Chimeric mice with humanized liver as a model for testing organophosphate and carbamate pesticide exposure

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Abstract

BACKGROUND: Diagnosis of acute intoxication with organophosphate (OP) or carbamate (CM) pesticides in humans is achieved by measuring plasma butyrylcholinesterase (BuChE) activity. However, BuChE activity is not an ideal biomarker in experimental animal models. The aim of this study was to establish an experimental mouse model for evaluating exposure to OP and CM pesticides by monitoring BuChE activity using chimeric mice in which the liver was reconstituted with human hepatocytes.

RESULTS: A single oral administration of acephate (300 mg/kg), chlorpyrifos (10 mg/kg), fenobucarb (300 mg/kg) or molinate (250 mg/kg) in chimeric mice led to inhibition of >95%, >95%, 28% and 60% of plasma BuChE activity after 7, 0.5, 0.5 and 7 h, respectively. Dose-dependent decreases in plasma BuChE activity were also observed for acephate and chlorpyrifos. A 5-day repeated-dose study with 10 or 30 mg/kg acephate found a constitutive reduction in plasma BuChE activity to 80% and 70% of pre-dose levels, respectively.

CONCLUSION: Changes in plasma BuChE activity in chimeric mice with humanized liver clearly reflected the exposure levels of OP and CM pesticides. These results suggest that the humanized-liver mouse model may be suitable for estimating levels of exposure to these pesticides in humans.

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Keywords: acephate; butyrylcholinesterase; chlorpyrifos; fenobucarb; humanized-liver mice; molinate

1 INTRODUCTION

Organophosphorus (OP) and carbamate (CM) pesticides are used worldwide as insecticides in agriculture, veterinary practice and public health. However, because of their easy accessibility and widespread use, intoxication is common, ranging from chronic (low-level exposure from application activities) to acute (high-level exposure from accidental release or deliberate ingestion). The main mechanism of acute OP and CM intoxication is the inhibition of acetylcholinesterase (AChE) at cholinergic synapses by covalent modification of the active site serine residue, leading to the accumulation of acetylcholine (ACh), uncontrolled activation of cholinergic receptors (cholinergic crisis), and, in serious cases, death. OPs and CMs are potent inhibitors of AChE not only in the central nervous system, but also on the membrane of red blood cells (RBCs). In addition, they target butyrylcholinesterase (BuChE) in plasma. Therefore, the inhibition of AChE in RBCs and BuChE in plasma are both used as proxy measurements for cholinergic AChE inhibition and as early biomarkers for environmental exposure to OPs and CMs in humans. Generally, RBC AChE assays are more reliable than plasma BuChE assays because synaptic AChE inhibition underlies the clinical effects of OP toxicity, and RBC AChE is kinetically similar to synaptic AChE. However, plasma BuChE is the more commonly used biomarker for subclinical, asymptomatic or low-level exposure to OPs, even though its inhibition is not known to be directly involved in neuronal cholinergic processes. Plasma BuChE activity is also established as a routine liver function test in clinical settings, and greater reliability and reproducibility of the plasma BuChE assay have been indicated within and between laboratories compared with the RBC AChE assay. Therefore, the measurement of plasma BuChE levels is faster and easier than the assessment of RBC AChE activity. The relationship between RBC AChE and plasma BuChE in OP handlers in Washington State, USA, who participated in a statewide cholinesterase monitoring program.
program, was reported to show a weak negative correlation. By contrast, the inhibition of brain AChE activity correlated well with that of plasma BuChE activity in neonatal and adult rats exposed to methyl parathion, parathion and chlorpyrifos. Thus, the evidence supporting and contradicting the use of plasma BuChE as a surrogate marker remains inconclusive, and its relevance is still debated.

