JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

In Vitro Metabolism of *trans*-Permethrin and Its Major Metabolites, PBalc and PBacid, in Humans

Tomoyuki Takaku,* Kazuki Mikata, Masayoshi Matsui, Kazuhiko Nishioka, Naohiko Isobe, and Hideo Kaneko

Environmental Health Science Laboratory, Sumitomo Chemical, Co., Ltd. 1-98, 3-Chome, Kasugade-naka Konohana-ku, Osaka, 554-8558 Japan

ABSTRACT: To estimate the metabolic profile of *trans*-permethrin in humans, a comparison of the *in vitro* metabolism of *trans*-permethrin in humans and rats was conducted using hepatic microsomes, and cytochrome P450 and UDP-glucuronyltransferase isoforms, which catalyze the metabolism of 3-phenoxybenzyl alcohol (PBalc) and 3-phenoxybenzoic acid (PBacid), respectively. In humans and rats, the major metabolic reaction of *trans*-permethrin in microsomal incubations was the cleavage of ester linkage to give PBalc, followed by oxidation to 4′-OH-PBalc, 4′-OH-PBacid, and PBacid. As to 4′-hydroxylation of PBalc, several CYPs were able to catalyze the reaction, and CYP2E1 was identified as a predominant isoform. PBacid and its conjugates (glucuronide and glycine) are major urinary metabolites of *trans*-permethrin in mammals. PBacid is also a metabolite of several pyrethroids, and has been used as a biomarker of human exposure to pyrethroids. Our study indicated that there was no difference in glucuronyltransferase activity of PBacid between humans and rats, and that only UGT1A9 can catalyze the glucuronidation of PBacid among human UGTs. Some UGT1A9 variants are known to have poor glucuronidation activity. From these results, it was assumed that deficiency or polymorphism of UGT1A9 might affect the profile of PBacid and its conjugates in urine collected from persons exposed to *trans*-permethrin or other pyrethroids. These results are helpful for understanding the metabolism of *trans*-permethrin in humans and determining methods for quantification of target analytes for assessment of human exposure to *trans*-permethrin and other pyrethroids that give PBacid and its conjugates as urinary metabolites.

KEYWORDS: *in vitro* metabolism, pyrethroid, P450, UGT

INTRODUCTION

Permethrin [3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate] (Figure 1) is a pyrethroid pesticide widely used throughout the world to control various insects for crop protection and prevention of disease spread.[1] The toxicity and metabolism of pyrethroids in animals have been well investigated and reported.[2−4]

In recent years, biological monitoring of pyrethroids has been conducted in terms of risk assessment of human exposure to pyrethroids. In these studies, the concentrations of some metabolites of pyrethroids in urine were monitored as a marker of human exposure to pyrethroids, because parent pyrethroids are not excreted into urine due to their high lipophilicity and rapid metabolism. For permethrin, PBacid has been used as a marker of human exposure.[5,6] PBacid is a common metabolite resulting from the oxidation of the hydrolytic product of many pyrethroids, and is excreted in urine in its conjugated and free forms. Therefore, it is significant to understand the metabolic behavior of PBacid in humans when using the compound as a marker for assessment of exposure to permethrin.

In *in vitro* human metabolism studies, permethrin was hydrolyzed by hCE-1 (human carboxylesterase 1) and hCE-2 to PBalc,[7,8] and subsequently PBalc was oxidized by alcohol dehydrogenase and aldehyde dehydrogenase to PBacid.[9] PBacid was rapidly excreted in urine as a free form, glucuronide, and a glycine conjugate in animals.[2] PBalc is also hydroxylated to 4′-OH-PBalc. In addition to the characterization of enzymes concerning the hydrolysis of *trans*-permethrin and the oxidation from PBalc to PBacid as described above, cytochrome P450 isoforms that catalyze the oxidation of *trans*-permethrin in humans (CYP1A1, CYP1A2, CYP2C8, CYP2C9, and CYP2C19) have been studied;[10] however, the enzymes responsible for the 4′-hydroxylation of PBalc and glucuronidation of PBacid in humans and the comparative metabolic profiles of *trans*-permethrin between humans and rats have not been reported.

