J. Pestic. Sci. **46**(3), 290–296 (2021)
DOI: 10.1584/jpestics.J21-02

JOURNAL OF PESTICIDE SCIENCE

Society Awards 2021

(for high prospectiveness/prominent achievement)

Researches on the evaluation of pesticide safety in humans using a pharmacokinetic approach

Jun Abe

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

(Accepted May 24, 2021)

Similar to the pharmaceutical compounds, pesticides require human safety assessment for their registration and distribution; however, it is absolutely impossible to assess human safety by dosing humans with pesticides. Thus, how to appropriately evaluate the safety of pesticides in humans remains a great subject of debate. In this article, we present some examples of pesticide toxicity studies that identify species differences in toxicity and evaluate human safety by applying combinations of novel *in vivo*, *in vitro*, and *in silico* techniques to separately assess the key toxicodynamic (*i.e.*, sensitivity) and/or toxicokinetic (*i.e.*, exposure) factors. Because it is scientifically sound, the safety assessment strategy illustrated for three compounds in this article is expected to play an important role in the human safety assessment of agricultural compounds.

Keywords: Flumioxazin, Procymidone, Epyrifenacil, Human safety, Pharmacokinetics.

Introduction

It is generally agreed that pesticides can play a significant role in stabilizing food production, and thus they are regarded as efficient and indispensable buffers against the consequences of forthcoming population increases and resulting food shortages. Similar to pharmaceutical compounds, pesticides require human safety assessment for their registration and distribution; however, clinical trials of pesticides in humans are obviously not allowed, thus the safety of pesticides in humans has been evaluated by dividing the safe dose levels obtained in laboratory animals by a safety factor. Recently, many *in vitro* and *in silico* techniques for human safety evaluation have been developed, with great improvement in assay variability and accuracy. With the development of the techniques, the importance and necessity of using them for the indirect human safety assessment of non-pharmaceutical chemicals including pesticides has gained greater recognition. In this article, we present some researches carried out to identify the species differences in pesticide toxicity and, by applying a combination of these techniques, evaluate the human safety of some pesticides with known toxicity profiles that differ between species. More specifically, scientifically-sound human safety assessment could be achieved by combining several *in vitro* (*e.g.*, enzymes or cells), *in vivo* (*e.g.*, chimeric mice with humanized liver), and *in silico* (*e.g.*, PBPK modeling) techniques, which are described in this review.

1. Human safety assessment of the fungicide, procymidone

Procymidone (Sumilex, its structure presented in Fig. 1) is a commercially available fungicide with both protective and curative properties. It is used to control plant diseases such as fruit rot (*i.e.*, gray mold on fruits, vines, and vegetables) and Sclerotinia rot of kidney beans and vegetable crops.[1)] The metabolic pathways of procymidone in mammals are known.[2)] Procymidone is first hydroxylated at the methyl group of the imide ring. This hydroxylated metabolite is metabolized further in one of two ways: the hydroxymethyl group is conjugated to form a glucuronide or oxidized to form the carboxylic acid metabolite. Both the glucuronide of hydroxylated procymidone and

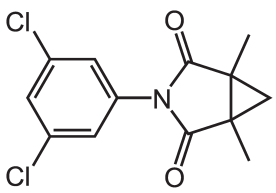

Fig. 1. Chemical structure of procymidone.

To whom correspondence should be addressed.
E-mail: abej@sc.sumitomo-chem.co.jp
Published online August 31, 2021

