# Tissue distribution and characterization of feline cytochrome P450 genes related to polychlorinated biphenyl exposure

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# ABSTRACT

Cats have been known to be extremely sensitive to chemical exposures. To understand these model species' sensitivity to chemicals and their toxicities, the expression profiles of xenobiotic-metabolizing enzymes should be studied. Unfortunately, the characterization of cytochrome P450 (CYP), the dominant enzyme in phase I metabolism, in cats has not extensively been studied. Polychlorinated biphenyls (PCBs) are known as CYP inducers in animals, but the information regarding the PCB-induced CYP expression in cats is limited. Therefore, in the present study, we aimed to elucidate the mRNA expression of the CYP1–CYP3 families in the cat tissues and to investigate the CYP mRNA expression related to PCB exposure. In cats, the greatest abundance of CYP1–CYP3 (CYP1A2, CYP2A13, CYP2C41, CYP2D6, CYP2E1, CYP2E2, CYP2F2, CYP2F5, CYP2J2, CYP2U1, and CYP3A132) was expressed in the liver, but some extrahepatic isozymes were found in the kidney (CYP1A1), heart (CYP1B1), lung (CYP2B11 and CYP2S1) and small intestine (CYP3A131). In cats, CYP1A1, CYP1A2 and CYP1B1 were significantly upregulated in the liver as well as in several tissues exposed to PCBs, indicating that these CYPs were distinctly induced by PCBs. The strong correlations between 3,3',4,4'-tetrachlorobiphenyl (CB77) and CYP1A1 and CYP1B1 mRNA expressions were noted, demonstrating that CB77 could be a potent CYP1 inducer. In addition, these CYP isoforms could play an essential role in the PCBs biotransformation, particularly 3–4 Cl-PCBs, because a high hydroxylated metabolite level of 3–4 Cl-OH-PCBs was observed in the liver.



**Keywords**: Cat, CYP, mRNA expression, PCBs, metabolism

# 1. Introduction

Cytochrome P450 (CYP) is a large family of heme-containing proteins that play an essential role in the oxidative metabolism of various endogenous compounds, drugs, and xenobiotics. Among several members of the CYP superfamily, the CYP1, CYP2, and CYP3 families are responsible for the biotransformation of most xenobiotics (Ogu and Maxa, 2000; Zanger and Schwab, 2013). Previous studies have revealed that the CYP expression and activity varied among genetic polymorphism, age, and animal species (Graham and Lake, 2008; Martignoni et al., 2006; Sadler et al., 2016; Tomaszewski et al., 2008; Zuber et al., 2002). The strong correlations between mRNA expression levels and enzyme activities of human CYPs (such as CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) have been described in the liver (Ohtsuki et al., 2012; Temesvári et al., 2012). As a result, the investigation of CYP1–CYP3 mRNA expression profiles provides valuable information for the prediction of environmental chemicals and pharmaceutical exposure effects for each species.

Domestic cat (*Felis catus*) is one of the most common pets worldwide. This species is highly sensitive to drugs and chemical exposures, probably due to insufficiencies observed in some parts of xenobiotic metabolism (Court, 2013b; Court and Greenblatt, 2000). Glucuronidation deficiency for phase II metabolism is well known in cats, but the study on CYPs, particularly CYP1–CYP3 families, has not extensively been clarified. Several studies have reported the genetic polymorphisms, metabolic activities, and protein and mRNA expressions of some feline CYPs, that is, CYP1A, CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, and CYP3A, have been reported (Honda et al., 2011; Komatsu et al., 2010;

Okamatsu et al., 2017a; Ono et al., 2019; Shah et al., 2007; Sugiyama et al., 2019; Tanaka et al., 2006), and most of these reviewed in the liver. However, the CYP expression is also found in extrahepatic tissues. The CYP expression patterns in the extrahepatic tissues, such as the brain, lung, and kidney, could be determined to understand the variation of controlling the tissue activity, susceptibility to xenobiotics and chemical exposures, as well as the local treatment outcome (Ferguson and Tyndale, 2011; Kamata et al., 2018; Knights et al., 2013). Therefore, the tissue-specific distribution of the CYP expression in cats should be characterized in detail.

CYPs can be induced or inhibited by several drugs and chemicals (Graham and Lake, 2008). Polychlorinated biphenyls (PCBs), which are man-made organohalogen compounds, are known to induce CYPs in humans and animals. Although dioxin-like PCBs (DL-PCBs) have been considered as aryl hydrocarbon receptor (AHR)-related CYP1A inducers (Korytko et al., 1999; Machala et al., 1998; McGraw and Waller, 2006), PCB mixtures are primarily metabolized by CYP2B and CYP3A in fish, rats, and humans (Connor et al., 1995; Koenig et al., 2012; Petersen et al., 2007). Furthermore, following CYP metabolism, the hydroxylated PCB (OH-PCB) metabolites are produced, which are more toxic than their parent compounds (Grimm et al., 2015; Tehrani and Van Aken, 2014). Interspecies differences in the OH-PCB metabolites retained in the blood and tissues have been noted. In numerous terrestrial mammals (e.g., dog, raccoon dog, and fox), the higher-chlorinated hydroxylated metabolites (6–8 Cl-OH-PCBs) were retained in the blood, whereas the lower-chlorinated hydroxylated metabolites (3–4 Cl-OH-PCBs) were mostly detected in the blood of cats (Mizukawa et al., 2013). Moreover, several findings have indicated that the PCBs

exposure contribute to the etiopathogenesis of feline hyperthyroidism (Peterson, 2012; Walter et al., 2017). The causes of feline hyperthyroidism and OH-PCB retention are relatively complicated, and the characterizations of phase I and II enzymes, the distribution and excretion of PCB exposure have been warranted. Considering the specific OH-PCB pattern in the blood and the lack of UGT1A6 and CYP2B in the liver of cats (Kakehi et al., 2015; Okamatsu et al., 2017b), the feline CYP expression pattern and induction by PCB exposure should be clearly understood to determine the risk of PCBs to felines, particularly domestic cats.

In the present study, we aimed to elucidate the existing isoforms of the CYP1–CYP3 families in various cat tissues including the liver, kidney, heart, lung, small intestine (duodenum, jejunum, and ileum), and brain (cerebrum, cerebellum, hypothalamus, midbrain, pons, and medulla). Furthermore, we investigated the CYP mRNA expression related to PCB exposure in cats using the cDNA cloning and quantitative real-time RT-PCR (qRT-PCR) techniques. Additionally, to estimate the possible PCB congener-induced CYPs, the correlation between the CYP mRNA expression and DL-PCBs toxic equivalent (TEQ) in the liver was analyzed.

## 2. Materials and Methods

### *2.1. Sample collection*

The animal experiment was carried out in the animal facility that is recognized by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all animal treatments were ethically approved by Hokkaido University, Japan (approval number 14-0054 and 14015). Eight male cats (24–28 months old, *Felis catus*) were purchased

from Kitayama Labes Co., Ltd., Japan. They were fed in a 14/10-h light/dark cycle at room temperature (24±5 °C) with a relative humidity of 50±10%. The cats were also fed with water ad libitum and 80 g of food per day. Cat food contained about 3,917 kcal/kg of energy including; 44.5% protein, 9.7% fat, and 2.3% fiber (Nosan Co., Japan). After the 2-month acclimatization, all cats were divided into two groups: control (n = 4, 4.1±0.6 kg) and exposure (n = 4, 4.3±0.3 kg). The cats were intraperitoneally injected with corn oil and a mixture of twelve PCBs (IUPAC no. 18, 28, 70, 77, 99, 101, 118, 138, 153, 180, 187, and 202) in corn oil at a dose of 0.5 mg (each congener)/kg (BW) once in the control and exposure groups, respectively. These twelve PCBs, including DL-PCBs and non-dioxin-like PCBs (NDL-PCBs), have been detected in serum and hair of pet cats and dogs (Ali et al., 2013; González-Gómez et al., 2018; Mizukawa et al., 2013; Serpe et al., 2018); and in house dust (DellaValle et al., 2013; Wang et al., 2013; Whitehead et al., 2014). The exposure dose was adjusted from the PCB dose given to the dogs in a previous study (Korytko et al., 1999). After a 5-day exposure period, the cats were anesthetized with pentobarbital and euthanized by KCl injection. The dissections were performed by a qualified veterinarian. All tissues were collected under aseptic conditions and stored at –80 °C until further analysis. The contents of the small and large intestines were removed, after which they were washed with phosphate-buffered saline, cut into segments and kept in –80 °C until analysis. To prevent RNase contamination and to preserve cellular RNA stability, all equipment used for the dissection were washed with RNase Quite (NACALAI TESQUE, INC., Japan) and the samples used for mRNA expression studies were kept in RNAlater (Sigma-Aldrich Co., St. Louis, MO, USA).