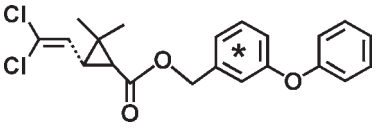

Figure 1. Chemical structure of *trans*-[phenoxyphenyl-^{14}C]permethrin.

In the present study, to compare metabolic profiles (route and rate) between rats and humans, the *in vitro* metabolism of *trans*-[phenoxyphenyl-^{14}C]permethrin by hepatic microsomes from rats and humans was investigated. In addition, the P450 isoforms responsible for the 4′-hydroxylation of PBalc were identified. Furthermore, glucuronidation of PBacid in humans was examined and the UGT isoforms responsible for the glucuronidation of PBacid were determined. These results

Received: January 5, 2011
Accepted: April 1, 2011
Revised: March 28, 2011
Published: April 01, 2011

Table 1. Gradient Program Used in HPLC Analyses

time [min]	0	5	5.1	20	35	40
acetonitrile [%]	5	5	35	35	100	100

provide insight into the understanding of the metabolism of *trans*-permethrin in humans and also provide information that will be useful when assessing the exposure to permethrin and other pyrethroids by measuring the PBacid metabolite and its conjugates as a marker of exposure.

MATERIALS AND METHODS

Chemicals. *trans*-[Phenoxyphenyl-^{14}C]permethrin and [phenoxyphenyl-^{14}C]PBalc were chemically synthesized at Amersham Biosciences UK Limited (Cardiff, South Wales) with a specific activity of 4.44 GBq/mmol. *trans*-[Phenoxyphenyl-^{14}C]permethrin was purified by preparative thin-layer chromatography (TLC) developed with hexane:diethyl ether (5:1, v/v) and by high-performance liquid chromatography (HPLC) with acetonitrile:0.1% (v/v) acetic acid in water (20:80, v/v) as mobile phase prior to use. [Phenoxyphenyl-^{14}C]PBalc was purified by preparative TLC developed with hexane:diethyl ether (5:1, v/v) prior to use. The radiochemical purities of the labeled compounds were 100.0%. Four authentic metabolite standards, unlabeled PBalc, PBacid, 4′-OH-PBalc, and 4′-OH-PBacid, were synthesized in our laboratory and used for the identification of metabolites. Other chemicals were reagent grade unless otherwise noted in the text.

Enzyme Preparations. The livers were collected from both male and female Crj:CD(SD) rats (Charles River Japan Inc., Kanagawa, Japan). Each liver was weighed, and homogenized in a Potter-Elvehjem homogenizer with a 3-fold volume of 0.05 M Tris-HCl (pH 7.4) buffer. Each liver homogenate was adjusted to 50 mL with Tris-HCl buffer and centrifuged at 10000*g* for 20 min and 105000*g* for 45 min. The precipitate was collected as the microsomal fraction. Pooled human liver microsomes were purchased from KAC Co., Ltd. Recombinant human and rat cytochrome P450s were purchased from BD Biosciences.

***In Vitro* Metabolism of *trans*-Permethrin and PBalc with Human, Male Rat, and Female Rat Liver Microsomes.** *trans*-[Phenoxyphenyl-^{14}C]permethrin (2.7−98 μM) or [phenoxyphenyl-^{14}C]PBalc (2.7−98 μM) was incubated with or without 3 mM NADPH in the presence of human, male rat, and female rat liver microsomes (1 mg/mL) for 60 min at 37 °C. All incubations were carried out in 100 mM phosphate buffer (pH 7.4). Control experiments were performed without liver microsomes. After incubation, the reaction was terminated by addition of acetonitrile and the mixtures were stored on ice for 10 min and centrifuged at 10000*g* for 5 min. The supernatant was analyzed by HPLC.

***In Vitro* Metabolism of PBalc with Baculovirus-Insect Cell-Expressed Human P450s.** [phenoxyphenyl-^{14}C]PBalc (9.0 μM) was incubated with 3 mM NADPH in the presence of baculovirus-insect cell-expressed human CYPs (1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1, and 3A4) for 60 min at 37 °C. All incubations were carried out in 100 mM phosphate buffer (pH 7.4). Control experiments were performed without CYPs. After incubation, the reaction was terminated by addition of acetonitrile and the mixtures were stored on ice for 10 min and centrifuged at 10000*g* for 5 min. The supernatant was analyzed by HPLC.