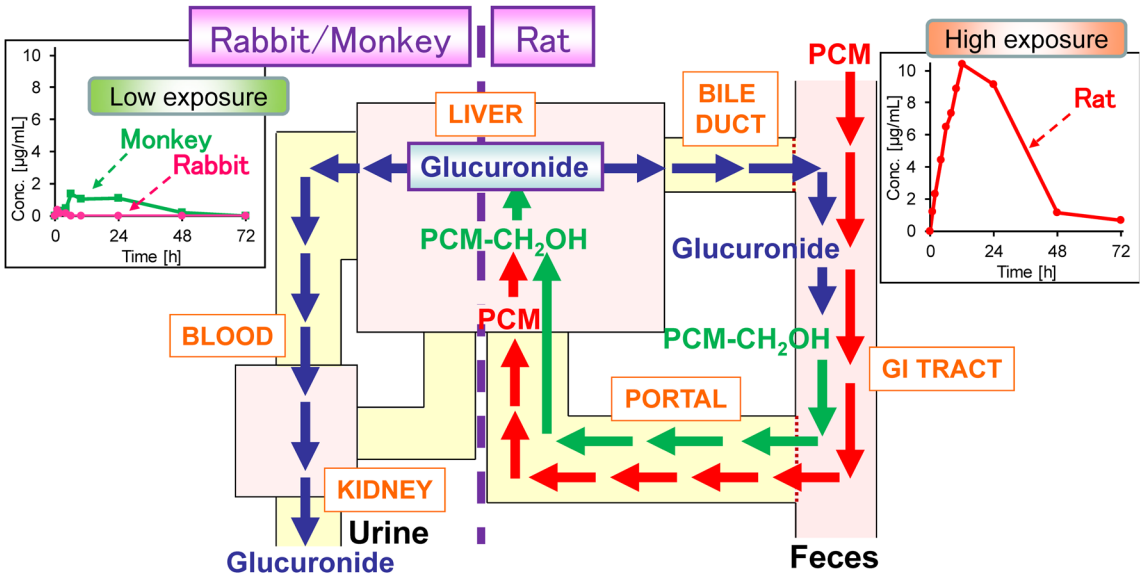

Fig. 2. Summary of the metabolic fate of procymidone in rats, rabbits, and monkeys, focusing on the species differences of the exposure.

the carboxylic acid metabolite are more hydrophilic and thus more readily excreted than the hydroxylated procymidone. As a major toxicity of procymidone, external genital abnormality (a reproductive toxicity) has been observed in male rats, but not in rabbits and monkeys.[3] The key mechanism of the species differences in the toxicity has already been identified. *In vivo* experiments in rabbits and monkeys showed that the hydroxylated metabolite, which has antiandrogenic activity, is conjugated in liver, transferred to blood, and then rapidly excreted in urine (Fig. 2). On the other hand, the kinetics of the hydroxylated metabolite was found to be different in rats, where the glucuronide was shown to be excreted in bile and deconjugated in the gastrointestinal tract and reabsorbed, resulting in recycling through the enterohepatic circulation and increased exposure in rats (Fig. 2).[3] The species difference in the maternal exposure to the hydroxylated metabolite results in a significant difference in fetal exposure.[4] From these results, it was suggested that the species difference in the developmental toxicity of procymidone is mainly due to variation in the level of exposure to the causal substance, hydroxylated metabolite, and this variation stems from interspecies differences in the biliary excretion route of the glucuronide. To assess the teratogenic risk associated with procymidone in humans, the excretion route and rate of formation of glucuronides, which are key factors involved in the mechanism of the toxicity must be clarified. To compare the metabolic profiles of procymidone in humans and rats, we planned to conduct *in vivo* experiments using chimeric mice.

Chimeric mice with human or mouse hepatocytes were provided by PhoenixBio Co., Ltd. (Hiroshima, Japan). ^{14}C-Labeled procymidone was orally administered to the chimeric mice and other laboratory animals, and the plasma concentrations of procymidone and its metabolites were measured.[5,6] The pharmacokinetic parameters of chimeric mice and other laboratory animals are summarized in Table 1. When the parameters are com-

Table 1. Pharmacokinetic parameters of procymidone and its hydroxylated metabolites after single oral administration of 14C-procymidone at 62.5 mg/kg. Values are of the mean plasma concentrations of 3 animals.

(A) Procymidone

Parameter	Rat[5]	Chimeric mice with rat hepatocytes[6]	Rabbit[5]	Monkey[5]	Chimeric mice with human hepatocytes[6]
C_{max} [μg equiv./mL]	6.80	4.80	N.D.	2.90	3.01
T_{max} [hr]	4	1	N.D.	10	2
AUC_{0-72hr} [μg equiv.hr/mL]	89.87	48.3	0	73.59	44.3

(B) Hydroxylated procymidone

Parameter	Rat[5]	Chimeric mice with rat hepatocytes[6]	Rabbit[5]	Monkey[5]	Chimeric mice with human hepatocytes[6]
C_{max} [μg equiv./mL]	10.42	9.57	0.33	1.35	1.77
T_{max} [hr]	12	12	1	6	1
AUC_{0-72hr} [μg equiv.hr/mL]	332.06	120.8	0.875	40.2	36.1

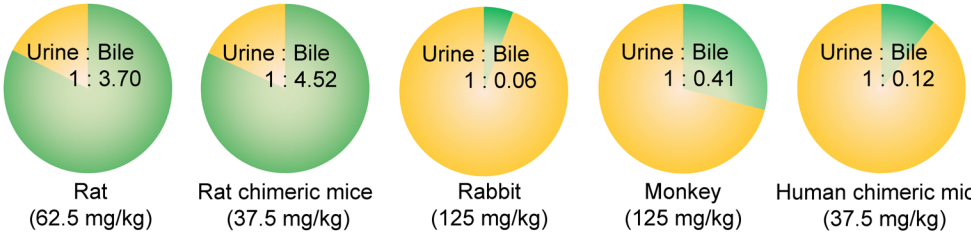

Fig. 3. The urinary and biliary excretion ratios of the glucuronide metabolite.

