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Tissue Distribution, Excretion and Pharmacokinetics of the Environmental Pollutant Dibenzo[*def,p*]chrysene in Mice

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Abstract

Dibenzo[*def,p*]chrysene (DBP), a representative example of the class of polycyclic aromatic hydrocarbon (PAH), is known to induce tumors in multiple organ sites including the ovary, lung, mammary glands, and oral cavity in rodents. The goal of this study was to test the hypothesis that the levels of DBP and its metabolites that reach and retain the levels for an extended time in the target organs as well as the capacity of these organs to metabolize this carcinogen to active metabolites that can damage DNA may account for its tissue selective tumorigenicity. Therefore, we used the radiolabeled [³H] DBP to accurately assess the tissue distribution, excretion, and pharmacokinetics of this carcinogen. We also compared the levels of DBPDE-DNA adducts in a select target organ (ovary) and nontarget organs (kidney and liver) in mice treated orally with DBP. Our results showed that after 1 week, 91.40 ± 7.23% of the radioactivity was recovered in the feces; the corresponding value excreted in the urine was less than 2% after 1 week. After 24 h, the stomach had the highest radioactivity followed by the intestine and the liver; however, after 1 week, levels of the radioactivity in these organs were the lowest among tissues examined including the ovary and liver; the pharmacokinetic analysis of DBP was conducted using a one compartment open model. The level of (–)-*anti-trans*-DBPDE-*dA* in the ovaries (8.91 ± 0.08 adducts/10⁷ dA) was significantly higher ($p < 0.01$) than the levels of adducts in kidneys (0.69 ± 0.09 adducts/10⁷ dA) and livers (0.63 ± 0.11 adducts/10⁷ dA). Collectively, the results of the tissue distribution and pharmacokinetic analysis may not fully support our hypothesis, but the capacity of the target organs vs nontarget organs to metabolize DBP to active intermediates that can damage DNA may account for its tissue selective tumorigenicity.

Graphical abstract

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The authors declare no competing financial interest.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are generated from incomplete combustion of organic matter; their environmental sources include air pollution, tobacco smoke, and diet.¹ Dibenzo[*def,p*]chrysene (also known as dibenzo[*a,l*]pyrene; DBP), a representative carcinogen of this class of compounds, has been identified in mainstream cigarette smoke, soil, and diesel exhaust particulate matter.^{2–5} Although the levels of DBP are lower than those of benzo[*a*]pyrene (B[*a*]P) in samples derived from environmental sources,⁶ DBP is the most potent carcinogenic PAH found in the environment to date in rodent model systems.^{7–9} DBP is ~100-fold more potent in inducing lung adenomas than B[*a*]P.¹⁰ Administration of DBP by i.p., oral gavage, or topical application onto the oral cavity is known to induce tumors in multiple organ sites including the ovary, lung, mammary gland, and oral cavity.^{11–15} Consistent with our finding, Buters et al. had reported that following a single dose administered intragastrically to mice, DBP induced tumors in several organs, but the ovary was the most susceptible tissue.^{11,14} Furthermore, we showed that oral application of its diol epoxide (DBPDE) induces tumors exclusively in the tongue and other oral tissues of mice; it induced more tumors in the oral tissues at lower doses than DBP did at a higher dose.^{14,15} DBP is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B).¹⁶

It is generally accepted that PAHs exert their mutagenic effects and initiate the carcinogenic process through the generation of active metabolites which can lead to the formation of covalent DNA adducts that can cause miscoding and mutations.¹⁷ Three distinct pathways have been described in the activation of DBP:^{18,19} (1) the radical cation pathway (mediated by cytochrome P450 peroxidases and other peroxidases) to yield depurinating adducts; (2) the diol epoxides pathway (mediated by cytochrome P4501A1/1A2 and 1B1) to yield stable bulky diol epoxide-DNA adducts; and (3) the PAH *ortho*-quinone pathway (mediated by aldo-keto reductases), which results in bulky stable DNA adducts, depurinating adducts, and oxidatively modified DNA lesions.^{14,19} Recently, we reported that DBP and its active metabolites, diol epoxides, can result in the formation of DBPDE-dA (major) and -dG (minor) adducts in mouse oral and ovarian tissues; these results suggest that DBP can be metabolized to its Fjord region diol epoxides which can react with DNA in the target organs of mice.^{14,20,21} Taken together, we concluded that the mechanism by which DBP exerts its tumorigenicity is mainly through the formation of its diol epoxides.

