Considerations for Exposure to Diazinon and Chlorpyrifos

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Goals of This Presentation

The purpose of this brief presentation is to share with you what we have learned about human genetic variability in paraoxonase 1 (PON1) and the consequences of this variability with respect to gene/environment interactions, specifically the role of PON1 in protecting against exposure to organophosphorus insecticides, particularly diazinon/diazoxon and chlorpyrifos/chlorpyrifos oxon.

PON1 is a high density lipoprotein (HDL) associated enzyme of 354 amino acids that plays a significant role in the hydrolysis of the highly toxic diazinon metabolite diazoxon. (Also the toxic metabolite of chlorpyrifos, chlorpyrifos oxon).

The presentation also describes the results of experiments carried out in a mouse model. These experiments were designed to provide information on the physiological consequences of the PON1 genetic variability in human populations.

Detoxicaton of OP Insecticides

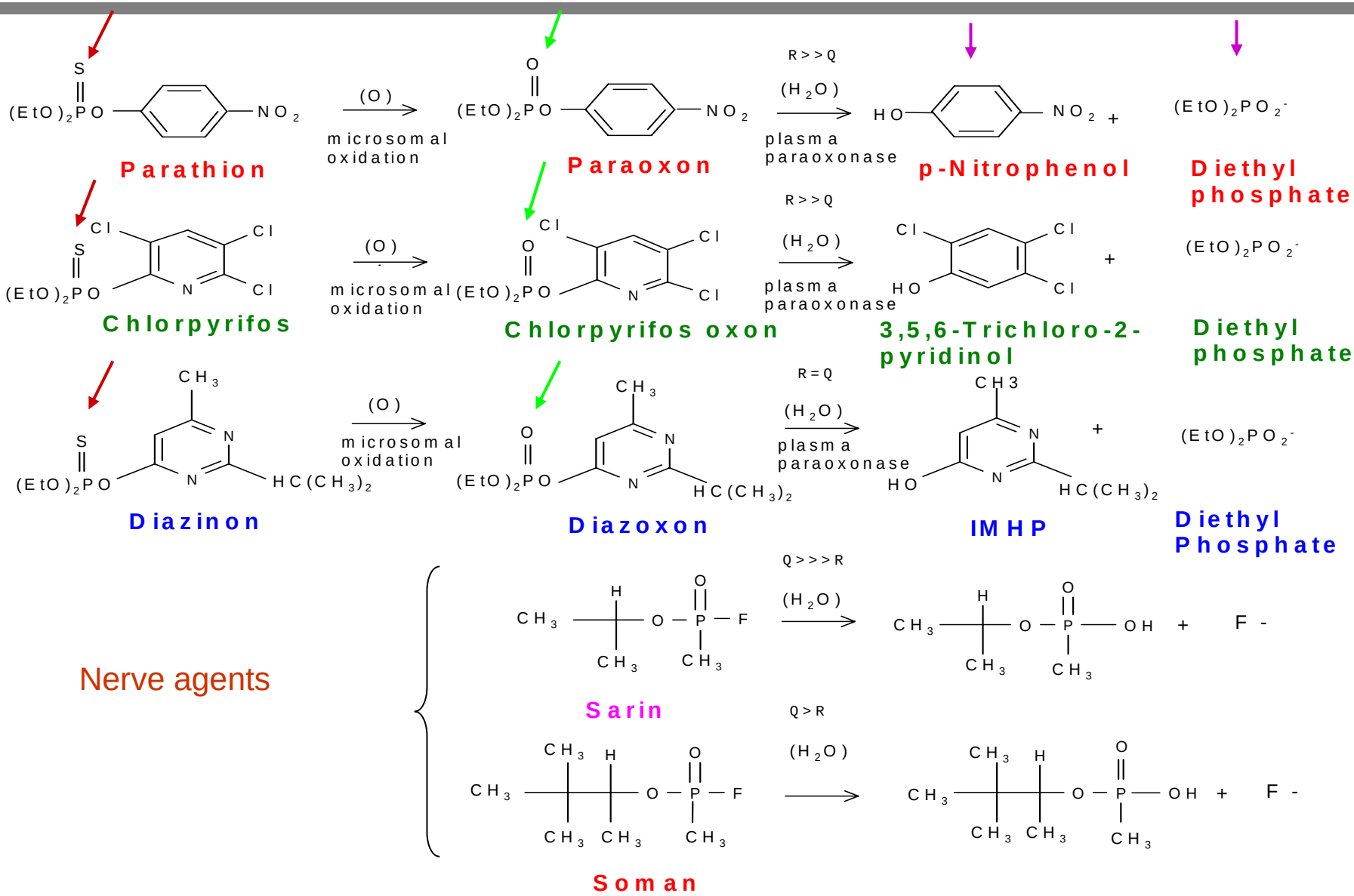
The commonly used organophosphorus insecticides parathion, chlorpyrifos and diazinon are manufactured as organoposphorothioates. These compounds are very poor inhibitors of cholinesterases. In organisms (target and non-target) the thioate is converted to an oxon form by cytochromes P450. Also, as discussed below, actual exposures include both parent thioate residues as well as the highly toxic oxon forms.

It was thought that mammals could detoxify the oxons as rapidly as they were formed. However, in recent years, it has become apparent that there is considerable variability in different individuals' plasma paraoxonase (PON1) levels that are controlled developmentally and genetically.

The following slides will elaborate on these factors and the consequence of high vs. low plasma PON1 levels.

An additional concern based on recent findings of researchers from North Carolina State University is that the thioates are suicide substrates for the P45O enzymes that catalyze the oxidative desulfuration of the parent compounds. Of particular interest is the inactivation of cytochromes P450 3A4 and 1A2 that are important in the metabolism of testosterone and estradiol.

Cytochrome P450-Paraoxonase (PON) pathway for Organophosphate Detoxification



Davies et al., Nature Genetics 1996

Problems with Safety Tests

- Most if not all safety tests were carried out with highly pure parent compounds (usuallly >99%).
- Exposures may contain a significant percentage of highly toxic oxon form of the OP.
- The oxon form is a much more potent inhibitor of cholinesterase than parent compound
- The genetic and developmental variability of sensitivity to the oxon component is significant
- Thioates are suicide substrates for P450s

Concerns about Product Safety Tests

One of the important factors to consider is how the safety tests were carried out with respect to what we now know about the genetically and developmentally variable sensitivity to diazinon/diazoxon exposures.

Safety tests were carried out with highly pure parent compounds, which at the time were the types of tests required by regulatory agencies.

Examples of Purity of Parent Compounds Used for Safety Tests

Safety studies with diazinon used parent compound of 99.5% purity..

For details see: *The reconsideration of approvals of the active constituent diazinon, registrations of products containing diazinon and approval of their associated labels. Part 2 Preliminary Review Findings Volume 2 of 2 Technical Reports, June 2006. Australian Pesticides & Veterinary Medicines Authority. Canberra Australia*

Safety studies with chlorpyrifos oxon used parent compound of very high purity.

Nolan RJ, Rick DL, Freshour NL, Saunders JH. (1984) Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol; 73: 8–15.

Literature Survey of Oxon Values in Leaf Foliar Residues

Table 1 Oxon levels in total pesticide residues taken from dislodgeable leaf foliar residue and dermal exposure studies

	Pesticide (units)	Oxon[a]	Thioate	Total OP	Oxon (%)
Ralls et al. (1966)	Diazinon (ppm[d])	0.05	0.25	0.3	17
Kansoug and Hopkins (1968)	Diazinon[b]	ND[c]	-	-	ND
Wolfe et al. (1975)	Parathion (ng/cm^2)[d]	8	106	114	7
Kraus et al. (1977)	Azinphosmethyl (%)[d]	0.05	99.95	100	0.05
Nigg et al. (1977)	Ethion (ng/cm^2)[d]	42	285	327	13
Spear et al. (1977a)	Parathion (ng/cm^2)[d]	84	29	113	74
	Parathion (μg)[e]	145	39	184	79
Spear et al. (1977b)	Parathion (ng/cm^2)[d]	229	8	237	97
Maddy and Meinders (1987)	Azinphosmethyl (μg)[e]	ND	-	-	ND
Costello et al. (1989)	Malathion (μg)[e]	659	2301	2960	22
Schneider et al. (1990)	Azinphosmethyl (ng/cm^2)[d]	0.008	0.31	0.32	2.5
	Azinphosmethyl (μg)[e]	272	1450	1722	16
Spencer et al. (1991)	Azinphosmethyl (%)[d]	15	85	100	15
McCurdy et al. (1994)	Azinphosmethyl[b]	-	-	-	2.3

[a]Based on the highest value reported in study.
[b]Units or values not given in study.
[c]ND, none detected.
[d]Foliar residue measurement.
[e]Dermal monitoring measurement.

Yuknavage et al., J. Toxicol. Environ. Health 1997; 51:35-55

Oxon Residues in Exposures

Real-life exposures, contain variable levels of highly toxic oxon components. In the study by Ralls et al., the oxon content of the diazinon residues represented 17% of the total residue. In light of what is now known, it makes sense for safety tests to include a range of oxon contents that include percentages of oxon likely to be encountered in actual exposures.

[Ralls, J. W., Gilmore, D. R., and Cortes, A. 1966. Fate of radioactive *O,O*-diethyl *O*-(2-iso-propyl-4-methylpyridmidin-6-yl) phosphorothioate on field-grown experimental crops. *J. Agric. Food Chem.* 14:387–392.]

Inhibition of ChE By CPS/CPO

TABLE 1

Inhibition of brain AChE activity

OP	K_a (M)	k_2 (min^{-1})	k_i (M^{-1} min^{-1})
Chlorpyrifos	$(2.84 \pm 1.03) \times 10^{-4}$	0.82 ± 0.21	$(3.22 \pm 0.48) \times 10^{3}$
Chlorpyrifos oxon	$(7.31 \pm 2.52) \times 10^{-7}$	2.21 ± 0.60	$(3.18 \pm 0.23) \times 10^{6}$

Dissociation constants (K_a), phosphorylation constants (k_2) and bimolecular rate constants (k_i) were calculated from Main plots like those in figure 1. Data are means and standard errors from three experiments.

Chlorpyrifos oxon (CPO, the toxic metabolite of chlorpyrifos) inhibits brain cholinesterase at approximately 1000-times the rate of chlorpyrifos (CPS). This is an important observation in light of the importance of the PON1 polymorphism in detoxifying parent organophosphorothioates (e.g., chlorpyrifos and diazinon) and the oxon contents of residues.