Species differences in plasma esterases exist between humans, mice and rats; higher BuChE and lower carboxylesterase activities have been reported in humans. These differences should be considered in experimental animal models for the accurate extrapolation of results to humans and for the prediction of specific features of compound drug metabolism in different species. To overcome these interspecies difference issues, we developed a chimeric mouse model with humanized liver (Hu-liver). In this model, highly immunodeficient NOG mice expressing a Herpes simplex virus type 1 thymidine kinase (HSV-TK) transgene in the liver via a mouse albumin enhancer/promoter (TK-NOG) are first treated with ganciclovir to ablate hepatocytes. Human hepatocytes are then transplanted to replace the mouse hepatocytes. The chimeric mouse with reconstituted Hu-liver produces human hepatic proteins, including drug-metabolizing enzymes and secretory proteins. We recently showed that plasma BuChE activity in Hu-liver chimeric mice was higher than activity in non-Hu-liver TK-NOG mice. These human-like features raised the possibility that the Hu-liver chimeric mouse might be a good surrogate model for human OP or CM pesticide exposure. In this study, we evaluated plasma BuChE activity in Hu-liver chimeric mice exposed to four pesticides; two OP insecticides (acephate and chlorpyrifos), one CM insecticide (fenobucarb) and one thiocarbamate herbicide (molinate). Changes in plasma BuChE activity in Hu-liver chimeric mice clearly reflected the exposure levels of OP and CM pesticides. These results suggest that the Hu-liver mouse model may be a suitable model for estimating exposure levels to these pesticides in humans.

2 MATERIALS AND METHODS

2.1 Chemicals

Acephate (O,S-dimethyl N-acetylphosphoramoiooiothioate), chlorpyrifos (O,O-dioyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), fenobucarb (O-sec-butylphenyl methylcarbamate) and molinate (S-ethyl perhydroazepin-1-carbothioate) were purchased from Wako Pure Chemical Industries (Osaka, Japan); all were 99% pure. Acephate was dissolved in saline, and other chemicals were suspended in 0.5% w/v methyl cellulose 400 solution (Wako). Control experiments were conducted with appropriate vehicles. All dosing solutions were prepared immediately before use.

2.2 Animals

Hu-liver chimeric mice were prepared as follows. Adult 7–8-week-old male TK-NOG mice were given 0.06 mg/mL Val-ganciclovir (ValGCV; Sigma-Aldrich, St. Louis, MO, USA) for 2 days to ablate hepatocytes expressing the HSV-TK transgene. One week after the administration of ValGCV, alanine aminotransferase (ALT) levels in plasma were measured using an automated clinical chemistry analyzer (Fuji Dri-Chem 7000; Fuji Photo Film, Tokyo, Japan). Liver-injured TK-NOG mice with ALT levels exceeding 600 U/L received human hepatocyte transplantation. Cryopreserved human hepatocytes were purchased from Biopredic International (BPI, Rennes, France) and Triangle Research Labs (TRL, Research Triangle Park, NC, USA). A total of 0.5–1 x 10^6 human hepatocytes in 50 μL of Williams’ medium E (Thermo Fisher Scientific, Waltham, MA, USA) were injected intrasplenically using a 0.5-mL insulin syringe with a permanently attached needle (29 G x 0.5 inch; Terumo Corporation, Tokyo, Japan). Successful engraftment was evaluated by measuring blood levels of human albumin (hAlb) with a human albumin ELISA quantitation kit (Bethyl Laboratories, Montgomery, TX, USA). The degree of replacement of mouse hepatocytes by human hepatocytes was assessed by the morphometric analysis of liver sections described previously. Mice were allowed ad libitum access to a radiation-sterilized (30 kGy) pellet diet (CLEA Rodent Diet CA-1; CLEA Japan, Tokyo, Japan) and tap water. Room temperature was maintained at 24°C with a 12/12 h light/dark cycle. All mouse studies were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals of the Central Institute for Experimental Animals (CIEA), and the experimental protocols were approved by the Animal Care Committee of CIEA (Permit Number: 11029A). All surgery was performed under isoflurane anesthesia, and all efforts were made to minimize suffering. The experimental use of commercially available cryopreserved human hepatocytes was approved by the Research Ethics Committee of CIEA.

