



Determination and prediction of permeability across intestinal epithelial cell monolayer of a diverse range of industrial chemicals/drugs for estimation of oral absorption as a putative marker of hepatotoxicity

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ABSTRACT

Apparent permeability coefficients (P_{app}) across a human intestinal epithelial Caco-2 cell monolayer were measured for a range of industrial/drug chemicals. A predictive equation for determining *in vitro* P_{app} values of fifty-six substances was set up using multivariate regression analysis based on *in silico*-estimated physicochemical properties (molecular weights and water distribution coefficients for apical and basal pH environments) ($r = 0.77$, $p < 0.01$). Predicted $\log P_{app}$ values of a secondary set of 34 compounds were correlated with the measured values. Under the medicinal $\log P_{app}$ values associated with their reported fraction absorbed, a significant inverse non-linear correlation was found between the logarithmic transformed values of observed P_{app} values and reported hepatic no-observed-effect levels of industrial chemicals ($r = -0.55$, $p < 0.01$, $n = 29$). *In vitro* determination and/or *in silico* prediction of permeability across intestinal cells could be effective for estimating oral absorption as a putative indicator for hepatotoxicity.

1. Introduction

Current experimental testing methods for estimation of the human risks of industrial chemicals generally require toxicological studies in experimental animals. Such studies include repeated oral doses to rodents for 28 days and employ procedures that adhere to guidance such as Organization for Economic Co-operation and Development test guidelines. Although big toxicity databases have been widely set up, limited numbers of chemicals only possess adequate toxicokinetic data *in vivo* regarding parameters (such as oral absorption rates) for assessing human potential hazards [1]. The *in vitro* permeability assay for oral absorption in pharmaceutical research is a kind of established methods and is principally based on using human colon cancer cell line Caco-2 systems [2–6]. Studies that attempted to predict the permeability of drugs and druglike chemicals across Caco-2 cell monolayers have been performed as part of preclinical drug development [7–9]. However, little information has been provided on the oral absorption of industrial chemicals through gastrointestinal absorption and/or the

mucosa, which is a necessary phase before such chemicals could exert their potential toxicity. It would be of great benefit for industrial chemicals if it were possible to derive the oral absorption parameters *in vivo* of general chemicals from established *in vitro* permeability values.

In the present study, we evaluated the permeability of a broad range of general chemical substances (for which the oral absorption is not commonly investigated) using a pH-dependent Caco-2 monolayer system. A multivariate prediction equation derived from the permeability coefficients of 56 disparate compounds was proposed. The input parameters for this equation were the *in silico* physicochemical properties of the compounds. This prediction equation was then used to estimate the permeability of a secondary set of 34 compounds. We report herein that the Caco-2 cell permeability coefficients of 28 industrial chemicals and acetaminophen were inversely associated with their hepatic no-observed-effect levels (NOELs).

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Table 1
Measured Permeability Coefficients of 56 Compounds with Their Physicochemical Properties and Reported Fraction Absorbed (F_a) and/or Hepatic No-observed-effect Levels (NOEL).