HPLC Analysis. HPLC was carried out on a system consisting of an L-6200 HPLC intelligent pump (Hitachi, Ltd., Tokyo, Japan), an L-4000 UV detector (Hitachi, Ltd., Tokyo, Japan), and a Radiomatic 610TR RI detector (PerkinElmer, Inc., MA, USA) fitted with an Atlantis dC18 column (4.6 mm i.d. × 150 mm, Waters, MA, USA). The mobile phase consisted of acetonitrile and 0.1% (v/v) acetic acid in water. A flow rate of 1 mL/min was used. For the separation, the gradient program shown in Table 1 was used. Retention times of *trans*-permethrin and authentic standards are shown in Table 2.

Table 2. HPLC Retention Times of Permethrin and Authentic Standards

metabolite	retention time [min]
4′-OH-PBalc	14.9
4′-OH-PBacid	17.4
PBalc	26.9
PBacid	28.3
trans-permethrin	37.3

***In Vitro* Assay of Glucuronyltransferase Activity toward PBacid.** [Glucuronyl-^{14}C]UDP-glucuronic acid (3 μM) was incubated with or without 100 μM PBacid in the presence of human and female rat liver microsomes (1 mg/mL) for 30 min at 37 °C. All incubations were carried out in 100 mM Tris-HCl buffer (pH 7.4). After incubation, the reaction was terminated by addition of acetonitrile and the mixtures were stored on ice for 10 min and centrifuged at 10000*g* for 5 min. The supernatant was analyzed by TLC.

***In Vitro* Metabolism of PBacid with Baculovirus-Insect Cell-Expressed Human UGTs.** [Glucuronyl-^{14}C]UDP-glucuronic acid (1.5 μM) was incubated with 100 μM PBacid in the presence of baculovirus-insect cell-expressed human UGTs (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17) for 30 min at 37 °C. All incubations were carried out in 100 mM Tris-HCl buffer (pH 7.4). After incubation, the reaction was terminated by addition of acetonitrile and the mixtures were stored on ice for 10 min and centrifuged at 10000*g* for 5 min. The supernatant was analyzed by TLC.

TLC Analysis. Precoated silica gel 60 F 254 TLC plates (20 × 20 cm, 0.25 thickness, Merck, Germany) were employed, with ethyl acetate:acetone:acetic acid:water (4:1:1:1, v/v/v/v) as solvent system. Radioactive compounds on TLC plates were detected by autoradiography using imaging plates (Fuji Photo Film, Tokyo, Japan). They were contacted with TLC plates at room temperature and then processed with a fluorescent image analyzer (FLA-5000, Fuji Photo Film).

RESULTS AND DISCUSSION

Comparative *in Vitro* Metabolism of *trans*-[Phenoxyphenyl-^{14}C] permethrin. *trans*-[Phenoxyphenyl-^{14}C]permethrin was incubated with human, male rat, and female rat liver microsomes each at concentrations of 2.7, 4.5, 17, 46, and 98 μM. In each incubation solution, PBalc, PBacid, and 4′-OH-PBalc were detected. PBalc (resulting from the hydrolysis of *trans*-permethrin) was the major metabolite in both humans and rats (Figure 2). The metabolites produced by human microsomes were nearly identical to those produced by rat liver microsomes, although 4′-OH-PBacid was not detected in a human liver microsomal incubation. The rates of metabolic formation were almost similar between humans and rats (Figure 2). In this study, human and rat liver microsomes metabolized *trans*-permethrin mainly through hydrolysis, not oxidation, suggesting that the rapid hydrolysis is the major metabolic route of *trans*-permethrin in both humans and rats.