pared among species, higher exposure (*i.e.*, *AUC* and C_{max}) was observed in rats and rat chimeric mice whereas the exposure was rather low in rabbits, monkeys, and human chimeric mice for both procymidone and its hydroxylated metabolite. In the previous articles on procymidone metabolism in intact rats,[5] delayed T_{max} and higher exposure to the hydroxylated metabolite were caused by the entero-hepatic circulation of its glucuronide. In the present research, the delayed T_{max} for the hydroxylated metabolite was well reproduced in rat chimeric mice and reported to result in higher exposure to this metabolite, suggesting this animal model with rat or human hepatocytes is a useful model to investigate species differences in the pharmacokinetic, metabolic, and excretion profiles. On the other hand, the plasma concentration of the hydroxylated metabolite in human chimeric mice shifted synchronously to that of procymidone. As a result of species differences in the transition from the plasma concentration of the hydroxylated metabolite to that of procymidone, the *AUC* of the hydroxylated metabolite was two to five times larger in rat chimeric mice than in human chimeric mice.

To further elucidate the excretion route of the glucuronide, bile duct-cannulation studies were performed with chimeric mice and other laboratory animals.[6] Metabolite profiles were analyzed in each species, and the excretion of the glucuronide was evaluated in urine and bile. The excretion ratios of the glucuronide are summarized in Fig. 3. The biliary excretion ratio of glucuronide was high in rat chimeric mice (4.52), like rats (3.70), but low in human chimeric mice (0.12).

In conclusion, the pharmacokinetics of the hydroxylated metabolite in urine and bile clearly show that the main excretion route of the glucuronide differs between rat chimeric mice and human chimeric mice, which results in lower exposure to the hydroxylated metabolite in humans (and as previously observed in rabbits and monkeys) than in rats. Because of the lower exposure to the hydroxylated metabolite in humans, which is a key teratogenicity trigger in rats, it is concluded that the teratogenicity of procymidone may be much lower in humans than in rats.

2. Human safety assessment of the herbicide, flumioxazin

Flumioxazin (Sumisoya, its structure presented in Fig. 4) is an *N*-phenylimide herbicide that is widely used for controlling annual broadleaf weeds in soybean crops.[7] The herbicidal activity of flumioxazin was shown to be due to its inhibition of protoporphyrinogen oxidase (PPO), one of the key enzymes in porphyrin biosynthesis. PPO is an essential enzyme involved in the synthesis of chlorophyll in plants. Inhibition of the enzyme in plant cells causes accumulation of intermediate tetrapyrroles, including protoporphyrin IX (PPIX), and then photoreactions that produce oxygen free radicals to destroy the cell membranes.[8,9]

It is well known that PPO exists also in mammals, and works as a key enzyme in heme biosynthesis.[10] Regarding the toxicity of PPO inhibitors in mammals, similar key events of PPO inhibition and further accumulation of PPIX leading to cell damage may also occur. Thus, it is believed that PPO inhibitors can cause toxicity especially in PPO-generating organs such as the liver and can occasionally induce hepatocellular tumors as a consequence of hepatotoxicity.[11,12] Also, PPO catalyzes the oxidation of protoporphyrinogen IX to PPIX during heme biosynthesis in both plants and mammals. In mammals, the inhibition of PPO blocks the production of hemoglobin and erythrocytes, resulting in anemia.[13–15] Since the molecular mechanism of heme biosynthesis is common to all mammals, the possibility that PPO inhibitors are hepatotoxic and/or cause anemia in humans must be considered, especially when the toxicity of PPO inhibitors is observed in rodents and other mammals. Flumioxazin has developmental toxicity in rats, causing embryonic lethality, teratogenicity (mainly ventricular septal defects, VSD, and wavy ribs), and growth retardation.[16,17] The mechanism of this developmental toxicity in rats has already been shown to be embryonic anemia resulting from PPO inhibition and following the elimination of heme biosynthesis.[18] Even though all mammals have PPO for heme biosynthesis, which is essential for vital activity, a remarkable species difference in the toxicity caused by flumioxazin-induced PPO inhibition has been reported. Oral administration of flumioxazin at 3,000 mg/kg, which was two orders of magnitude larger than the teratogenic dose of 30 mg/kg in rats, was shown not to cause developmental toxicity in rabbits.[16] Thus, further mechanistic studies were warranted to assess the human safety of flumioxazin and evaluate the possibility

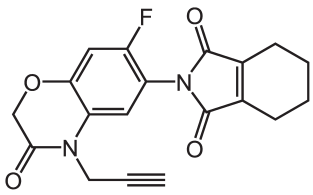

Fig. 4. Chemical structure of flumioxazin.