### *2.2. Cloning and quantitative real-time PCR*

The total RNAs of the liver, kidney, heart, lung, intestine (duodenum, jejunum, and ileum), and brain (cerebrum, cerebellum, hypothalamus, midbrain, pons, and medulla) were extracted and cleaned using TRI Reagent® (SIGMA Life Science, USA) and the NucleoSpin® kit (MACHEREY-NAGEL, Germany), respectively. The integrity and quality of mRNA isolates were examined via 1.5% agarose gel electrophoresis and checked using the 28S and 18S ribosomal RNA bands. The amount of RNA was measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., USA), and RNA was synthesized for the cDNA cloning-technique using the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Life Science Department, Osaka, Japan). The CYP1–CYP3 genes were selected from the National Center for Biotechnology Information (NCBI) databases and plasmids were constructed by using the TOPO TA Cloning kit (Invitrogen, USA). The nucleotide sequences of the PCR product and plasmid were performed by FASMAC Company (FASMAC Co., Ltd., Japan). All CYP1–CYP3 primers, reference gene primers, and primer sequences as well as the PCR efficacy are shown in the Supplementary Table 1. The qRT-PCR (StepOnePlus Real-Time PCR System, Applied Biosystems, USA) was performed using a 10-μL PCR reaction mixture containing 5 μL of Fast SYBR Green Master Mix (Applied Biosystems, USA), 0.4 μL of 5-μM forward and reverse primers (Thermo Fisher Scientific, Life Technologies Japan Ltd., Japan), 20 ng of cDNA of each tissue, and 2.2 μL of distilled water. The qRT-PCR condition for all target genes was 95 °C for 20 s, followed by 40 cycles of 95 °C for 3 s, and 60 °C for 30 s.

### *2.3. Chemical analysis*

Previous reports have described the measurement method for PCBs and OH-PCBs (Eguchi et al., 2014; Nomiyama et al., 2017). Briefly, 2.5 g of four liver samples from PCB-exposed cats were spiked with the $^{13}C_{12}$-labeled internal standards (detailed information about the internal standards are given in Supplementary Table 2), denatured by 6 M of HCl, and homogenized with 2-propanol and 50% methyl *t*-butyl ether/hexane. After centrifugation, the organic phase was partitioned into neutral and phenolic fractions using 1 M of KOH in 50% ethanol/water. Using gel permeation chromatography (GPC), the lipid in neutral fraction was removed. Thereafter, the GPC fraction containing PCBs was passed through an activated silica gel column. The phenolic fraction was acidified using sulfuric acid and extracted twice using 50% methyl *t*-butyl ether/hexane. Next, the extracted solution containing OH-PCBs was passed through a deactivated silica gel (5% $H_2O$ deactivated) column and then derivatized to methylated compounds (MeO-PCBs) overnight using trimethylsilyl-diazomethane. After removing the lipid using GPC, the derivatized solution was passed through the activated silica gel column, and the MeO-PCBs were eluted with 10% dichloromethane/hexane. A gas chromatograph (6890 series, Agilent Technologies) coupled to a high-resolution (>10,000) mass spectrometer (JMS-800D, JEOL) was used to identify and quantify the target PCBs and metabolites.

### *2.4. Quality assurance for chemical analysis*

The target compounds were quantified using the isotope dilution method for the corresponding $^{13}C_{12}$-internal standards (Eguchi et al., 2014; Nomiyama et al., 2017). A procedural blank was analyzed with each batch of 8 samples using the same protocol that was applied to the samples, to detect any contamination from solvents and glassware. The

limit of quantification was defined as the concentration of target compounds that produced a signal to noise ratio (S/N) of 10. Hepatic samples were showed remarkably low recoveries (38±6% for PCBs and 23±4% for OH-PCBs) because of ion suppression due to the matrix contained within liver tissues. However, these samples with high matrix effects were corrected for the influence for foreign substances by $^{13}C_{12}$-labelsd internal standards in this study. MeO-PCBs were quantified when the retention times matched that of the standards within ±0.1 min, the S/N was greater than 10, and the deviation of ion intensity ratio was within 15% of the standards.

### *2.5. Data and statistical analysis*

Sequence alignments were examined using the MEGA7 software. In addition, the gene copy number for each cDNA sample was calculated using standard curves and normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin (ACTB) copy numbers. The result of this experiment was presented as the mean ± standard deviation. A statistical analysis was performed using JMP Pro 13 (SAS Institute, USA). The levels of CYP mRNA expressions in the control and exposure groups were tested for normality using the Shapiro-Wilk test and checked for homogeneity of variance using the Levene's test. Wilcoxon test was used to determine the significant differences between the control and exposure groups. Furthermore, the correlations between CYP mRNA expression and DL-PCB TEQ in the liver were analyzed using Spearman's correlation. $P$ value < 0.05 was considered significant.

## 3. Results

### *3.1. Tissue distribution of CYP isoforms in cats*

The mRNA expressions (copy number of target CYP isoform/copy number of reference genes, GAPDH and ACTB) of 17 CYP isoforms in the hepatic and extrahepatic tissues of the control group are given in the Supplementary Figs. 1 and 2, and the Supplementary Tables 3 and 4. The expression levels (mean ± SD) of CYP1–CYP3 were as follows: liver, 0.0004±0.0004 to 27.16±16.20; small intestine, 0.000007±0.00001 to 14.90±4.37; lung, 0.00003±0.00004 to 5.84±4.43; kidney, 0.00004±0.00004 to 5.05±1.69; heart, 0.00002±0.00003 to 0.18±0.10; and brain, undetectable to 0.21±0.14. Although most of CYPs were predominantly expressed in the liver, the expressions of some CYP isoforms were greater than those in the liver, including CYP1A1 (5.05±1.69), CYP1B1 (0.18±0.10), CYP2B11 (5.84±4.43) and CYP2S1 (2.24±1.48), and CYP3A131 (14.90±4.37) in the kidney, heart, lung, and jejunum, respectively. On the other hand, all CYP1–CYP3 existed relatively low (copy number of CYP isoform to copy number of reference genes < 1) in the heart and six parts of the brain. In addition, the comparison of CYP isoforms (% of copy number/copy number of reference genes) in the tissues of cats in the control group is shown in Fig. 1.

Fig. 1

*3.1.1. Liver*

The CYP3A family, especially CYP3A132, was most abundant (45.20% [GAPDH normalization] and 54.09% [ACTB normalization]) in the liver, followed by CYP2 (46.32% [GAPDH normalization] and 34.55% [ACTB normalization]) and CYP1 (8.48% [GAPDH normalization] and 11.35% [ACTB normalization]). However, CYP1B1 and CYP2B11 were hardly detected (<0.01%).

*3.1.2. Kidney*

More than 95 % of all CYP expressions in the kidney were CYP1A1 (96.17% [GAPDH normalization] and 95.35% [ACTB normalization]), followed by CYP2 (3.47% [GAPDH normalization] and 4.00% [ACTB normalization]), and CYP3A (0.11% [GAPDH normalization] and 0.18% [ACTB normalization]). Only a scarce number of CYP3A12, CYP1A2, and CYP2D6 (<0.01%), on the contrary, were found.

