Biomarkers of Sensitivity and Exposure in Washington State Pesticide Handlers

Jonathan N. Hofmann, Matthew C. Keifer, Harvey Checkoway, Anneclaire J. De Roos, Federico M. Farin, Richard A. Fenske, Rebecca J. Richter, Gerald van Belle, and Clement E. Furlong

Abstract Organophosphate (OP) and *N*-methyl-carbamate (CB) insecticides are widely used in agriculture in the US and abroad. These compounds – which inhibit acetylcholinestersase (AChE) enzyme activity – continue to be responsible for a high proportion of pesticide poisonings among US agricultural workers. It is possible that some individuals may be especially susceptible to health effects related to OP/CB exposure. The paraoxonase (PON1) enzyme metabolizes the highly toxic oxon forms of some OPs, and an individual's PON1 status may be an important determinant of his or her sensitivity to these chemicals. This chapter discusses methods used to characterize the PON1 status of individuals and reviews previous epidemiologic studies that have evaluated PON1-related sensitivity to OPs in relation to various health endpoints. It also describes an ongoing longitudinal study among OP-exposed agricultural pesticide handlers who are participating in a recently implemented cholinesterase monitoring program in Washington State. This study will evaluate handlers' PON1 status as a hypothesized determinant of butyrylcholinesterase (BuChE) inhibition. Such studies will be useful to determine how regulatory risk assessments might account for differences in PON1-related OP sensitivity when characterizing inter-individual variability in risk related to OP exposure. Recent work assessing newer and more sensitive biomarkers of OP exposure is also discussed briefly in this chapter.

Keywords Agriculture · Cholinesterase · Farm workers · Gene–environment interaction · Organophosphates · Paraoxonase (PON1) · Pesticides

1 Introduction

Since the 1970s, the use of organophosphate (OP) and *N*-methyl-carbamate (CB) insecticides has increased dramatically in the US (Reigart and Roberts, 1999). In 2007, approximately 589,000 lbs of azinphos-methyl, chlorpyrifos, and carbaryl

J.N. Hofmann (✉)
Division of Cancer Epidemilogy and Genetics, National Cancer Institute, Bethesda, MD, USA
e-mail: hofmannjn@mail.nih.gov

S.T. Reddy (ed.), *Paraoxonases in Inflammation, Infection, and Toxicology*, Advances in Experimental Medicine and Biology 660, DOI 10.1007/978-1-60761-350-3_3,

(three common OP/CB insecticides) were applied in apple orchards in Washington State alone (WASS, 2007). OP/CBs are also widely used to treat other crops including pears, cherries, grapes, hops, and potatoes (WSUCE, 2001; WASS, 2008; Smith, 2006).

Acute effects of OP/CB exposure have been well documented; inhibition of neuronal acetylcholinesterase (AChE) enzyme activity is the main mechanism of OP/CB toxicity (Ecobichon, 2001). AChE hydrolyzes the neurotransmitter acetylcholine, and thereby plays a critical role in regulating nerve transmissions in the central and peripheral nervous systems (Ecobichon, 2001). Cholinesterases (ChE) are also found in blood in two different forms; AChE is associated with red blood cell membranes, and butyrylcholinesterase (BuChE) is present in serum (Wilson, 2001). Both AChE and BuChE inhibition are considered to be surrogate markers of early biologic effects related to OP/CB exposure (USEPA, 2000). Generally, AChE inhibition is considered to be a better marker of toxicity, whereas BuChE inhibition is a more sensitive marker of exposure because it is inhibited more effectively than AChE by most OP/CBs including chlorpyrifos, diazinon, and malathion (Lotti, 1995, 2001; Yuknavage et al. 1997).

2 ChE Monitoring in Washington State

In 2004, the Washington State Department of Labor and Industries (L&I) initiated a ChE monitoring program for agricultural pesticide handlers who are exposed to toxicity class I or II OP/CBs (WAC, 2004). In this program, handlers (e.g., agricultural workers who mix, load, or apply pesticides) are tested for AChE and BuChE activities annually prior to the OP/CB spray season (i.e., at baseline), and during the spray season if they are exposed for 30 or more hours in a 30-day period. AChE or BuChE inhibition from baseline levels can lead to work practice evaluations or removal from continued OP/CB exposure (with wage protection) depending on the degree of ChE inhibition observed. The goals of this monitoring program are to identify and correct unsafe work practices, and to prevent further exposure among handlers with ChE inhibition before they experience symptoms of pesticide-related illness.

3 Health Effects of OP/CB Exposure

Among agricultural workers in the US, OP/CBs continue to be responsible for a high proportion of pesticide poisonings due to their high toxicity and widespread use in agriculture (Reigart and Roberts, 1999). In an analysis of acute pesticide poisonings among US agricultural workers from 1998 to 2005, Calvert et al. noted that OP/CBs were implicated more frequently than any other class of pesticides (Calvert et al. 2008). In addition to acute poisonings, there is also growing concern about a variety of health endpoints that may be associated with chronic exposure to OP/CB insecticides (McCauley et al. 2006). In particular, there is some evidence of associations between OP exposure and chronic neurologic effects (Kamel et al.

2005; Kamel and Hoppin, 2004; Rothlein et al. 2006) and various types of cancer (Alavanja et al. 2004).