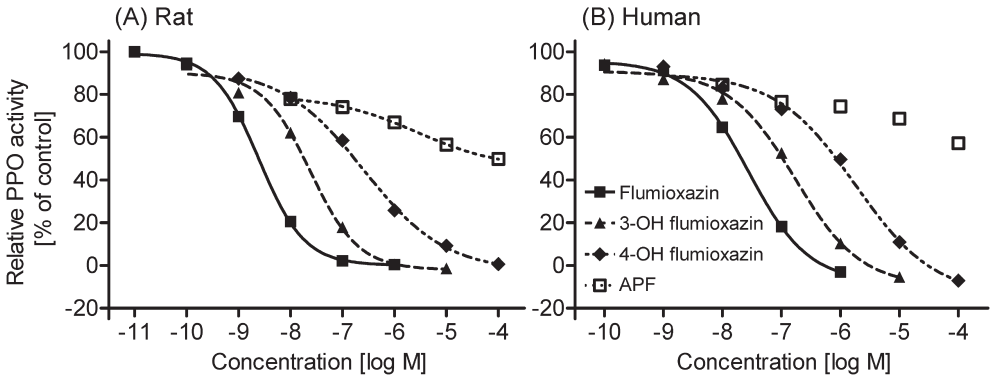

Fig. 5. Inhibitory curves of flumioxazin, 3-OH flumioxazin, 4-OH flumioxazin, and APF against protoporphyrinogen oxidase (PPO) in (A) rat and (B) human mitochondrial fractions.

of its developmental toxicity in humans.

First, we focused on identification of the toxic agent. Metabolism study with pregnant/non-pregnant rats and rabbits revealed that the major metabolites in the fetus are 3-OH flumioxazin, 4-OH flumioxazin, and 6-amino-7-fluoro-4-(2-propynyl)-2*H*-1,4-benzoxazin-3(4*H*)-one (APF).[19,20] The inhibitory activity of these three metabolites as well as the parent flumioxazin were tested in a PPO inhibition assay with mitochondrial fractions of rat liver, the results of which demonstrated the significant high inhibitory activity of flumioxazin followed by 4-OH flumioxazin (14.0-times weaker), 3-OH flumioxazin (150-times weaker), and non-active APF[20] as shown in Fig. 5A. From the quantitative evaluation in dynamics and kinetics of metabolites, we concluded that the agent causing embryonic anemia is the parent compound flumioxazin.

Next, we arranged to assess the human safety. The PPO inhibition assay was also conducted with liver mitochondrial fractions to compare the sensitivity between rats and humans. The results showed rats were more sensitive than humans, and the IC_{50} of flumioxazin was 4.56 times larger in humans than in rats, with lower inhibitory activity of the metabolites[20] as shown in Fig. 5B. The details underlying species differences have been revealed. A molecular dynamics study (*i.e.*, docking simulation study) of flumioxazin in several species clearly demonstrated that the affinity of substrate binding to PPO varied among species[21] and was inconsistent with the species differences in PPO inhibitory activity observed in the *in vitro* assay.

Since PPO exists in complex and highly organized structures in mitochondria and thus accessibility of flumioxazin to PPO in cells can be different from that in the *in vitro* mitochondrial extract model, further confirmation of the species difference in a cell-based test system was sought and obtained. Rat, rabbit, monkey, and human hepatocytes were exposed to flumioxazin, and accumulation of PPIX resulting from PPO inhibition was monitored. The results (Fig. 6) showed similar tendencies to those shown in the mitochondrial assays, that the PPO inhibitory activity of flumioxazin was stronger in rats than in humans. The results suggested that the higher-order structure of PPO in cells does not affect its inhibition, and both the cell-based hepatocyte assay and the isolated mitochondria assay can adequately evaluate the inhibitory activity of chemicals, thus indicating that the inhibition is three to five times more sensitive in rats than in humans. For further evaluation of the effect of species difference in dynamics on the downstream outcome of heme biosynthesis inhibition, we investigated the impact of flumioxazin-induced PPO inhibition on heme biosynthesis in human and rat erythroid cells.[22] Treatment with flumioxazin led to dose-dependent accumulation of PPIX in both human erythroleukemia (K562) cells and rat erythroleukemia (REL) cells; however, differences in heme biosynthesis were observed between species. As presented in Fig. 7, heme biosynthesis was not affected in human K562 cells by flumioxazin up to 5.0 μM, whereas a statistically-significant decrease in heme production was observed in rat REL cells. To complete the human risk assessment, a physiologically-based pharmacokinetic (PBPK) model was developed for pregnant women, and human fetal exposure to flumioxazin was estimated.[23] By the *in silico* technique, the maximum concentration of flumioxazin in human embryos was estimated to be 1.92 μM at an exposure level equivalent to a maternal dose of 1,000 mg/kg/day. This concentration is lower than the no-effect level of 5.0 μM for heme production in human erythroid cells. From all these results, we concluded that human