compound	Cas No.	P_{app}^a , nm/s	molecular weight	logP	log D_{apical}	log D_{basal}	reported human F_a , %	hepatic NOEL, mg/kg/day
2-aminobiphenyl	90-41-5	576 ± 11 ^a	169	3.05	3.05	3.05		100
3-aminobenzenesulfonic acid	121-47-1	21 ± 3 ^a	173	-4.26	-5.65	-5.71		1000
5-amino-2-chlorotoluene-4-sulfonic acid	88-53-9	20 ± 2 ^a	222	-3.17	-3.31	-3.33		1000
3-aminophenol	591-27-5	513 ± 23 ^a	109	-0.05	-0.06	-0.05		240
aniline	62-53-3	544 ± 26	93	1.03	1.01	1.03		
atenolol	29122-68-7	5 ± 1	266	0.01	-2.97	-1.56	50 [7]	
atomoxetine	83015-26-3	27 ± 1	255	4.21	1.01	2.33	100 [8]	
benzimidazole	51-17-2	730 ± 6	118	0.24	0.13	0.24		
benzoic acid	65-85-0	1490 ± 160	122	1.55	-0.42	-1.92		
benzylamine	642-72-8	16 ± 1	309	4.39	1.81	2.63	100 [23]	
bisphenol A	80-05-7	321 ± 13 ^a	228	4.48	4.48	4.47		200
caffeine	58-08-2	544 ± 12	194	0.95	0.95	0.95	100 [24]	
2-chloroaniline	95-51-2	893 ± 26	128	1.74	1.74	1.74		
cotinine	486-56-6	412 ± 29	176	1.02	1.01	1.02	90 [8]	
3-cyanopyridine	100-54-9	569 ± 70 ^a	104	0.58	0.58	0.58		5
dexamethasone	50-02-2	95 ± 14	392	2.63	2.63	2.63	90 [8]	
diclofenac	15307-86-5	756 ± 6	296	4.14	2.25	0.77	82 [25]	
dihydrocodeine	125-28-0	24 ± 1	301	2.99	0.47	0.95		
diphenylamine	122-39-4	151 ± 11	169	3.53	3.53	3.53		
1,3-dinitrobenzene	99-65-0	536 ± 35	168	1.51	1.51	1.51		
2,3-dimethylaniline	87-59-2	624 ± 30 ^a	121	1.90	1.87	1.90		12
2,4-dimethylaniline	95-68-1	661 ± 20 ^a	121	1.92	1.88	1.92		2
3,4-dimethylaniline	95-64-7	541 ± 58 ^a	121	2.01	1.93	2.01		50
3,5-dimethylaniline	108-69-0	674 ± 113 ^a	121	2.08	2.03	2.08		10
hippuric acid	495-69-2	6 ± 1	179	-0.46	-2.49	-3.79		
2-hydroxybenzimidazole	615-16-7	507 ± 16	134	-0.97	-1.52	-1.53		
4-hydroxybiphenyl	92-69-3	441 ± 28	170	3.89	3.89	3.88		
isophthalonitrile	626-17-5	805 ± 8 ^a	128	1.48	1.48	1.48		8
lenalidomide	191732-72-6	7 ± 1	259	-1.03	-1.03	-1.04	90 [26]	
lucifer yellow	67769-47-5	7 ± 1	445	-4.80	-13.1	-13.3	0 [27]	
m-cresol	108-39-4	851 ± 35 ^a	108	2.21	2.21	2.21		100
2-mercaptobenzimidazole	583-39-1	673 ± 18 ^a	150	0.64	-3.74	-3.76		2
metoprolol	51384-51-1	34 ± 1	267	2.20	-0.80	0.61	98 [7]	
midazolam	59467-70-8	318 ± 19	326	4.54	4.20	4.49	60 [28]	
mono(2-ethylhexyl) phthalate	4376-20-9	467 ± 18	278	4.93	3.16	1.67		
monobutyl phthalate	131-70-4	318 ± 8	222	3.35	1.58	0.09		
N,N-dimethylaniline	121-69-7	999 ± 129	121	2.17	2.07	2.16		
N-ethylaniline	103-69-5	660 ± 10 ^a	121	2.10	2.04	2.10		5
nicotine	54-11-5	57 ± 6	162	2.07	0.23	1.56	100 [8]	
nifedipine	21829-25-4	424 ± 44	346	2.21	2.21	2.21	100 [8]	
3-nitroaniline	99-09-2	520 ± 50 ^a	138	1.92	1.92	1.92		15
2-nitrotoluene	88-72-2	576 ± 46	137	2.28	2.28	2.28		
N-methylaniline	100-61-8	463 ± 50 ^a	107	1.59	1.55	1.59		5
o-cresol	95-48-7	905 ± 64	108	1.82	1.82	1.82		
p-cresol	106-44-5	507 ± 45	108	2.21	2.21	2.21		
phthalimide	85-41-6	933 ± 69	147	0.30	0.30	0.30		
p-hydroxybenzoic acid	99-96-7	609 ± 30	138	1.73	-0.10	-1.61		
pomalidomide	19171-19-8	466 ± 57	273	-0.03	-0.03	-0.04	73 [29]	
progesterone	57-83-0	113 ± 20	315	4.18	4.18	4.18		
propranolol	525-66-6	29 ± 3	259	3.07	0.15	1.57	90 [24]	
quetiapine	111974-69-7	38 ± 4	384	2.61	2.29	2.60	73 [30]	
terephthalonitrile	623-26-7	573 ± 13 ^a	128	1.48	1.48	1.48		20
thalidomide	50-35-1	235 ± 19	258	0.36	0.36	0.34		
tolbutamide	64-77-7	1220 ± 110	270	2.58	2.06	0.71	88 [25]	
trimethylamine	75-50-3	33 ± 1	59	0.76	-1.77	-1.43		
warfarin	81-81-2	1210 ± 50	308	2.33	1.83	0.49	98 [8]	

Observed P_{app} value represents the mean of triplicate determinations with standard deviation in this study. Physicochemical properties were calculated using the SPARC physicochemical calculator as mentioned in Materials and Methods. NOEL values for hepatotoxicity of chemical substances were obtained from the Hazard Evaluation Support System Integrated Platform [12].

