Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism

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Objectives **The Q192R polymorphism of paraoxonase (PON1) has been shown to affect hydrolysis of organophosphorus compounds. The Q192 and R192 alloforms exhibit equivalent catalytic efficiencies of hydrolysis for diazoxon, the oxon form of the pesticide (DZ). However, the R192 alloform has a higher catalytic efficiency of hydrolysis than does the Q192 alloform for chlorpyrifos oxon (CPO), the oxon form of the pesticide chlorpyrifos (CPS). The current study examined the relevance of these observations for in-vivo exposures to chlorpyrifos and chlorpyrifos oxon.**

Methods **Using a transgenic mouse model we examined the relevance of the Q192R polymorphism for exposure to CPS and CPO *in vivo*. Transgenic mice were generated that expressed either human $PON1_{Q192}$ or $PON1_{R192}$ at equivalent levels, in the absence of endogenous mouse *PON1*. Dose–response and time course experiments were performed on adult mice exposed dermally to CPS or CPO. Morbidity and acetylcholinesterase (AChE) activity in the brain and diaphragm were determined in the first 24 h following exposure.**

Results **Mice expressing $PON1_{Q192}$ were significantly more sensitive to CPO, and to a lesser extent CPS, than were mice expressing $PON1_{R192}$. The time course of inhibition following exposure to 1.2 mg/kg CPO revealed maximum inhibition of brain AChE at 6–12 h, with $PON1_{R192}$, $PON1_{Q192}$, and $PON1^{-/-}$ mice exhibiting 40, 70 and 85% inhibition, respectively, relative to control mice. The effect of PON1 removal on the dose–response curve for CPS exposure was remarkably consistent with a PBPK/PD model of CPS exposure.**

Conclusion **These results indicate that individuals expressing only the $PON1_{Q192}$ allele would be more sensitive to the adverse effects of CPO or CPS exposure, especially if they are expressing a low level of plasma $PON1_{Q192}$.**
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Introduction

Paraoxonase (PON1) is a high density lipoprotein (HDL) associated enzyme with a broad range of activities, including metabolism of oxidized lipids [1–3], toxic insecticide metabolites [4], and a number of drugs [5]. PON1 activity in an individual is determined largely by two factors: the levels of PON1 in their plasma and the amino acid (glutamine or arginine) present at position 192 [5]. The authors have previously defined the term, 'PON1 Status' that incorporates both of these factors, and designed a high throughput two-substrate assay that provides an individual's functional position 192 phenotype and their plasma PON1 levels [6,7].

PON1 plays an important role in the detoxification of organophosphorus compounds, including the oxon forms of the OP insecticides chlorpyrifos (CPS) and diazinon (DZ). Species with high serum paraoxonase levels are more resistant to OP exposures than species with low paraoxonase levels [8,9], and exogenous administration of paraoxonase to rats or mice provides significant protection against the toxicity of OP compounds [10–13].

Studies of mice lacking PON1 due to targeted gene disruption (*PON1* knockout mice) demonstrated that PON1 plays a dominant role in the detoxication of chlorpyrifos oxon (CPO) and diazoxon (DZO), and

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contributes to the detoxication of the respective parent compounds CPS and DZ [14,15]. *PON1* knockout mice were much more sensitive than wild-type mice to exposure of either CPO or DZO, and resistance to these compounds could be restored by injection of purified PON1 protein [14,15]. However, the *PON1* knockout mice were not more sensitive than wild-type mice to paraoxon, and injection of purified human PON1 protein failed to provide protection against this compound [15].

In adult humans, plasma levels of PON1 are highly variable, with as high as 13-fold differences in PON1 protein levels [16] and as much as 100-fold differences in plasma activity, depending on the substrate used [7,17–19]. Newborns have very low PON1 levels [20–23], and do not express mature PON1 levels until between 6 to 24 months of age [20]. A recent prospective study of *in utero* pesticide exposure in humans showed a significant association of maternal PON1 levels with reduced head circumference in the offspring of CPS-exposed mothers, suggesting that CPS has a detrimental effect on fetal neurodevelopment among mothers who have low PON1 activity [24]. These studies taken together indicate that adults with low PON1 levels and children less than 2 years old are at greater risk for toxicological consequences following exposure to CPS/CPO or DZ/DZO than individuals with higher PON1 levels.

A number of single nucleotide polymorphisms (SNPs) have been discovered in human *PON1*, including SNPs in the 5′ regulatory region that affect plasma PON1 levels, and coding-region SNPs that result in amino acid substitutions at L55 M and Q192R. Early studies examining the rates of paraoxon hydrolysis among individuals demonstrated that paraoxonase activity was distributed polymorphically in human populations [21,25,26].

The Q192R polymorphism was shown to be responsible for this polymorphic distribution of paraoxonase activity [27,28]. Allele frequencies of the Q192 allele range from about 0.3 for Asian populations to around 0.7 for Caucasian/Northern European populations [29].

The Q192R polymorphism affects the catalytic efficiency of hydrolysis of a number of PON1 substrates, including CPO, the toxic oxon metabolite of CPS [15], the nerve agents soman and sarin [17] and a number of drugs, activating some [30] while inactivating others [31,32]. The relevance of the different catalytic efficiencies of hydrolysis was demonstrated *in vivo* in a study involving reconstitution of $PON1_{Q192}$ or $PON1_{R192}$ in the plasma, but not liver, of *PON1* knockout mice [15]. $PON1_{R192}$ had significantly higher catalytic efficiency for hydrolysis of CPO than $PON1_{Q192}$ and provided significantly better protection against exposure *in vivo* [15]. In contrast, the Q192 and R192 alloforms had equivalent catalytic efficiencies of hydrolysis of DZO and the two alloforms provided equivalent protection against exposure to this OP. For paraoxon hydrolysis, the catalytic efficiencies of both alloforms were low, and neither alloform was able to provide protection against a paraoxon exposure *in vivo* [15]. Thus, the extent of protection afforded by the PON1 Q192R alloforms *in vivo* was determined by their respective catalytic efficiencies.