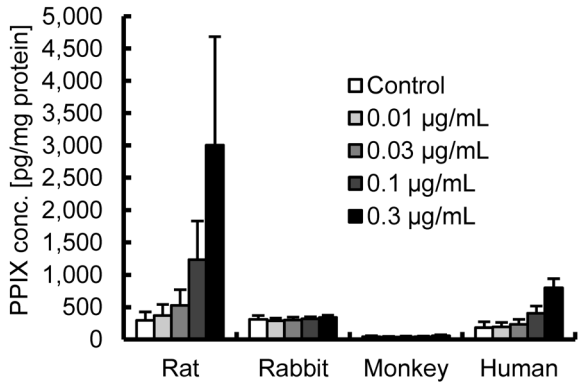

Fig. 6. Flumioxazin-induced accumulation of protoporphyrin IX (PPIX) in hepatocytes of rats, rabbits, monkeys, and humans.

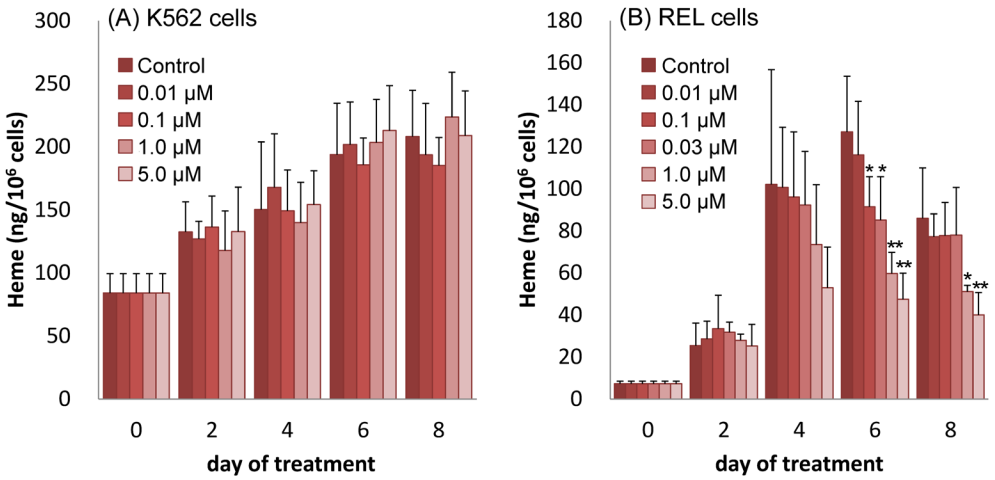

Fig. 7. Effect of flumioxazin on heme biosynthesis in (A) human erythroleukemia K562 cells and (B) rat erythroleukemia REL cells. *: $p<0.05$ and **: $p<0.01$ by Dunnett's test.

exposure could not result in levels that would produce toxicological effects and thus flumioxazin was not a developmental toxicity issue in humans.

3. Human safety assessment of the herbicide, epyrifenacil

Epyrifenacil (S-3100, with structure shown in Fig. 8) is a new herbicide under development. The herbicidal mechanism of epyrifenacil is similar to that of flumioxazin, which is the inhibition of PPO. As mentioned above, PPO inhibitors may cause accumulation of PPIX leading to cell damage in PPO-generating organs such as the liver[11,12] and/or cause anemia resulting from inhibition of heme biosynthesis.[13–15]

First, the results of a subchronic 90-day toxicity study in laboratory animals[24] demonstrated that adverse outcomes caused by epyrifenacil were limited to hepatotoxicity and anemia in rats and mice, which were thought to be consequences of PPO inhibition. In these toxicological observations, hepatotoxicity was considered to be a more important risk and its exploration in detail was considered necessary since it may lead to neoplastic transformation when exposure is prolonged. As presented in Fig. 8, epyrifenacil has an ester bond in its structure, which is expected to be easily cleaved and thereby produce the carboxylic acid metabolite (CA). An *in vitro* metabolism study proved that ester cleavage in epyrifenacil was very rapid in mice, rats, and humans,[24] which suggests that epyrifenacil, when dosed orally, is mostly metabolized to CA by the first-pass effect, and CA is the major metabolite in mammals. In order to conclude that CA is the main cause of epyrifenacil-induced hepatotoxicity in rodents, we also conducted an *in vitro* PPO inhibition assay. The results showed that CA inhibited PPO in rodents to a similar extent as the parent epyrifenacil.[24] From these results, we concluded that the principal cause of epyrifenacil-induced hepatotoxicity in rodents is CA.

