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Extrapolation of Hepatic Concentrations of Industrial Chemicals Using Pharmacokinetic Models to Predict Hepatotoxicity

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Abstract

In this review, we describe the absorption rates (Caco-2 cell permeability) and hepatic/plasma pharmacokinetics of 53 diverse chemicals estimated by modeling virtual oral administration in rats. To ensure that a broad range of chemical structures is present among the selected substances, the properties described by 196 chemical descriptors in a chemoinformatics tool were calculated for 50,000 randomly selected molecules in the original chemical space. To allow visualization, the resulting chemical space was projected onto a two-dimensional plane using generative topographic mapping. The calculated absorbance rates of the chemicals based on cell permeability studies were found to be inversely correlated to the no-observed-effect levels for hepatoxicity after oral administration, as obtained from the Hazard Evaluation Support System Integrated Platform in Japan ($r = -0.88$, $p < 0.01$, n = 27). The maximum plasma concentrations and the areas under the concentration-time curves (AUC) of a varied selection of chemicals were estimated using two different methods: simple one-compartment models (i.e., high-throughput toxicokinetic models) and simplified physiologically based pharmacokinetic (PBPK) modeling consisting of chemical receptor (gut), metabolizing (liver), and central (main) compartments. The results obtained from the two methods were consistent. Although the maximum concentrations and AUC values of the 53 chemicals roughly correlated in the liver and plasma, inconsistencies were apparent between empirically measured concentrations and the PBPK-modeled levels. The lowest-observed-effect levels and the virtual hepatic AUC values obtained using PBPK models were inversely correlated ($r = -0.78$, $p < 0.05$, n = 7). The present simplified PBPK models could estimate the relationships between hepatic/plasma concentrations and oral doses of general chemicals using both forward and reverse dosimetry. These methods are therefore valuable for estimating hepatotoxicity.

***Key words*:** PBPK modeling, Caco-2 permeability, Hepatotoxicity, No-observed-effect level, Lowest-observed-effect level

INTRODUCTION

There is a wide variety of human-made chemicals in the environment. Organic compounds originating from myriad natural processes and industrial sources (1) are also ubiquitous in the human environment. Because long-term exposure to some volatile organic compounds may increase the risk of cancer or birth defects, estimation of the exposures to such compounds is a research area that can have a significant positive impact on human health (2). Estimation of health risks due to chemical substances has historically followed guidelines on investigatory studies with experimental animals. The general toxicities of industrial chemicals have been extensively investigated by administering repeated oral doses of the chemicals to rodents. Nonetheless, in general, repeated-dose toxicity studies for many chemicals may require significant cost and time. For animal welfare, the current movement has been focused on developing alternative methods such as *in vitro* studies or *in vitro* studies mimmicking on *in vivo* studies.

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Recently developed high-throughput *in vitro* screening assays combined with *in silico* computational models might provide suitable alternative methods to conventional animal testing. However, the pharmacokinetics and/or toxicokinetics (including intestinal absorption) of industrial chemicals are not generally considered when evaluating their toxicological potential. Although extensive toxicity databases have been set up worldwide, only a limited numbers of industrial chemicals possess adequate *in vivo* toxicokinetic data related to pharmacokinetic parameters, such as absorption rates, for assessing potential hazards in humans (3). This fact highlights the urgent and significant need to develop more efficient and informative tools to determine toxicity.

To interpret toxicity data obtained from high-throughput *in vitro* screening, scientists at the United States regulatory authorities have recommended the inclusion of both dosimetry and exposure data supported by complex multicompartment pharmacokinetic models using full-scale physiologically based pharmacokinetic (PBPK) modeling (4,5). Furthermore, recent studies have proposed extrapolation of *in vitro* toxicokinetic screening data to predict *in vivo* toxicokinetics based on chemical structure-based properties (6,7). Against this background, our research group established a simplified systematic PBPK modeling with input parameters derived from *in vitro* and *in vivo* data and the literature (8,9). Because of the utility and simplicity, these models could also be used by the regulatory authorities and the industrial researchers for risk assessment.