^a Results (without SD values) of 17 compounds are reported in our study [10].

2. Materials and methods

2.1. Materials and chemical properties

The chemicals tested for permeability in the Caco-2 cell system (shown in Tables 1 and 2) were of analytical grade and were obtained from Fujifilm Wako Pure Chemical (Osaka, Japan), Tokyo Chemical Industry (Tokyo, Japan) or from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum,

nonessential amino acids, penicillin–streptomycin–amphotericin B suspension, and Hank's balanced salt solution (HBSS) were obtained from Fujifilm Wako Pure Chemical. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 2-morpholinoethanesulfonic acid monohydrate (MES) were purchased from Sigma-Aldrich. Cell culture dishes (100 mm) and Transwell plates (12-well, pore size: 0.4 μ m, growth area: 1.12 cm²) were obtained from Corning (Corning, NY, USA).

The broad diversity of the tested chemical substances is illustrated

Table 2
Predicted and Observed $\log P_{app}$ Values of a Secondary Set of 34 Compounds and Their Reported Fraction Absorbed (F_a) and/or Hepatic NOEL Values.

compound	CAS No.	molecular weight	$\log D_{apical}$	$\log D_{basal}$	predicted ^a $\log P_{app}$	observed P_{app} nm/s	observed $\log P_{app}$	reported human F_a , %	hepatic NOEL mg/ kg/day
acetaminophen	103-90-2	151	0.09	0.09	2.42	319 ± 14	2.50	100 [23]	250 [11]
azamethiophos	35575-96-3	325	2.58	2.58	2.14	402 ± 18	2.60		
bisphenol F	620-92-8	200	3.61	3.60	2.66	415 ± 21	2.62		100
bisphenol S	80-09-1	250	1.26	1.11	2.29	503 ± 35	2.70		200
carbamazepine	298-46-4	236	3.64	3.64	2.54	380 ± 14	2.58		
4-chloro- <i>o</i> -cresol	1570-64-5	143	2.51	2.51	2.71	754 ± 39	2.88		250
2-chlorophenol	95-57-8	129	2.13	2.07	2.73	752 ± 83	2.88		200
4-chlorophenol	106-48-9	129	2.26	2.26	2.73	431 ± 37	2.63		500
cimetidine	51481-61-9	252	-0.79	-0.58	1.92	17 ± 2	1.22	68 [7]	
coumarin	91-64-5	146	0.85	0.85	2.52	806 ± 54	2.91	100 [13]	
4- α -cumylphenol	599-64-4	212	4.99	4.99	2.77	195 ± 34	2.29		100
dabigatran	211915-06-9	472	0.26	-1.19	1.97	38 ± 17	1.58		
disopyramide	3737-09-5	340	-0.70	0.79	1.17	14 ± 3	1.16	83 [7]	
7-ethoxycoumarin	31005-02-4	190	1.94	1.94	2.50	750 ± 48	2.88		
3-ethylphenol	620-17-7	122	2.75	2.75	2.80	515 ± 50	2.71		300
4-ethylphenol	123-07-9	122	2.76	2.75	2.81	437 ± 23	2.64		100
flvoxamine	54739-18-3	318	1.85	2.88	1.69	23 ± 3	1.37		
2-hydroxybiphenyl	90-43-7	170	3.72	3.72	2.76	334 ± 29	2.52		
3-hydroxybiphenyl	580-51-8	170	3.88	3.88	2.78	284 ± 22	2.45		
7-hydroxycoumarin	93-35-6	162	0.24	0.01	2.49	1030 ± 170	3.01		
itopride	122898-67-3	358	0.15	1.40	1.29	12 ± 3	1.09		
lovastatin	75330-75-5	405	4.04	4.04	2.05	21 ± 1	1.32	31 [7]	
mefenamic acid	61-68-7	241	3.78	2.29	3.11	1804 ± 83	3.26		
2-mercaptoimidazole	872-35-5	100	-4.91	-4.91	2.02	91 ± 3	1.96		
methotrexate	59-05-2	454	-4.60	-7.46	2.03	11 ± 2	1.04	20 [24]	
2-methoxy-4-nitroaniline	97-52-9	168	1.71	1.71	2.54	552 ± 70	2.74		100
mirtazapine	85650-52-8	265	2.10	3.03	1.93	46 ± 3	1.66	80 [7]	
olanzapine	132539-06-1	312	3.00	3.29	2.12	35 ± 3	1.54		
omeprazole	73590-58-6	345	1.60	1.63	1.96	674 ± 69	2.83	95 [7]	
<i>p</i> -aminobenzoic acid	150-13-0	137	0.40	-1.08	3.06	587 ± 40	2.77		
<i>p</i> -phenetidine	156-43-4	137	1.42	1.46	2.59	582 ± 31	2.76		160
pravastatin	81093-37-0	425	-0.11	-1.57	2.08	9 ± 1	0.95	13 [31]	
4- <i>sec</i> -butylphenol	99-71-8	150	3.70	3.70	2.82	402 ± 19	2.60		300
verapamil	52-53-9	455	0.58	2.01	0.97	23 ± 1	1.36	100 [7]	