Cytochrome P450 Isoforms Catalyzing the Oxidation of [Phenoxyphenyl-^{14}C]PBalc in Humans. According to *in vitro* metabolism of *trans*-[phenoxyphenyl-^{14}C]permethrin in human, male rat, and female rat liver microsomal incubations, PBalc was detected as the major metabolite, which appeared to be further metabolized to PBacid, 4′-OH-PBalc, and 4′-OH-PBacid in rats *in vivo*.[2] This would indicate that 4′-OH-PBalc is mainly

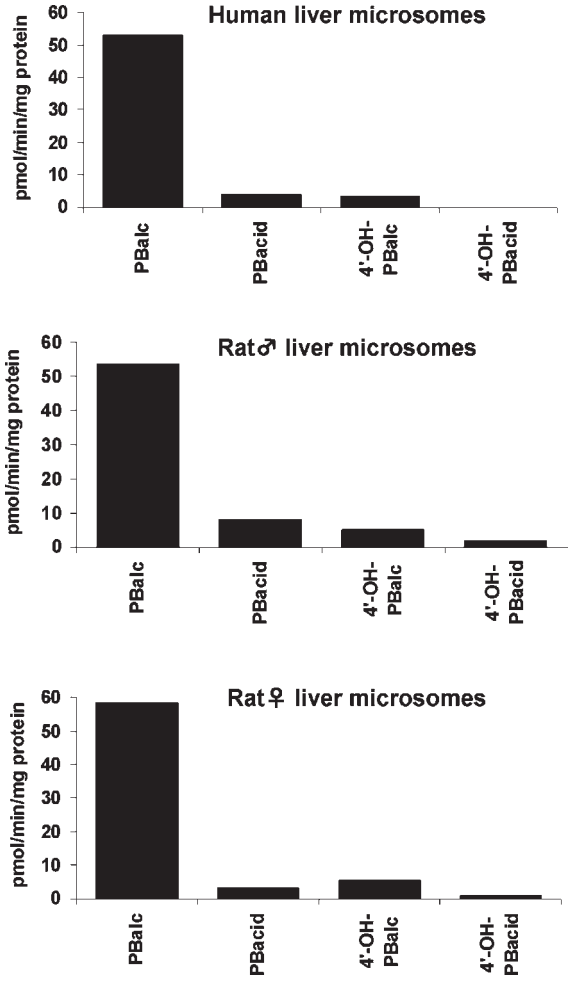

Figure 2. Rates of metabolite formation of *trans*-permethrin with human, male rat, and female rat microsomes. The PBalc, PBacid, 4′-OH-PBalc, and 4′-OH-PBacid formed were determined using HPLC.

produced from PBalc, not monohydroxylated *trans*-permethrin *in vivo*. Therefore, it is significant to determine the cytochrome P450 isoforms responsible for the 4′-hydroxylation of PBalc for the understanding of the fate of *trans*-permethrin in humans, although it is reported that the metabolism of PBalc to PBacid is catalyzed by alcohol dehydrogenases and aldehyde dehydrogenase.[9]

[Phenoxyphenyl-^{14}C]PBalc was incubated with baculovirus-insect cell-expressed human cytochrome P450s. CYP2E1 catalyzed mainly the oxidation of PBalc to 4′-OH-PBalc. CYP2C19 and CYP2D6 also catalyzed this reaction (Figure 3). Interestingly, the cytochrome P450s catalyzing the 4′-hydroxylation of PBalc (CYP2E1, CYP2C19, and CYP2D6) were slightly different from those metabolizing *trans*-permethrin (CYP1A1, CYP1A2, CYP2C8, CYP2C9, and CYP2C19).[10]

Kinetic analyses of [phenoxyphenyl-^{14}C]PBalc by baculovirus-insect cell-expressed human cytochrome P450s were conducted. The kinetic parameters of [^{14}C]PBalc with human CYP2C19, CYP2D6, and CYP2E1 were analyzed. Table 3 shows the kinetic parameters for 4′-OH-PBalc formation by recombinant CYP enzymes. The K_m values were ranked as CYP2C19 > CYP2D6 > CYP2E1, and the V_{max} values were ranked as CYP2C19 > CYP2E1 > CYP2D6. CYP2E1 showed the highest intrinsic clearance (V_{max}/K_m value). Based on the present results

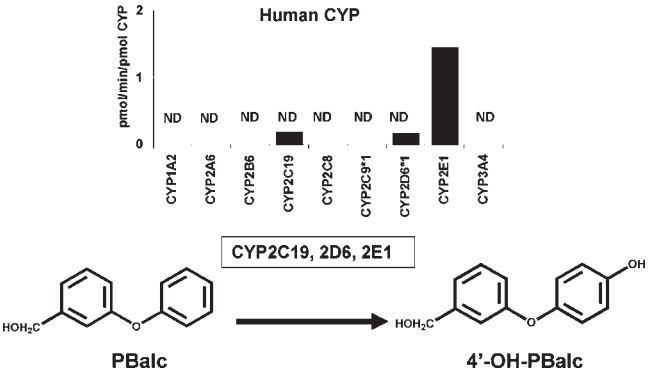

Figure 3. Rates of [phenoxyphenyl-^{14}C]4′-OH-PBalc formation with baculovirus-insect cell-expressed human P450s from [phenoxyphenyl-^{14}C]PBalc. The formed 4′-OH-PBalc was determined using HPLC.