A five-year project has started in April 2017, sponsored by the Ministry of Economy and Trade Industry in Japan. The aim is to develop a high-accuracy *in silico* hazard prediction system based on toxicity mechanism using accumulated *in vivo* data over the past 40 years under the Chemical Substance Control Law and the results of *in vitro* studies in toxicology, along with artificial intelligence technology and human expertise (AI-based Substance Hazard Integrated Prediction System, https://ai-ships.net/en/project.html). In this review, the absorption rates (Caco-2 cell permeability) and estimated plasma/hepatic pharmacokinetics of a variety of chemicals are evaluated as a part of this national project. The pharmacokinetic parameters were estimated using two approaches to model different chemicals orally administered to rats. The models made use of the reported or experimentally determined plasma pharmacokinetics data. If a successful method could be established to derive the *in vivo* absorption parameters of general chemicals from the measured *in vitro* permeability values, it would be of great benefit. Chemical risk assessment involves the prediction of hepatotoxicity, and the simplified PBPK models used here could predict the hepatic as well as the plasma concentrations of drugs and other chemicals after oral ingestion using both forward and reverse dosimetry.

ORAL ABSORPTION OF CHEMICALS AND THEIR HEPATOTOXICITY

The *in vitro* permeability assay is an established method in pharmaceutical research and is based on human models that use Caco-2 colon cancer cell line to estimate the oral absorption of drugs and other substances (10-14). However, little information is available on the absorbability of industrial chemicals through the mucosa and/or gastrointestinal absorption, which is a necessary step before such chemicals can exert their toxicological potential.

The procedures used for preparing *in vitro* monolayers of human intestinal Caco-2 cells have been described previously (15). For experimental use, Caco-2 cells were seeded on permeable polycarbonate membranes at a density of 1.0×10^5 cells/cm^2 and were cultured for 3-4 weeks. The apparent permeability coefficients (P_{app}, nm/s) for time-dependent *in vitro* absorption from the apical side of the Caco-2 monolayer [10 mM 2-morpholinoethanesulfonic acid monohydrate (pH 6.0)] to the basal side of the Caco-2 monolayer [10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4)] were calculated as described previously (15) (Fig. 1).

To investigate the relevance of pH-dependent Caco-2 monolayer assays in the *in vivo* absorption rate, the relationship was examined between the measured log P_{app} values for the drugs and their reported absorbabilities (fraction absorbed, F_a) in humans. To ensure that the substances analyzed exhibited a diversity of chemical structures, the structures defined by 196 chemical descriptors in a chemoinformatics tool were calculated for 50,000 randomly generated molecules in the original chemical space (15). To allow visualization, the resulting chemical space underwent projection onto a two-dimensional plane using generative topographic mapping (Fig. 2). In this figure, closer plots in the chemical space could indicate more similarity in the chemical properties. A wide range of apparent permeability coefficients (P_{app}) (approximately 10-1,000 nm/s)

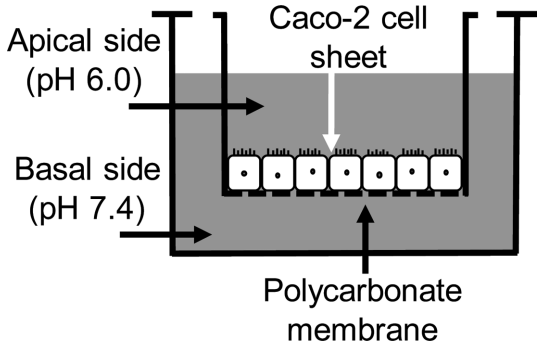

Fig. 1. Apparatus for intestinal epithelial permeability testing. The Caco-2 cell monolayer system was used for a variety of general chemicals.

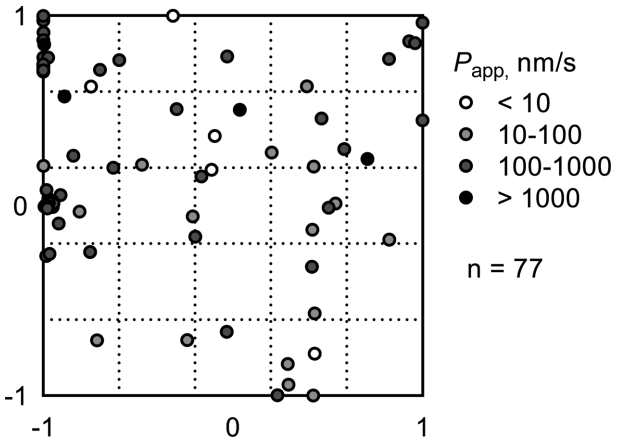

Fig. 2. Coordinate values in a two-dimensional plane illustrating a variety of chemical structures. The density of the circles indicates apparent permeability values of the chemicals across an intestinal epithelial cell monolayer.