Since the molecular mechanism of heme biosynthesis is common to all mammals, the possibility of epyrifenacil-induced hepatotoxicity in humans must be considered. To conduct a precise risk assessment, we planned to look for evidence of species differences in the dynamics (*i.e.*, PPO inhibitory activity) and kinetics (*i.e.*, uptake by the liver) of PPO inhibition separately. Mitochondrial PPO inhibition assay showed a significant species difference in the inhibitory activity by CA. The IC_{50} in humans was 8.3 times larger than in rats and 13.9 times larger than in mice, demonstrating that the sensitivity to PPO inhibition by CA is less in humans than in rodents (Fig. 9). The details underlying species differences have not been revealed; however as mentioned above, a molecular dynamics study of flumioxazin clearly demonstrated the species difference in the binding affinity of the substrate to PPO,[21] so the binding affinity of CA for PPO was deemed to vary similarly.

In view of the kinetics, we have obtained some preliminary data which suggest the contribution of active uptake and accumulation of CA in mouse livers. Since CA, the ester-cleaved metabolite of epyrifenacil, contains a carboxylic acid moiety, it is highly likely to be in its ionized form, a COO^- anion, under physiological conditions. It is known that uptake of such anions into organs *via* organic anion transporters (OATs) or organic anion transporting peptides (OATPs) leads to significant exposure to the chemicals.[25,26] It is also reported that there are species differences in the active transport of chemicals and thereby species differences in exposure and toxicity.[27–29] In the *in vitro*

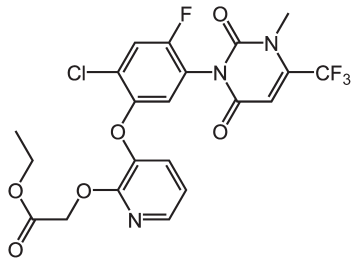

Fig. 8. Chemical structure of epyrifenacil.

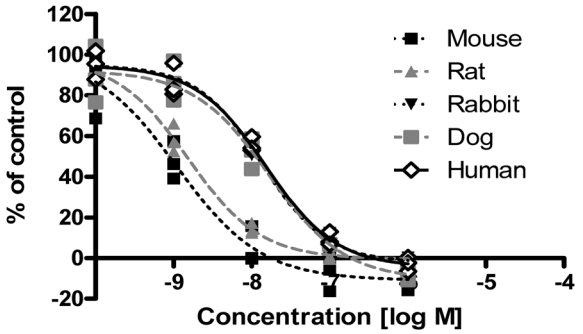

Fig. 9. Concentration–response curves of PPO inhibition by CA expressed as relative PPO activity (*y*-axis) *versus* the logarithm of the concentration of CA (*x*-axis).

metabolism study, no quantitative and qualitative species differences in metabolite production and metabolic degradation were present, thus we focused on evaluating species differences in the hepatic uptake of CA as one of the predominant toxicity factors. As shown in Fig. 10, the results of *in vitro* assays using hepatocytes from rodents and humans demonstrated that the uptake of CA is 6 to 13 times larger in mice and two times larger in rats than in humans.[24] This result clearly indicates that exposure to CA in the liver is smaller in humans than in rodents, especially mice. Since species differences were proved in both dynamics and kinetics, it was concluded that the risk of CA-induced hepatotoxicity was lower in humans than in rodents. Our research provides only phenomenological results at this time, thus further mechanistic researches to explain the significant differences among species are highly desired.

Concluding remarks

It is always debatable whether it is appropriate to evaluate the safety and toxicity of pesticides directly in humans; however, it is absolutely impossible to assess the human safety of pesticides by direct dosing of humans. In order to address the dilemma, many *in vivo*, *in vitro*, and *in silico* techniques for evaluating safety in humans have been recently developed with great improvement in variability and accuracy. For procymidone, as explained above, the biliary excretion and consequent high exposure to the causal metabolite in plasma were identified as the key toxicity factors, and the low risk to humans of exposure was confirmed using a novel animal model of humanized chimeric mice. For flumioxazin, lower PPO inhibitory activity in human mitochondria and consequent higher no-effect level of no less than 5.0 μM in human erythroid cells were determined in *in vitro* assays, and then, using the *in silico* PBPK technique, it was confirmed that the concentration of flumioxazin in human fetus does not reach the no-effect level even at the extremely high dose level. For epyrifenacil, the levels of hepatic exposure to the causal metabolite and inhibition of the target enzyme were evaluated *in vitro* and were significantly different between mice and humans. The safety assessment strategy illustrated for the three compounds in this article to separately assess the key toxicodynamic factor (*i.e.*, sensitivity) and/or toxicokinetic factor (*i.e.*, exposure) is scientifically-sound approach, thus it is expected to play an important role in the human safety assessment of agricultural compounds.