To extend these observations to a more general in-vivo model, *PON1* knockout mice and *PON1* knockout mice whose *PON1* genes were replaced with either human $PON1_{R192}$ ($hPON1_{R192}$) or $PON1_{Q192}$ ($hPON1_{Q192}$) were exposed to either CPO or its parent compound CPS. Expression of the $hPON1_{R192}$ or $hPON1_{Q192}$ transgenes resulted in endogenous production of the human PON1 alloforms in both liver and plasma, at physiological levels that were both stable over time and equivalent between the two transgenic lines. We report here that mice expressing $hPON1_{Q192}$ were significantly more sensitive to CPO/CPS exposure than were mice expressing equivalent levels of $hPON1_{R192}$.

Materials and methods

Generation of $hPON1_{R192}$ and $hPON1_{Q192}$ transgenic mice

Mice expressing either of the two human $PON1_{R192}$ or $PON1_{Q192}$ transgenes ($hPON1_{R192}$ or $hPON1_{Q192}$) in place of endogenous mouse *PON1* were generated as described [20]. Mice were produced by microinjection of C57Bl/6J wild-type mouse eggs with either a 80 kb fragment of DNA containing $hPON1_{R192}$ (along with 12 kb of 5′-flanking region and 33 kb of 3′-flanking region), or with a 45 kb fragment of a BAC clone (Genbank #AC004022) containing $hPON1_{Q192}$ (along with 10 kb of 5′-flanking region and 10 kb of 3′-flanking region) [15,16]. Three mice carrying the $hPON1_{R192}$ transgene were identified by PCR, with one founder transmitting the transgene to its offspring. Of three mice identified by PCR as carrying the $hPON1_{Q192}$ transgene, one $hPON1_{Q192}$ transgenic line was identified that expressed nearly equivalent levels of human PON1 as the $hPON1_{R192}$ transgenic mouse line, as measured by Northern blot analysis and plasma arylesterase activity. These two lines of $hPON1_{Q192}$ and $hPON1_{R192}$ mice were estimated by Southern blot analysis to carry two and one copies of the respective transgenes. Northern blot analysis indicated that transgene expression was present in the liver but not in any other organs examined (Shih *et al.*, unpublished data). To eliminate the contribution of mouse PON1, these transgenic mice were then crossed with *PON1* knockout ($PON1^{-/-}$) mice to generate 'humanized' transgenic mice that express equivalent levels of either $hPON1_{Q192}$ or $hPON1_{R192}$ in the absence of mouse PON1. All mice were of the equivalent congenic B6.129 strain background, as the *PON1* knockout mice used for the crosses were $>96\%$

C57Bl/6J, backcrossed five times from the original C57Bl/6J × 129/svEv strain background. The $hPON1_{Q192}$ and $hPON1_{R192}$ mice used in these studies were hemizygous for the respective transgenes.

Mice were housed in modified SPF (specific pathogen-free) facilities with a 12 h dark–light cycle and free access to food and water. Animal use protocols were approved by the Institutional Animal Care and Use Committee at the University of Washington. All animal experiments were carried out in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health.

Exposure of mice to organophosphorus compounds

CPS (99.5% purity) or CPO (98% purity), purchased from Chem-Service (West Chester, Pennsylvania, USA), were dissolved in acetone (5 µl/g body weight) and applied on the shaved (4 cm^2) back of 8–12-week-old mice. Control animals received acetone only. At various times (15 min, 1 h, 3 h, 4 h, 6 h, 12 h, or 24 h) after treatment, mice were euthanized and brains and diaphragms were collected and stored at −70°C. At the time of sacrifice, each mouse was assigned a morbidity score ranging from 0 to 4, based on the scoring system detailed in Table 1. Immediately prior to sacrifice, approximately 0.1 ml of blood was collected from the saphenous vein into a heparinized microcapillary hematocrit tube. Plasma, obtained by centrifugation for 5 min, was frozen at −70°C until analysis. For all of the mice in this study, blood was collected from the saphenous vein at least 24 h prior to the experiment, for the purpose of measuring PON1 levels.

PON1 activity assays

Arylesterase activities in the plasma of mice were measured in a microtiter plate reader (SPECTRAmax Plus, Molecular Devices, Sunnyvale, California, USA) as previously described [20]. Appearance of plasma PON1 was determined by measuring the initial rates of hydrolysis of phenylacetate (arylesterase activity). The rate of hydrolysis of phenylacetate under the assay conditions used is unaffected by the Q192R polymorphism, and is related linearly to PON1 expression levels. For the assays, 2 µl plasma were diluted in 400 µl assay buffer (0.9 mM calcium chloride; 9.0 mM Tris-HCL, pH 8.0), and 100 µl of diluted plasma (equivalent to 0.5 µl whole plasma) were added to each of three wells of a UV-transparent 96-well plate. The reaction was initiated by the addition of 100 µl of phenylacetate substrate solution (6.52 mM phenylacetate; 0.9 mM calcium chloride; 9.0 mM Tris-HCl, pH 8.0). Absorbance of phenol was monitored continuously at 270 nm for 4 min at room temperature. Arylesterase values expressed in mOD/min were converted to units of activity (µmol of phenol formed per min) per ml plasma using the molar extinction coefficient for phenol (1.310 $mM^{-1} cm^{-1}$) and correcting for path-length and sample dilution.