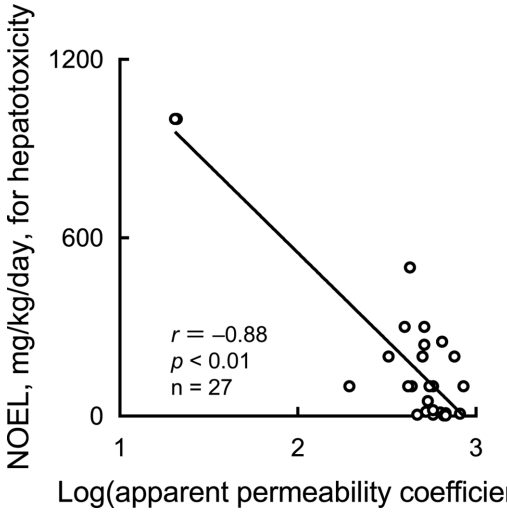

Fig. 3. The inverse relationship between hepatic NOEL values of the industrial chemicals reported in a rat database and the apparent permeability data measured using Caco-2 permeability assays.

across human intestinal epithelial Caco-2 cell monolayers was obtained for a diverse range of drugs/industrial chemicals. There was no evident relationship between the apparent permeability coefficients and the location on the chemical space represented by the two-dimensional plane in this study. Under the present conditions, the oral administration no-observed-effect levels (NOEL) given in the Hazard Evaluation Support System Integrated Platform (HESS) in Japan (16) and the chemical absorbance rates obtained from *in vitro* cell permeability data were found to be inversely correlated ($r = -0.88$, $p < 0.01$, n = 27, Fig. 3).

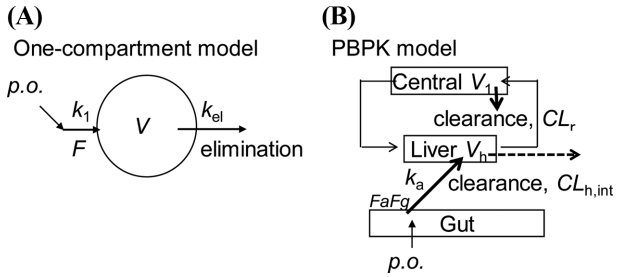

Fig. 4. Schematic representations of one-compartment (A) and PBPK (B) models to simulate virtual oral administrations of the chemicals. F, F_a, and F_g: bioavailability, k_1, and k_a: absorption constants, V, V_1, and V_h: volumes of distribution, k_{el}: elimination constant, and CL_h and CL_r: clearances.

HEPATIC CONCENTRATIONS OF CHEMICALS AND THEIR TOXICITIES

The current study employed two modeling systems, the simple one-compartment model (Fig. 4A) that was recently recommended by US authorities as a high-throughput toxicokinetic screening tool, and a simplified PBPK model (Fig. 4B) made up of chemical receptor (gut), metabolizing (liver), and central (main) compartments. Examples of the plasma concentration curves of individual general chemicals estimated by the two models after virtual oral doses were reported previously (8,17-28).

The simplified PBPK models were established as previously described (9,15). For some chemicals, a peripheral compartment was used in addition to the central compartment. The general procedure for PBPK modeling is as follows: the octanol-water partition coefficient (log P) and the plasma unbound fraction ($f_{u,p}$) of the general chemicals were estimated *in silico* using Chemdraw and the Simcyp simulator (29). The blood-to-plasma concentration ratio (R_b) and the liver-to-plasma concentration ratio ($K_{p,h}$) were calculated from $f_{u,p}$ and log P values (30):

$$K_{p,h} = \frac{0.02289 \cdot P + 0.72621}{0.00396 \cdot P + 0.960581} \times \frac{1 + f_{u,p}}{2}$$

