity of the two compounds. Hence the computational model can help in the design of safer chemicals, but knowledge of the underlying mechanism of toxicity of a compound and its metabolites is critical to the correct interpretation of the results.

The agreement between the predicted and experimental rates of reaction is not expected on the basis of the results of kinetic experiments on CYP-mediated reactions. It has been shown that hydrogen-atom abstraction is not the rate-limiting step in the CYP catalytic cycle (34). We and others, however, have shown that the isotope effects for CYP-mediated reactions can be observed for CYP-mediated hydrogen-atom transfers (35) even if the hydrogen-atom transfer step is not rate limiting. The observation of a linear correlation between rates and calculated activation energies can be explained by at least two possible kinetic mechanisms: (i) A branched pathway could exist for an alternative product to be formed from the same enzyme-substrate complex as oxidation of the halogenated hydrocarbon. Since no alternative position for metabolism exists in most of the compounds studied, this alternative product is likely water. Atkins and Sligar (36) have shown that water formation from the two-electron reduction of the CYP-heme-iron-oxygen can unmask isotope effects in CYP-catalyzed reactions. This same effect could unmask the differences in intrinsic rates for the different halogenated hydrocarbons. (ii) The observed rates could reflect a combination of steps, including the oxidation step—i.e., the oxidation step is only partially masked. Thus, a percentage of the relative difference in rates may still be observed. Finally, a combination of these factors could lead to the observed results.

It is also surprising that a purely electronic model can account for the differences in the rates of biotransformation. Previous studies by Jones (37) showed that the interchange of different orientations of xylene inside cytochrome P450 active site is fast. However, for bulky molecules, such as benzo[*a*]pyrene and nicotine (38), the spatial interaction between the substrate and the apoprotein becomes significant and steric factors, in addition to electronic factors, influence the rate.

Lipophilicity is a common parameter used in structure-activity relationships (39, 40). CYPs, in general, prefer lipophilic substrates. The compounds we selected for study are all relatively lipophilic, and the possibility that lipophilicity plays a role must be considered, but electronic factors were shown to be the major determinants of rates.

In conclusion, we present a validated model that predicts rates of halogenated alkane metabolism *in vitro* and *in vivo* in humans and in rodents. These results provide a strategic tool in the design of safer halogenated alkanes. Furthermore, the ability to predict human metabolic rates provides a tool to predict the potential toxicity of compounds that cannot be tested in humans. This model may be important in decreasing the cost of chemical development and in the design of safer chemicals. Integration of this model into the process of chemical discovery and development and into the regulatory review process of existing chemicals may also decrease the costs of

¹¹There are two oxidation sites in each molecule, each of which results in the release of different number of fluoride ions. There is no literature report as to how many fluoride ions are released from each of these inhalation anesthetics. If we assume that oxidation at each position results in the release of the maximal mechanistically feasible number of fluoride ions and that the net rate of fluoride release (rate $[F^-]_{calc}$) is the sum of fluoride release rate from each site, with percent partition between two sites calculated according to their relative activation energies (10), the following correlation is obtained ($r^2 = 0.77$): $\ln[F^-]_{peak\ plasma} = 0.52 + 0.95 \ln \text{rate } [F^-]_{calc}$. Recent studies on the metabolism rates of HCFCs with multiple oxidation sites showed that regioselectivity is governed by the relative activation energies associated with each site (41).

chemical development and result in the design of safer chemicals.

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