2.3 Measurement of plasma BuChE activity

Between 25 and 50 μL of blood were collected in heparinized tubes and spun at 1300 g for 20 min at 4°C to separate plasma. BuChE activity was measured using a Fuji Dri-Chem 7000 automated clinical chemistry analyzer with a Fuji Dri-Chem slide CHE-P (Fuji Photo Film) according to the manufacturer’s instructions. Human plasma (P9523) was purchased from Sigma-Aldrich.

2.4 Chemical treatments

All chemicals were administered via oral gavage at 10 mL/kg body weight to achieve greater dosing volume accuracy. A dose range-finding study was conducted using non-Hu-liver TK-NOG mice that received neither the ValGCV administration nor the human hepatocyte transplantation. The purpose of this range-finding study was to: (1) investigate the susceptibility to OPs in an immunodeficient mouse strain that is not usually used in general toxicity studies, and (2) determine the exact half-maximal lethal dose (LD₅₀). We sought to find the non-lethal maximum dose using a small number of Hu-liver TK-NOG mice. The dose levels were determined from LD₅₀ data for each pesticide. Based on these findings, a time-course study was conducted in Hu-liver chimeric mice. The mice were given a single oral dose of OP (acephate, 300 mg/kg or chlorpyrifos, 10 mg/kg), CM (fenobucarb, 300 mg/kg) or thiocarbamate (molinate, 250 mg/kg). Time points for the time-course study were 0.25, 0.5, 1, 7 and 24 h for acephate dosing, and 0.5, 1, 2, 4 and 24 h for chlorpyrifos dosing. For fenobucarb and molinate, time points of 0.5, 1, 4, 7 and 24 h were used. The time to peak effect was evaluated by measuring plasma BuChE activity. For the dose–response studies of OP pesticides (0.1–300 mg/kg for acephate and 0.1–1 mg/kg for chlorpyrifos), plasma BuChE activity was measured before and after OP exposure. The repeated dose study with 10 and 30 mg/kg acephate was conducted for five consecutive days. Plasma BuChE activity was measured every 24 h in addition to before and 7 h after the first acephate exposure.

2.5 Statistical analysis

Statistical analyses were performed with Prism 5 software (GraphPad Software, La Jolla, CA, USA). Differences between non-Hu-liver
and Hu-liver TK-NOG groups were assessed using Student’s t-test, and P-values <0.05 were considered statistically significant. Correlations between plasma BuChE activity and plasma hAlb levels were assessed using linear regression with $r^2$ values as measures of goodness of fit. In time-course studies, significant differences between treated groups at each specific time point on the day of dosing were determined by one-way analysis of variance with post-dosing time points as one factor and pre-dosing as a repeated (within-subject) measure. Benchmark dose (BMD) modeling software (version 2.6.0; US Environmental Protection Agency, http://www2.epa.gov/bmds) was used to calculate doses estimated to produce a 10% decrease (BMD$_{10}$, i.e., 90% of control) at each endpoint. In all cases, the Hill model was used with a correction for non-equal variances when any were present.

### 3 RESULTS

#### 3.1 BuChE activity

In preliminary experiments, plasma BuChE activity, which is a well-known serum protein with species-specific differences between humans and mice, was measured in Hu-liver chimeric mice. Commercially available pooled human plasma was used as a representative human sample, and its BuChE activity was compared with that of Hu-liver chimeric mice and non-Hu-liver TK-NOG mice. Figure 1(A) shows that Hu-liver chimeric mice had significantly higher plasma BuChE activity (271.8 ± 67.0 U/L) than non-Hu-liver TK-NOG mice (28.3 ± 10.1 U/L), and the levels were comparable to those in the human sample (189 U/L). The plasma BuChE activity in Hu-liver chimeric mice was highly correlated with the level of plasma hAlb (Fig. 1B), suggesting that the plasma BuChE activity reflected the degree of replacement of mouse hepatocytes by human hepatocytes in this model (replacement index, RI%). Thus, we analyzed the relationship between plasma BuChE activity and RI% in 20 Hu-liver chimeric mice with varying plasma hAlb levels, and found a strong correlation between plasma BuChE activity and RI% (Fig. 1C).