Huff et al. J Pharmacol Exp Therapeutics 269:329-335(1994)

Some Concerns About the Parent Organophosphorothioates

For many years, it was thought that the parent organophosphorothioates were quite safe compounds, i.e. they are very poor inhibitors of cholinesterases. However, recent studies reported by Usamani and colleagues at Duke University (see next slide) noted that cytochrome P450 3A4 was inhibited during the bioactivation of parent organophosphorothioate compounds. Since this P450 is an important enzyme in testosterone metabolism, this raises a number of concerns about exposures to the parent compound, particularly questions about consequences of exposure during critical windows of development and affects on reproductive health.

Concerns about the parent thioates

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INHIBITION AND ACTIVATION OF THE HUMAN LIVER MICROSOMAL AND HUMAN CYTOCHROME P450 3A4 METABOLISM OF TESTOSTERONE BY DEPLOYMENT-RELATED CHEMICALS

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(Received October 16, 2002; accepted December 18, 2002)

“Preincubation of CYP3A4 with chlorpyrifos, but not chlorpyrifos-oxon, resulted in 98% inhibition of TST metabolism.”

Gene Frequency of PON1 Activity Polymorphism

Early studies of the genetic variability of serum paraoxonase (PON1) activities in different ethnic groups. Note in the next slide the different allele frequencies of the PON1 activity polymorphism in different populations. In populations of Northern European origin, approximately one-half of the populations were low metabolizers. Other populations of African or Asian origin had very few low metabolizers (For an excellent review of the early PON1 studies, see Geldmacher v.-Mallinckrodt and Diepgen, Toxicol and Environ Chem 1988; 18:79-196).

Examples of the Polymorphic Distribution of PON1 Activity in Different Populations

See below that this analysis will not resolve 3 phenotypes accurately

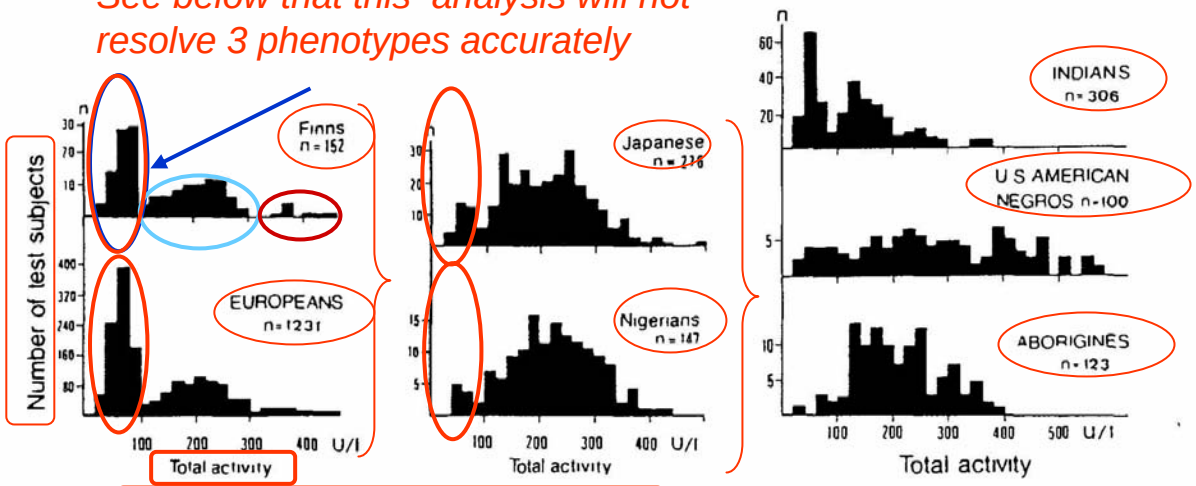


Fig. 2. Serum paraoxonase activity in different ethnic groups

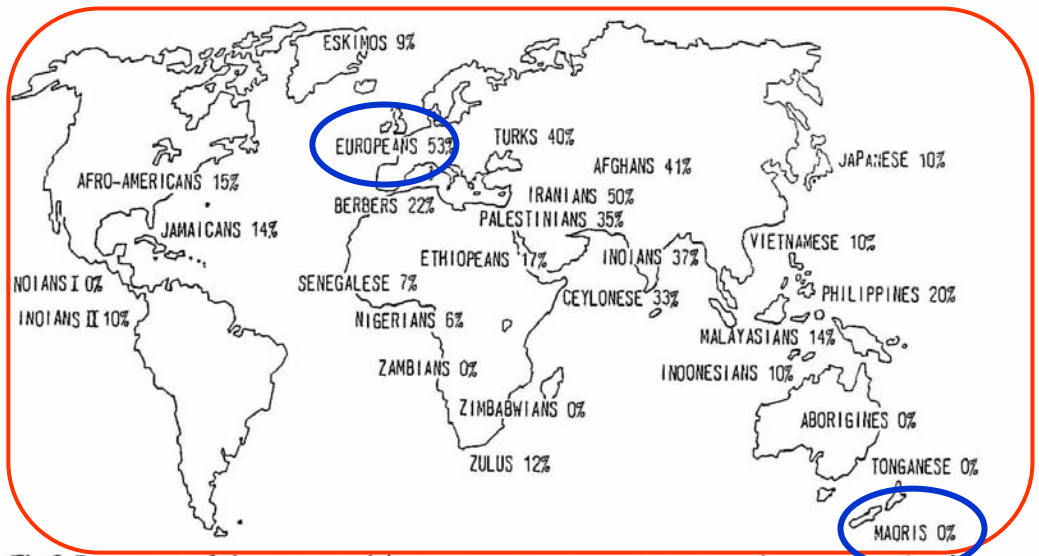


Fig. 3. Percentages of phenotypes with low serum paraoxonase activity in random samples taken from different ethnic groups

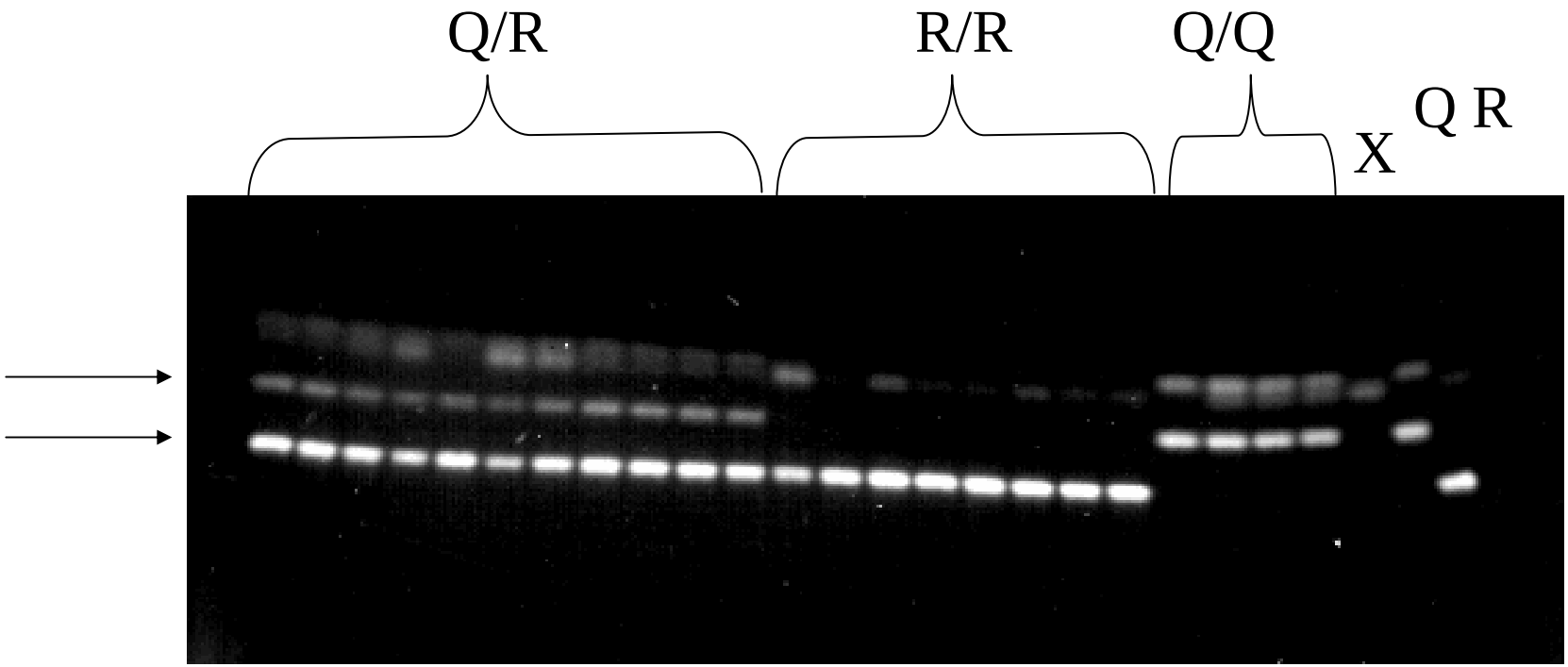
T.L. Diepgen & M. Geldmacher-v. Mallinckrodt. Arch. Toxicol. Suppl. 9, 154-158 (1986)

DNA Analysis of the PON1-192 Polymorphism

A DNA segment that includes the polymorphic site that specified which amino acid appears at position 192 is amplified by enzymes in a process referred to as a polymerase chain reaction (PCR). A method that has come to public attention through highly visible criminal trials. The resulting fragments of DNA are exposed to a specific restriction enzyme that will cut DNA containing the codon for Q, but not for R. The fragments are separated by an electrophoretic procedure, then stained and photographed.

In the next slide, the uncut polymerase chain reaction (PCR) product runs at the position of the upper arrow, while the cut sequence runs at the position of the lower arrow. The genotypes of the individuals are shown above their respective band patterns. X = no DNA in the amplification reaction. Q = DNA from a Q/Q homozygote, R from an R/R homozygote. The PON1-R192 allele was shown to be the high paraoxonase activity allele and the PON1-Q192 allele the low metabolizer allele. As noted below, we recommend not using this protocol, but instead, a functional analysis that provides additional information on PON1 levels which are as important or more important than the amino acid present at position 192.