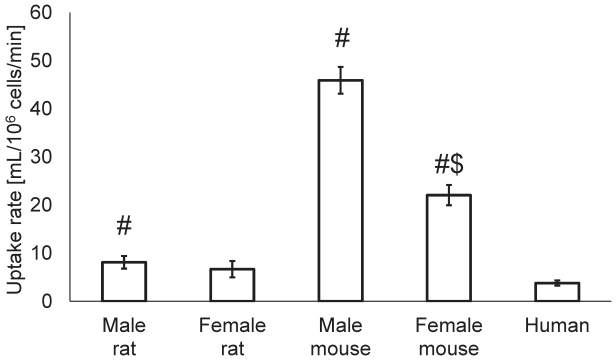

Fig. 10. Rate of active uptake of CA by hepatocytes. #: $p<0.01$ *vs.* humans, and $: $p<0.01$ *vs.* corresponding male group in the same species by Student *t*-test.

Acknowledgements

These researches were performed at Sumitomo Chemical Co., Ltd. I would like to express my deepest gratitude to Dr. Yoshitaka Tomigahara and Dr. Satoshi Kawamura, who designed the overall strategy of human risk assessment for procymidone and flumioxazin. I also express my appreciation to all my colleagues in the Environmental Health Science Laboratory.

References

1) Y. Hisada, K. Maeda, N. Tottori and Y. Kawase: Plant disease control by N-(3′,5′-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide. *J. Pestic. Sci.* **1**, 145–149 (1976).
2) K. Shiba, H. Kaneko, H. Yoshino, N. Kakuta, K. Iba, I. Nakatsuka, A. Yoshitake, H. Yamada and J. Miyamoto: Comparative metabolism of procymidone in rats and mice. *J. Pestic. Sci.* **16**, 27–33 (1991).
3) Y. Tomigahara, H. Tarui, H. Nagahori, K. Sugimoto, M. Mogi, K. Nishioka, S. Kawamura, N. Isobe, Y. Okuno and H. Kaneko: Species differences in the developmental toxicity of procymidone: Remarkable variation in pharmacokinetics, metabolism, and excretion. *J. Pestic. Sci.* **40**, 111–123 (2015).
4) H. Tarui, Y. Tomigahara, H. Nagahori, K. Sugimoto, M. Mogi, S. Kawamura, N. Isobe and H. Kaneko: Species differences in the developmental toxicity of procymidone: Placental transfer of procymidone in pregnant rats, rabbits, and monkeys. *J. Pestic. Sci.* **43**, 79–87 (2018).
5) Y. Tomigahara, H. Tarui, M. Matsui, M. Kurosawa, S. Kawamura and N. Isobe: Lack of human relevance for procymidone's developmental toxicity attributable to species difference in its kinetics and metabolism. *J. Pestic. Sci.* **43**, 114–123 (2018).
6) J. Abe, Y. Tomigahara, H. Tarui, R. Omori and S. Kawamura: Identification of metabolism and excretion differences of procymidone between rats and humans using chimeric mice: Implications for differential developmental toxicity. *J. Agric. Food Chem.* **66**, 1955–1963 (2018).
7) R. Yoshida, M. Sakaki, R. Sato, T. Haga, E. Nagaho, H. Oshio, K. Kamoshita: S-53482: A new *N*-phenyl phthalimide herbicide. *Brighton Crop Prot. Conf.-Weeds.*, 69–75 (1991).
8) K. Wakabayashi and P. Böger: Target sites for herbicides: Entering the 21st century. *Pest Manag. Sci.* **58**, 1149–1154 (2002).