*3.1.3. Lung*

CYP2B11 (89.26% [GAPDH normalization] and 94.03% [ACTB normalization]) was highest expressed in the lung, followed by CYP1 (0.95% [GAPDH normalization] and 0.97% [ACTB normalization]) and CYP3A (0.61% [GAPDH normalization] and 0.31% [ACTB normalization]), but only CYP2E2 showed very low expression level (<0.01% [GAPDH and ACTB normalization]).

*3.1.4. Heart*

In the heart, the dominant CYP isoform was CYP1B1 (49.09% [GAPDH normalization] and 37.79% [ACTB normalization]), and much lower mRNA expressions of CYP2 (36.31% [GAPDH normalization] and 50.26% [ACTB normalization]) and CYP3A (1.31% [GAPDH normalization] and 1.26% [ACTB normalization]) were noted.

*3.1.5. Small intestine*

In the three parts of the small intestine, nearly 100% of the CYP expressions were CYP3A131 (48.72% [GAPDH normalization] and 56.68% [ACTB normalization]) and CYP2C41 (42.15% [GAPDH normalization] and 38.42% [ACTB normalization]), while the CYP1 expression varied from 0.35% to 7.44 % (GAPDH normalization) and 0.29% to 2.60%

(ACTB normalization) in each part. However, the following expressions were sparsely found (<0.01% [GAPDH and ACTB normalization]): duodenum, CYP2E2 and CYP2D6; jejunum, CYP2E1, CYP2E2, CYP2A13, and CYP2D6; and ileum, CYP2E1, CYP2E2, and CYP2D6.

*3.1.6. Brain*

CYP2J2 (65.55% [GAPDH normalization] and 68.90% [ACTB normalization]) was the main isoform in all six parts of the brain, whereas the expressions of CYP1 and CYP3A were 1.74% to 10.72% (GAPDH normalization), 1.30% to 5.69% (ACTB normalization) and 1.60% to 7.38% (GAPDH normalization), 1.60% to 6.98% (ACTB normalization), respectively. Some CYP isoforms were scarcely observed, including CYP2D6 in the cerebrum and medulla and CYP2E2 in the pons (<0.01% [GAPDH and ACTB normalization]). Moreover, these two isoforms were undetectable in the hypothalamus.

***3.2. CYP mRNA expressions related to PCB exposure***

Comparing the control and exposure groups, the expressions of some CYP isoforms (copy number/reference genes) in each tissue were significantly changed ($P < 0.05$) (Fig. 2 and 3). The significant differences of the CYP1A1 expression between the two groups were found in the liver (5.9- to 6.7-fold), kidney (263.7- to 308.7-fold), lung (9.0- to 9.5-fold), heart (19.7- to 5.3-fold), cerebrum (1.7-fold), cerebellum (50.5- to 57.9-fold), hypothalamus (8.7- to 13.3- fold), midbrain (19.4- to 39.9-fold), Pons (8.6-fold), and medulla (10.1- to 12.2-fold). CYP1A2 showed the significant alteration in liver (2.9- to 3.7- fold), kidney (168.1- to 211.9-fold), heart (6.1-fold), cerebrum (0.6-fold), hypothalamus (45.4-fold), midbrain (2.9-fold), and medulla (2.0-fold) of the PCB-exposed cats compared with those in the control group. The significant CYP1B1 induction was also observed in the liver (37.7-fold), kidney

(35.2- to 46.8-fold), lung (25.8- to 37.7-fold), heart (9.7- to 25.6-fold), jejunum (7.7-fold), cerebrum (5.0- to 6.8-fold), cerebellum (5.0-fold), midbrain (3.5- to 7.0-fold), pons (5.6- to 7.8-fold), and medulla (6.5- to 8.0-fold) of PCB-exposed cats compared with those in the control group. Furthermore, the significant differences of the CYP2 expression between the cats in the control and exposure groups were noted as follows: CYP2A13, heart (2.8-fold) and midbrain (2.2-fold); CYP2B11, cerebrum (6.2- to 6.3-fold) and midbrain (1.7- to 2.1-fold); CYP2E2, duodenum (5.0- to 6.1-fold); CYP2F5, ileum (1.4- to 1.5-fold); CYP2J2, midbrain (1.7-fold); CYP2S1, kidney (4.1- to 4.6-fold) and heart (1.8-fold); and CYP2U1, lung (1.7-fold), heart (1.6-fold), medulla (0.7-fold) and pons (0.6-fold). However, CYP3A12 and CYP3A131 in the liver (0.4-fold and 1.58-fold, respectively) and CYP3A131 in the jejunum (0.3- to 0.7-fold) of cats in the exposure group were significantly downregulated compared with those in the control group.

Fig. 2. and Fig. 3.

### *3.3. PCB and OH-PCB levels in the liver*

Table 1 shows the PCB and OH-PCB levels in the liver samples of cats exposed to PCBs, and the Supplementary Table 5 depicts the details of all PCB and OH-PCB congener concentrations examined. The mean concentrations (mean ± SD) of the total PCBs and OH-PCBs in the liver were 527.86±462.41 and 209.85±97.46 ng/g, respectively. Among the 12 PCB parent compounds, CB118 (190±270) had the highest level, followed by CB101 (160±230), CB99 (150±230), CB153 (140±210), CB180 (140±200), CB202 (140±190), CB138 (130±190), CB187 (130±190), CB77 (130±160), CB28 (110±100), CB70 (94±150), and CB18 (64±68). The predominant hydroxylated metabolite in the cat liver was 4’OH-

CB18 (130±79), while much lower concentrations of the following were detected: 4′OH-CB61 (28±21), 4OH-CB70 (19±9.9), 4′OH-CB79 (7.6±2.1), 3OH-CB25 (6.1±2.7), 3′OH-CB28 (0.86±1.5), 4OH-CB26 (0.78±0.75), 4OH-CB97 (0.74±0.2), 3OH-CB101 (0.3±0.099), 3OH-CB118 (0.051±0.021), and 4OH-CB187 (0.011±0.019).

Table 1.

### *3.4. Relationship between CYP mRNA expression and DL-PCB TEQ in the liver*

Fig. 4 presents the Spearman' correlation coefficients between the CYP mRNA expression levels and TEQ of DL-PCBs (CB77 and CB118) in the liver. Significant correlations were noted between the CYP1A1 mRNA expression level and TEQ of CB77 ($\rho = 1.00$) and total TEQ ($\rho = 1.00$) ($P < 0.05$). Furthermore, the CYP1B1 mRNA expression level was significantly correlated with the TEQ of CB77 ($\rho = 1.00$) and total TEQ ($\rho = 1.00$) ($P < 0.05$).

Fig. 4

## 4. Discussion

### *4.1. Tissue expressions of feline CYP1-3 families*

In this study, we focused on the CYP1–CYP3 families because they are the most significant CYPs, which play an essential role in the xenobiotic metabolism in humans and animals (Zanger and Schwab, 2013). In fact, the data on the characterization of the feline CYP1–CYP3 families, including CYP1A1, CYP1A2, CYP2A, CYP2B11, CYP2D6, CYP2E, CYP3A131, and CYP3A132, in the liver have been published (Honda et al., 2011; Komatsu et al., 2010; Okamatsu et al., 2017a; Okamatsu et al., 2017b; Tanaka et al., 2006; Tanaka et al., 2005), but no information on the tissue distribution of all CYP1–CYP3 has been presented.

Therefore, after searching feline CYP databases from NCBI website and primer screening using the conventional PCR, 17 CYP isoforms were selected in this study including CYP1A1, CYP1A2, CYP1B1, CYP2A13, CYP2B11, CYP2C41, CYP2D6, CYP2E1, CYP2E2, CYP2F2, CYP2F5, CYP2J2, CYP2S1, CYP2U1, CYP3A12, CYP3A131, and CYP3A132. GAPDH and ACTB were used as the house keeping genes. Because the stability of reference genes differs among feline tissues (Kessler et al., 2009; Penning et al., 2007), the variations of the relative CYP expressions in the tissues were found in this study.