In spite of its remarkable carcinogenicity at multiple sites with varied potency in rodents and environmental occurrence, previous studies on pharmacokinetics and tissues distribution are incomplete.²² The goal of this study was to test the hypothesis that the levels of DBP and its metabolites that reach and retain them for an extended time in the target organs as well as the capacity of these organs to metabolize this carcinogen to active metabolites that can damage DNA may account for its tissue selective tumorigenicity. A preliminary physiological based pharmacokinetic model was reported in the literature using a nonradiolabeled DBP.²² However, to provide insights on the multiorgan carcinogenicity of this agent in mice, in this study we used the radiolabeled [³H] DBP to accurately assess the tissue distribution and pharmacokinetics of this carcinogen. To further test our hypothesis, we also compared the levels of DBPDE-DNA adducts in the target organ (ovary) and nontarget organs (kidney and liver) in mice treated orally with DBP. The results of the tissue distribution and pharmacokinetic analysis may not fully support our hypothesis, but the capacity of the target organs (e.g., ovary) vs nontarget organs (kidney and liver) to metabolize DBP to active intermediates that can damage DNA may account for its tissue selective tumorigenicity.

MATERIALS AND METHODS

Chemicals and Dosing Solution

The uniformly labeled [G-³H] DBP (4.1 Ci mmol⁻¹) was obtained from Moravek Biochemicals, Inc. (Brea, CA). Radiochemical and chemical purities as determined by HPLC were >98%. The nonradiolabeled DBP was prepared as described previously.²³

The [³H] DBP dosing solution was prepared in dimethyl sulfoxide (DMSO) at a target dose of 1.07 mg kg⁻¹ body weight with a volume of administration of 5 mL kg⁻¹ body weight. [³H] DBP (specific activity of 4.7 Ci/mmol) was mixed with nonradiolabeled DBP to obtain a target radioactivity and concentration of 1.07 mCi mL⁻¹ and 200 µg mL⁻¹ of dosing solution (specific activity of 1.512 Ci mmol⁻¹), respectively.

Animal

Six week old female B6C3F1 mice (Jackson Laboratories, Bar Harbor, ME) were fed with a semipurified, modified AIN-93 M diet (5% corn oil) and water *ad libitum* and quarantined for 1 week before treatments. The bioassays were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by Institutional Animal Care and Use Committee. A constant environmental temperature of 21 ± 2 °C was maintained with a relative humidity of 50% and 12 h light/dark cycle.

Determination of the Total Radioactivity and the Radioactivity Profile in Plasma, Urine, Feces, and Selected Tissues

The number of animals used for this experiment was 18 (17.3 ± 0.6 g), and 3 mice were sacrificed at each time point. Each mouse received a single dose of [³H] DBP (1.07 mg kg⁻¹ body weight) via gavage and were individually housed in glass Roth-type metabolism cages designed for the separate collection of urine and feces. At 40 min, 2 h, 6 h, 24 h, 72 h, and 1 week after carcinogen administration, mice were sacrificed by CO₂ asphyxiation. Blood was

collected in EDTA containing vials and centrifuged to obtain plasma specimen. Selected tissues including the ovary, mammary fat pat, uterus, liver, stomach, intestine, pancreas, spleen, kidney, bladder, and lung were collected. Feces and urine were collected at room temperature for each time intervals. Following the collection of each urine specimen, the cage was rinsed with 60% ethanol/H₂O, and the washing solutions were combined with the urine samples at each collection interval, but they were measured separately, and the values were added up for cumulative analysis.

Radioactivity was determined by liquid scintillation spectroscopy (Beckman Coulter, LS 6500) with samples mixed with 10 mL of Ultima Gold scintillation cocktail (PerkinElmer, Waltham, MA) in unquenched standard vials and counted for 5 min or to 2 σ error of 0.1%, whichever occurred first. Correction of matrix-specific quenching of radioactivity in all samples was conducted by spiking [³H] DBP into tissue samples prior to the measurement. All counts were converted to absolute radioactivity (dpm) by automatic quench correction based on the shift of the spectrum for the external standard. Samples that exhibited radioactivity less than or equal to twice the background values were considered as zero for all subsequent manipulations.