^a Predicted using the following equation: $\log P_{app} = 2.9 - 0.0032 \times (\text{molecular weight}) + 0.49 \times (\log D_{apical}) - 0.38 \times (\log D_{basal})$. Observed P_{app} value represents the mean of triplicate determinations with standard deviation in this study.

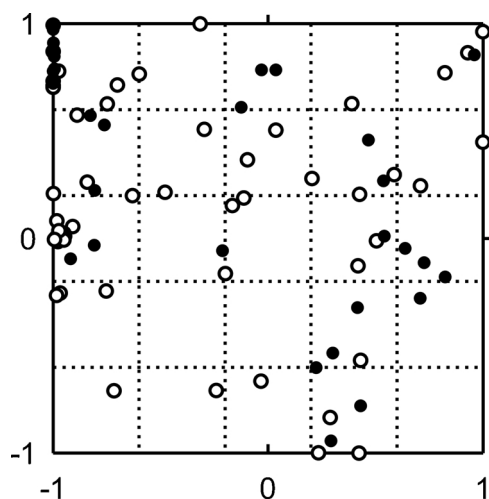


Fig. 1. Coordinate values in a two-dimensional plane illustrating variety in the chemical space for the primary set of 56 compounds (open circles) and the secondary set of 34 (solid circles) compounds evaluated using Caco-2 permeability assays.

in a two-dimensional plane depicting the wide chemical space (Fig. 1), as described previously [10]. Briefly, the structures described by 196 chemical descriptors were calculated using the chemoinformatics tool RDKit and projected using generative topographic mapping methods onto a two-dimensional plane [10]. In Fig. 1, closer plots in the illustrated chemical space could indicate some similarity in their material

properties. The molecular weights (MW), the octanol–water partition coefficients ($\log P$), and the octanol–water distribution coefficients for Caco-2 cell apical and basal pH environments ($\log D_{apical}$ and $\log D_{basal}$, respectively) of the tested chemical substances and drugs were calculated based on their chemical structures using the Sparc physico-chemical calculator (ARChem, Atlanta, GA, USA).

2.2. Permeation studies and permeability coefficients

The general procedures employed to prepare *in vitro* human intestinal Caco-2 monolayers were described previously [10]. Briefly, Caco-2 cells (American Type Culture Collection, Manassas, VA, USA; passages: 20–65) were cultured in DMEM supplemented with non-essential amino acids, penicillin–streptomycin–amphotericin B, and fetal bovine serum at 37 °C under a 5 % CO₂ atmosphere. For experimental use, the cells were seeded on permeable polycarbonate Transwell membranes at a density of 1.0×10^5 cells/cm² and were cultured for 21–28 days. Before and after the experiments, the integrity of the Caco-2 cell monolayers was evaluated by measuring the transepithelial electrical resistance (TEER) using a Voltohmmeter (Millicell ERS-2, Merck, Darmstadt, Germany); only Caco-2 cell monolayers with a TEER value of $> 200 \Omega\text{-cm}^2$ at pre- and post-incubations were used for the current experiments.

The apparent experimental permeability coefficients (P_{app} , nm/s) were calculated for time-dependent absorption *in vitro* from the apical side of the Caco-2 monolayer in HBSS with 10 mM MES (pH 6.0) to the basal side of the Caco-2 monolayer in HBSS with 10 mM HEPES (pH 7.4), as described previously [10] with slight modification. Briefly, 1–100 μM (dependent on the solubility of each substrate) of test

substance in a final concentration of < 0.1 % dimethyl sulfoxide (originally dissolved in dimethyl sulfoxide and diluted with Hank's balanced salt solution) was applied to the apical side of Caco-2 cells cultured on Transwell plates. Caffeine and lucifer yellow were used as positive and negative permeability controls, respectively. The amounts of the test substances in permeation samples from the basal sides were measured by high-performance liquid chromatography or liquid chromatography–mass spectrometry [10]. The experiment for each chemical substance was performed in triplicate determinations.