*4.1.1. CYP expressions in the liver*

In the present study, CYP isoforms were mainly expressed in the liver, and CYP3A132 was the most abundant isoform. On the other hand, CYP3A131 and CYP2E2 have been reported as the most dominant isoforms by early studies (Honda et al., 2011; Okamatsu et al., 2017a). The differences in the breed, age, and individual conditions could be the significant factors affecting CYP expressions (Zanger and Schwab, 2013). Moreover, the species difference affects the mRNA expression pattern of CYPs. The comparison of the mRNA expressions of the CYP1–CYP3 families in the liver of humans (Rodriguez-Antona et al., 2001), dogs (Martinez et al., 2013), and cats (present study) is shown in Fig. 4. The mRNA expression of CYP2, including CYP2A, CYP2D, and CYP2E, was dominant in the dog liver, whereas the major isoform in the cat and human livers was CYP3A. In humans, low CYP2A and CYP2D levels were expressed (0.13% and 0.18%, respectively), but the presence of these CYPs in dogs (25.14% and 25.17%) and cats (4.13% and 4.16%) suggested these CYPs are significant subfamily for the xenobiotic metabolism in dogs and cat compared with humans.

On the other hand, a lack of CYP2B in the cat liver was noted, and this could alter the metabolism process for some treatment drugs and environmental pollutants such as cyclophosphamide, antipyrine, DDT, and organohalogen compounds (Hedrich et al., 2016).

Fig. 5.

*4.1.2. CYP expressions in the kidney*

Some CYP isoforms were mainly expressed in the extrahepatic tissues. The abundance of CYP1A1 in the feline kidney, rat, and mouse kidneys (El-Sherbeni and El-Kadi, 2014; Meyer et al., 2002) suggested that this isoform plays an essential role in the xenobiotic metabolism in their kidneys (Androutsopoulos et al., 2009). CYP1A1 is also primarily expressed in the human kidney. It is used as an indicator for human renal cell tumor because it metabolizes carcinogens to their inactive derivatives and infrequently converts the chemicals to more potent carcinogens (Cheung et al., 1999; Meng et al., 2015). Therefore, the high-level of mRNA expression of feline CYP1A1 in the kidney may result in an active metabolite-induced renal toxicity. In rats, CYP activities, such as aromatic oxidation and oxidative dehalogenation, in the kidney were equal to or less than those in the liver (Lohr et al., 1998). At the same time, the previous study characterized the feline CYP1A1 functions and found that feline CYP1A1 contributed to theophylline 3-demethylation with high $V_{max}$ and low $K_m$, in contrast to that of rats (Tanaka et al., 2006). Thus, the species differences of activities in CYP1A1 isolated in the kidneys should be taken into consideration, and further study of these on cats is needed.

*4.1.3. CYP expressions in the lung*

CYP2B11 expression was found to be predominantly in the lung. It has been suggested that CYP2B11 to play a role in the local defense mechanism of cat's respiratory system (Okamatsu et al., 2017b). In humans, CYP2B6 (the human ortholog of feline CYP2B11) has been primarily expressed in the liver (Rendic, 2002), while CYP2B11 was significantly expressed in both the liver and intestine of dogs (Court, 2013a). Canine CYP2B11 is mainly responsible for the clearance of anesthetic drugs, especially propofol (Hay Kraus et al., 2000). Hence, feline CYP2B11 may not play a central role in the systemic metabolism and clearance of xenobiotics. In addition, a higher CYP2S1 level was found in the lung tissue compared with the liver. In the humans, CYP2S1 was highly expressed in the extrahepatic tissues that were exposed to the environment (e.g., skin, respiratory, urinary, and gastrointestinal tracts) and selectively played a role in pulmonary xenobiotic biotransformation (Bui and Hankinson, 2009; Hukkanen et al., 2002). Human CYP2S1 was suggested to have a potential role in the carcinogen metabolism and shown to be inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin through the AHR signaling pathway (Bui and Hankinson, 2009). Therefore, the CYP2S1 expression in the cat lung of cat may have a high metabolic rate for inhaled substances causing cancer.

*4.1.4. CYP expressions in the small intestine*

CYP3A131 and CYP2C41 were abundantly expressed in all parts of the small intestine. Previous reports (Honda et al., 2011; Okamatsu et al., 2017a) found that the feline CYP3A131 expression in the small intestine was comparable with that in the liver, suggesting the importance of CYP3A131 in xenobiotics that were orally exposed in cats. However, the

present study indicated that not only CYP3A131 but also CYP2C41 might play a major role in both hepatic and intestinal xenobiotic clearances. In humans, the high levels of mRNA and protein expressions of CYP2C and CYP3A4 were detected in the small intestine, and the contents of both CYPs decreased toward the small intestine's distal end (Zhang et al., 1999). In contrast, there was greater expression of feline CYP3A131 and CYP2C41 in the jejunum and ileum and then lower expressed upward the duodenum. The distributions of most CYPs varied as a function of position along the small intestine; however, the CYP expression pattern among these three parts of the small intestine is not significantly different in our study.

*4.1.5. CYP expressions in the heart and brain*

Comparing all tissues analyzed, CYP isoforms were hardly observed in the heart and six parts of the brain. In humans, the total CYP level in the brain was also low (approximately 0.5%–2% of that in the liver); thus, the CYP-mediated metabolism in the brain unlikely affects the systemic metabolite levels (Ferguson and Tyndale, 2011; Hedlund et al., 2001). Additionally, the metabolic capacity and xenobiotic clearance are relatively low in the mammalian heart compared with those in the liver (Chaudhary et al., 2009). These previous reports confirmed our results that the heart and brain may not play a role in systemic xenobiotic metabolism in cats.

In this study, the dominant CYP isoform in the cats' heart was CYP1B1. Similar to the hearts of humans, rats and mice, the CYP1B1 mRNA expression was also observed in relatively high amounts and associated with the biosynthesis of various intermediated types of arachidonic acid (Chaudhary et al., 2009; Elbekai and El-Kadi, 2006). In addition, the

cardiac expressions of CYP1A, CYP2B, CYP2C, CYP2D, and CYP2J are involved in the xenobiotic metabolism within the heart itself (Chaudhary et al., 2009; Thum and Borlak, 2000). Consequently, the CYP mRNA expression pattern in the cat heart indicated that the xenobiotic metabolism could occur in the heart, potentially influencing toxicological efficacy or adverse drug effects.

Although the total CYP levels in the brain are lower than those in the liver, the brain CYPs play an essential role in the local xenobiotic biotransformation, modulating brain activities in specific regions and cell types (Ferguson and Tyndale, 2011; Ghosh et al., 2016; Hedlund et al., 2001). In our results, CYP2J2 was the main isoform in all parts of the cats' brain. Furthermore, in the human brain, CYP2J2 was mainly expressed in the endothelial cells of the cortex and microvessels, while rat neuron cells in the cerebellum, hippocampus, cerebral cortex, and brain stem were the main regions of the CYP2J9 expression (Ghosh et al., 2016). CYP2J is responsible for the metabolism of endogenous fatty acids, whereas the CYP1A, CYP2C, and CYP2B subfamilies contribute to the metabolism of both endogenous and exogenous substances such as steroids, neurotransmitters, antidepressant drugs, and neurotoxins in the brain (Ferguson and Tyndale, 2011). Therefore, these CYP expression patterns in the cat brain could potentially have a significant impact differently on the local xenobiotic metabolism in each part.