PCR Analysis of $PON1_{192}$ Genotype



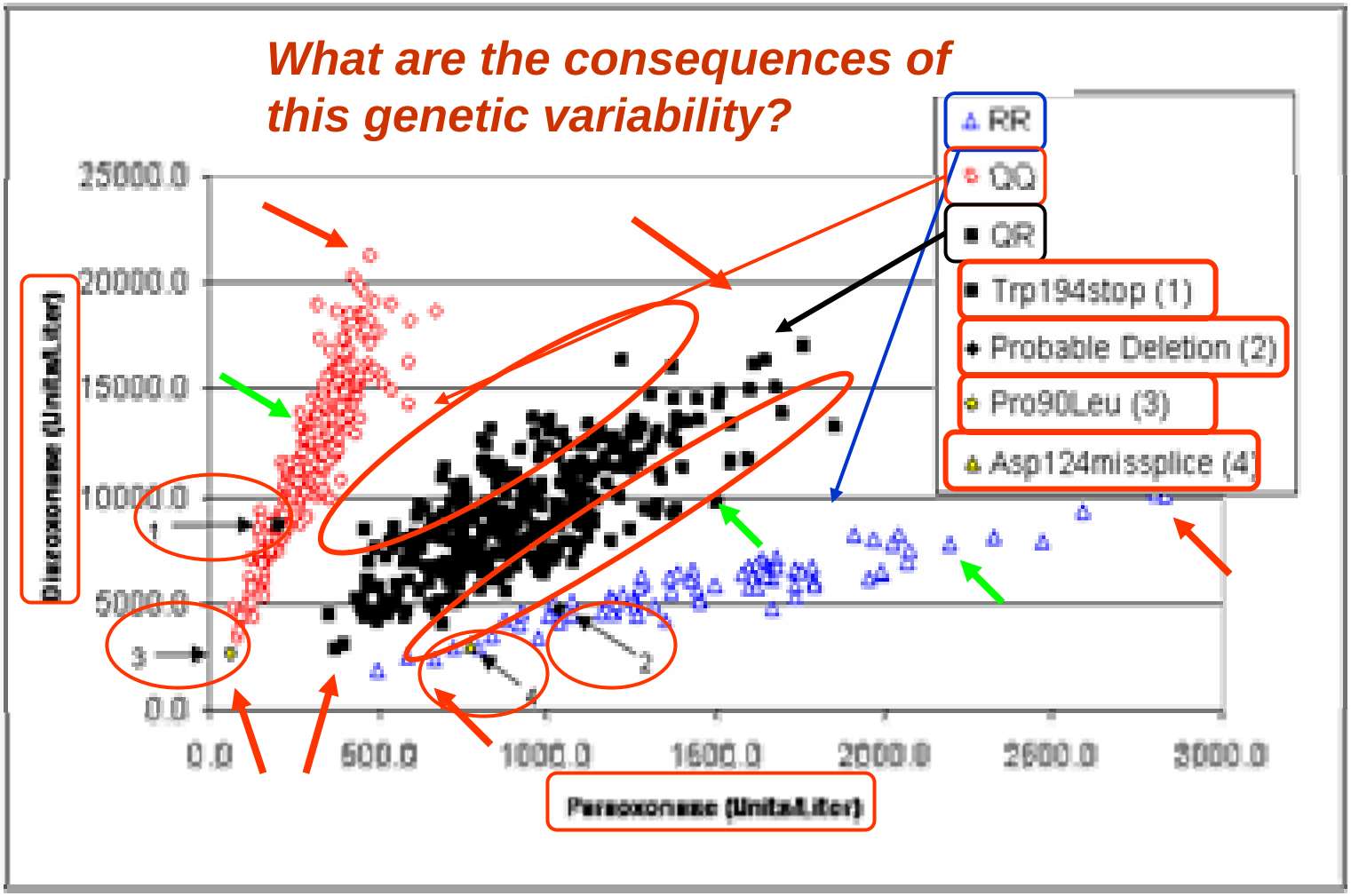
Humbert et al. Nature Genet 3:73-76

PON1 Status

Recently, much better functional two-substrate assays have been developed that separate populations into individuals with specific functional genotypes as will be described below. The assay also provides the level of enzyme present in the plasma of each individual. An important genetic variability in the amino acid present at position 192 of this 355 amino acid protein [glutamine (Q) or arginine (R)] determines whether the PON1 in an individual can hydrolyze paraoxon rapidly or slowly. Since the two so-called alloforms of paraoxonase (PON1-Q192 or PON1-R192) have different properties, this analysis provides the resolution of phenotypes shown in the slide. In the data shown in this slide, DNA analysis was also carried out. There were some discrepancies observed, where the DNA sequence was observed to specify a heterozygous genotype at position 192 (Q/R) where as the functional assay showed that only one alloform was present in the individual's plasma. Further studies involving sequencing the entire PON1 genes of these individuals elucidated the reason for the discrepancy. These individuals had PON1 genes that were defective at regions of the gene away from that analyzed by the DNA analysis protocol as noted in the slide. These observations serve to illustrate the accuracy of the functional 2-substrate assay.

[Richter, RJ and Furlong, CE. 1999. Determination of paraoxonase (PON1) status requires more than genotyping. Pharmacogenetics **9**:745-753; Jarvik GP, R Jampsa, RJ Richter, C Carlson, M Rieder, D Nickerson and CE Furlong. 2003. Novel Paraoxonase (PON1) nonsense and missense mutations predicted by functional genomic assay of PON1 status. Pharmacogenetics **13**:291-295.]

Determination of PON1 Status



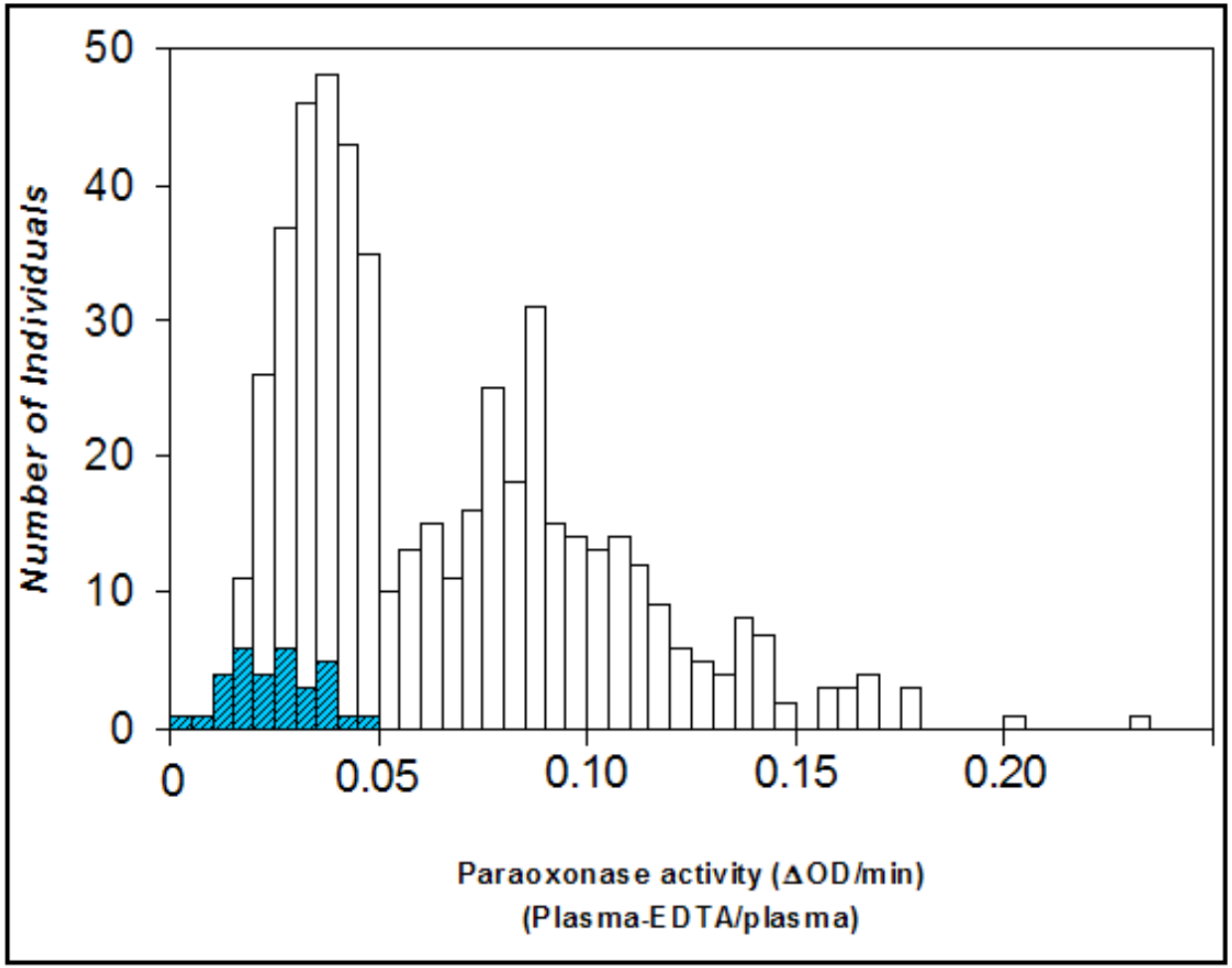
Newly discovered PON1 SNPs resolve anomalous individuals in the correlation of enzyme activities and PON1 Q192R genotypes

Jarvik et al. 2003. Pharmacogenetics 13:291-295

Why are young individuals more sensitive to OP compounds?

Developmental regulation of plasma PON1 levels is such that newborns have only 1/4th to 1/3rd the levels of plasma PON1 compared with adults.

[Cole TB, RL Jampsa, BJ Walter, TL Arndt, RJ Richter, DM Shih, A Tward, AJ Lusis, RM Jack, LG Costa, and CE Furlong. 2003. Expression of human paraoxonase (PON1) during development. Pharmacogenetics **13**:357-364 and references cited therein.]