To bleach, 1 $\times$ volume of 30% hydrogen peroxide was added dropwise with swirling into aliquots of urine or plasma samples. The solution was allowed to stand for 15 min at room temperature followed by incubations at 50–55 °C for 1 h to decompose excess peroxides. After cooling to room temperature, the samples were mixed with scintillation cocktail and counted. The total volumes of the urine samples were recorded; the total radioactivity was calculated based on the radioactivity of selected fractions of each sample. Air-dried feces were weighed and then pulverized. A portion of the feces were prepared in aqueous homogenate (ca. 25% w/w) and solubilized in Soluene-350 (PerkinElmer Inc., Waltham, MA) after incubations for 1–3 h at 50–60 °C. The solutions were then treated with 30% hydrogen peroxide as described above and mixed with liquid scintillation fluid for the analysis of radioactivity.

The total weights of individual tissues were recorded. For the stomach and intestine, the contents were removed before weighing. The tissue samples were solubilized in SOLVABLE (PerkinElmer Inc., Waltham, MA) and treated with 30% hydrogen peroxide as described above. The solutions were then mixed with liquid scintillation fluid for radioactivity measurements. Tissue concentrations of radioactivity were calculated as $\mu\text{g}/\text{mg}$ of ³H-equivalents of DBP.

Pharmacokinetic Analysis

Pharmacokinetics analysis was performed with Phoenix WinNonlin 6.3 (Pharsight Corp, Mt. View, CA) using a one-compartment model.

Analysis of DNA Adducts in Target and Nontarget Organs of Mice Treated with DBP

As reported in our previous studies, DBPDE-dA is the major DNA adduct detected in mice treated with DBP.¹¹ Therefore, in the present report, we carried out a time-course study in which mice were administered with 24 nmol DBP into the oral cavity 3 times per week for 5 weeks as we previously reported.¹¹ Three animals were sacrificed at 48 h after the last

administration of DBP. At termination, mice were sacrificed by CO₂ asphyxiation, and the target organ (ovary) and nontarget organs (kidney and liver) were collected for DNA adduct analysis. Samples collected in both studies were stored at -80 °C prior to the analysis.

The method used for the analysis of DBPDE-dA adducts by LC-MS/MS is identical to our previously published procedure.^{14,20,21} In brief, DNA was isolated from tissues using the Qiagen genomic DNA isolation procedure. The concentration of DNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Prior to enzymatic digestion, 150 pg of each [¹⁵N₅]-*anti-trans*- and [¹⁵N₅]-*anti-cis*-DBPDE-dA adducts were added to ~150 µg of DNA. DNA was hydrolyzed in the presence of 1 M MgCl₂ (10 µL/mg DNA) and DNase I (0.2 mg/mg DNA) at 37 °C for 1.5 h. Subsequently, nuclease P1 (20 µg/mg DNA) snake venom phosphodiesterase (0.08 unit/mg DNA) and alkaline phosphatase (2 units/mg DNA) were used. Aliquot of the DNA hydrolysate was subjected to dA base analysis by HPLC. The remaining supernatant was partially purified by solid phase extraction using an Oasis HLB column (1 cm³, 30 mg, Waters Ltd.). Then, the analysis was carried out on an API 3200 LC/MS/MS triple quadrupole mass spectrometer interfaced with an Agilent 1200 series HPLC using an Agilent extend-c18 5 µm 4.6 × 150 mm column. Adducts were monitored in multiple reaction monitoring (MRM) mode. The MS/MS transitions of *m/z* 604 → *m/z* 335 and *m/z* 609 → *m/z* 335 were monitored for targeted adducts and internal standards, respectively.

RESULTS

Fecal and Urinary Excretion

Following the oral administration of [³H] DBP (1.07 mg/kg) to mice, 8.50 ± 0.93% and 73.21 ± 9.58% (*n* = 3) of the radioactivity was eliminated through feces in 6 and 24 h, respectively (Figure 1). After 72 h and 1 week, the corresponding values recovered in the feces were 89.94 ± 7.00% and 91.40 ± 7.23%, respectively. The total radioactivity excreted in the urine was less than 2% after 1 week.