2.3. Statistical analysis

Univariate and multivariate linear regression analyses were performed using Prism software (GraphPad Software, San Diego, CA, USA). The relationships among $\log P_{app}$ values of chemicals experimentally determined *in vitro*, their physicochemical properties estimated *in silico*, and reported *in vivo* toxicological properties [the no-observed-effect level (NOEL)] for hepatotoxicity taken from the Hazard Evaluation Support System Integrated Platform in Japan and literature were investigated [11,12].

3. Results

The P_{app} values of more than 50 disparate types of chemicals (Fig. 1) were measured and are shown in Table 1. The observed P_{app} values of 56 compounds varied in the range 5–1490 nm/s. The physicochemical properties (MW, $\log P$, $\log D_{apical}$, and $\log D_{basal}$) of the 56 chemicals were estimated using *in silico* methods and are shown in Table 1. To investigate the feasibility of establishing a predictive equation, we carried out various analyses to identify the relationships between $\log P_{app}$ values and the compounds' physicochemical parameters. Univariate linear regression analyses revealed that, under the present conditions, the observed $\log P_{app}$ values (Table 1) were correlated with the corresponding MW ($r = 0.48$, $p < 0.01$, $n = 56$, Fig. 2A), $\log P$ values ($r = 0.31$, $p < 0.05$, $n = 56$, Fig. 2B), $\log D_{apical}$ values ($r = 0.53$, $p <$

0.01 , $n = 56$, Fig. 2C), and $\log D_{basal}$ values ($r = 0.41$, $p < 0.01$, $n = 56$, Fig. 2D). Because $\log P$ values univariately showed a low correlation coefficient, further analyses were performed with the rest of three chemical parameters, MW, $\log D_{apical}$ and $\log D_{basal}$ values. Bivariate analyses established that $\log P_{app}$ values were correlated with the MW and $\log D_{apical}$ values ($r = 0.67$, $p < 0.01$, $n = 56$, Fig. 2E), MW and $\log D_{basal}$ values ($r = 0.66$, $p < 0.01$, $n = 56$, Fig. 2F), and $\log D_{apical}$ and $\log D_{basal}$ values ($r = 0.60$, $p < 0.01$, $n = 56$, Fig. 2G) in combination. Moreover, $\log P_{app}$ values were multivariately correlated with the MW, $\log D_{apical}$, and $\log D_{basal}$ values in combination ($r = 0.77$, $p < 0.01$, $n = 56$; Fig. 2H), which led to the following equation: Predicted $\log P_{app}$ value = $2.9 - 0.0032 \times (\text{MW}) + 0.49 \times (\log D_{apical}) - 0.38 \times (\log D_{basal})$. These results suggest that multiple physicochemical properties are the determinants of the permeability coefficient of a variety of chemicals in the pH-dependent Caco-2 monolayer assays.

To verify the multivariate prediction equation, $\log P_{app}$ values for a secondary set of 34 compounds (Table 2) were predicted using the above equation *in silico* before P_{app} values were measured in *in vitro* experiments. The Caco-2 cell permeability coefficients of these additional 34 compounds were determined and are shown in Table 2. Estimated $\log P_{app}$ values were well correlated with the experimentally observed $\log P_{app}$ values ($r = 0.78$, $p < 0.01$, $n = 34$; Fig. 3). Under the present conditions, the predicted P_{app} values of 23 and 27 of the 34 additional compounds were within twofold and threefold errors, respectively, of the experimentally observed values. Under these conditions, predicted $\log P_{app}$ values of some medicines, namely olanzapine, lovastatin, methotrexate, pravastatin, and cimetidine were over-estimated in comparison with the observed values, presumably because of partly contributions of active efflux pump in the experimental environment.