#### 3.2 OP pesticides

##### 3.2.1 Dose range-finding study with non-Hu-liver TK-NOG mice

In the dose range-finding study using non-Hu-liver TK-NOG mice, no deaths occurred in the acephate-treated mice; one death occurred in the 30 mg/kg dose chlorpyrifos-treated group (Table 1). Based on these results, time-course studies using Hu-liver chimeric mice were conducted with 300 mg/kg acephate and 10 mg/kg chlorpyrifos.

##### 3.2.2 Time-course study

Basal levels of plasma BuChE activity in non-Hu-liver TK-NOG and Hu-liver chimeric mice were 36.0 ± 0.0 and 299.3 ± 11.3 U/L, respectively (Fig. 2A, Pre). A single oral dose of 300 mg/kg acephate led to the gradual inhibition of plasma BuChE activity to 5.0 ± 0.0 U/L (approximately a seven-fold reduction) and 6.3 ± 2.5 U/L (approximately a 48-fold reduction) in non-Hu-liver TK-NOG and Hu-liver chimeric mice, respectively. The time to peak inhibition was 7 h post dosing for acephate (Fig. 2A). The recovery of plasma BuChE activity to pre-dosing levels, represented as the percent of control BuChE activity, was evident after 14 days for acephate (Fig. 2B). In the chlorpyrifos study, the basal plasma BuChE activity levels were 32.0 ± 12.7 and 240.0 ± 17.8 U/L in non-Hu-liver TK-NOG and Hu-liver chimeric mice, respectively (Fig. 2C, Pre). Unlike the plasma BuChE inhibition kinetics observed with acephate, a single oral dose of 10 mg/kg chlorpyrifos led to a rapid inhibition of plasma BuChE activity in both non-Hu-liver TK-NOG and Hu-liver chimeric mice to 15.0 ± 0.0 U/L (approximately a two-fold reduction) and 20.0 ± 2.5 U/L (approximately a 12-fold reduction), respectively. Time to peak inhibition was 0.5 h post dosing for chlorpyrifos (Fig. 2C). The plasma BuChE activity recovered to pre-dosing levels with no significant difference 14 days post dosing (Fig. 2D).

| Compound | LD$_{50}$ in mice (mg/kg) | Dose (mg/kg) | No. of mice used | No. of mice died |
|----------|--------------------------|-------------|-----------------|-----------------|
| Acephate | 361 (Ref. 22)            | 300         | 4               | 0               |
|          |                          | 150         | 4               | 0               |
| Chlorpyrifos | 60 (Ref. 23)        | 30          | 3               | 1               |
|          |                          | 10          | 3               | 0               |
Figure 2. Time-course studies for the organophosphates (OPs) acephate and chlorpyrifos. Time-course studies to determine plasma butyrylcholinesterase (BuChE) inhibition by acephate (A) and chlorpyrifos (C) in non-Hu-liver TK-NOG mice (open bars, n = 4) and Hu-liver chimeric mice (filled bars, n = 4). Values are expressed as the mean ± SD. Time of peak inhibition was 7 and 0.5 h for 300 mg/kg acephate and 10 mg/kg chlorpyrifos, respectively. Recovery of plasma BuChE activity after acephate (B; AC, n = 7) and chlorpyrifos (D; CP, n = 9) dosing. Values are expressed as the mean percentage of pre-dosing control (Pre) ± SD.