Population study of paraoxonase activity in 531 random donors (White columns) and 31 samples of newborns (Hatched columns)

From: Mueller et al, Am J Hum Genet 35:393-408 1983

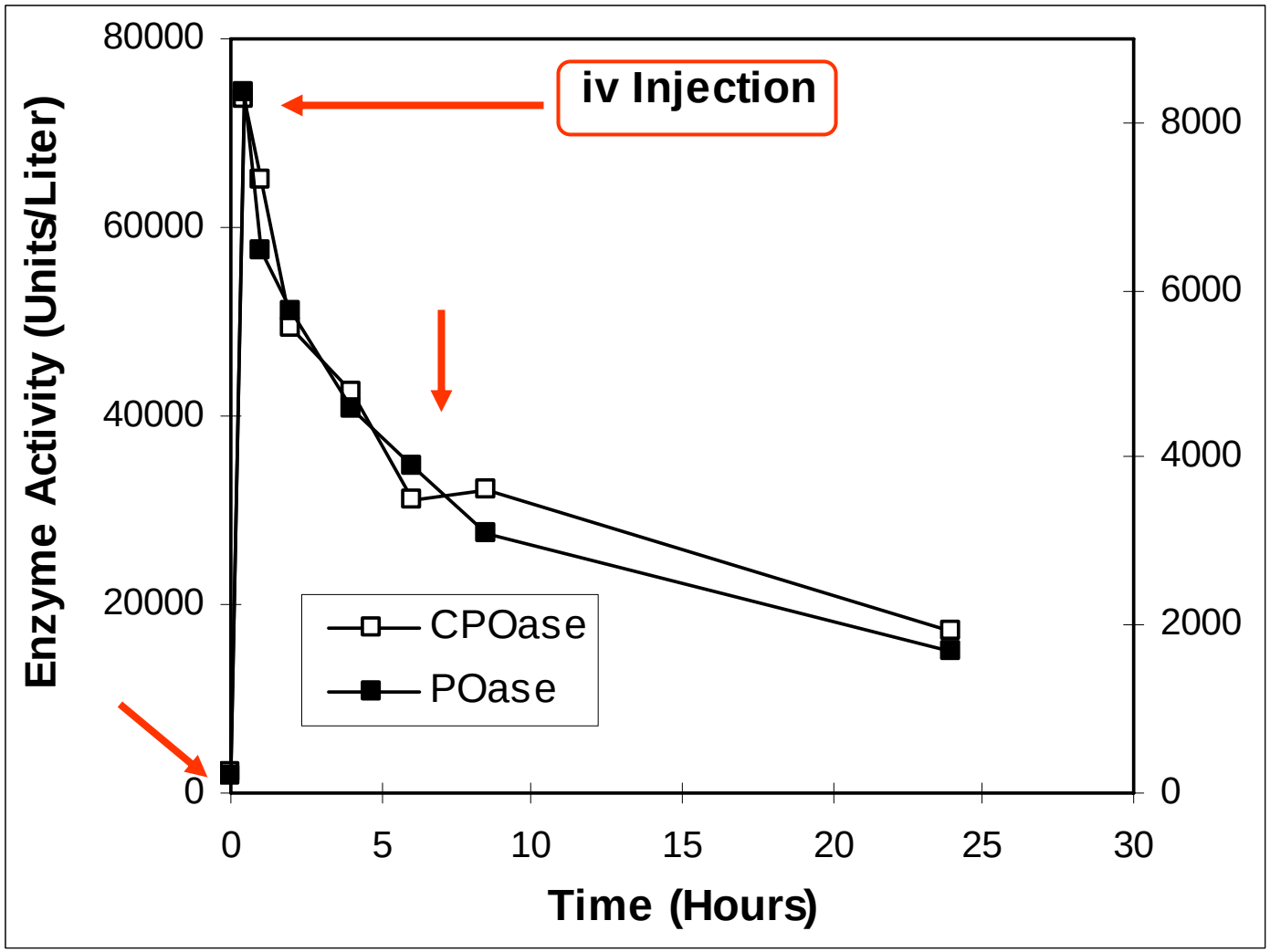
What are the consequences of high PON1 levels?

Early studies on the effects of high PON1 levels on resistance to OP exposure involved the injection of purified rabbit PON1 into mice and challenging the mice with a dermal exposure to OPs. The early studies were mostly carried out with chlorpyrifos oxon or chlorpyrifos.

To test whether PON1 protects against OP exposure, we first determined the most suitable route of administration of purified rabbit PON1 into mice. Injection via the iv route was chosen for the experiment on the next slide. At time zero, purified rabbit PON1 was injected into mice via the tail vein and rates of PON1 hydrolysis of chlorpyrifos oxon (CPOase) and paraoxon (POase) were monitored over time.

(Li et al., J Toxicol and Environ Health 1993; 40:337-346).

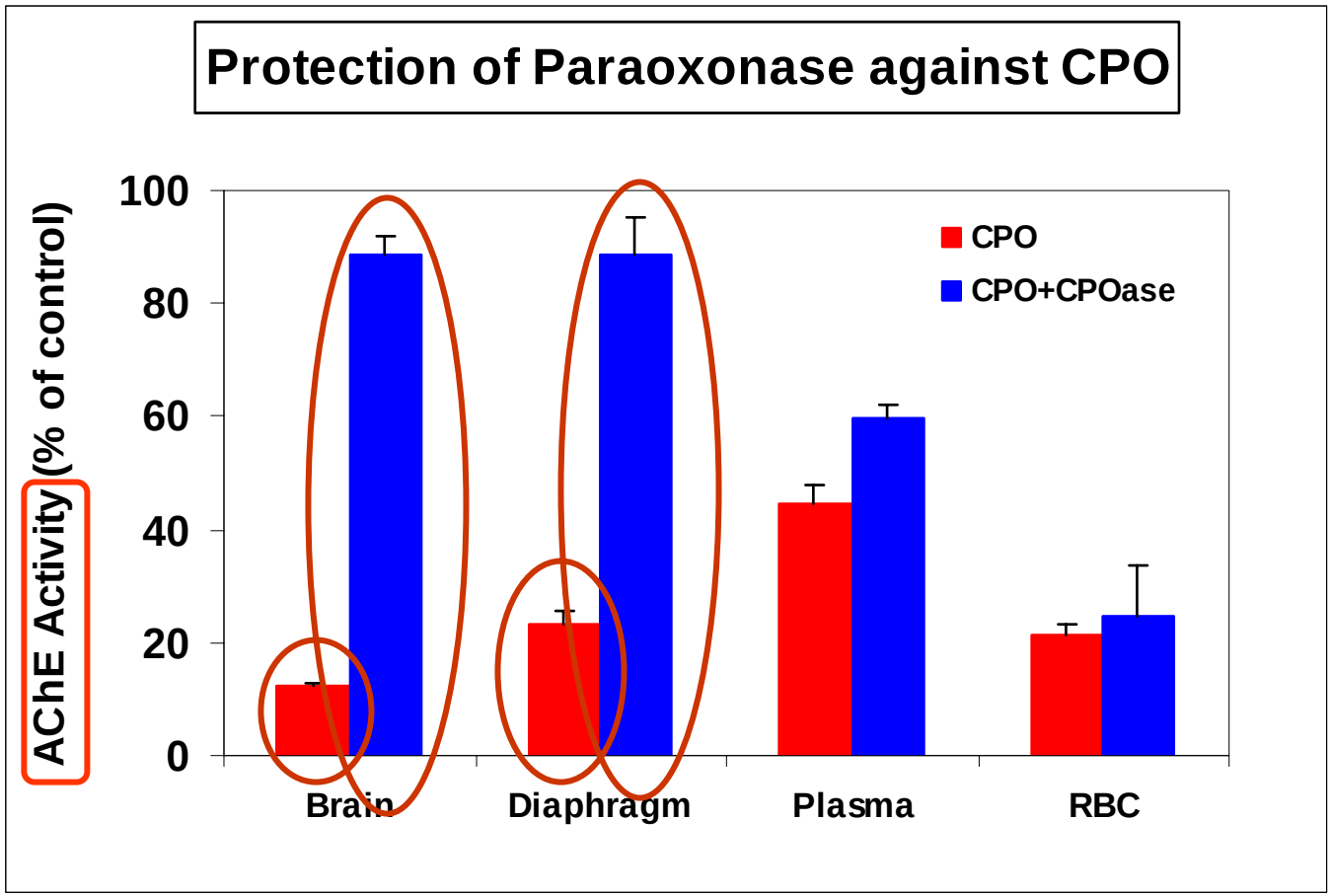
Plasma levels of PON1 can be increased by injecting purified rabbit PON1



Injected PON1 Protects Against OP Exposure

The next slide shows the results of dermal exposure to chlorpyrifos oxon (CPO, 14 mg/kg) of mice injected with purified rabbit PON1 compared with mice not receiving purified rabbit PON1. It is clear from the slide that high levels of plasma PON1 provided excellent protection against cholinesterase inhibition in the brain and diaphragm.

High PON1 levels are protective against exposure to CPO (14 mg/kg)



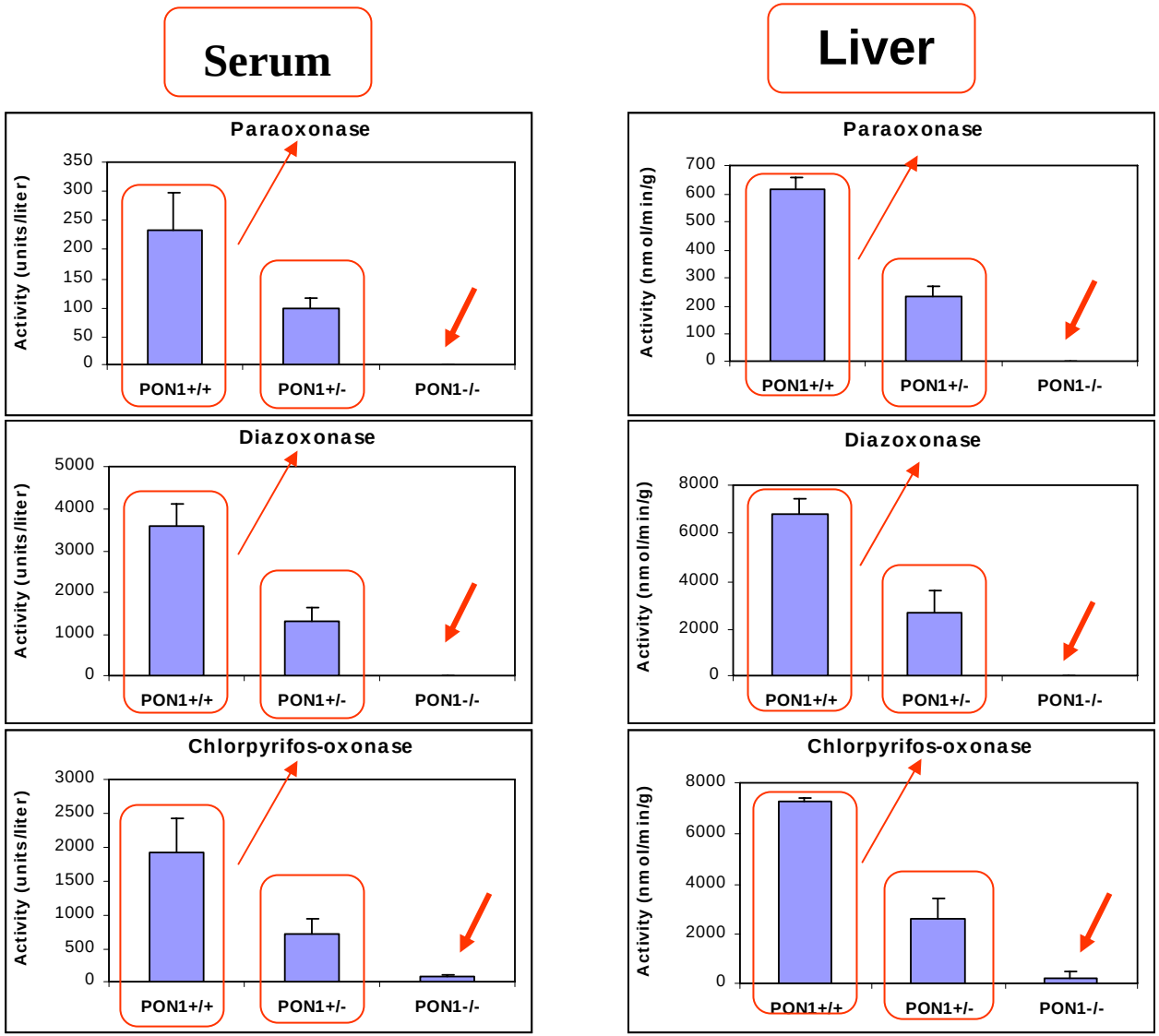
What are the consequences of low PON1 levels?