Table 1 Morbidity scoring system

Morbidity score	Criterion/criteria
0	Normal behavior
1	Hypoactivity
2	Hindlimb paresis; immobility; heavy, irregular breathing
3	Tremors; eye secretions; hypothermia
4	Death

Acetylcholinesterase activity assays

Brain and diaphragm acetylcholinesterase (AChE) activities were measured using a microtiter plate assay based on the method of Ellman *et al.* [33], essentially as described [15,36,37], with minor modifications. Tissues were homogenized in 9 volumes of ice-cold 0.1 M sodium phosphate buffer (PB), pH 8.0, then diluted in the same buffer to 4 mg/ml (brain) or 25 mg/ml (diaphragm). For triplicate assays, 35 µl of diluted tissue homogenate were added to 315 µl 0.1 M PB, pH 8.0, and 100 µl of this were added to each of three wells of a 96-well plate. Following initiation of the kinetic assay by addition of 100 µl of freshly-prepared 2 × substrate mix (2.0 mM acetylthiocholine; 0.64 mM 5,5′-dithio-bis-nitrobenzoic acid (DTNB); 0.1 M PB pH 8.0), the formation of 5-thio-2-nitrobenzoate was monitored continuously for 10 min at room temperature by measuring absorbance at 412 nm in a microtiter plate reader (SpectraMax Plus, Molecular Devices). The initial rates of 5-thio-2-nitrobenzoate formed during the assay were calculated using an extinction coefficient of 13.6 $mM^{-1} cm^{-1}$. AChE activity was expressed as U/g of wet tissue (U = µmol of acetylthiocholine hydrolyzed per minute).

Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling

The PBPK/PD model was originally developed to describe the time-course of metabolism of CPS and CPO, and the major metabolite trichloropyridinol (TCP), as well as the inhibition of target esterases in adult rats and humans. For a detailed description of the model structure and original estimates for physiological and metabolic parameters see [36]. Briefly, in this model CYP450-mediated activation of CPS to CPO and detoxification to TCP occurs only in the liver and is described as a Michaelis–Menten process. PON1-mediated metabolism of CPO occurs in the liver and blood and is likewise described as a Michaelis–Menten process. Interaction of CPO with AChE, butyrylcholinesterase and carboxylesterase is modeled as a second-order process occurring in the liver, blood, diaphragm and brain.

The PBPK/PD rat model was used to simulate brain and diaphragm AChE inhibition in $PON^{-/-}$ mice exposed dermally to a broad range of CPS doses. To get a sense for how well the existing rat model predicts effects in mice, physiological parameters such as cardiac output, blood

flow and organ volumes were scaled allometrically to body weight, and A-esterase (PON1) values in the model were set to zero. In addition, the dermal permeability coefficient was optimized to fit the experimental data. Other than adjusting body weight for the mice, no other parameter modifications were conducted.

Statistical analysis

Statistical analysis of the data was performed with the SPSS software package, using multivariate ANOVA to determine statistically significant effects of genotype, dosage, and time on the relevant dependent variable (AChE activity or morbidity). Results are presented as mean ± SEM.

Results

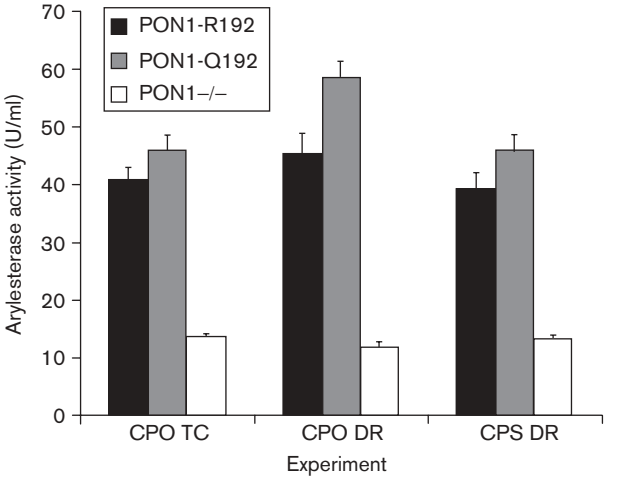
The experiments that follow examine the differences between the PON1 Q192R alloforms in protecting against exposure to CPO or its parent compound CPS. Figure 1 shows that the two transgenic strains expressing either human $PON1_{Q192}$ or human $PON1_{R192}$ in place of mouse PON1 express nearly equivalent levels of plasma hPON1, with the *hPON1*$_{Q192}$ transgenic mice expressing slightly higher levels than the *hPON1*$_{R192}$ mice. Plasma PON1 levels were determined by measuring rates of phenylacetate hydrolysis, a substrate unaffected by the Q192R polymorphism. There was a low level of arylesterase activity (~10 U/ml) in the plasma of the *PON1*$^{-/-}$ mice, demonstrating the presence of other esterases capable of hydrolyzing phenylacetate. There was no measurable paraoxonase activity in the plasma of the *PON1*$^{-/-}$ mice (data not shown).