### *4.2. The expressions of CYP related to PCB exposure in the liver*

To date, PCB-induced CYP expressions in cats have not yet been reported. The PCB exposure with the experimental dose statistically induced CYP1A1, CYP1A2, and CYP1B1

expressions in both hepatic and extrahepatic tissues (including the kidney, heart, and lung and some parts of the brain), suggesting these CYP isozymes play a fundamental role in the PCBs metabolism in cats. The CYP1 family is generally induced by polycyclic and halogenated aromatic hydrocarbons including dioxin and DL-PCBs involved in the AHR-mediated signaling pathway. The AHR functions contribute to a broad range of physiological roles, including immune function, organ development, reproduction, and steroid signaling modulation (Beischlag et al., 2008). Subsequently, the CYP1A1, CYP1A2, and CYP1B1 expressions should be considered a biomarker not only for the exposure of DL-PCBs (CB77 and CB118) but also for the related toxic effects of these 12 PCB congeners in cats. Furthermore, CYP2B and CYP3A most probably play a major role in the metabolism of NDL-PCBs via the constitutive androstane receptor and pregnane X receptor (PXR) in humans and other animals (Ariyoshi et al., 1995; Gahrs et al., 2013; Koenig et al., 2012; Korytko et al., 1999; Petersen et al., 2007). The inductions of ethoxyresorufin-O-deethylase (CYP1A1) and pentoxyresorufin-O-dealkylase (CYP2B) activities as well as the levels of NDL-PCB (0–20.5 ng/g) were reported in the livers of two purpose-bred mongrel dogs (age, 12–15 months; weight, 12–27 kg) after PCB exposure (25 mg/kg) (Korytko et al., 1999). The NDL-PCB levels (>0.0074-160 ng/g) in the livers of PCB-exposed cats (24 mg/kg) were relatively higher than those of PCB-exposed dogs, but findings from our study and a previous study (Okamatsu et al., 2017b) suggest that, feline CYP2B does not exist in the liver. These results suggest that NDL-PCBs might be metabolized by only hepatic CYP1A1 in cats and dogs. However, it is possible that the absence of CYP2B expression in cats might be

responsible for the reduced biotransformation efficiency of NDL-PCB biotransformation in the cat liver.

*4.2.1. The downregulation of CYP3 related to PCB exposure*

Relative to the cats in the control group, CYP3A12 and CYP3A131 had statistically lower mRNA expression levels in the liver of PCB-exposed cats, suggesting that PCBs induce a repressor, which is related to the PXR-mediated repression of CYP3A. Although CYP3A downregulation by PCBs in both *in vivo* and *in vitro* studies has not been reported, the hepatic CYP3A expression was suppressed by the parathyroid hormone in rats and during the inflammation by interleukin 6 in the human cell line (Jover et al., 2002; Watanabe et al., 2017). Multiple signaling pathways such as phosphatidylinositol 3-kinase, protein kinases C and A, and nuclear factor kappa B have been reported to be involved in the PXR inactivation, leading to the CYP3A repression (Ding and Staudinger, 2005; Jover et al., 2002; Watanabe et al., 2017). In humans, cattle, and fish, the expressions of CYP3A protein and activity were induced by PCBs (Koenig et al., 2012; Machala et al., 1998; Petersen et al., 2007; Pondugula et al., 2009). Thus, cats could have a specific PCB metabolism as well as toxic effects compared with other animals that may be consistent with the finding of a specific pattern of OH-PCB metabolites in the cat blood (Mizukawa et al., 2013).

***4.3. The upregulation of CYP1 related to PCB exposure in the kidney***

Xenobiotic biotransformation by CYP occurs not only in the liver but also in the kidney (Lohr et al., 1998). In our results, the kidneys of PCB-exposed cats presented significantly higher mRNA expression levels of CYP1A1, CYP1A2, and CYP1B1 than those in the

control group, and the AHR signaling pathway may have contributed to this induction. In the previous reports of mice and rats (Anders, 1980; Lohr et al., 1998), PCBs (Aroclor 1254) slightly induced the renal oxidase activity (2.1-fold), whereas dioxin (TCDD) produced a drastic oxidase activity induction (55.6–60.4-fold), suggesting that DL-PCBs have a higher potential to induce the renal CYP1 mRNA expressions compared with NDL-PCBs. In addition, CYP1A1 in the human kidney is one of the most active CYPs in metabolizing procarcinogens to highly reactive intermediates that can cause cancer and other toxicities (Meng et al., 2015). Therefore, the toxicological consequences of the CYP mRNA inductions in the kidneys of PCB-exposed cats should be considered as well.

### *4.3. The expressions of CYP2 family related to PCB exposure in extrahepatic tissues*

Unlike other tissues, the intestinal CYP1A mRNA expressions were not altered by PCBs, suggesting that, gut flora might possibly be causing the specific expression of CYP in the intestines of PCB-exposed cats. The metabolic capacity of enteric bacteria has been reported to alter the expression and activity of CYP in the liver as well as in the small intestine (Bezirtzoglou, 2012; Björkholm et al., 2009; Ishii et al., 2012). However, the upregulation of the CYP2 family were observed in the intestines and other tissues of PCB-exposed cats. In humans, the CYP2 family is significantly responsible for the CYP-mediated drug metabolism as well as cytokine signaling and inflammatory response. CYP2A, CYP2B, CYP2F, and CYP2S are a CYP cluster on the same chromosome (Bui and Hankinson, 2009; Hoffman et al., 2001). The expressions of these CYPs and CYP2E have been found to be involved in the xenobiotic metabolism and synthesis of cholesterol, steroids, and lipids (Lewis et al., 2003;

Thelen and Dressman, 2009; Zhang et al., 2002). In addition, the CYP2J2 and CYP2U1 inductions in the midbrain and heart could be associated with lipid metabolism and inflammatory responses because CYP2J2 can activate the nuclear peroxisome proliferator-activated receptor α, a controller of lipid metabolism and inflammatory responses (Xu et al., 2013). CYP2U1 also plays a physiological role in the fatty acid signaling processes and arachidonic acid signaling pathway modulation (Dhers et al., 2017; Siller et al., 2014). Hence, the upregulation of these CYPs in the extrahepatic tissues of PCB-exposed cats indicated that the PCB exposure may activate the lipid metabolism and inflammatory responses in cats. Thus, further studies on the adverse effects and toxicological risks related to the PCB exposure with the analysis of the lipid pathway and immune function in the feline hepatic and extrahepatic tissues are required.

### *4.4. The levels of PCB and OH-PCB in the liver*

We also analyzed the PCB and OH-PCB levels in the liver samples of PCB-exposed cats. The average concentration (mean ± SD) of the total PCBs in the liver was 1270±2020 ng/g. In contrast the two purpose-bred mongrel dogs (age, 12–15 months; weight, 12–27 kg) from the previous study (Korytko et al., 1999) had lower PCB levels (42.51–104.75 ng/g) in the liver, even though they were exposed to PCBs at almost the same levels (25 mg/kg) relative to our study. Comparing the PCB levels in the liver of cats and dogs, the higher residue of PCBs in the cat liver suggested that cats have a weaker metabolism for PCBs than dogs. However, the differences in the administration route and the exposure period of PCB between the dog study and our study may be responsible for the differences in the residual levels of

PCB observed in the liver of dogs and cats. Among the OH-PCBs, 3–4 Cl-OH-PCBs were mainly presented in the cat liver. Especially, 4′OH-CB18 was the predominant hydroxylated metabolite in the liver of PCB-exposed cats, while CB18 had the lowest level. These results demonstrated that feline CYP, especially CYP1A1, CYP1A2, and CYP1B1, could mainly metabolize 3–4 Cl-PCBs and may have a low-affinity binding with 5–8 Cl-PCBs.

### *4.5. TEQ of DL-PCBs correlations with CYP1 mRNA expressions in the liver*

Since DL-PCBs have been known as an inducer for the AHR-CYP1 signaling pathway (Girolami et al., 2015; Larigot et al., 2018), the relationship between the TEQ of DL-PCBs (CB77 and CB118) and CYP mRNA expression in the liver was investigated. The relative mRNA expression levels of CYP1A1 and CYP1B1 revealed the significant positive correlations with CB77 and the total TEQ. These results implied that these CYP expressions could be related to the DL-PCB level.

Toxic equivalency factor (TEF) indicates the degree of toxicity of dioxins and dioxin-like compounds (including PCBs) compared to the most toxic form of dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin, which is given a reference value of 1. The TEF values are determined based on in vitro and in vivo studies of dermal toxicity, immunotoxicity, reproductive deficits, teratogenicity, endocrine toxicity, and carcinogenicity promotion (Ahlborg et al., 1994; van den Berg et al., 2006). Comparing DL-PCBs in this study, CB77 presented the strongest correlations with the CYP1A1 and CYP1B1 mRNA expressions. Moreover, the higher TEF value of CB77 (0.0001) compared with that of CB118 (0.00003), suggests that CB77 may be the most potent inducer of CYP1A1 and CYP1B1 in the liver.