The consequences of low levels of plasma PON1 were examined in genetically modified mice that were devoid of liver and plasma PON1.

Drs. Shih and Lusis (UCLA) generated mice devoid of PON1. Mice with only one copy of the PON1 gene have ~50% of the PON1 activity levels (paraoxonase, diazoxonase and chlorpyrifos oxonase). These mice have proven to be invaluable in understanding the physiological role of PON1 in detoxifying specific OP compounds as well as the role of PON1 in protecting against vascular disease.

(Shih DM, Gu L, Xia Y-R, Navab M, Li W-F, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. 1998. Mice lacking serum paraoxonase are also susceptible to organophosphate toxicity and atherosclerosis. Nature 394:284-287)

PON1 activity levels in *PON1*$^{+/+}$, *PON1* $^{+/-}$, and *PON1*$^{-/-}$ mice



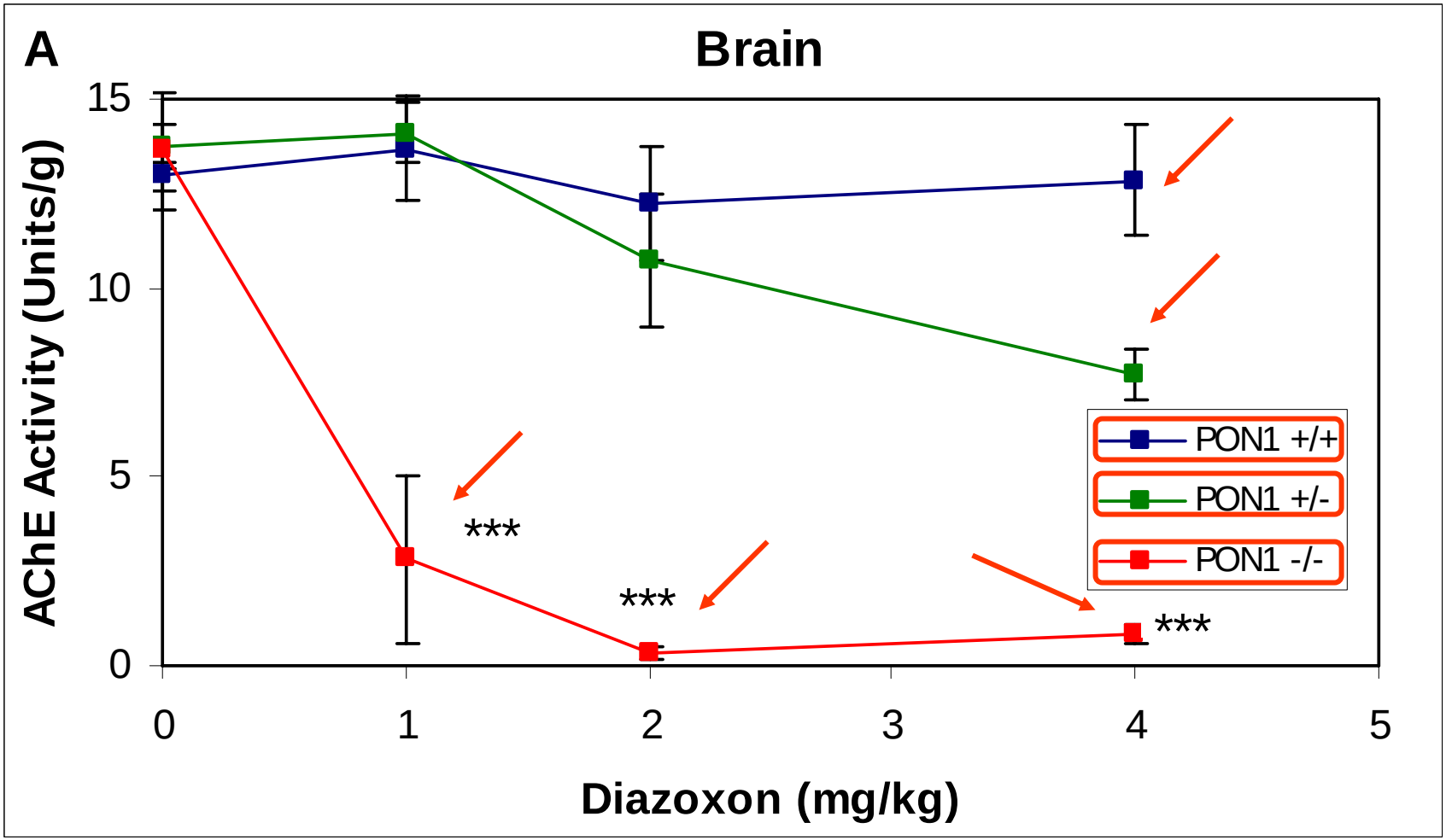
W-F Li, Dissertation, University of Washington

Role of PON1 in Modulating OP Exposures

The dose response curves for the PON1 deficient mice are dramatically changed for dermal exposure to diazoxon (next slide) but much less so to exposure to the parent compound diazinon. $PON1^{-/-}$ mice lacking both PON1 genes were killed by dermal exposures (4 mg/kg) that had no measurable inhibition of brain cholinesterase in normal mice as well as by half that dose. Mice exposed to one-fourth the dose (1 mg/kg) of diazoxon exhibited significant signs of OP intoxication. On the other hand, the differences in sensitivity to the parent compound diazinon were less dramatic (following slide). These observations took us back to one of our earlier papers that included a literature survey of the levels of oxon in residues (Yuknavage et al. 1997, slide after next) and re-emphasized the importance of the PON1 genetic variability in modulating exposure to the oxon component as well as a role in detoxifying the parent compound.

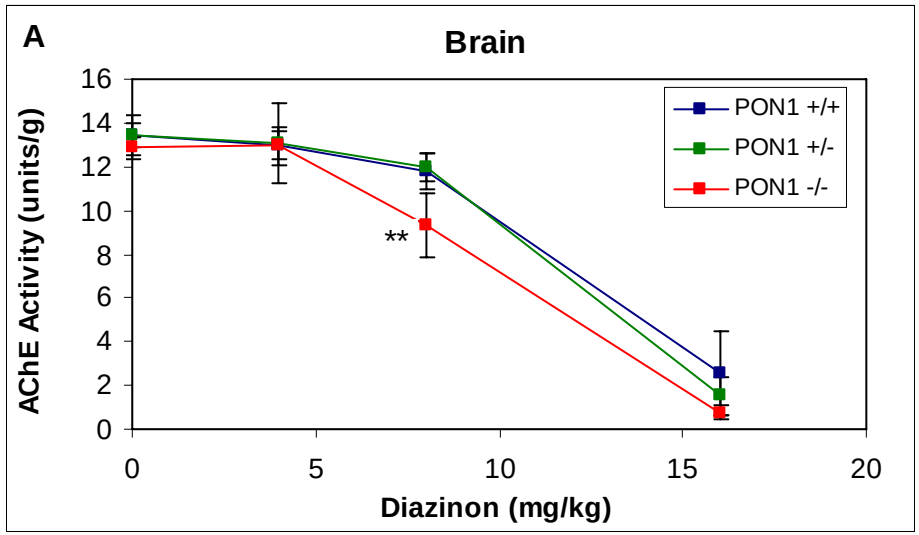
(Li W.-F., L.G. Costa, R.J. Richter, T. Hagen, D.M. Shih, A. Tward, A.J. Lusis and C.E. Furlong. 2000. Catalytic efficiency determines the *in vivo* efficacy of PON1 for detoxifying organophosphates. Pharmacogenetics **10**:767-780.)

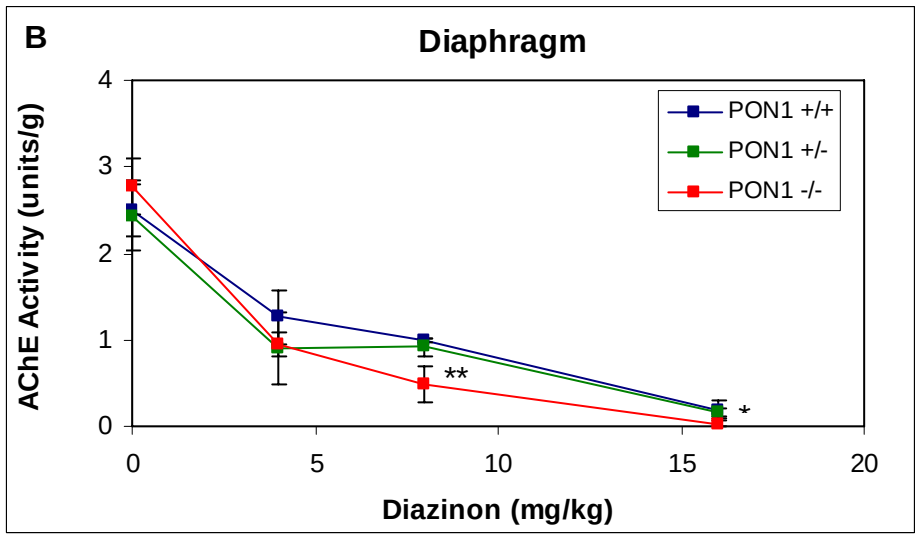
Diazoxon is more toxic to *PON1*$^{-/-}$ than to *PON1*$^{+/+}$ or *PON1*$^{+/-}$ mice



Li et al. 2000. Pharmacogenetics 10:767-780

Diazinon Toxicity in *PON1*$^{+/+}$ & $^{-/-}$ Mice





Li et al. Pharmacogenetics 10:767-780.