Dose–response of CPO on morbidity and AChE inhibition

PON1$^{-/-}$ mice, *hPON1*$_{Q192}$ mice and *hPON1*$_{R192}$ mice were exposed dermally to CPO (0, 0.5, 1.0, 1.5, 2.0, or 3.0 mg/kg) dissolved in acetone. Six hours after the initial exposure, when AChE inhibition was near its maximum level, mice were sacrificed by cervical dislocation and the extent of AChE inhibition in brain and diaphragm was assessed by measuring total AChE activity. AChE activities in the diaphragms and brains of vehicle-treated mice were equivalent among the three lines of mice (Fig. 2d). With CPO exposure, *PON1*$^{-/-}$ mice were highly sensitive to AChE inhibition in both brain (Fig. 2a) and diaphragm (Fig. 2b), exhibiting 90% inhibition of AChE in both brain and diaphragm at a dose of 1.5 mg/kg.

In contrast, *hPON1*$_{R192}$ mice had only a 20% reduction in brain AChE at this dose, and the dose–response curves for AChE inhibition in brain and diaphragm were both shifted significantly to the right relative to that seen in the *PON1*$^{-/-}$ mice. The *hPON1*$_{Q192}$ mice were significantly more sensitive than *hPON1*$_{R192}$ mice to AChE inhibition in both brain and diaphragm, with the dose–response curves intermediate between those for the *PON1*$^{-/-}$ and *hPON1*$_{R192}$ mice. The approximate IC_{50} values for inhibition of brain AChE in *PON1*$^{-/-}$, *hPON1*$_{Q192}$, and *hPON1*$_{R192}$ mice were 1.0, 1.3, and 2.4 mg/kg, respectively. For diaphragm AChE, the respective IC_{50} values were approximately 0.9, 1.1, and 1.8 mg/kg. Morbidity associated with CPO exposure was also assessed at 6 hr using the scoring system outlined in Table 1. In contrast to the *hPON1*$_{R192}$ mice, which exhibited only minimal outward signs of toxicity at the higher doses, both *PON1*$^{-/-}$ and *hPON1*$_{Q192}$ mice had significantly higher morbidity associated with exposure to CPO. At 3 mg/kg, two of the *PON1*$^{-/-}$ and one of the *hPON1*$_{Q192}$ mice died from CPO exposure, whereas none of the *hPON1*$_{R192}$ mice died at this dose. Tests of between-subjects effects by multivariate ANOVA revealed significant effects of genotype on morbidity ($P < 0.001$), brain AChE inhibition ($P < 0.001$), and diaphragm AChE inhibition ($P < 0.05$), with a highly significant effect ($P < 0.0001$) of dosage on the three parameters, and a significant interaction between genotype and dosage ($P < 0.01$).