Tissue Distribution of Radioactivity

Table 1 shows the levels of ³H-equivalent of DBP and its metabolites in multiple tissues of mice. We found that tissues in the digestive tract including the stomach and intestine reached their highest levels of radioactivity after 40 min, followed by the lung after 2 h. The highest radioactivity for the mammary, liver, pancreas, kidney, and blood was reached after 6 h, and that for the ovary, uterine, spleen, and bladder was reached after 24 h. However, after 1 week, the levels of radioactivity in the stomach and intestine dropped below 2% of the radioactivity that was measured at 24 h, but more than 20% of the radioactivity was retained after 1 week in the ovary, mammary glands, lung, and liver. A comparison of radioactivity in these tissues at 24 h and 1 week is shown in Figure 2; at 24 h, 6.41 ± 0.88% of the total radioactivity was detected in these tissues.

Pharmacokinetics of [³H] DBP

We had constructed the plasma level–time curve for [³H] DBP given in a single oral dose (1.07 mg kg⁻¹ body weight) to describe the absorption and elimination rate process, and it is

depicted graphically in Figure 3; the values were plotted in a log (plasma level) versus time curve, and a linear plot was observed on the elimination phase from 6 h to 1 week ($\gamma^2 = 0.999$). Therefore, the pharmacokinetic analysis of DBP was conducted using a one compartment open model using Phoenix WinNonlin 6.3 (Pharsight Corp, Mt. View, CA). Data obtained in most of the tissues including the ovaries, mammary tissues, uterine tissues, liver, pancreas, spleen, kidney, bladder, lung, and plasma fit well with a first order absorption model. However, it is not surprising that data obtained from the stomach and intestine fit better with the equation $C(T) = (D/V)e^{-K_{10}T}$ because following its oral administration, DBP is expected to be in a direct contact with tissues in the digestive tract. Detailed pharmacokinetic parameters including maximum concentration ($C_{\max}$), time needed to reach $C_{\max}$ ($T_{\max}$), absorption constant (K_{01}), elimination constant (K_{10}), K_{01} half-life ($T_{1/2 K01}$), K_{10} half-life ($T_{1/2 K10}$), area under the curve (AUC), apparent volume of distribution (V_d), lag time (T_{lag}), and clearance (CL_F) in this study are shown in Table 2. The absorption and elimination of [^3H] DBP in each tissue of the mouse after oral administration are graphically shown in Figure 4. The simulated $T_{\max}$ showed that the radioactivity associated with DBP reached the $C_{\max}$ in each tissues are in the order similar to the data presented in Table 1, but the $T_{\max}$ for the lung was much later at about 10 h.

Analysis of DNA Adducts in Target (Ovary) and Nontarget Organs (Kidney and Liver) of Mice Treated with DBP

To test our hypothesis and to better understand the tissue selective tumorigenicity of DBP, we determined the levels of DBPDE-dA adduct in ovaries, kidneys, and livers of mice treated orally with DBP using a LC-MS/MS method (Figure 5).²⁹ In this bioassay, mice were administered multiple oral doses of DBP to mimic, to some extent, the carcinogenesis animal bioassay (24 nmol of DBP, 3 times a week for 5 weeks); DNA were isolated from the ovary, kidney, and liver, and the level of (–)-*anti-trans* adducts in the ovary (8.91 ± 0.08 adducts/ 10^7 dA) was significantly higher ($p < 0.01$) than the levels of adducts in the kidney (0.69 ± 0.09 adducts/ 10^7 dA) and the liver (0.63 ± 0.11 adducts/ 10^7 dA).