As noted above, and as seen in the following repeat slide, actual exposures may contain very significant levels of oxon residues.

In the study by Ralls et al., the oxon content of the diazinon residues represented 17% of the total residue. (Ralls, J. W., Gilmore, D. R., and Cortes, A. 1966. Fate of radioactive *O,O*-diethyl *O*-(2-iso-propyl-4-methylpyridmidin-6-yl) phosphorothioate on field-grown experimental crops. *J. Agric. Food Chem.* 14:387–392.

Literature Survey of Oxon Values in Leaf Foliar Residues

Table 1 Oxon levels in total pesticide residues taken from dislodgeable leaf foliar residue and dermal exposure studies

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[a]Based on the highest value reported in study.
[b]Units or values not given in study.
[c]ND, none detected.
[d]Foliar residue measurement.
[e]Dermal monitoring measurement.

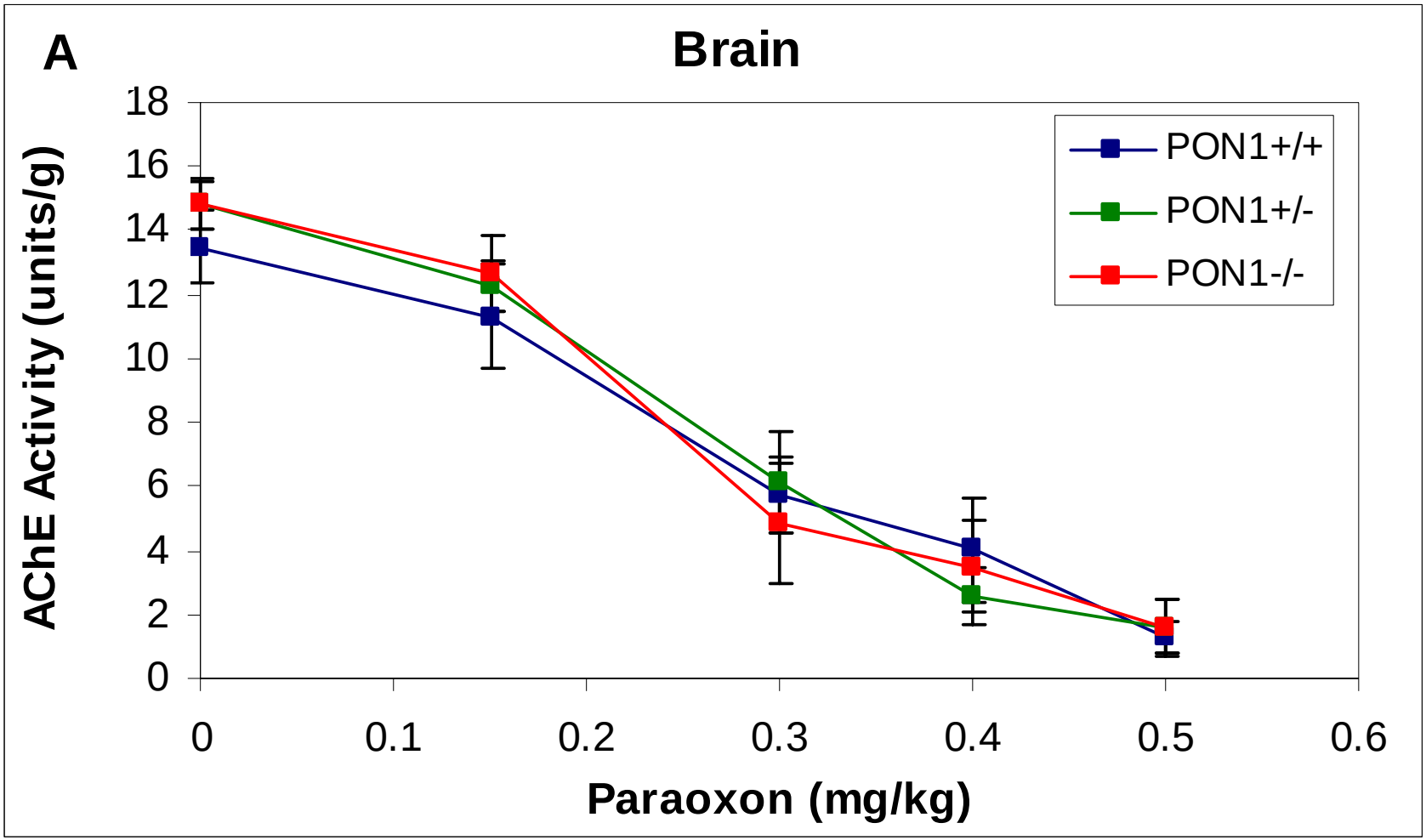
Yuknavage et al., J. Toxicol. Environ. Health 1997; 51:35-55

The Importance of the Mouse Genetic Model

The next slide shows the most surprising result from the series of dermal exposure experiments with the PON1 knockout mice. It was assumed for nearly 50 years that high levels of PON1 would protect against paraoxon toxicity and conversely, low PON1 levels would render individuals sensitive to this OP. As seen in the next slide, we observed no significant differences in paraoxon sensitivity between wild type mice, PON1 hemizygous mice and PON1 knockout mice. The reason for this will become clear in the slide after next.

(Li et al., 2000. Pharmacogenetics, 10:767-779).

Paraoxon toxicity is not influenced by *PON1* status



Li et al., Pharmacogenetics 2000

Catalytic Efficiency, the Key to Understanding the Ability of PON1 to Protect Against OP Exposure

The next slide provides an explanation for the results seen when the PON1 deficient mice are injected with either purified human PON1-192 alloform (PON1-Q192 or PON1-R192) or saline and exposed dermally to the indicated organophosphates (chlorpyrifos oxon, diazoxon and paraoxon).

PON1-192 alloforms (Q102 or R192) were purified from human plasma from PON1 Status-typed individual human plasma samples. The purified PON1 was injected into the PON1 deficient mice to determine the effectiveness of each alloform to protect against exposure to chlorpyrifos oxon, diazoxon and paraoxon. The degree of protection provided by each alloform was closely related to the catalytic efficiency of the specific alloform for the given OP. PON1-R192 provided better protection against chlorpyrifos oxon exposure, both alloforms protected nearly equally as well against diazoxon exposure with PON1-R192 protecting a bit better and neither protected against paraoxon exposure, in agreement of a lack of increased sensitivity of PON1 null mice to paraoxon exposure.

Thus resistance to diazoxon exposure should be governed primarily by an individual's plasma PON1 levels, whereas resistance to chlorpyrifos oxon exposure depends on plasma PON1 levels as well as position PON1-192 genotype with PON1-R192 providing the best protection.

Li W.-F., L.G. Costa, R.J. Richter, T. Hagen, D.M. Shih, A. Tward, A.J. Lusis and C.E. Furlong. 2000. Catalytic efficiency determines the *in vivo* efficacy of PON1 for detoxifying organophosphates. Pharmacogenetics **10**:767-780.)

Catalytic efficiency determines the *in vivo* efficacy of PON1 for detoxifying organophosphates

Catalytic efficiencies of PON1 192Q and PON1 192R enzymes

Chlorpyrifos-oxon Hydrolysis

	PON1Q192	PON1R192
Km (mM)	0.54	0.25
Vmax (units/mg)	82	64
Vmax/Km	152	256

Diazoxon Hydrolysis

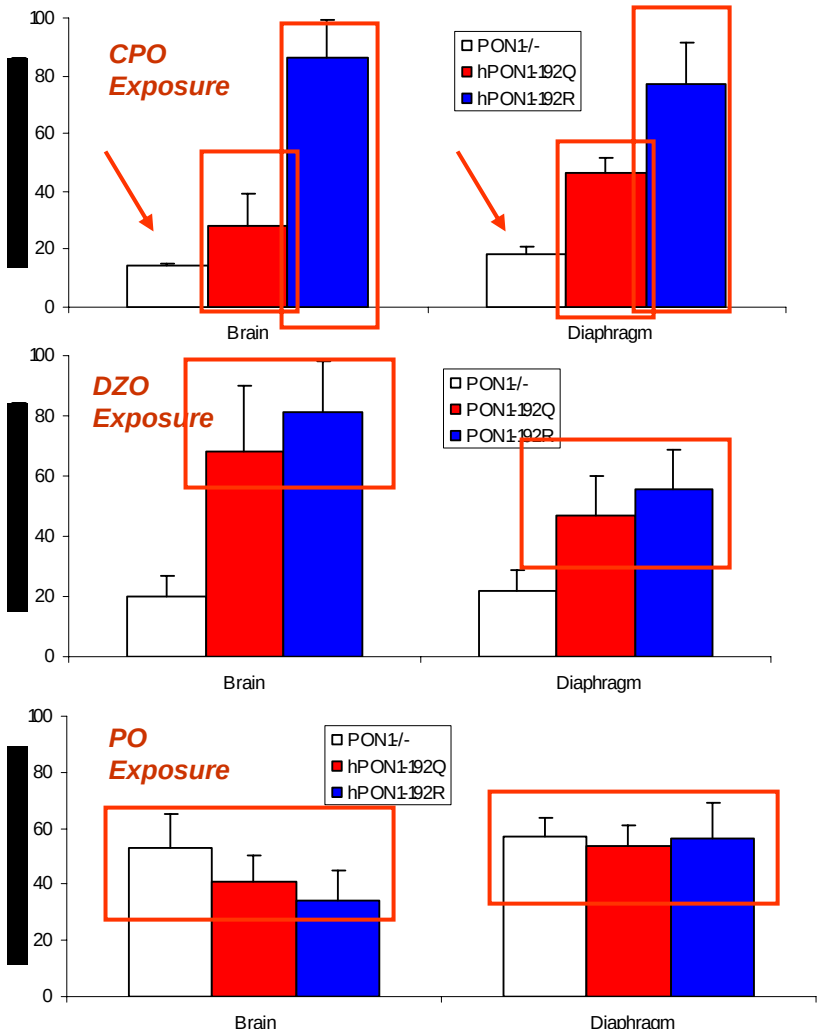
	PON1Q192	PON1R192
Km (mM)	2.98	1.02
Vmax (units/mg)	222	79
Vmax/Km	75	77