Fig. 1

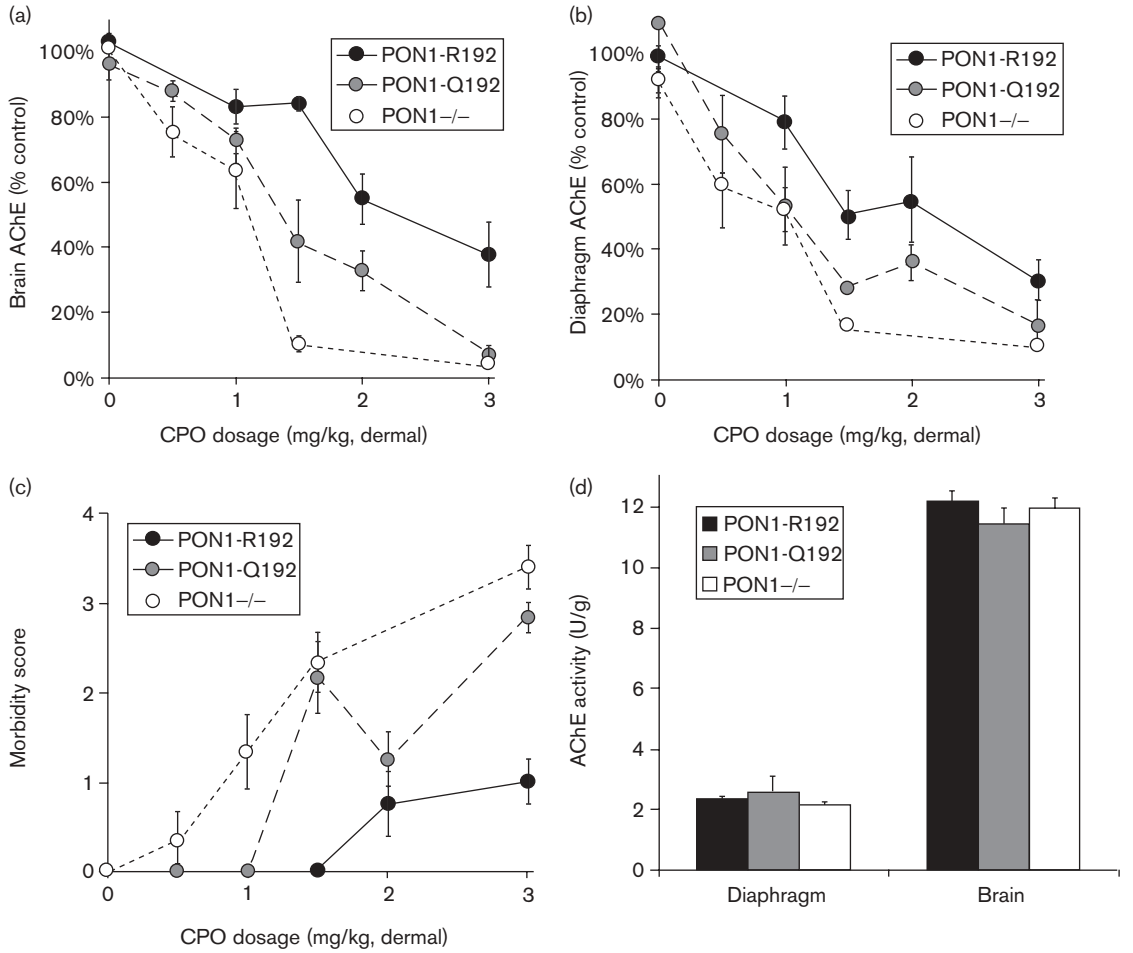


Plasma PON1 levels (arylesterase activity) in the *hPON1*$_{Q192}$, *hPON1*$_{R192}$, and *PON1*$^{-/-}$ mice used in the CPO time course (CPO TC), CPO dose response (CPO DR) and CPS dose response (CPS DR) experiments. Plasma arylesterase activity was measured in all mice at least 24 h prior to dosing by measuring initial rates of hydrolysis of phenylacetate. Since the arylesterase activity of PON1 is unaffected by the Q192R polymorphism, it provides an unbiased measure of PON1 levels. Data are shown as mean ± SEM, with $n = 24$ to 39 mice.

Time course of morbidity and AChE inhibition following CPO exposure

The time course of AChE inhibition following exposure to CPO was determined by exposing *PON1*$^{-/-}$, *hPON1*$_{Q192}$ and *hPON1*$_{R192}$ mice to CPO (1.2 mg/kg, dermal), followed by sacrifice at 1, 3, 6, 12, or 24 h post-exposure (Fig. 3). Immediately prior to sacrifice,

Fig. 2

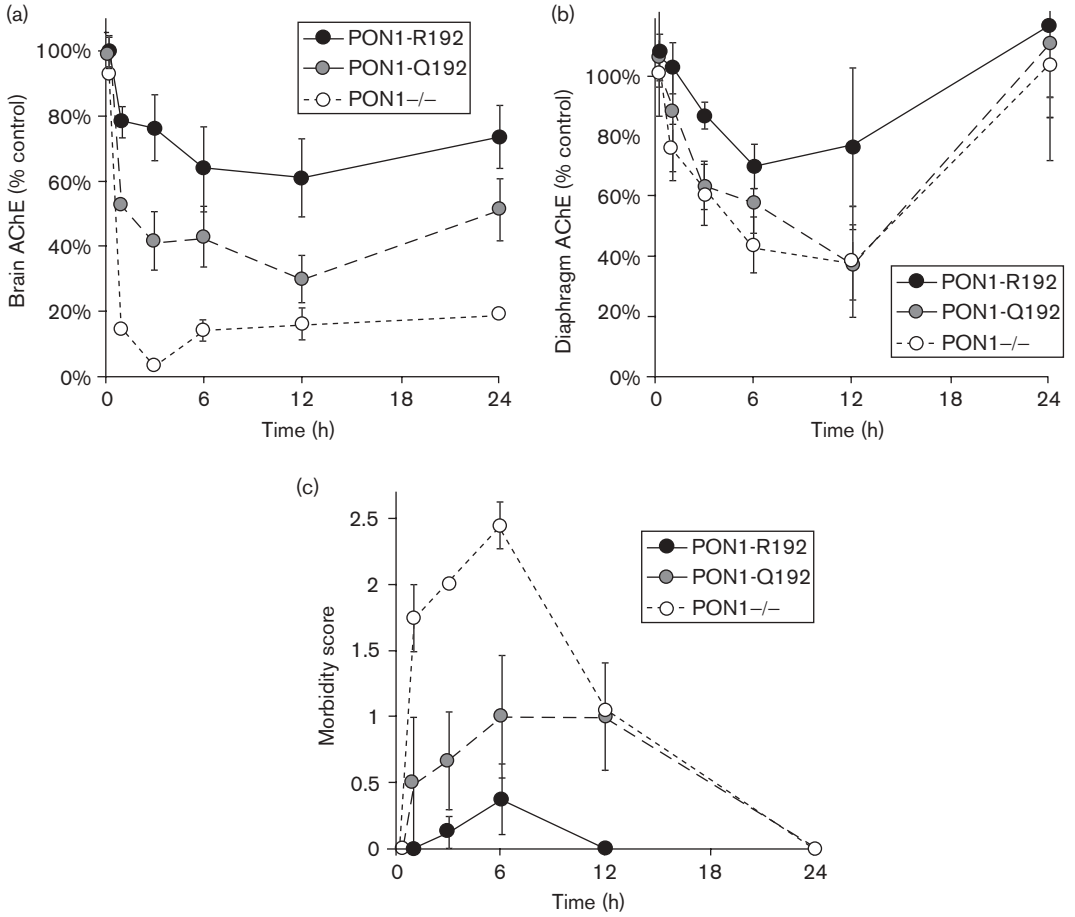


Dose–response curves of CPO on morbidity and on AChE activity in the brain and diaphragm of $hPON1_{Q192}$, $hPON1_{R192}$, and $PON1^{-/-}$ mice at 6 h post-exposure. (a) Dose–response of CPO on inhibition of brain AChE activity; (b) Dose–response of CPO on inhibition of diaphragm AChE activity; (c) Dose–response of CPO on morbidity, scored as shown in Table 1; (d) AChE activities (units/g) measured in brain and diaphragm of control mice exposed to vehicle (acetone). Data are shown as mean ± SEM, with $n = 6$–8 mice.