DISCUSSION

DBP is the most powerful PAH carcinogen (the ovary, lung, mammary, skin, and oral cavity) known to date; its remarkable genotoxicity has been attributed to the sterically hindered fjord region diol epoxides ($\pm$)-*anti*-DBPDE.^{11,14,24–27} Buters et al. found DBP administered intragastrically induced ovarian tumors in over 70% of the mice, which is the highest among all targeted organs.¹¹ We have also shown that topical application of DBP onto the oral cavity can induce cancer in the oral cavity but primarily in mouse ovary.¹⁴ In addition, we were the first to develop a sensitive LC-MS/MS method and use it to detect DBPDE-DNA adducts in the ovaries as well as in oral tissues of mice treated with DBP.^{14,20,21}

In the present article, by conducting a radiotracer experiment we provide further evidence that DBP and its metabolites can be distributed to the target tissues after intragastric administration. Radiotracer experiments have high sensitivity and are relatively easy to employ; the radioactivity in tissues can reveal the degree of transient exposure of tissues to the compound that allow us to predict the biological fate of the compound in a specific

tissue. In an attempt to investigate whether the tissue distribution and retention of orally administered DBP and its excretion may, in part, account for its tissue selective tumorigenicity *in vivo*, we conducted a radiotracer experiment to assess the distribution of DBP among various tissues including its target organs in mice. Crowell et al. reported that fecal excretion of DBP primarily comprises DBP with small quantities of conjugated hydroxylated metabolites, but urinary excretion was dominated by conjugated tetraol metabolites which were derived from the Fjord region diol epoxides.²⁸ Although the pharmacokinetics of DBP in mice after oral administration has been investigated by this group,^{22,28} it was not conducted by a radiotracer method, and thus the parameters reported by these investigators are not expected to be entirely consistent with our pharmacokinetic analysis. Furthermore, these investigators used a dose (15 mg/kg B.W. in corn oil) which is much higher than that employed in our previous carcinogenesis experiment.¹⁵ Therefore, it is not surprising that the $C_{\max}$ values reported in the present study are much lower than those reported by this group (3.5–13.3 μM). We have also observed that in general $T_{\max}$ is longer (10 h in plasma) in our model than that reported by this group (2–4 h). On the basis of a previous study,²⁹ it is possible that the vehicle (DMSO) used in our study and the differences of the strain of mice used in these two studies may account for the varied results. Upon using the radiolabeled DBP, the pharmacokinetic parameters reported in the present study should accurately reflect the fate of DBP in our previous carcinogenesis bioassay.¹⁵

The liver showed the highest uptake among tissues other than stomach and intestine, which are the portal of entry for oral administration; on the basis of its AUC and $C_{\max}$, our results support that the liver is the major organ for the metabolism and elimination of DBP. Kidney and mammary tissues also showed higher uptake compared with that of the other tissues; the kidney is a major organ for excretion, and the mammary fat-pad favors the accumulation of lipophilic compounds such as DBP. Furthermore, our results showed a much shorter half-life (about 10 h) of DBP in digestive tract than that in other tissues (about 50 h); this finding also supports that DBP can be retained in various target and nontarget organs within the body. In fact, consistent with the detection of DBP-DNA adducts in the target organs,^{14,20,21} appreciable amounts of the radioactivity (>20% of the radioactivity found at 24 h) were retained in its target tissues including the ovary after 1 week.

Since DBP can be delivered to target (ovarian) and nontarget (kidney and liver) organs by oral administration and it can be retained for an extended time, the results of tissue distribution do not fully support the preferential carcinogenicity of DBP in the ovary. Therefore, a follow-up study was performed, and we demonstrate that the capacity of target vs nontarget organs to metabolize DBP to active intermediates (Fjord region diol epoxide) that can damage DNA may account for its tissue selective tumorigenicity.

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ABBREVIATIONS

Cl_F	clearance
C_{max}	maximum concentration
dA	deoxyadenosine
DBP	dibenzo[<i>def,p</i>]chrysene, also known as dibenzo-[<i>a,l</i>]pyrene
DBPDE	(±)- <i>anti</i> -11,12-dihydroxy-13,14-epoxy-11,12,13,14-tetrahydrodibenzo[<i>def,p</i>]chrysene
dG	deoxyguanosine
K₀₁	absorption constant
K₁₀	elimination constant
PAH	polycyclic aromatic hydrocarbon
T_{max}	time needed to reach C _{max}
T_{1/2 K01}	K ₀₁ half-life
T_{1/2 K10}	K ₁₀ half-life
V_d	apparent volume of distribution
T_{lag}	lag time

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Figure 1.
Mean cumulative excretion of total radioactivity in mice excreta.