3.2.3 Dose–response study
Dose-dependent decreases in plasma BuChE activity were assessed at peak effective time points in response to acephate and chlorpyrifos treatments of 0.1–300 and 0.1–3 mg/kg, respectively. The dose–response curves for each OP are presented in Fig. 3(A,B). Analysis of BuChE inhibition by single oral OP dose revealed that the BMD10 and lower limit of the BMD (BMDL)10 were 1.9 and 1.5 mg/kg for acephate, and 0.12 and 0.07 mg/kg for chlorpyrifos, respectively (Fig. 3C,D).

3.2.4 Repeated-dose study
The effect of consecutive exposures to acephate was evaluated in Hu-liver chimeric mice by measuring the inhibition of plasma BuChE activity. A 5-day repeated-dose study with 10 and 30 mg/kg acephate found a constitutive reduction in plasma BuChE activity to ~80% and 70% of pre-dosing levels, respectively (Fig. 4). At peak effective time points after the first exposure, treatment with 10 and 30 mg/kg acephate led to ~70% and 50% levels of pre-dosing plasma BuChE activity, respectively. Twenty-four hours after the first exposure, plasma BuChE activities recovered to 80% and 70% of pre-dosing levels, and the inhibition levels were maintained during repeated exposures. Furthermore, at peak effective time points after the last exposure, we confirmed the inhibition of plasma BuChE activity to be at similar levels to those observed after the first exposure to each dose (Fig. 4, at 96 h). Dotted lines indicate expected values after each exposure.

3.3 CM and thiocarbamate pesticides
Doses were determined from LD50 data for fenobucarb,22 and reported non-lethal dose in Hu-liver chimeric mice for molinate.23 Time-course studies using Hu-liver chimeric mice were conducted with 300 mg/kg fenobucarb and 250 mg/kg molinate. A single oral dose of 300 mg/kg fenobucarb led to a 28.2% maximal inhibition of plasma BuChE activity 0.5 h post dosing (Fig. 5A). Unlike the plasma BuChE inhibition kinetics observed with fenobucarb, a single oral dose of 250 mg/kg molinate led to a gradual inhibition of plasma BuChE activity, with maximal inhibition of 60.5% observed 7 h post dosing in Hu-liver chimeric mice (Fig. 5B).

4 DISCUSSION
The findings of this study suggest that the Hu-liver TK-NOG chimeric mouse, which has similar drug metabolism profiles and plasma BuChE activity to those of humans, may be a good surrogate model for estimating levels of OP and CM human exposures. Plasma esterase activity varies among species. There are three major plasma esterase classes: paraoxonase, carboxylesterase and BuChE. Both paraoxonase and carboxylesterase activities in human plasma are lower than in rat and mouse plasma, whereas the activity of BuChE is extremely high in human plasma.17,24 We measured plasma BuChE activity in both non-Hu-liver TK-NOG and Hu-liver chimeric mice using an automated biochemical analyzer (range: 5–500 U/L) and found that the average BuChE activity (271.8 ± 66.9 U/L; n = 75) in Hu-liver chimeric mice was ~10 times higher than in non-Hu-liver TK-NOG mice.
Figure 3. Dose–response studies for the organophosphates (OPs) acephate and chlorpyrifos. Dose–response of plasma butyrylcholinesterase (BuChE) inhibition 7 h after acephate dosing (A, $n = 8$) and 0.5 h after chlorpyrifos dosing (B, $n = 5$) in Hu-liver chimeric mice. Values are expressed as the mean percentage of control ± SD. Benchmark dose modeling for acephate (C) and chlorpyrifos (D), using the Hill model with a 0.95 confidence level. Benchmark dose for 10% inhibition of plasma BuChE activity lower one-side confidence limit of the benchmark dose.