## 5. Conclusions

Our study is the first extensive and statistical study to elucidate the various tissue distribution of the CYP mRNA expressions in cats. CYP3A was the dominant subfamily in the liver, but some CYP isoforms such as CYP1A1 and CYP2B11 were greatly expressed in the extrahepatic tissues. Our data showed that CYP1A1, CYP1A2 and CYP1B1 mRNA expressions could be clearly induced by PCBs and may be strongly induced by CB77 in several tissues. In addition, these isozymes may play a significant role in the metabolism of PCBs, particularly 3–4 Cl-PCBs, in the cat liver.

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## Conflict of interest

There are no conflicts of interest to declare.

**Figure legends**

**Fig. 1.** Comparison of the relative CYP isoforms in the tissues of cats in the control group (Reference genes: GAPDH [a] and ACTB [b])

**Fig. 2.** Comparison of CYP mRNA expressions (mean ± SD) in the tissues of cats between control and exposure groups ($P < 0.05$, Reference gene: GAPDH)

**Fig. 3.** Comparison of CYP mRNA expressions (mean ± SD) in the tissues of cats between control and exposure groups ($P < 0.05$, Reference gene: ACTB)

**Fig. 4.** The Spearman's correlation coefficients (ρ) between the DL-PCB TEQ and the relative CYP mRNA expression levels in the liver of PCB-exposed cats ($P < 0.05$)

**Fig. 5.** Comparison of the relative mRNA expressions of the CYP1–CYP3 families in liver of cats (copy number/copy number of GAPDH), humans (copy number/copy number of ACTB) (Rodriguez-Antona et al., 2001), and dogs (copy number/copy number of GAPDH) (Martinez et al., 2013)

**Table 1.** Mean concentration of PCBs and OH-PCBs (ng/g wet weight, mean ± SD) in the liver of PCB-exposed cats (n=4).

| **PCB congeners** | **Level** (ng/g wet weight) |
|---|---|
| CB18 | 64±68 |
| CB28 | 110±100 |
| CB70 | 94±150 |
| CB77 | 130±160 |
| CB99 | 150±230 |
| CB101 | 160±230 |
| CB118 | 190±270 |
| CB138 | 130±190 |
| CB153 | 140±210 |
| CB180 | 140±200 |
| CB187 | 130±190 |
| CB202 | 140±190 |
| Total PCBs | 1270±2020 |
| **OH-PCB congeners** | **Level** (ng/g wet weight) |
| 4'OH-CB18 | 130±79 |
| 3OH-CB25 | 6.1±2.7 |
| 4OH-CB26 | 0.78±0.75 |
| 3'OH-CB28 | 0.86±1.5 |
| 4'OH-CB25/4'OH-CB26/4OH-CB31* | 1.5±1.6 |
| 4'OH-CB61 | 28±21 |
| 4OH-CB70 | 19±9.9 |
| 4'OH-CB79 | 7.6±2.1 |
| 4OH-CB97 | 0.74±0.2 |
| 3OH-CB101 | 0.3±0.099 |
| 4OH-CB107/4OH-CB108* | 0.72±0.02 |
| 3OH-CB118 | 0.051±0.021 |
| 4OH-CB101/4OH-CB120* | 0.77±0.31 |
| 4OH-CB187 | 0.011±0.019 |
| Total OH-PCBs | 200±120 |

*These OH-PCB congeners could not separate completely by the retention time.

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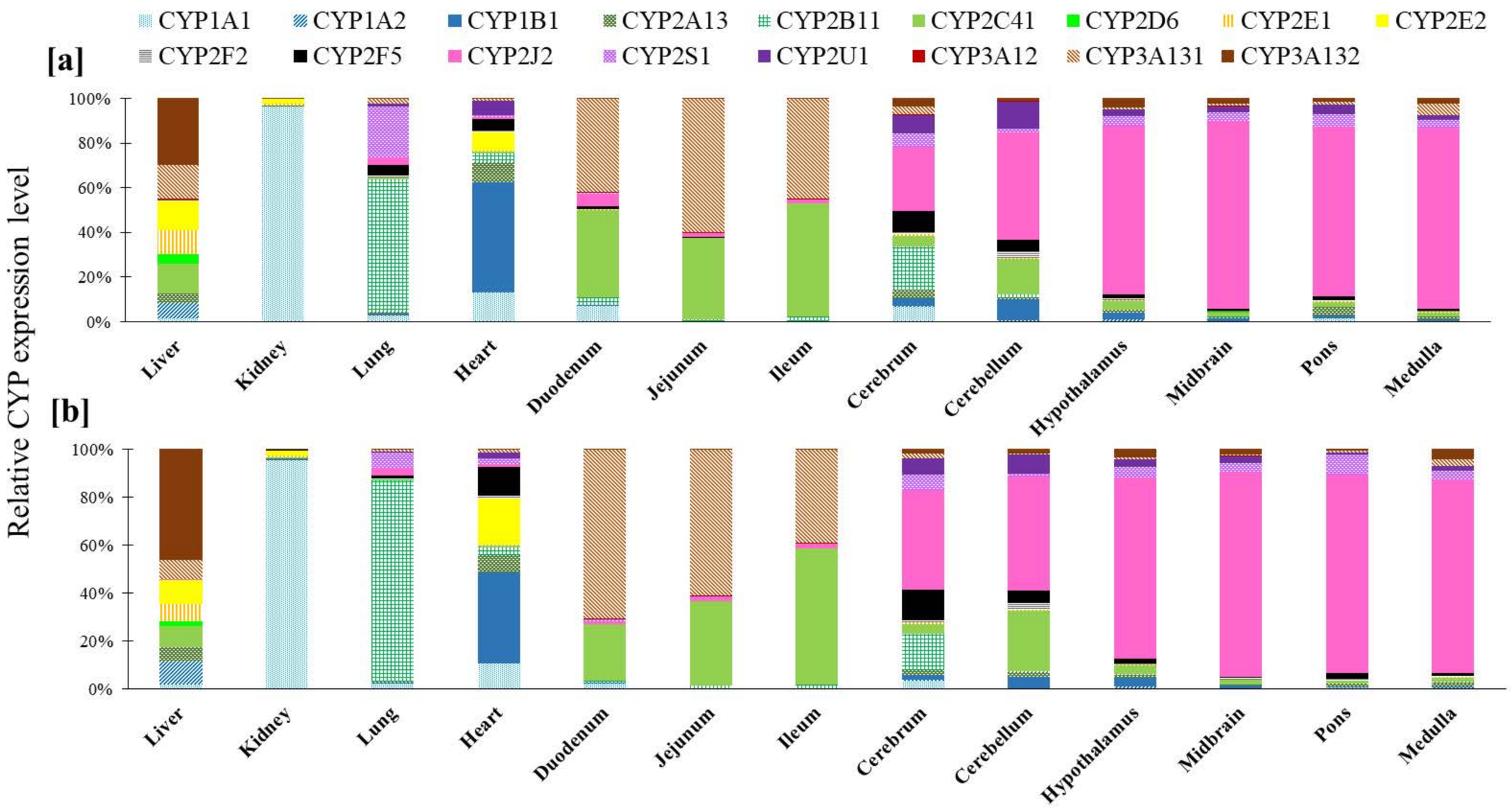
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CYP1A2
CYP1B1
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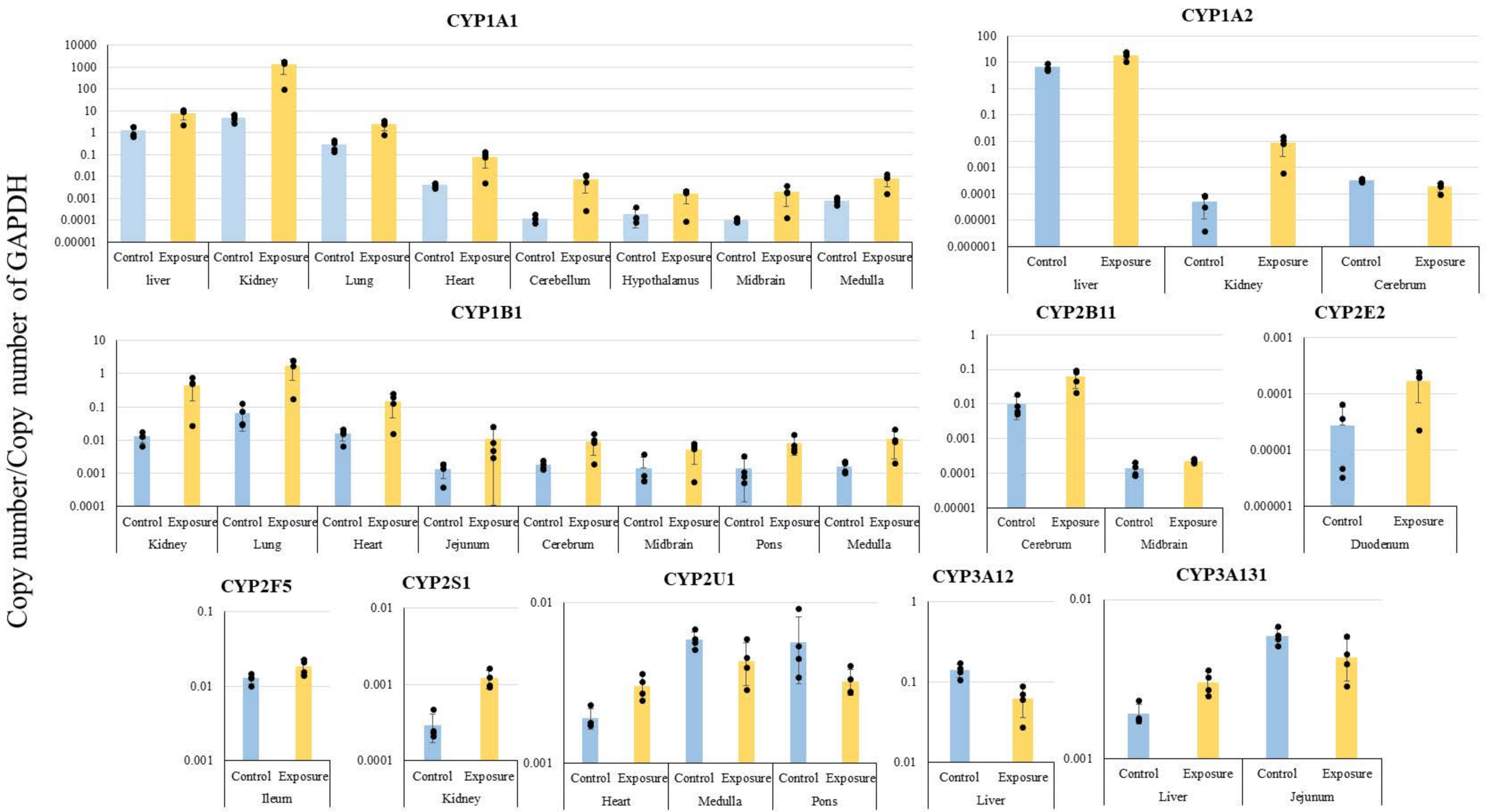
CYP2F2
CYP2F5
CYP2J2
CYP2S1
CYP2U1
CYP3A12
CYP3A131
CYP3A132
[a]
[b]
Relative CYP expression level
100%
80%
60%
40%
20%
0%
Liver
Kidney
Lung
Heart
Duodenum
Jejunum
Ileum
Cerebrum
Cerebellum
Hypothalamus
Midbrain
Pons
Medulla