morbidity associated with CPO exposure was scored as described in Table 1 for each mouse. Maximal inhibition of AChE occurred between 6 and 12 h following exposure, with the exception of brain AChE in the $PON1^{-/-}$ mice, which declined steeply within the first 3 h (Fig. 3a). At 24 h, brain AChE activity had not yet returned to normal levels, but diaphragm AChE activity had recovered to 100% of control levels (Fig. 3a and b). $PON1^{-/-}$ mice had significantly higher inhibition of brain AChE (80–90% inhibition) than did the $hPON1_{Q192}$ or $hPON1_{R192}$ mice, and $hPON1_{Q192}$ mice had an intermediate phenotype that was significantly different from that seen in the $hPON1_{R192}$ mice (Fig. 3a). At 1.2 mg/kg, brain AChE was inhibited by 75% in the $hPON1_{Q192}$ mice, in contrast to the 40% inhibition of AChE in the $hPON1_{R192}$ mice. In the diaphragm, the time course of AChE inhibition was similar between the $PON1^{-/-}$ and $hPON1_{Q192}$ mice, and AChE inhibition in both of these lines of mice was significantly greater than that seen in the $hPON1_{R192}$ mice (Fig. 3b).

The time course of morbidity following CPO exposure generally followed the time course of AChE inhibition, with maximal morbidity observed at 6 h (Fig. 3c). Whereas $hPON1_{R192}$ mice exhibited mostly normal behavior, $PON1^{-/-}$ mice exhibited signs of toxicity that included immobility, hindlimb paresis, and in some cases hypothermia or tremors. The $hPON1_{Q192}$ mice had an intermediate phenotype, usually exhibiting hypoactivity and in some cases immobility or hindlimb paresis. Tests of between-subjects effects by multivariate ANOVA revealed significant effects of genotype on morbidity ($P < 0.05$), brain AChE inhibition ($P < 0.001$), and diaphragm AChE inhibition ($P < 0.05$), with a significant effect ($P < 0.005$) of dosage on the three parameters. Significant interactions ($P < 0.05$) occurred between

Fig. 3



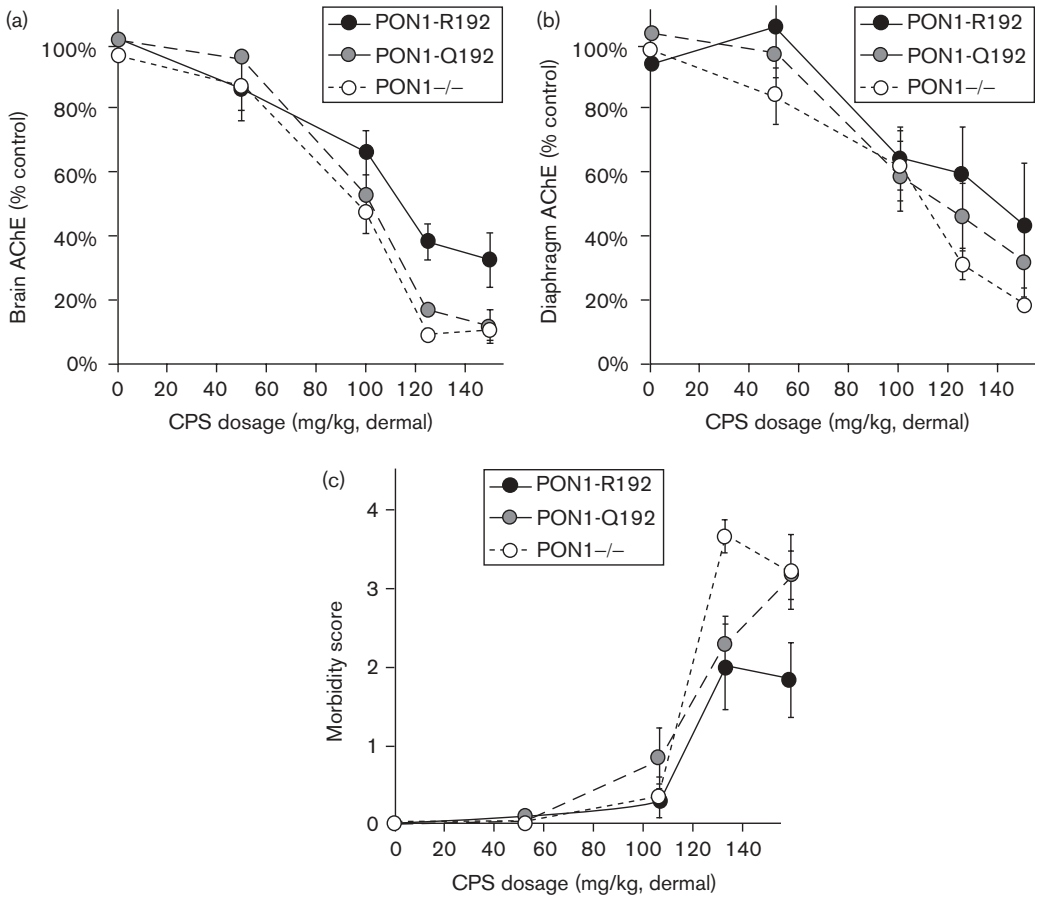
Time-course of morbidity and of AChE inhibition in brain and diaphragm following exposure of $hPON1_{Q192}$, $hPON1_{R192}$, and $PON1^{-/-}$ mice to CPO (1.2 mg/kg, dermal). (a) Time-course of inhibition of brain AChE activity following CPO exposure; (b) Time-course of inhibition of diaphragm AChE activity following CPO exposure; (c) Time-course of morbidity following CPO exposure, scored as shown in Table 1. Data are shown as mean ± SEM, with $n = 4–9$ mice.

genotype and dosage, and between dosage and time, with the exception of diaphragm ChE inhibition, which did not show a significant interaction between genotype and dosage.