Figure 4. Constitutive reduction of plasma butyrylcholinesterase (BuChE) activity by repeated dosing with acephate. Two doses of acephate (●: 10 mg/kg, $n = 5$; ○: 30 mg/kg, $n = 6$) were administered every 24 h for 5 consecutive days (arrows). Plasma BuChE activity was measured every 24 h before acephate exposure and 7 h after first and final acephate exposures. Observed values are indicated as solid circles and lines, and expected values as dotted circles and lines.

higher than that in non-Hu-liver TK-NOG mice (28.3 ± 10.1 U/L; $n = 36$). In non-Hu-liver TK-NOG mice, BuChE activity was close to the lower detection limit of this assay system, hindering the accurate measurement of plasma BuChE activity inhibition and, therefore, the assessment of OP exposures. In contrast, BuChE activity in most Hu-liver chimeric mice was within the reference interval of 70–420 U/L and, therefore, this model readily assessed OP exposures by measuring plasma BuChE activity in the same clinical test.

It is generally difficult to determine toxic doses of OP and CM in human volunteers for ethical reasons. However, some human studies have investigated the effects of single oral dose of acephate or chlorpyrifos. In one human acephate study, seven volunteers who received single oral doses of acephate at 1.2 mg/kg body weight (men) and 1 mg/kg body weight (women) did not show any changes in RBC AChE activity. Furthermore, there were no changes in vital signs or electrocardiography, hematology, clinical chemistry, urinalysis or physical examination parameters.

Thus, although this was the only dose tested in humans, the no observed adverse effect level (NOAEL) was set at 1.2 mg/kg. In the current study, we observed dose-dependent decreases in plasma BuChE activity following a single oral treatment of acephate. The doses estimated to produce a 10% decrease at each endpoint were 1.9 mg/kg ($\text{BMD}_{10}$) and 1.5 mg/kg ($\text{BMDL}_{10}$) for plasma BuChE. Although plasma BuChE activity represents the no observed effect level (NOEL), the $\text{BMDL}_{10}$ value (1.5 mg/kg) for plasma BuChE in Hu-liver TK-NOG mice was very close to the NOAEL (1.2 mg/kg) established in humans. Similarly, a chlorpyrifos study with six human volunteers found a 15% decrease in plasma BuChE activity 24 h after a single oral dose of 0.3 mg/kg chlorpyrifos. The time-course study with Hu-liver chimeric mice indicated that the peak effective time was within 1 h after chlorpyrifos dosing, and the decreased activities recovered slightly after 24 h. Considering these kinetics, 24 h after a single oral dose of 0.3 mg/kg chlorpyrifos in Hu-liver chimeric mice, plasma BuChE activity will not decrease by more than 20%; this estimate is comparable with findings in humans. These results suggest that the Hu-liver mouse model is suitable for estimating exposure levels of these pesticides in humans. Another study with human volunteers reported that blood plasma and erythrocyte cholinesterase activity did not fall below 90% of baseline levels during dosing in five volunteers (weight range: 73–92 kg) who ingested 1 mg of analytical-grade chlorpyrifos (dose range: 0.011–0.014 mg/kg), indicating that the NOAEL should be set at 0.014 mg/kg. By using Hu-liver TK-NOG mice, we could obtain the $\text{BMD}_{10}$ (0.12 mg/kg) and $\text{BMDL}_{10}$ (0.07 mg/kg) values for plasma BuChE, and the values were high compared with the NOAEL. These discrepancies underscore the
difficulty in setting doses to find the NOAEL in humans. Therefore, comparisons of compounds using the BMD may be inherently more accurate than comparisons based on the NOAEL. 28

In clinical practice, plasma BuChE measurements remain a mainstay for the fast initial screening of OP exposure. However, plasma BuChE inhibition is not directly measured; rather, it is compared with a ‘normal’ value. 29 Measuring pre-exposure levels would certainly improve precision, but this is only practically possible under experimental conditions. Stefanidou et al. emphasized the importance of plasma BuChE activity, which is superior to RBC AChE activity even though it is considered to be an indicator of subclinical, asymptomatic, or low-level exposure. The advantage of using BuChE activity as a biomarker of long-term exposure to OP insecticides in farmers, greenhouse workers, spray applicators and workers in the pesticide industry is that measurements made before, during and after exposure are convenient, reliable and cost-effective. 8 In in vivo experimental conditions using Hu-liver chimeric mice, despite large variations in plasma BuChE activity, the inhibition rate of plasma BuChE activity remained constant, depending on the exposure level.