Paraoxon Hydrolysis

	PON1Q192	PON1R192
Km (mM)	0.81	0.52
Vmax (units/mg)	0.57	3.26
Vmax/Km	0.71	6.27

Li et al. 2000. Pharmacogenetics 10:767-780

Protection afforded PON1$^{-/-}$ mice by injecting human PON1 192Q orPON1 192R enzymes



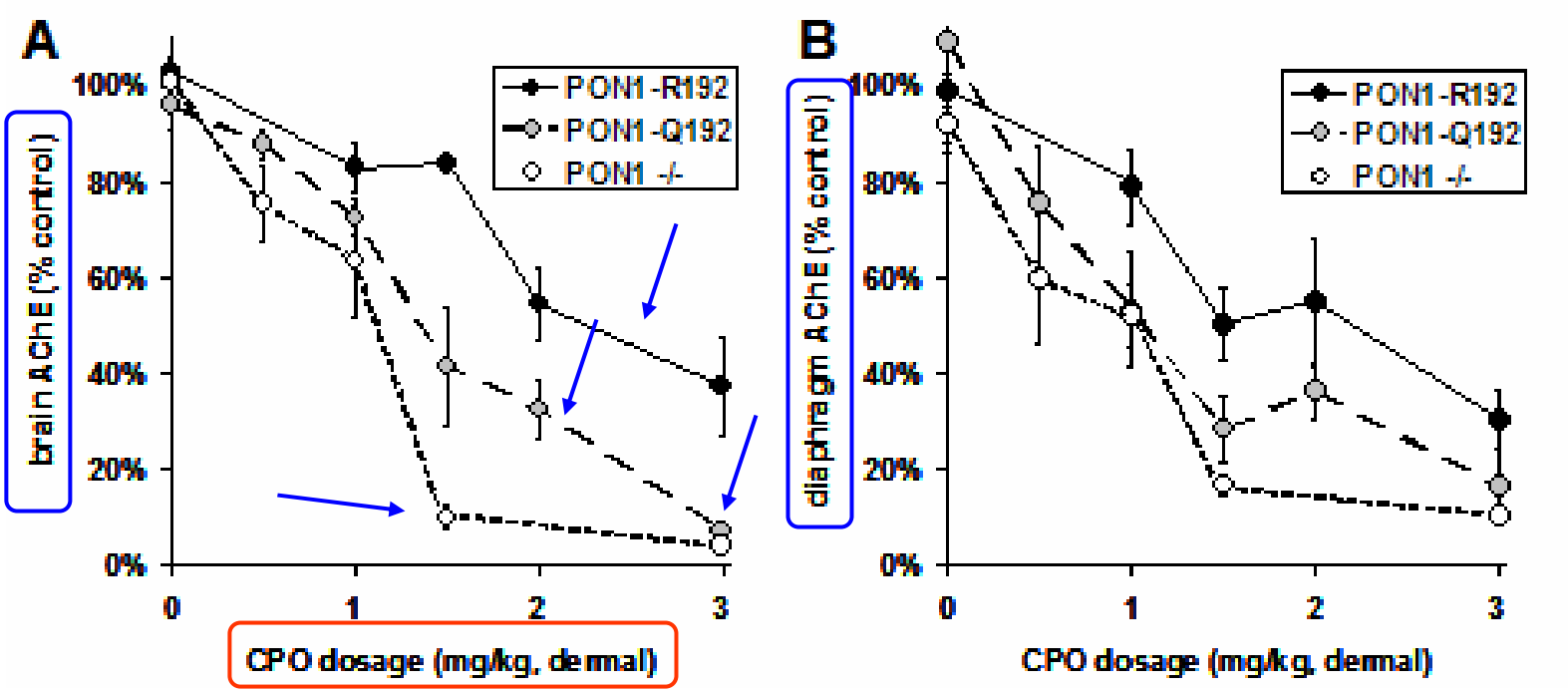
Further Development of the Mouse Genetic Model

Further insights into the ability of PON1 to protect against exposure to chlorpyrifos oxon were obtained from studies with "PON1 humanized mice". These mice were generated by Dr. Diana Shih and collaborators at UCLA. Essentially, these mice have their mouse PON1 replaced with human PON1-R192 or PON1-Q192. From the original "founder mice", animals that expressed the same levels of each PON1-192 alloform were chosen for establishing colonies. By choosing animals producing the same levels of each alloform in their plasma, the efficacy in protecting against OP exposure could be tested at any time without having to inject purified human paraoxonase, i.e. they were designed genetically to produce their own human PON1s in the absence of mouse PON1.

The next slide shows that the animals expressing human PON1-R192 were much more resistant to cholinesterase inhibition by chlorpyrifos oxon exposure than PON1 deficient animals with PON1-Q192 expressing animals demonstrating intermediate sensitivity except at high doses, where the PON1-Q191 mice were essentially as sensitive as the PON1 deficient mice. This is a very significant observation, since ~50% of individuals of Northern European origin are homozygous for PON1-Q192.

[Cole TB, Walter BJ, Shih DM, Tward AD, Lusis AJ, Timchalk C, Richter RJ, Costa LG, Furlong CE. 2005. Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism. Pharmacogenet and Genomics 15:589-598].

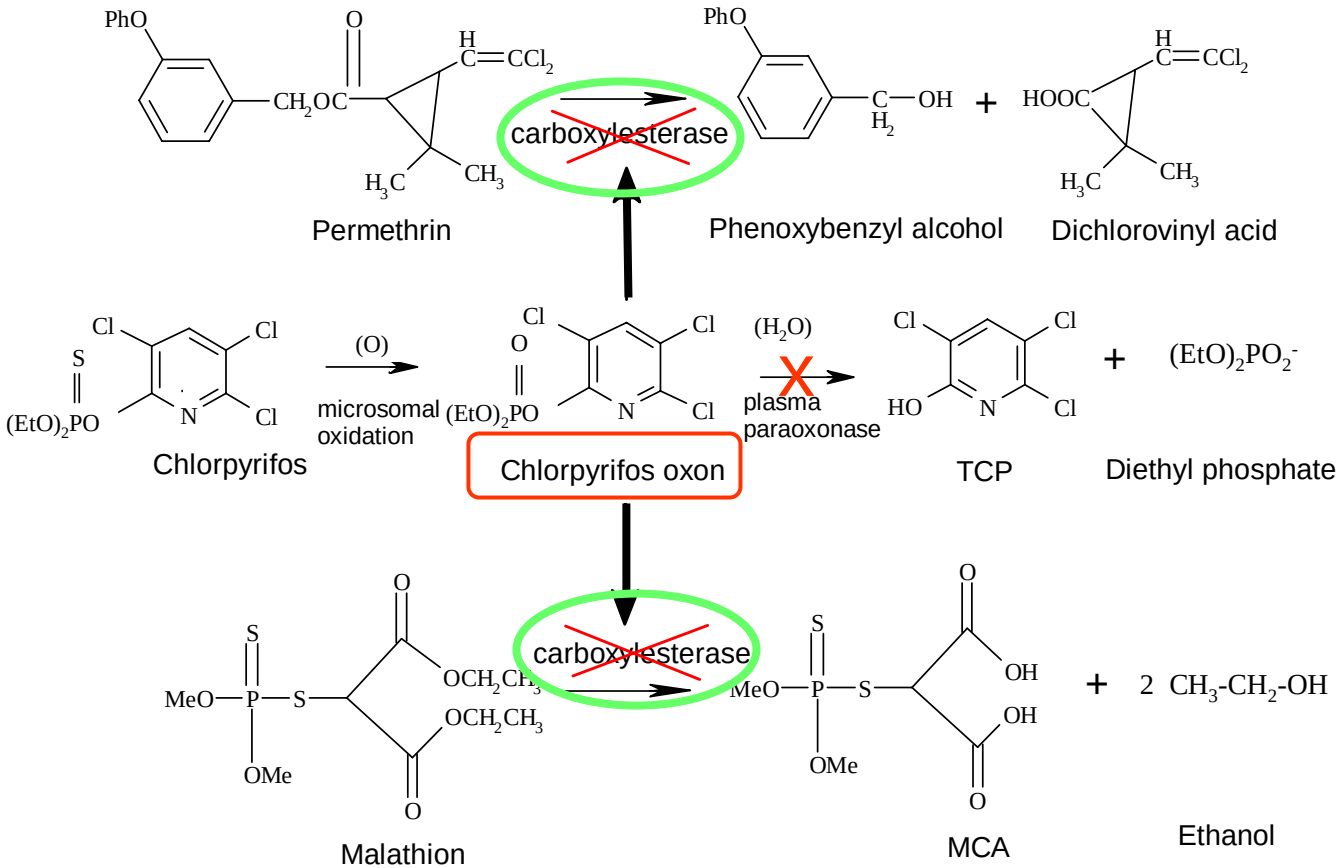
Dose Response for Chlorpyrifos Oxon Exposure of 21d PON1 Humanized Mice (Q192;R192) Compared with PON1 Null Mice



Important since approximately 50% of many populations are homozygous for PON1Q$_{192}$

What about Mixed Exposures?

Experiments were designed to examine interactions between insecticides. Specific organophosphate compounds such as chlorpyrifos oxon, diazoxon and tricresyl phosphate are irreversible inhibitors of carboxylesterase, which is important in the detoxication of malathion and pyretyroids.