Table 3. Kinetic Parameters for 4′-OH-PBalc Formation by Recombinant CYP Enzymes

	CYP2E1	CYP2D6	CYP2C19
K_m (μM)	24	29	272
V_{max} (pmol/min/pmol of CYP)	3.6	1.3	11.2
V_{max}/K_m (μL/min/pmol of CYP)	0.150	0.046	0.041

together with the liver expression level of CYP2E1 in humans,[11] it was concluded that CYP2E1 is the CYP most responsible for the monohydroxylation of PBalc to 4′-OH-PBalc.

Glucuronyltransferase activity toward PBacid. One of the major *in vivo* urinary metabolites in rats was the glucuronide of PBacid.[2] In addition, in regard to exposure to pyrethroid pesticides, the urine concentration of PBacid and PBacid glucuronide has been examined in humans.[5,6] However, there have been no reports about species differences in glucuronide of PBacid in humans and rats. Thus, in the present study the *in vitro* glucuronyltransferase activity toward PBacid was examined in humans and rats and the UGT isoforms that catalyzed glucuronidation were identified. Figure 4 shows the glucuronyltransferase activity of PBacid by human and rat liver microsomes, suggesting that a difference in qualitative glucuronyltransferase activity between humans and rats was not detected. To determine the UGT isoforms catalyzing the glucuronidation of PBacid, [glucuronyl-^{14}C]UDP-glucuronic acid was incubated with baculovirus-insect cell-expressed human UGTs (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17). It was demonstrated that only UGT1A9 was able to catalyze the glucuronidation of PBacid (Figure 5). Olson et al. reported that there are three UGT1A9 variants (UGT1A9^{33Thr}, UGT1A9^{167Ala}, and UGT1A9^{183Gly}) and the prevalence of UGT1A9 variants is very low in Caucasians, African-Americans, and Asians.[12] On the other hand, the effects of UGT1A9^{256Asn} and UGT1A9^{483Asp} were examined for propofol metabolism.[13] Propofol is used as an anesthetic agent, and it is excreted after glucuronidation by UGT1A9. As the activity of UGT1A9^{256Asn} for propofol glucuronidation was relatively low compared to UGT1A9, Takahasi et al. proposed that care should be taken with patients with UGT1A9^{256Asn} when treated with propofol.[13] These studies indicated that there are some poor metabolizers due to polymorphism in UGT1A9. If PBacid is glucuronidated slowly, PBacid might be excreted more in the free form or

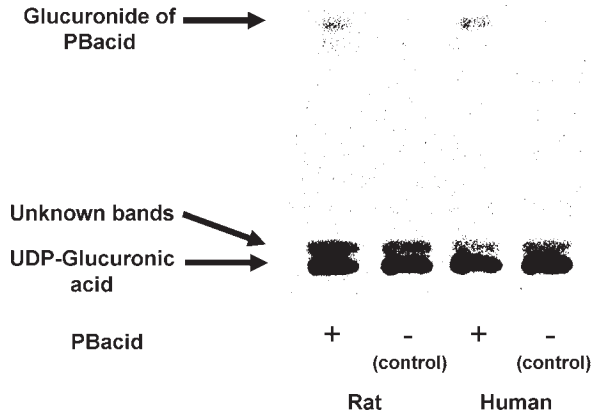

Figure 4. TLC autoradiogram of *in vitro* glucuronidation of PBacid in human, male rat, and female rat liver microsomal incubations.