To investigate the relevance of *in vitro* pH-dependent Caco-2 monolayer systems to *in vivo* absorption rates, the relationship was examined between the measured $\log P_{app}$ values for pharmaceutical drugs and their reported absorption (fraction absorbed, F_a) in humans (Fig. 4). A significant sigmoidal correlation was observed between the

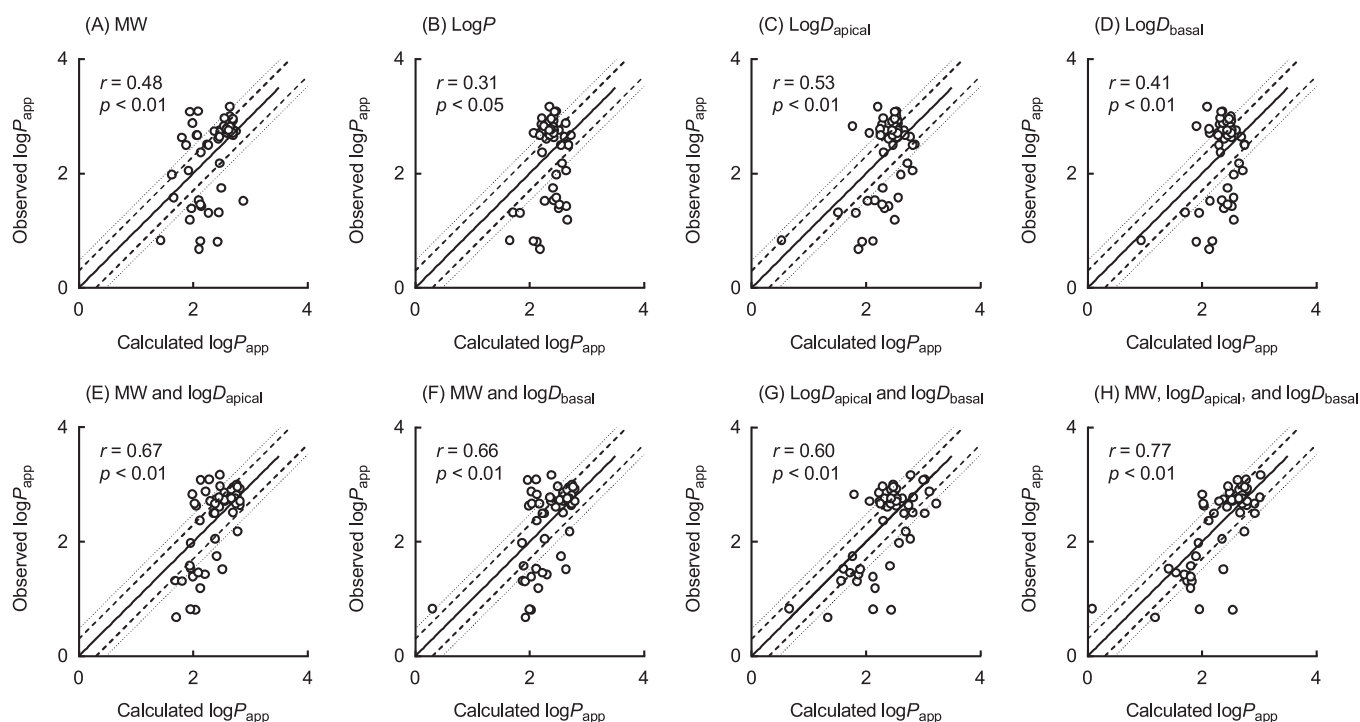


Fig. 2. Relationships between $\log P_{app}$ values experimentally observed in the Caco-2 cell system and those calculated using univariate (A–D), bivariate (E–G) and multivariate (H) linear regression analyses of the primary set of 56 compounds, as a function of physicochemical properties (MW, $\log P$, $\log D_{apical}$, and $\log D_{basal}$). Each observed $\log P_{app}$ value represents the mean of triplicate determinations with standard deviation as shown in Table 1. Solid and dashed/dotted lines indicate linear regression and twofold/threefold ranges, respectively.

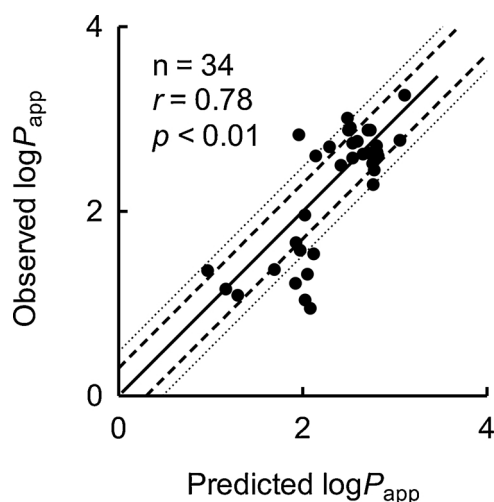


Fig. 3. The relationship between $\log P_{app}$ values of the secondary set of 34 compounds calculated using multivariate linear regression analysis and those of experimentally observed in the Caco-2 cell system. The multivariate prediction equation set up using the dataset shown in Fig. 2H was applied to the secondary set of 34 compounds in this Figure: Predicted $\log P_{app} = 2.9 - 0.0032 \times (\text{MW}) + 0.49 \times (\log D_{apical}) - 0.38 \times (\log D_{basal})$. Each observed $\log P_{app}$ value represents the mean of triplicate determinations with standard deviation as shown in Table 2. Solid and dashed/dotted lines indicate linear regression and twofold/threefold ranges, respectively.