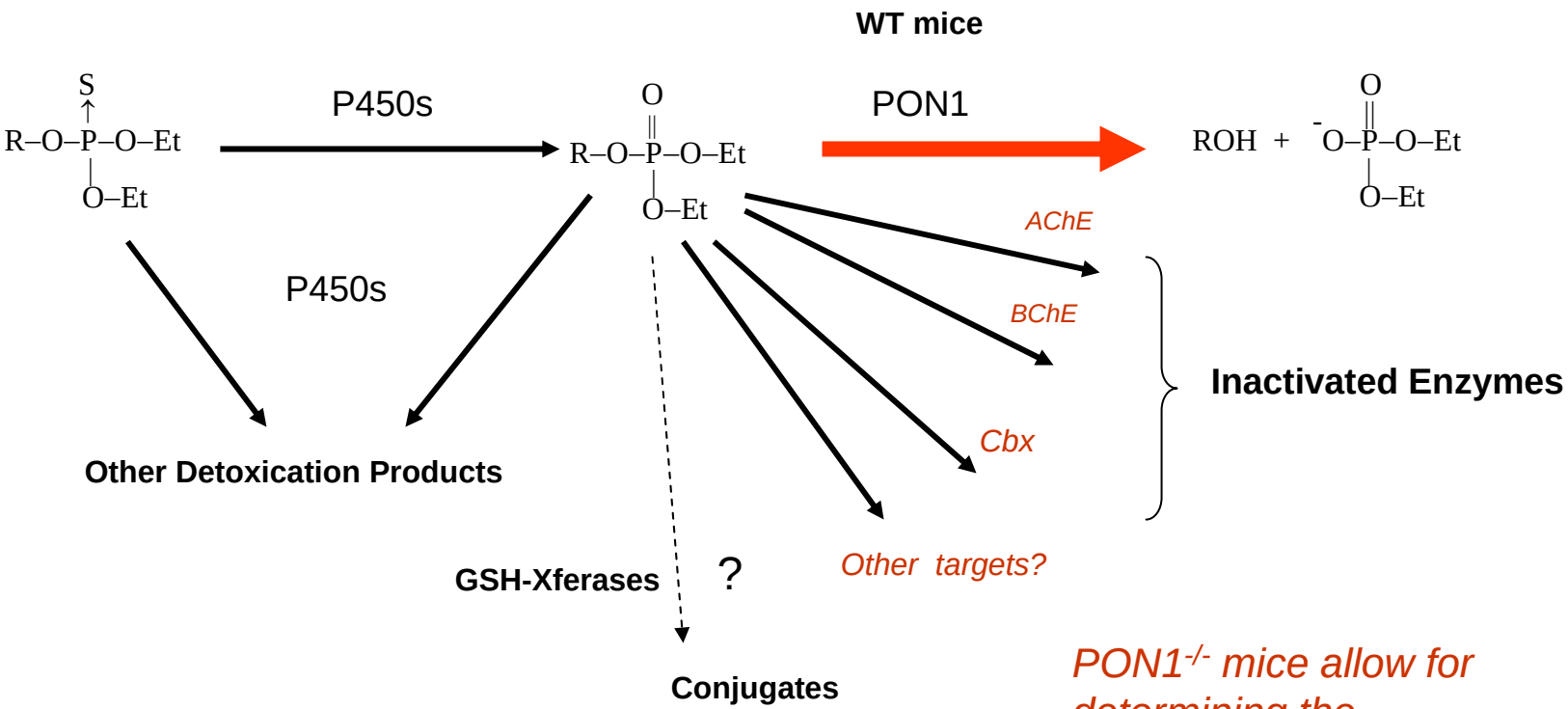


Conclusion: Prior exposure to chlorpyrifos oxon potentiates sensitivity to malaoxon

Other Advantages of the PON1$^{-/-}$ Mice

PON1 has such a significant impact on the detoxication of the oxons of diazinon and chlorpyrifos that it is difficult to examine the contributions of other enzymes and pathways to the detoxication of these compounds. It will be much easier to examine the contributions of these other enzymes and pathways in the PON1 deficient mice.

Detoxication of OPs in PON1 knockouts



PON1$^{-/-}$ mice allow for determining the contributions of other pathways to detoxication and metabolism

Summary of Observations Bearing on Exposures to Diazinon/Diazoxon (DZS/DZO) and Chlorpyrifos/ Chlorpyrifos Oxon (CPS/CPO).

There are significant genetic and developmental differences in individual sensitivities to OP exposure. Newborns have low PON1 levels which contribute to their increased sensitivity to exposure.

Within populations of adults, there is significant variability in PON1 levels (~15 fold) which based on animal model studies, indicate a significant variability in sensitivity to OP exposure.

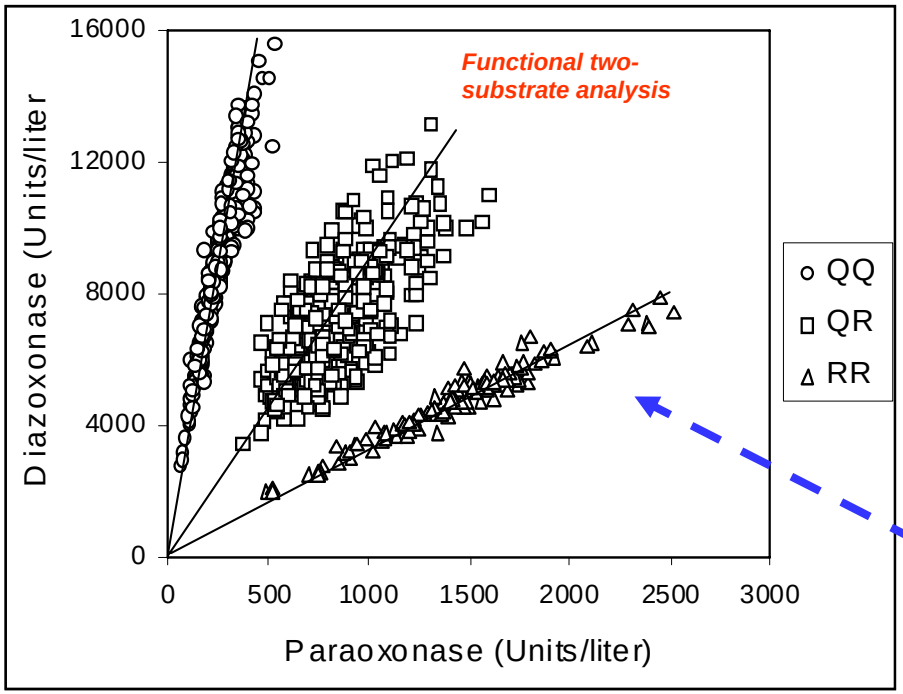
The genetic and developmental variability of PON1 are primarily reflected in sensitivity to the oxon contents of the exposure that have not been considered in product safety studies.

Sensitivity to CPS/CPO exposures is governed not only by variability in PON1 levels but also by the PON1-192 Q/R polymorphism with the PON1-R192 alloform protecting better than the PON1-Q192 alloform against exposure.

Catalytic efficiency of hydrolysis (oxon inactivation) is the key for determining whether PON1 can protect against a given OP compound.

Exposure to the parent compounds can inhibit cytochrome P450 3A4, an enzyme that is very important in hormone metabolism.

The Bottom Line



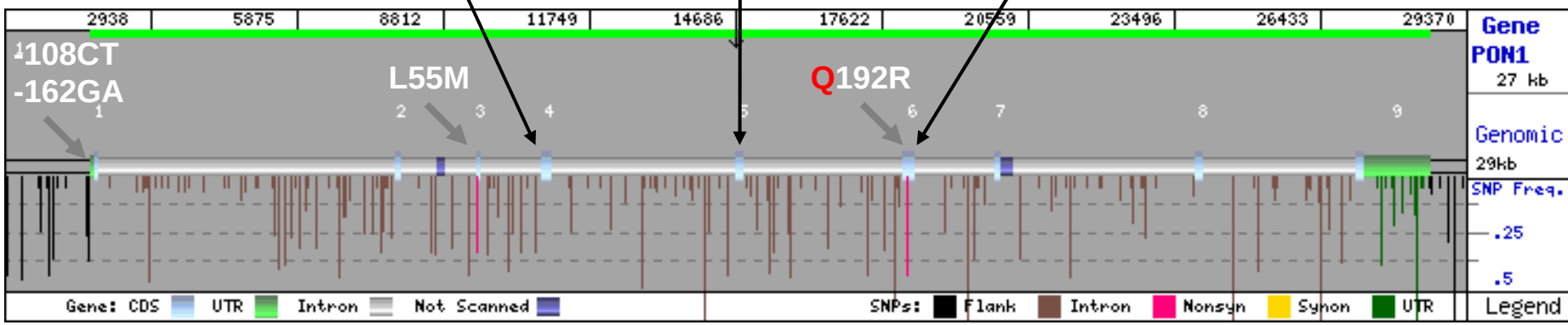
A lot of things can happen between the gene encoding PON1 and the final PON1 protein product in the plasma. The high throughput two substrate assay provides the determination of the end result of all of the processes from transcription to the HDL particle and is the method of choice for studies of genetic variation of *PON1*.

PON1

P90L
(low activity)

124 missplice
(low activity)

W194X
(premature stop)



Research Needs

- More Data are needed on oxon content of residues (completely ignored in safety testing)
- Data are needed on residue ratios (DZO/DZS) and persistence over time and along product line (wool processing)
- Development of realistic (DZO/DZS) exposure models including genetic variability - iterate humanized mouse data with PBPK/PD models
- Data are needed on DZO/DZS effects on developing fetus
- Identify longer-term biomarkers of exposure
- Better endpoints of exposure than ChE inhibition (microarray analysis of effects on gene expression in different tissues/organs)
- Effects of DZS exposure on reproductive health
- Identification of other targets of DZS/DZO

I hope that this presentation has been useful for you. Additional publications from our research laboratory are listed at the end of this presentation.

There are plans to generate a paraoxonase resource web site that will provide many more references to earlier research and work done in other laboratories. When this site becomes available, a link will be provided.

The next slide lists our many collaborators who have helped explore the different facets of PON1 genetic variability. The following slides include additional references to our studies on organophosphates. If you need to contact me for further information or suggestions for additional research questions, my email address is clem@u.washington.edu and phone is 206-543-1193. My mailing address is: CE Furlong, Div. Medical Genetics, Box 357720, University of Washington, Seattle, WA 98195-7720.

PON1 collaborators

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  TB Cole

- **Genetics, purification & expression**
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  R Jampsa
  T Hagen
  VH Brophy

- **Pathology studies**
  CP Brewer

- **Mouse behavior studies**
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  J Fisher
  B Walter
  T Burbacher

- **Development/Toxico-genomics**
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  J Furlong, M Katze
  G Geiss

- **Genomics**
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  C Carlson
  M Rieder

Parkinson's Studies
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Samir Kelada
Gary Franklin

- **Cardiovascular studies**
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UCLA

- **Pon1$^{-/-}$ and transgenic mice**
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  DM Shih
  A Tward

UC Berkeley

- **Mother/Infant Study**
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  N Holland
  A Bradman

PNNL, Batelle

PBPK/PD Modeling
C Timchalk

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P42ES04696

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