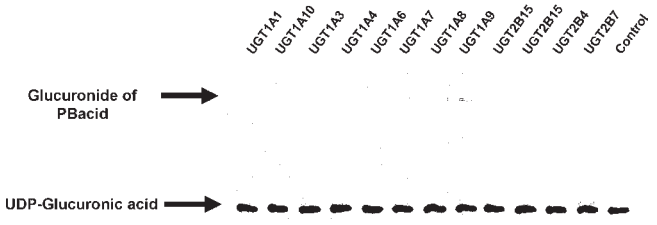

Figure 5. TLC autoradiogram of *in vitro* glucuronidation of PBacid in human UGT incubations.

metabolized via another route, such as PBacid to 4′-OH-PBacid, or PBacid to PBacid−glycine.

Biological Monitoring of Metabolites in Urine. Because PBacid has been used as a marker for pyrethroids, many detection methods have been developed for PBacid in human urine.[5,14] In these methods, PBacid (and the glucuronide of PBacid) in urine was analyzed by GC/MS,[5] or PBacid−glycine was selected as a target analyte for immunoassay.[14] In rat metabolism study, PBacid-free (10%), glucuronide of PBacid (14.9%), PBacid−glycine (4.4%), and sulfate of 4′-OH-PBacid (42.8%) are detected in urine.[2] The present study demonstrated that UGT1A9 catalyzed the glucuronidation of PBacid (Figure 5), and it is known that there are polymorphisms in UGT1A9.[12,13] When PBacid is not glucuronidated effectively due to polymorphisms, more PBacid−glycine might be excreted than usually. In that case, the total amount of free form of PBacid and its conjugates, including PBacid−glycine in urine, would be required for evaluating human pyrethroid exposure.

In addition, as for more accurate permethrin exposure monitoring, it was demonstrated that measurement of the sulfate of 4′-OH-PBacid in urine would be required. The present study showed that PBalc was generated equally in both human and rat microsomes (Figure 2) and PBalc was hydroxylated to 4′-OH-PBalc by many CYPs (CYP2E1, CYP2D6, and CYP2C19). Using both PBacid and 4′-OH-PBacid as target analytes for monitoring, exposure of permethrin in humans could be evaluated more accurately.

CONCLUSION

The *in vitro* metabolism of *trans*-permethrin with human, male rat, and female rat liver microsomes was investigated. The study showed that the main metabolic pathway of *trans*-permethrin was the hydrolysis of the ester linkage to give PBalc in humans and rats, and the metabolites produced by both rat and human microsomes were nearly identical except for 4′-OH-PBacid, which was not detected in a human liver microsomal incubation. In addition, the human cytochrome P450 isoforms responsible for 4′-hydroxylation of PBalc were identified, and it was indicated that 4′-OH-PBalc would be mainly produced from PBalc, not *via* monohydroxylated *trans*-permethrin *in vivo*. It was also shown that several types of P450 isoforms catalyzed the 4′-hydroxylation of PBalc in humans. This suggests that the metabolic behavior of PBalc is not affected by genetic polymorphisms of P450 isoforms in humans, although it is well-known that genetic polymorphisms exist in particular P450 isoforms in humans,[15] and the lack of a certain enzyme involved in the metabolism of a chemical occasionally causes adverse effects.[16]

In the present study, the *in vitro* glucuronyltransferase activity toward PBacid in humans and rats was examined and the human UGT isoforms that catalyzed glucuronidation were identified. No difference of glucuronyltransferase activity between humans and rats was detected *in vitro*, and human UGT1A9 catalyzed the glucuronidation of PBacid. It is known that UGT1A9 variants (UGT1A9^{33Thr}, UGT1A9^{167Ala}, UGT1A9^{183Gly}, UGT1A9^{256Asn} and UGT1A9^{483Asp}) exist,[12,13] and the activity of some UGT1A9 variants is relatively low.[13] This suggests that the change of metabolic profile of PBacid in a poor metabolizer due to polymorphisms in UGT1A9 should be taken into account for more accurate estimation of exposure to permethrin. Given that sulfate of 4′-OH-PBacid is excreted mainly in rat urine, and that the metabolic pathways of trans-*permethrin* were similar in humans and rats in this study, it might be appropriate to use PBacid and 4′-OH-PBacid as biomarkers of permethrin exposure in humans for more accurate evaluations.

AUTHOR INFORMATION

Corresponding Author

*E-mail: takakut@sc.sumitomo-chem.co.jp. Tel: +81-6-6466-5321. Fax: +81-6-6466-5442.