Figure 2.
Tissue distribution of the radioactivity in various tissues at 24 h and 1 week after DBP administration.

Figure 3.
Time-plasma concentrations profile of DBP.

Figure 4.

Data and model simulation of the concentration of DBP in various tissues of mice administered via oral gavage.

Figure 5. Comparative DNA adducts levels in the ovaries, kidneys, and livers of DBP-treated mice. * statistically significant ($P < 0.01$); compared to ovaries.

Table 1

Tissue Distribution of Radioactivity Administered by Oral Gavage of [^3H] DBP in Mice at 1.07 mg/kg Body Weight

ng/mg tissue ^a									
time	ovary	mammary	uterine	liver	stomach	intestine	pancreas		
40 min	0.011 ± 0.003	0.014 ± 0.004	0.014 ± 0.002	0.179 ± 0.061	6.355 ± 0.574	4.120 ± 1.956	0.123 ± 0.039		
2 h	0.090 ± 0.010	0.052 ± 0.024	0.032 ± 0.004	0.477 ± 0.223	5.895 ± 1.094	3.424 ± 2.134	0.145 ± 0.044		
6 h	0.122 ± 0.029	0.201 ± 0.075	0.096 ± 0.058	0.548 ± 0.215	4.445 ± 1.309	1.516 ± 0.524	0.175 ± 0.074		
24 h	0.125 ± 0.039	0.186 ± 0.045	0.128 ± 0.028	0.396 ± 0.053	3.369 ± 0.917	0.781 ± 0.060	0.124 ± 0.017		
72 h	0.059 ± 0.015	0.062 ± 0.019	0.051 ± 0.004	0.149 ± 0.017	0.104 ± 0.020	0.054 ± 0.013	0.055 ± 0.014		
168 h	0.024 ± 0.001	0.053 ± 0.016	0.024 ± 0.003	0.080 ± 0.017	0.014 ± 0.001	0.013 ± 0.001	0.018 ± 0.001		

time	spleen	kidney	bladder	lung	blood ^b
40 min	0.047 ± 0.019	0.052 ± 0.020	0.013 ± 0.005	0.075 ± 0.039	0.034 ± 0.020
2 h	0.073 ± 0.018	0.135 ± 0.046	0.037 ± 0.013	0.232 ± 0.033	0.070 ± 0.030
6 h	0.122 ± 0.043	0.224 ± 0.062	0.112 ± 0.057	0.148 ± 0.042	0.139 ± 0.047
24 h	0.135 ± 0.012	0.151 ± 0.025	0.138 ± 0.020	0.139 ± 0.021	0.110 ± 0.004
72 h	0.053 ± 0.005	0.097 ± 0.011	0.064 ± 0.016	0.059 ± 0.002	0.060 ± 0.009
168 h	0.024 ± 0.005	0.026 ± 0.005	0.018 ± 0.003	0.028 ± 0.012	0.015 ± 0.002

^aResults are the mean ± SD expressed as ^3H -equivalents of DBP obtained from groups of 3 mice.

^bResults in blood are expressed in ng/ μL .