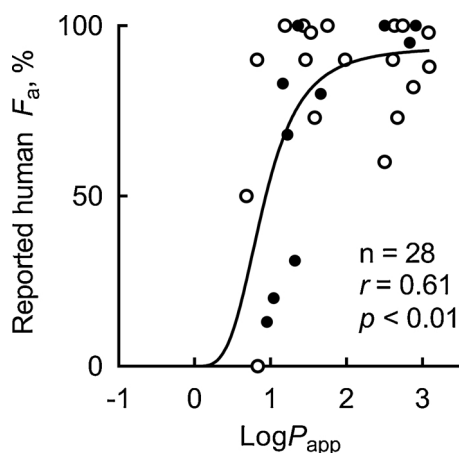


Fig. 4. The relationship between observed $\log P_{app}$ values and fraction of oral absorption (F_a) values of medicines reported in humans among the primary set of 56 compounds (open circles) and the secondary set of 34 (solid circles) compounds. Solid line indicates non-linear regression curve: Reported human $F_a = 94 \times (\log P_{app})^{3.6} / (0.69 + (\log P_{app})^{3.6})$.

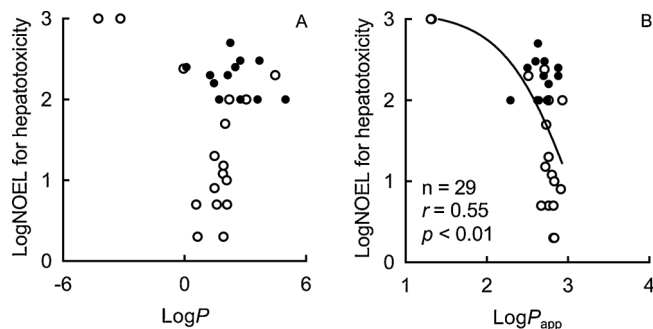


Fig. 5. Relationships between hepatic NOEL values of industrial chemicals and acetaminophen reported in rats and their chemical lipophilicity ($\log P$, A) and apparent permeability data (P_{app} , B) among the primary set of 56 compounds (open circles) and the secondary set of 34 (solid circles) compounds.

experimental $\log P_{app}$ and reported F_a values ($r = 0.61$, $p < 0.01$, $n = 28$); a similar nonlinear shape has been previously reported for this relationship [5,13]. Furthermore, under the present conditions, a significant inversely non-linear relationship was found between the logarithmic transformed values of observed P_{app} and reported hepatic NOELs of industrial chemicals and acetaminophen ($r = -0.55$, $p < 0.01$, $n = 29$; Fig. 5B), but not with the calculated $\log P$ ($r = -0.27$, $p = 0.2$, $n = 29$; Fig. 5A).

4. Discussion

Conditions that mimic the *in vitro* pH gradient found between the gastrointestinal lumen and plasma have been shown to well reflect human oral absorption of drugs in the gut [6,14]. Furthermore, simple pH-dependent Caco-2 monolayer systems have proven advantageous in predicting *in vivo* drug absorption as a part of pharmaceutical research [2,5,13,15–19]. It has been reported recently that Caco-2 permeability coefficients for 768 diverse drugs and druglike compounds could account for passive diffusion across the mucosal epithelium [9] using a minimal set of physicochemical descriptors (octanol–water $\log D$, pKa, hydrogen bonding potential, and molecular size), a model has been successfully set up to predict Caco-2 permeability coefficients [9]. However, the pharmacokinetics and/or toxicokinetics of industrial chemicals are not usually investigated as part of their extensive acute toxicity studies [1]. Therefore, the relationship between P_{app} and the hepatic NOEL values of chemical substances were examined in the present study.