REFERENCES

(1) Casida, J. E.; Quistad, G. B. Golden age of insecticide research: past, present, or future? *Annu. Rev. Entomol.* **1999**, *43*, 1–16.

(2) Gaughan, L. C.; Unai, T.; Casida, J. E. Permethrin Metabolism in Rats. *J. Agric. Food Chem.* **1977**, *25*, 9–17.

(3) Verschoyle, R. D.; Barnes, J. M. Toxicity of natural and synthetic pyrethrins to rats. *Pestic. Biochem. Physiol.* **1972**, *2*, 308–311.

(4) Tomigahara, Y.; Mori, M.; Shiba, K.; Isobe, N.; Kaneko, H.; Nakatsuka, I.; Yamada, H. Metabolism of tetramethrin isomers in rat. I. Identification of a sulphonic acid type of conjugate and reduced metabolites. *Xenobiotica* **1994**, *24*, 473–484.

(5) Wang, D.; Kamijima, M.; Imai, R.; Suzuki, T.; Kameda, Y.; Asai, K.; Okamura, A.; Naito, H.; Ueyama, J.; Saito, I.; Nakajima, T.; Goto, M.; Shibata, E.; Kondo, T.; Takagi, K.; Takagi, K.; Wakusawa., S. Biological monitoring of pyrethroid exposure of pest control workers in Japan. *J. Occup. Health* **2007**, *49*, 509–514.

(6) Barr, D. B.; Olsson, A. O.; Wong, L. Y.; Udunka, S.; Baker, S. E.; Whitehead, R. D.; Magsumbol, M. S.; Williams, B. L.; Needham, L. L. Urinary concentration of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999−2002. *Environ. Health Perspect.* **2010**, *118*, 742–748.

(7) Ross, M. K.; Borazjani, A.; Edwards, C. C.; Potter, P. M. Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. *Biochem. Pharmacol.* **2006**, *71*, 657–669.

(8) Yang, D.; Pearce, R. E.; Wang, X.; Gaedigk, R.; Wan, Y. J.; Yan, B. Human carboxylesterases HCE1 and HCE2: Ontogenic expression, inter-individual variability and differential hydrolysis of oseltamivir, aspirin, deltamethrin and permethrin. *Biochem. Pharmacol.* **2009**, *77*, 238–247.

(9) Jonghoon, C.; Randy, L. R.; Ernest, H. In vitro human metabolism of permethrin: the role of human alcohol and aldehyde dehydrogenases. *Pestic. Biochem. Phys.* **2002**, *73*, 117–128.

(10) Scollon, E. J.; Starr, J. M.; Godin, S. J.; DeVito, M. J.; Hughes, M. F. In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome p450 isoforms. *Drug Metab. Dispos.* **2009**, *37*, 221–228.

(11) Rodrigues, A. D. Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. *Biochem. Pharmacol.* **1999**, *57*, 465–480.

(12) Olson, K. C.; Dellinger, R. W.; Zhong, Q.; Sun, D.; Amin, S.; Spratt, T. E.; Lazarus, P. Functional characterization of low-prevalence missense polymorphisms in the UDP-glucuronosyltransferase 1A9 gene. *Drug Metab. Dispos.* **2009**, *37*, 1999–2007.

(13) Takahashi, H.; Maruo, Y.; Mori, A.; Iwai, M.; Sato, H.; Takeuchi, Y. Effect of D256N and Y483D on propofol glucuronidation by human uridine 5′-diphosphate glucuronosyltransferase (UGT1A9). *Basic Clin. Pharmacol. Toxicol.* **2008**, *103*, 131–6.

(14) Ahn, K. C.; Kim, H. J.; McCoy, M. R.; Gee, S. J.; Hammock, B. D. Immunoassays and Biosensors for Monitoring Environmental and Human Exposure to Pyrethroid Insecticides. *J. Agric. Food Chem.* **2010**in press.

(15) Bozina, N.; Bradamante, V.; Lovrić, M. Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer risk. *Arh. Hig. Rada Toksikol.* **2009**, *60*, 217–242.

(16) Rogers, J. F.; Nafziger, A. N.; Bertino, J. S., Jr. Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. *Am. J. Med.* **2002**, *113*, 746–50.