The physiological values, such as the hepatic blood flow rate (Q_h) in rats (0.853 L/h) were obtained from the literature (31). The volume of the systemic circulation (V_1), the absorption rate constant (k_a), and the *in vivo* hepatic intrinsic clearance ($CL_{h,int}$) were evaluated using nonlinear regression analysis fitting techniques, as described previously (15). The renal clearance (CL_r) was set at a minimum value as compared to the hepatic clearance (CL_h). Finally, to perform modeling, the following system of differential equations (8) was solved:

$$\frac{dX_g}{dt} = -k_a \cdot X_g \text{ when at } t = 0,\ X_g(0) = F_a \cdot F_g \cdot dose$$

$$V_h \frac{dC_h}{dt} = Q_h \cdot C_b - \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} + k_a \cdot X_g - CL_{h,int} \cdot \frac{C_h}{K_{p,h}} \cdot f_{u,p}$$

$$V_1 \frac{dC_b}{dt} = -Q_h \cdot C_b + \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} - k_{12} \cdot V_1 \cdot C_b + k_{21} X_{peripheral} - CL_r \cdot C_b$$

where X_g and $X_{peripheral}$ are the amounts of the chemicals in the gut and peripheral compartments, respectively; C_h and C_b are the hepatic and blood chemical concentrations, respectively; V_h and V_1 are the volumes of the liver and the central compartment, respectively; F_a and F_g are availability; k_{12} and k_{21} are rate constants; and Q_h is the hepatic blood flow rate of the systemic circulation in the hepatic compartment.

The calculated maximum plasma concentrations and areas under the concentration-time curves (AUC) of 34 diverse chemicals (15) and an updated selection of 70 chemicals (unpublished) obtained using the one-compartment models and our simplified PBPK models were found to be consistent ($r > 0.95$). However, for 53 chemicals as shown in Table 1 (15) [and an updated selection of 70 chemicals (unpublished)], there were only approximate correlations (r = ~0.4-0.6) between the hepatic and plasma concentrations and AUC values (15) as estimated using our PBPK model. Moreover, the liver and plasma concentrations of the tested chemicals estimated by the PBPK models differed considerably from the reported empirical values.

Among the compounds with a broad diversity of structures that were used in the PBPK modeling, seven had reported hepatoxicity NOEL or lowest-observed-effect level (LOEL) values in the HESS database (Table 2) (16). The LOEL values in rats for oral administrations of 2-mercaptobenzimidazole (CAS No. 583-39-1), 4-nonylphenol (84852-15-3), paraacetaldehyde (123-63-7), 1,2,3-trimethylbenzene (526-73-8), 1,2,4-trimethylbenzene (95-63-6), *m*-cresol (108-39-4), and bisphenol A (80-05-7) were of the order of 10, 250, 300, 300, 300, 300, and 600 mg/kg/day, respectively. The main PBPK model parameters, namely, absorption rate constants, distribution volumes, and hepatic intrinsic clearance values, for these seven compounds are shown in Table 2. Among the simple PBPK modeling results shown in Table 2 for virtual oral administration of 1.0 mg/kg to rats, the highest values for the maximum plasma concentration and AUC were observed

Table 1. Names of the chemicals tested in the current PBPK modeling

Acetaminophen	Chlorpyrifos	Itopride	Omeprazole	Thalidomide
Acrylonitrile	*m*-Cresol	Lenalidomide	Oseltamivir	Tolbutamide
Alprazolam	*p*-Cresol	Losartan	Paraacetaldehyde	Toluene
Aniline	Dabigatran	Melengestrol acetate	Pemafibrate	Trichloroethylene
Apixaban	Dextromethorphan	2-Mercaptobenzimidazole	PF-04937319	Trimethylamine
Apomorphine	Dichlorodiphenyltrichloroethane	Metoprolol	Pomalidomide	1,2,3-Trimethylbenzene
Atomoxetine	Dichloromethane	Midazolam	Rivaroxaban	1,2,4-Trimethylbenzene
Azithromycin	1,4-Dioxane	Molinate	Styrene	Verapamil
Benzydamine	Disopyramide	Mono(2-ethylhexyl) phthalate	Tetrabromobisphenol A	Warfarin
Bisphenol A	Edoxaban	Nicotine	2,3,5,6-Tetrafluorobenzylalcohol	
Caffeine	Fluvoxamine	4-Nonylphenol	Tetramethylammonium	

These chemicals are those examined in our previous report (15).