Dose–response of CPS on morbidity and AChE inhibition

The contribution of PON1 for protecting against exposure to the parent organophosphorothioate, CPS, was assessed by constructing dose–response curves of CPS exposure on brain and diaphragm AChE inhibition. $PON1^{-/-}$ mice, $hPON1_{Q192}$ mice and $hPON1_{R192}$ mice were exposed dermally to CPS (0, 50, 100, 125, or 150 mg/kg) dissolved in acetone. Six hours after the initial exposure, mice were sacrificed by cervical dislocation and the extent of AChE inhibition in brain and diaphragm was assessed as described above. A nearly 100-fold higher dosage of CPS (Fig. 4) was required to produce an effect equivalent to that observed with CPO (Fig. 2). With CPS exposure, $PON1^{-/-}$ mice and $hPON1_{Q192}$ mice were significantly more sensitive than $hPON1_{R192}$ mice to AChE inhibition in both brain and diaphragm (Fig. 4a and b), albeit to a lesser extent than seen with exposure to CPO. Higher dosages of CPS (175–300 mg/kg) were lethal, with 32% of the $PON1^{-/-}$ mice (seven mice), 33% of the $hPON1_{Q192}$ mice (eight mice), and 8% of the $hPON1_{R192}$ mice (two mice) dying before 6 h. Morbidity following CPS exposure was highly variable and had a different presentation than that associated with CPO exposure, with more subtle signs of morbidity, especially at the lower doses (Fig. 4c). Tests of between-subjects effects by multivariate ANOVA revealed significant effects of genotype on brain AChE inhibition ($P < 0.05$), and diaphragm AChE inhibition ($P < 0.05$), but not on morbidity. There were highly significant effects ($P < 0.0001$) of dosage on the three parameters,

Fig. 4



Dose–response curves for CPS inhibition of AChE activity in the brain and diaphragm of $hPON1_{Q192}$, $hPON1_{R192}$, and $PON1^{-/-}$ mice at 6 h post-exposure. (a) Dose–response of CPS on inhibition of brain AChE activity; (b) Dose–response of CPS on inhibition of diaphragm AChE activity; (c) Dose–response of CPO on morbidity, scored as shown in Table 1. Data are shown as mean ± SEM, with $n = 6$–8 mice.

and no significant interactions between genotype and dosage; indicating that the effect of genotype on acetylcholinesterase inhibition was not dependent on the dosage.

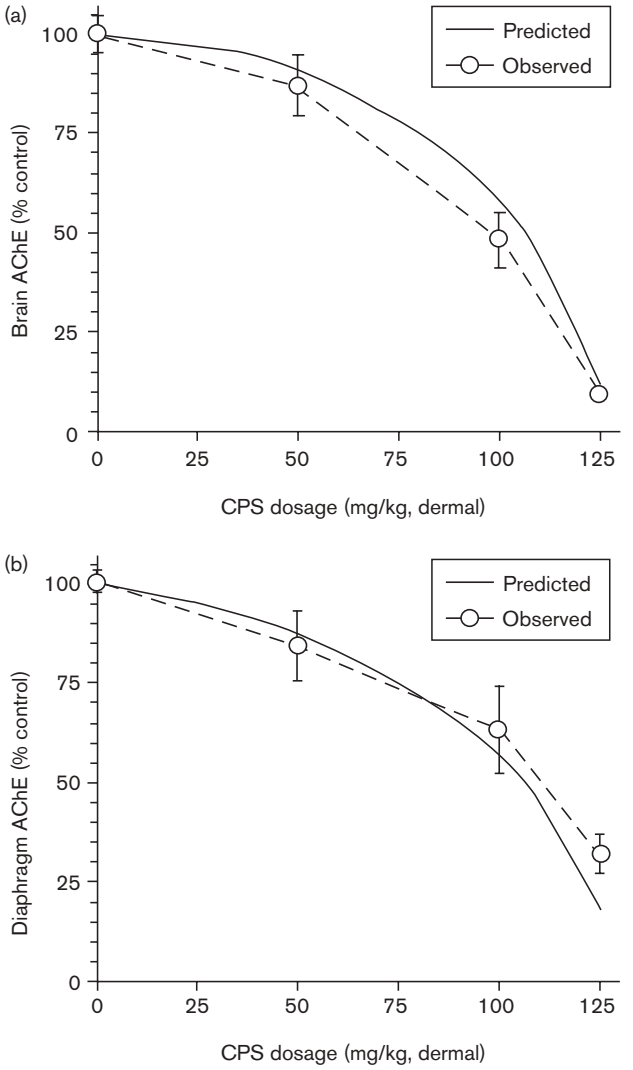
PBPK/PD model

One objective of our studies was to modify the current PBPK/PD model for CPS [36] to more adequately describe both the biokinetic time-course of CPS and CPO and the inhibition of target esterases following dermal exposure to these OPs, using mice that express no PON1 activity ($PON1^{-/-}$ mice). Initial simulations of diaphragm and brain AChE showed dose-dependent inhibition in the $PON1^{-/-}$ mice following dermal exposure to a broad range of CPS doses. Figure 5 shows the close agreement between the values of brain and diaphragm AChE observed in the dose response of $PON1^{-/-}$ mice in the current study and the dose–response curves predicted by the PBPK/PD model. The close correspondence between the predicted and observed results in the $PON1^{-/-}$ mice provides a starting point for refining the PBPK/PD model to take into account differences in detoxication efficacy due to the $PON1_{Q192R}$ polymorphism.