Table 2

Kinetic Parameters of Total ³H-DBP Equivalents from Tissues of Mice

	plasma ^a	ovaries ^a	mammary ^a	uterine ^a	liver ^a
<i>C</i> _{max} (ng/g)	134.70 ± 11.51 ^c	146.15 ± 14.03	201.25 ± 61.99	121.05 ± 19.84	524.15 ± 63.22
<i>T</i> _{max} (h)	10.02 ± 2.10	12.46 ± 2.23	9.78 ± 9.72	17.96 ± 5.07	4.31 ± 2.52
<i>K</i> ₀₁ (h ⁻¹)	0.33 ± 0.11	0.26 ± 0.08	0.43 ± 0.82	0.15 ± 0.08	1.08 ± 1.00
<i>K</i> ₁₀ (h ⁻¹)	0.014 ± 0.002	0.013 ± 0.002	0.014 ± 0.009	0.014 ± 0.005	0.015 ± 0.003
<i>T</i> _{1/2 K01} (h)	2.11 ± 0.73	2.68 ± 0.81	1.60 ± 3.03	4.75 ± 2.51	0.64 ± 0.60
<i>T</i> _{1/2 K10} (h)	49.05 ± 7.45	51.55 ± 9.20	49.99 ± 30.94	49.90 ± 16.96	45.26 ± 10.65
AUC ^d	10982.6 ± 1139.9	12780.9 ± 1464.1	16267.2 ± 5920.9	11160.8 ± 2184.0	36392.5 ± 6342.1
<i>V</i> _d (mL)	137.9 ± 16.2	124.52 ± 16.70	94.88 ± 42.10	138.02 ± 36.24	38.40 ± 5.78
<i>T</i> _{lag} (h)	2.6 × 10 ⁻⁵ ± 0.30	0.41 ± 0.14	1.56 ± 1.22	0.14 ± 0.49	0.31 ± 0.34
<i>Cl</i> _f	1.95 ± 0.20	1.67 ± 0.19	1.32 ± 0.48	1.92 ± 0.38	0.59 ± 0.10

	stomach ^b	intestine ^b	pancreas ^a	spleen ^a	kidney ^a
<i>C</i> _{max} (ng/g)	6578.0 ± 678.1	3470.3 ± 644.3	162.21 ± 13.86	127.85 ± 23.85	203.26 ± 17.73
<i>T</i> _{max} (h)			2.58 ± 5.26	9.44 ± 4.78	7.69 ± 1.97
<i>K</i> ₀₁ (h ⁻¹)			1.91 ± 7.10	0.38 ± 0.31	0.50 ± 0.20
<i>K</i> ₁₀ (h ⁻¹)	0.048 ± 0.008	0.09 ± 0.03	0.014 ± 0.002	0.012 ± 0.004	0.013 ± 0.002
<i>T</i> _{1/2 K01} (h)			0.36 ± 1.35	1.82 ± 1.49	1.40 ± 0.57
<i>T</i> _{1/2 K10} (h)	14.53 ± 2.44	7.97 ± 2.80	48.53 ± 7.96	59.07 ± 20.75	53.28 ± 8.40
AUC	137885 ± 21011	42981.1 ± 11664.5	11784.4 ± 1480.0	12171.6 ± 2834.6	17235.5 ± 1954.9
<i>V</i> _d (mL)	3.25 ± 0.34	5.72 ± 0.99	127.15 ± 14.89	149.83 ± 37.64	95.44 ± 10.71
<i>T</i> _{lag} (h)			2.9 × 10 ⁻⁵ ± 2.4	2.73 × 10 ⁻⁵ ± 0.70	0.15 ± 0.25
<i>Cl</i> _f ^e	0.16 ± 0.02	0.50 ± 0.14	1.82 ± 0.23	1.76 ± 0.41	1.24 ± 0.14

	bladder ^a	lung ^a
<i>C</i> _{max} (ng/g)	141.30 ± 14.57	202.27 ± 79.89

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	lung ^a	bladder ^a
T_{max} (hr)	10.31 ± 10.21	17.81 ± 3.23
K_{01} (h ⁻¹)	0.27 ± 0.49	0.14 ± 0.05
K_{10} (h ⁻¹)	0.020 ± 0.018	0.015 ± 0.003
$T_{1/2 K01}$ (h)	2.54 ± 4.54	4.86 ± 1.59
$T_{1/2 K10}$ (h)	34.37 ± 30.34	45.72 ± 9.13
AUC	12349.5 ± 6545.4	12166.1 ± 1462.8
V_d (mL)	85.93 ± 55.62	116.02 ± 19.18
T_{lag} (h)	2.98 × 10 ⁻⁶ ± 1.6	0.23 ± 0.27
Cl_F	1.73 ± 0.92	1.76 ± 0.21

^aData were fitted to the equation $C(T) = ((DK_{01})/(V(K_{01} - e^{-K_{10}T} - e^{-K_{01}T})))$.

^bData were fitted to the equation $C(T) = (D/V)e^{-K_{10}T}$.

^cResults were expressed by the mean ± SD.

^dThe unit is h·ng/g.

^eThe unit is ng/(h·ng/g).