Table 2. PBPK parameters and modeling results of seven compounds after virtual oral administration (1.0 mg/kg) and their reported hepatic NOEL and LOEL values in rats

Substrate	CAS No.	Log P_{app}	PBPK parameters			PBPK modeling results				NOEL	LOEL
			k_a, 1/h	V_1, L	$CL_{h,int}$, L/h	C_{max}, ng/mL	AUC, ng h/mL	Liver C_{max}, ng/g	Liver AUC, ng h/g	mg/kg/day	
2-Mercaptobenzimidazole	583-39-1	2.8	1.9	1.6	0.1	172	2500	2550	11600	2	10
4-Nonylphenol	84852-15-3	NA	1.4	1.2	48.4	203	1350	2440	6570	15	250
Paraacetaldehyde	123-63-7	NA	2.9	0.2	0.1	1070	7930	840	5960	100	300
1,2,3-Trimethylbenzene	526-73-8	NA	1.4	1.6	7.3	30	180	630	1130	30	300
1,2,4-Trimethylbenzene	95-63-6	NA	1.5	1.7	7.6	29	175	683	1110	100	300
m-Cresol	108-39-4	2.9	2.9	0.1	2.0	459	421	913	668	100	300
Bisphenol A	80-05-7	2.5	3.5	2.6	62.4	15	66	1070	373	200	600

NA, not available. Data were obtained from our previous report (15).

for paraacetaldehyde (1,070 ng/mL and 7,930 ng h/mL, respectively). In contrast, the highest values for the maximum concentration and AUC in the livers were seen with 2-mercaptobenzimidazole (2,550 ng/mL and 11,600 ng h/mL, respectively), which also had the lowest hepatic LOEL value in this group. Further analysis showed that the LOEL values given in the HESS database and the hepatic AUC values that we obtained from the PBPK modeling were inversely correlated ($r = -0.78$, $p < 0.05$, n = 7). This significant example indicated the importance of simulating the hepatic levels of general chemicals because those with high hepatic concentrations are more likely to be potent hepatotoxic compounds.

CONCLUSION AND FUTURE STUDY

In this review, the broad chemical diversity of the substances tested is illustrated in a two-dimensional plane depicting the chemical space (Fig. 2). We determined the permeability across the intestinal epithelial cell monolayers (in a pH-dependent Caco-2 cell system) of a diverse range of industrial chemicals/drugs. These permeability values provided a good estimation of oral absorbance as a putative marker of hepatotoxicity. Further analysis revealed that the cell permeability coefficients of the industrial chemicals were inversely correlated to their hepatic NOELs (Fig. 3), suggesting that the estimation of oral absorbance could be a useful tool to indicate hepatotoxicity *in vivo*. Moreover, the hepatic LOELs of chemicals and the virtual hepatic AUCs derived using PBPK modeling were also inversely correlated (Table 2). Therefore, using PBPK models to estimate hepatic chemical concentrations following oral administration could also be useful as an indicator of hepatotoxicity *in vivo*. The present simplified PBPK models could estimate the relationships between hepatic/plasma concentrations of chemicals and their oral doses using both forward and reverse dosimetry and provide a useful approach to predict hepatotoxicity. Estimation of the parameters for simplified PBPK modeling (Fig. 4B) of additional chemicals will be carried out in future research projects by our research group. To develop a more precise hepatotoxicity prediction system based on PBPK modeling, it would be valuable to establish supportive equations to predict the key parameters for this simple PBPK modeling approach. In future, in-depth forward and reverse dosimetry assessments of industrial chemical levels would be facilitated by adopting more human PBPK models (9,32) based on the above-described animal PBPK modeling techniques. In conclusion, evaluation of chemical exposure levels in the liver after oral doses should be one of the key primary steps, and mechanistic metabolic activations and their modifications (33) will be critical to understanding the potential risk from multiple exposures (34) in the field of chemical toxicology.

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CONFLICT OF INTEREST

There is no conflict of interest.

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