**Fig. 1.**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liver
Kidney
Lung
Heart
Cerebellum
Hypothalamus
Midbrain
Medulla
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Kidney
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Kidney
Lung
Heart
Jejunum
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Midbrain
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Heart
Medulla
Pons
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Liver
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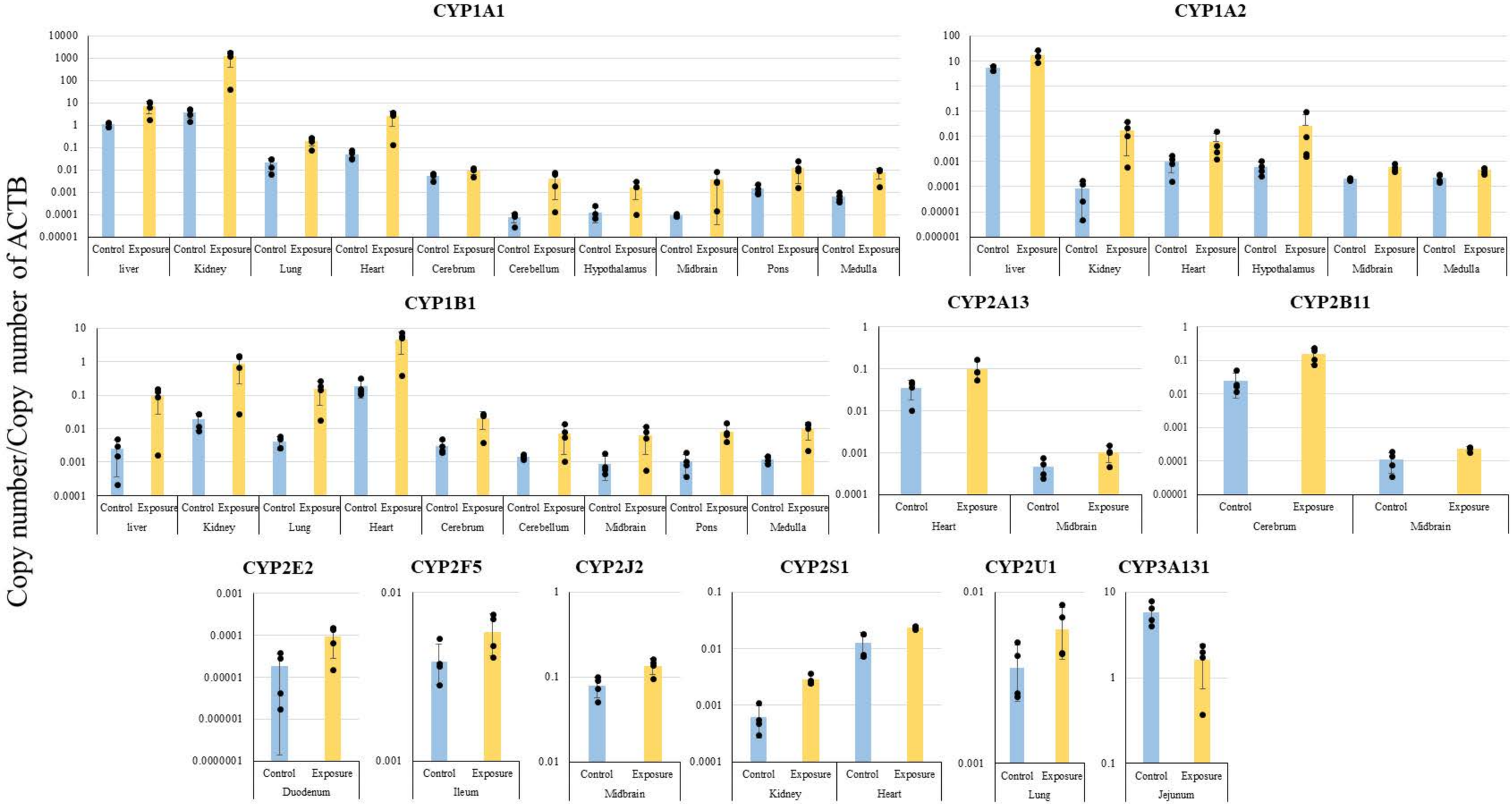
**Fig. 2.**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Medulla
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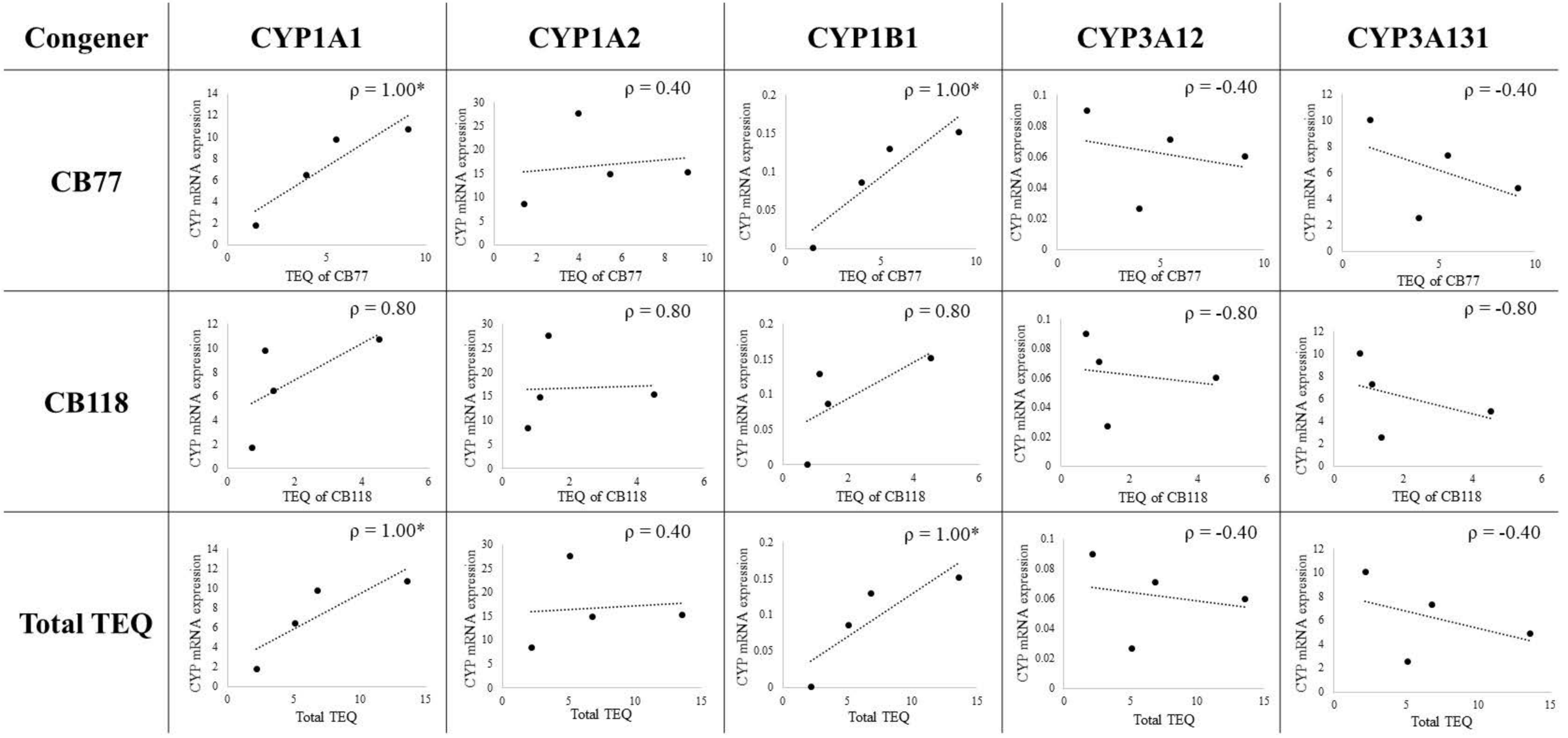
**Fig. 3.**