9) G. F. Hao, Y. Zuo, S. G. Yang and G. F. Yang: Protoporphyrinogen oxidase inhibitor: An ideal target for herbicide discovery. *Chimia (Aarau)* **65**, 961–969 (2011).
10) H. A. Dailey: "Conversion of Coprotoporphyrinogen to Protoheme in Higher Eukaryotes and Bacteria. Biosynthesis of Heme and Chlorophylls," McGraw-Hill Publishing Co. New York: 123–161 (1990).
11) A. G. Smith and J. R. Foster: The association between chemical-induced porphyria and hepatic cancer. *Toxicol. Res.* **7**, 647–663 (2018).
12) M. P. Holsapple, H. C. Pitot, S. M. Cohen, A. R. Boobis, J. E. Klaunig, T. Pastoor, V. L. Dellarco and Y. P. Dragan: Mode of action in relevance of rodent liver tumors to human cancer risk. *Toxicol. Sci.* **89**, 51–56 (2006).
13) Butafenacil (PC Code 122004). Section 3 Registration for Application of Butafenacil to Cotton. Memorandum from United States Environmental Protection Agency (USEPA). July 2003.
14) European Food Safety Authority: Conclusion on the peer review of the pesticide risk assessment of the active substance pyraflufen-ethyl. *EFSA J.* **13**, 4001 (2015).
15) Saflufenacil. Human-Health Risk Assessment in Support of Tolerances for Residues in/on Barley, Wheat, Grass, and Olives. Memorandum from United States Environmental Protection Agency (USEPA). June 2014.
16) S. Kawamura, T. Kato, M. Matsuo, M. Sasaki, Y. Katsuda, A. M. Hoberman and M. Yasuda: Species difference in developmental toxicity of an *N*-phenylimide herbicide between rats and rabbits and sensitive period of the toxicity to rat embryos. *Cong. Anom.* **35**, 123–132 (1995).
17) S. Kawamura, T. Yoshioka, T. Kato, M. Matsuo and M. Yasuda: Histological changes in rat embryonic blood cells as a possible mechanism for ventricular septal defects produced by an *N*-phenylimide herbicide. *Teratology* **54**, 237–244 (1996).
18) S. Kawamura, T. Kato and A. G. Fantel: Close link between protoporphyrin IX accumulation and developmental toxicity induced by *N*-phenylimide hervicides in rats. *Birth Defects Res. B* **101**, 429–437 (2014).
19) Y. Tomigahara, M. Matsui, H. Matsunaga, N. Isobe, H. Kaneko, I. Nakatsuka, A. Yoshitake and S. Yamane: Metabolism of 7-fluoro-6-(3,4,5,6-tetrahydrophthalimido)-4-(2-propynyl)-2*H*-1,4-benzoxazin-3(4*H*)-one (S-53482) in rat. 1. Identification of a sulfonic acid type conjugate. *J. Agric. Food Chem.* **47**, 305–312 (1999).
20) J. Abe, N. Isobe, K. Mikata, H. Nagahori, Y. Naito, H. Saji, M. Ono and S. Kawamura: Flumioxazin metabolism in pregnant animals and cell-based protoporphyrinogen IX oxidase (PPO) inhibition assay of fetal metabolites in various animal species to elucidate the mechanism of the rat-specific developmental toxicity. *Toxicol. Appl. Pharmacol.* **339**, 34–41 (2018).
21) A. Arakawa, M. Otani, K. Iwashita and K. Yamazaki: Molecular dynamics mechanism to generate species differences in inhibition of protoporphyrinogen oxidase by flumioxazin. *Comput. Toxicol.* **1**, 12–21 (2017).
22) S. Kawamura, M. Otani, T. Miyamoto, J. Abe, R. Ihara, K. Inawaka and A. G. Fantel: Different effects of an *N*-phenylimide herbicide on heme biosynthesis between human and rat erythroid cells. *Reprod. Toxicol.* **99**, 27–38 (2021).
23) T. Takaku, H. Nagahori and Y. Sogame: Metabolism and physiologically based pharmacokinetics modelling of flumioxazin in pregnant animals. *Toxicol. Appl. Pharmacol.* **277**, 242–249 (2014).
24) K. Matsunaga, S. Fukunaga, J. Abe, H. Takeuchi, S. Kitamoto, Y. Tomigahara: Risk assessment of hepatotoxicity by a new herbicide, epyrifenacil, in humans by comparison of the dynamics and kinetics of its causal metabolite in rodents. Accepted to *J. Pestic. Sci.*
25) T. T. Li, J. X. An, J. Y. Xu and B. G. Tuo: Overview of organic anion transporters and organic anion transporter polypeptides and their roles in the liver. *World J. Clin. Cases* **7**, 3915–3933 (2019).
26) T. Mikkaichi, T. Suzuki, M. Tanemoto, S. Ito and T. Abe: The organic anion transporter (OATP) family. *Drug Metab. Pharmacokinet.* **19**, 171–179 (2004).
27) X. Qi, E. Wagenaar, W. Xu, K. Huang and A. H. Schinkel: Ochratoxin A transport by the human breast cancer resistance protein (BCRP), multidrug resistance protein 2 (MRP2), and organic anion-transporting polypeptides 1A2, 1B1 and 2B1. *Toxicol. Appl. Pharmacol.* **329**, 18–25 (2017).
28) L. Zou, A. Stecula, A. Gupta, B. Prasad, H. C. Chien, S. W. Yee, L. Wang, J. D. Unadkat, S. H. Stahl, K. S. Fenner and K. M. Giacomini: Molecular mechanisms for species differences in organic anion transporter 1, OAT1: Implications for renal drug toxicity. *Mol. Pharmacol.* **94**, 689–699 (2018).
29) M. Bartels, C. Brown, G. Chung, M. Chan, C. Terry, S. Gehen and M. Corvaro: Review of the pharmacokinetics and metabolism of triclopyr herbicide in mammals: Impact on safety assessments. *Regul. Toxicol. Pharmacol.* **116**, 104714 (2020).