Discussion

One of the major goals of the environmental genome project is aimed at identifying biomarkers of susceptibility. The discovery and analysis of single nucleotide polymorphisms (SNPs) associated with risk for specific diseases is one of the major benefits of this effort [37]. Our earlier studies on the human PON1 polymorphisms demonstrated that a SNP at codon 192 that specified Arg or Gln in PON1 affected the catalytic efficiency of hydrolysis of specific substrates [15] and has been reported by some research groups but not others to be a marker for risk for vascular disease [29,38]. A second C/T polymorphism in the 5′ regulatory region at position –108 in a consensus Sp1 binding site has been shown by

Fig. 5



PBPK/PD model predictions. Comparison of the effects of CPS exposure on AChE inhibition in *PON1*$^{-/-}$ mice, as predicted by the PBPK/PD model [36], to those observed in the current study over a realistic range of CPS doses (25 to 125 mg/kg). (a) inhibition of brain AChE; (b) inhibition of diaphragm AChE. Data are shown as mean ± SEM.

our research group [18,19] and others [39,40] to have a major influence on *PON1* expression and plasma levels. Toxicology studies by our research group in a mouse model system have demonstrated that for the case of CPS/CPO exposures, both position 192 genotype and PON1 levels are important determinants of sensitivity [15].

In the current study, transgenic mice expressing human $PON1_{Q192}$ were more sensitive than mice expressing human $PON1_{R192}$ to the toxicity associated with exposure to CPS or its metabolite, CPO. The use of transgenic mice with equivalent levels of PON1 in both liver and plasma allowed direct comparison of the relative importance of the two $PON1_{Q192R}$ alloforms for OP detoxification *in vivo*, without the complication of variability in PON1 levels seen in normal human populations. The results indicate that adults carrying the R192 alloform would be approximately twice as resistant to the effects of CPO compared to adults carrying the Q192 alloform. Our previous results indicated that there is also considerable variability in PON1 levels, both among adults (at least 13-fold among individuals of the same genotype), and across early postnatal development [20]. The experimental results seen in *PON1*$^{-/-}$ mice corresponded closely to those predicted by a PBPK/PD model of CPS exposure developed for the human and rat [36]. This PBPK/PD model for CPS dosimetry behaves in a manner consistent with what is generally known about the toxicity, pharmacokinetics, and pharmacodynamics of CPS and its metabolites, CPO and TCP [36]. The model was previously shown to provide an accurate estimation of changes in CPS and TCP levels over time, as demonstrated by comparing data predicted by the model to that observed in humans who were exposed to known concentrations of CPS [36]. The close correspondence between the results observed in the *PON1*$^{-/-}$ mice and those predicted by the model provides a starting point for modifying the PDPK/PD model of human exposure to simulate the risk implications of variability in PON1 status. A previous study coupled Monte Carlo analysis with the PBPK/PD model of CPS metabolism to estimate a theoretically delivered dose of CPO to the brain [41]. This model simulation predicted that the $PON1_{Q192R}$ polymorphism would have the greatest impact on CPS metabolism and detoxification at dose levels greater than 0.5 mg/kg, and that at lower-level exposures other esterase detoxification pathways would be capable of compensating for the inter-individual differences in CPOase activity due to the $PON1_{Q192R}$ polymorphism [41]. Consistent with this prediction, the current study demonstrated differences in toxicity between the $hPON1_{Q192}$ and $hPON1_{R192}$ mice across CPS doses ranging from 50 to 150 mg/kg and CPO doses ranging from 0.5 to 3 mg/kg, doses that were well above the threshold of effect predicted for the Q192R polymorphism [41]. A pronounced effect of the Q192R polymorphism was observed even with low-level exposures producing relatively low (< 20%) inhibition of brain acetylcholinesterase.

Mice expressing $hPON1_{Q192}$ were particularly sensitive to the toxicity associated with exposure to CPO. CPS, the parent organophosphorothioate compound of CPO, is converted to CPO in the liver via CYP450-mediated oxidative desulfuration [42]. Because organophosphorus compounds can also be converted to their oxon forms by non-enzymatic processes in the environment, the oxon

forms can represent a significant contaminant in foliar residues [43]. Thus, human exposures are likely to involve a mixture of CPS and CPO. Our data indicate that CPO is about 100 times more toxic than CPS *in vivo*, and biochemical data indicate that CPO inactivates AChE 1000 times faster than CPS [44,45]. Thus, CPO contamination as low as 1% would represent a significant contaminant in terms of toxicity associated with exposure.

Conclusion

The relevance of the Q192R polymorphism for protecting against *in vivo* exposure to CPS and CPO was addressed through the use of transgenic mice expressing equivalent levels of either of the two human allozymes in the absence of endogenous mouse PON1. The heightened sensitivity to both CPO and CPS of mice carrying the human $PON1_{Q192}$ allozyme indicates that individuals expressing the $PON1_{Q192}$ allele would be more sensitive than individuals expressing the $PON1_{R192}$ allele to the adverse effects of CPO or CPS exposure, especially if they have a low level of plasma PON_{Q192}. These findings are particularly significant due to the prevalence of the $PON1_{Q192}$ allele (0.3 to 0.7 depending on the population) in the human population [29].

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