One prospective cohort study found that patients with mild to moderate chlorpyrifos poisoning had severely inhibited BuChE on admission; in contrast, BuChE was not severely inhibited in 52% of patients with dimethoate poisoning who died. 30 It is more likely that the pharmacodynamics differences are due variable effect of these pesticides on AChE and BuChE inhibition, with chlorpyrifos being particularly potent. 31 Chlorpyrifos rapidly decreases BuChE activity, while dimethoate reduces it gradually. In this experimental study, we observed a similar difference in pharmacodynamics; chlorpyrifos and fenobucarb decreased BuChE activity rapidly, while, acephate and molinate reduced BuChE activity more slowly. Rapid elimination of BuChE activity after chlorpyrifos exposure was similar to that seen in patients who ingested chlorpyrifos intentionally. 30 Because of these differences in pharmacodynamics, plasma BuChE activity on admission is useful when the OP pesticide has been identified and when its sensitivity and specificity are known. In other words, if the pharmacodynamic properties of the pesticide are not sufficiently investigated, plasma BuChE activity is unlikely to be clinically useful. Our findings suggest that Hu-liver chimeric mice could yield information on the pharmacodynamic properties of each pesticide by following a stepwise in vivo BuChE inhibition assay: (1) determine the non-lethal maximum dose in non-Hu-liver (or preferably Hu-liver chimeric) mice; (2) determine the most effective time with non-lethal maximum dose in Hu-liver chimeric mice; and (3) examine the dose–response relationship using various doses at the most effective time. In many cases of self-harm, it is possible to identify the ingested pesticide; however, it is difficult to know exactly the amount ingested and the elapsed time to hospital admission. Although the variable time from ingestion to admission may be obscuring a relationship, most clinical samples obtained upon patient admission are likely to be taken 1–6 h after ingestion. 30 Therefore, the accumulation of time-course and dose–response data for each pesticide obtained from Hu-liver chimeric mice, the so-called pharmacodynamic data panel, ideally including plasma pesticide concentrations, will increase the significance of plasma BuChE activity in clinical toxicology.

The degree of chimerism in chimeric mice with human hepatocytes can be evaluated by the RI% utilizing plasma hAlb. 38,32 Here, we determined that BuChE activity closely correlated with hAlb levels in Hu-liver chimeric mice of various chimeric ratios. We also observed a significant correlation between the RI% and plasma BuChE activity, which indicated that plasma BuChE activity could serve as a potential alternative marker for estimating the RI%. (Fig. 1C). This method of utilizing an automated analyzer is an easier and faster method than the hAlb ELISA method currently used for estimating the RI%.

In summary, the levels of plasma BuChE activity in Hu-liver chimeric mice were comparable with those in humans, and BuChE activity was inhibited by exposure to the OP pesticides acephate and chlorpyrifos, and the CM pesticide fenobucarb and the thiocarbamate molinate. We demonstrated that changes in plasma BuChE activity in Hu-liver chimeric mice clearly reflected the exposure levels of acephate and chlorpyrifos. These results suggest that the Hu-liver chimeric mouse may be a good surrogate model for estimating the levels of anti-cholinesterase pesticides in human exposures.

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AUTHOR CONTRIBUTIONS
H.S. designed the experiments. K.K. and H.S. performed in vivo experiments. H.S. performed in vitro analyses. N.M. and H.Y. analyzed in vitro data. K.K. performed histological analyses. H.S., M.N., and H.Y. wrote the manuscript. All authors read and revised the manuscript.

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