Congener
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CB77
CB118
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ρ = 1.00*
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CYP mRNA expression
TEQ of CB77
TEQ of CB118
Total TEQ

**Fig. 4.**

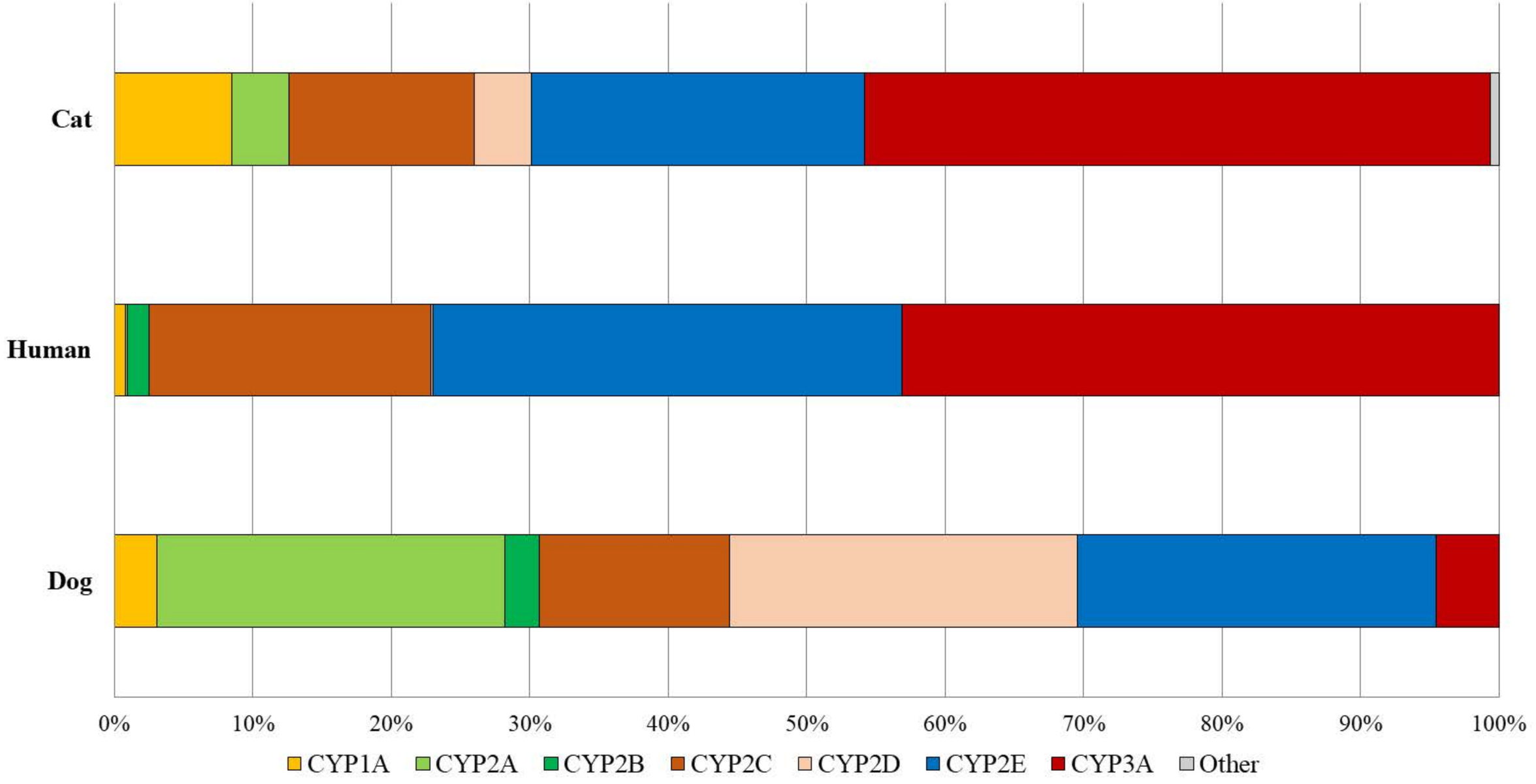
Cat
Human
Dog
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
CYP1A
CYP2A
CYP2B
CYP2C
CYP2D
CYP2E
CYP3A
Other

**Fig. 5.**