In our previous report, suitable concentrations of albumin for *in vitro* assays of drug oxidations by human liver microsomal cytochrome P450 2C enzymes could be multivariately estimated using the drugs' physicochemical properties in combination [20]. In the current study, multivariate regression analysis with three physicochemical properties in combination (reflecting the experimental apical and basal pH conditions in the current monolayer cell assays) showed that the *in silico* predicted and *in vitro* measured P_{app} values of a total of 90 chemicals were well correlated (Fig. 2). These results suggest that our proposed multivariate regression equation using the physicochemical properties of compounds in combination could predict the permeability coefficients across the Caco-2 cell sheets of a wide variety of chemicals. Analysis of the combined 90 tested chemical substances allowed us to update the multivariate equation as follows: Predicted $\log P_{app}$ value = $3.0 - 0.0038 \times (\text{MW}) + 0.41 \times (\log D_{apical}) - 0.30 \times (\log D_{basal})$. The reason why some predicted $\log P_{app}$ values of drugs were out of threefold areas are not known under the present conditions, presumably because of some contributions of active efflux/influx pump in the actual experimental Caco-2 environment. Predictions for any uptake/efflux transport potential of substances in the current models using simple physiological parameters may have some limitation at present and would be another big project expected in this research area. In another viewpoint, a multivalent equation fortified with more chemical descriptors might be solved for good prediction in future.

The P_{app} values obtained from experiments in this study could reflect the *in vivo* intestinal absorption of known medicines (Fig. 4), although some absolute values were different in *in vitro* systems (Tables 1 and 2). Our current inverse correlation between logarithmic transformed values of reported NOEL and measured P_{app} values of general chemicals was able to apply for a drug, acetaminophen (Fig. 5). The *in vivo* oral absorption, rather than partition coefficients, is considered to be one of the many determinant factors predicting the pharmacokinetics and/or potential hepatotoxicity (Fig. 5) of intentionally or unintentionally orally ingested chemical substances. In the present study, if one (5-amino-2-chlorotoluene-4-sulfonic acid) and two points, respectively, from two compounds implying moderate absorption would be omitted in correlation assays shown in Fig. 5B, both the non-linear correlation coefficients were still significant ($r = -0.49$, $p < 0.01$, $n = 28$; and $r = -0.39$, $p < 0.05$, $n = 27$). Under the present relationship

analyses, although NOEL values of chemicals are generally determined in discreet numbers dependent on animal dosing levels, continuous variable *in vitro* apparent permeability data (P_{app}) of industrial chemicals would be one of the diverse determinant factors predicting *in vivo* potential hepatotoxicity, in comparison with chemical lipophilicity ($\log P$). It could be of use to have more NOEL values of chemicals from any toxicity/regulatory databases with similar evaluation criteria to help correlations between the P_{app} and NOEL values to the toxicity conclusions. Anyway, it should be noted that chemical exposure levels *via* intestinal absorption after oral doses should be one of the primary key steps and following species-specific metabolic activations in livers and their mechanistically modifications would be the secondary critical points to understand potential hepatic risk from multiple exposures in chemical toxicology. Gastrointestinal epithelial Caco-2 cells have been also reportedly used in the other toxicological research such as cytotoxic effects of pesticides in combination [21] or gene expression profiles by nanosilver [22].

Consequently, being able to predict the permeability of a diverse range of industrial chemicals across the intestinal epithelial cell monolayer using their physicochemical properties in combination could be of use for estimating systemic exposure *via* oral absorption as one of putative toxicokinetic markers of hepatotoxicity. With a view to predicting hepatic toxicity after oral absorption of chemicals as a part of risk assessment, simple physiologically based pharmacokinetic models (consisting of gut, liver, and central compartments) were recently used to estimate the plasma/hepatic concentrations of chemicals after virtual oral doses [10]. In conclusion, the *in vitro* determination and/or *in silico* prediction of permeability coefficients across the intestinal cell monolayer of a diverse range of industrial chemicals/drugs demonstrated in the current study represent useful tools for estimating oral absorption as a possible indicator of hepatotoxicity *in vivo*.

CRediT authorship contribution statement

Yusuke Kamiya: Data curation, Formal analysis, Methodology. **Hiroka Takaku:** Investigation. **Rio Yamada:** Investigation. **Chisato Akase:** Investigation. **Yuto Abe:** Investigation. **Yuko Sekiguchi:** Investigation. **Norie Murayama:** Validation. **Makiko Shimizu:** Validation. **Masato Kitajima:** Software. **Fumiaki Shono:** Funding acquisition. **Kimoto Funatsu:** Funding acquisition. **Hiroshi Yamazaki:** Project administration, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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