4 Sensitivity to OP/CB Toxicity

It is possible that some individuals may be especially susceptible to health effects related to OP/CB exposure. High density lipoprotein (HDL)-associated paraoxonase (PON1) is thought to be one important determinant of an individual's sensitivity to some OP insecticides, based primarily on evidence from studies in animal models (Cole et al. 2005; Li et al. 2000; Shih et al. 1998). PON1 hydrolyzes the highly toxic oxon forms of several widely used OPs, including chlorpyrifos and diazinon. Studies in transgenic mice have clearly demonstrated that low plasma PON1 activity is associated with greater brain AChE inhibition following exposure to chlorpyrifos oxon and diazoxon (the oxon forms of chlorpyrifos and diazinon) (Li et al. 2000). Also, a Q/R polymorphism at position 192 in the *PON1* coding region affects the catalytic efficiency of the enzyme for chlorpyrifos oxon metabolism. In a study by Cole et al. of mice expressing equivalent levels of the different alloforms of humanized PON1, greater brain AChE inhibition was observed among mice with the Q alloform relative to the R alloform following chlorpyrifos oxon exposure (Cole et al. 2005).

Several important points should be considered regarding PON1-mediated sensitivity to OP exposure: (1) PON1 status is most relevant for protecting against direct exposure to the oxon forms of OP insecticides (Li et al. 2000; Shih et al. 1998); (2) most – if not all – OP exposures include oxon residues (CalEPA, 1996; Yuknavage et al. 1997); and (3) the safety studies for OPs such as chlorpyrifos were carried out with the highly pure parent organophosphorothioate (Nolan et al. 1984).

5 Characterizing PON1 Status

An individual's functional PON1 Q192R genotype can be determined using a two-substrate enzymatic analysis. In this analysis, paraoxonase (POase) and diazoxonase (DZOase) activities are measured in plasma samples, and the results are plotted on a graph (Fig. 1). Methods for these assays and the two-substrate enzymatic analysis have been described previously (Furlong et al. 1989; Jarvik et al. 2000; Richter and Furlong, 1999). Previous studies have found that there is generally excellent agreement between predicted Q192R genotype determined by the two-substrate analysis and observed genotype using polymerase chain reaction (PCR) assays (Jarvik et al. 2003).

In addition to assessing functional Q192R genotype, it is also useful to characterize the level of plasma PON1 activity by measuring arylesterase (AREase) activity in plasma samples using phenylacetate as the substrate. AREase activity is considered to be a good surrogate for PON1 concentration in plasma since its rate of hydrolysis is not affected by the Q192R polymorphism (Furlong et al. 1993, 2006).

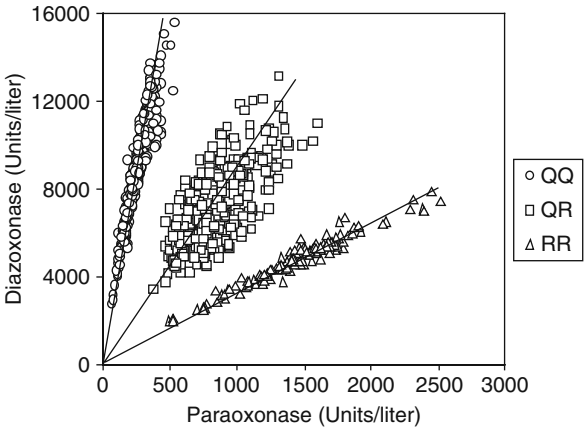

Fig. 1 Example of the two-substrate enzyme activity distribution plot for determination of PON1 status [figure reproduced from Richter et al., 2004 with permission]

6 Previous Epidemiologic Studies

Despite convincing evidence in animal models, relatively few epidemiologic studies have evaluated PON1 status as a determinant of OP sensitivity. Mackness et al. conducted a case–control study of self-reported chronic ill health among sheep dippers exposed primarily to diazinon (Mackness et al. 2003). Cases were individuals who believed that their chronic ill health was due to exposure to sheep dip, and controls were sheep dippers who were believed to be in good health. The investigators found that farmers in the lowest quintile of DZOase activity had a 2.5-fold higher risk of chronic ill health. Another study by Lee et al. evaluated *PON1* Q192R genotype among OP-exposed fruit farm workers in South Africa (Lee et al. 2003). Relative to RR individuals, those who were either QR heterozygotes or QQ homozygotes were almost three times as likely to report multiple ($\geq$2) symptoms of chronic OP toxicity (e.g., abdominal pain, headache, gait disturbance, and limb numbness, among other symptoms). In a case–control study of acute OP intoxication, Sozmen et al. (2002) found that cases had a significantly higher frequency of the *PON1* Q192 alloform and lower POase activity than controls. They also found that POase activity was lower among cases with low BuChE activity upon hospital admission relative to cases with higher BuChE activity, suggesting a protective effect of PON1 against BuChE inhibition.

7 PON1 Status and BuChE Inhibition Among Pesticide Handlers

In 2006, we began a study to evaluate BuChE inhibition in relation to PON1 status among OP-exposed agricultural pesticide handlers in Washington State. Pesticide handlers in the statewide ChE monitoring program are recruited through two collaborating clinics in eastern Washington during the OP/CB spray season (April–July).

Blood samples for determination of PON1 status and self-reported OP/CB exposure information are collected at the time of follow-up ChE testing. Data from the 2006 and 2007 spray seasons have been analyzed, and results have recently been published (Hofmann et al. 2009). We focused on the outcome of BuChE inhibition in this analysis for several reasons. First, BuChE is more sensitive than AChE to inhibition by many OPs including chlorpyrifos, which was the most widely used OP among study participants (Amitai et al. 1998; Lotti, 2001). Second, there was little evidence of substantial AChE inhibition among study participants or among all handlers in the statewide monitoring program. Among the 472 handlers in the state monitoring program who had baseline tests and at least one follow-up test in 2006, mean AChE inhibition was 1.8%, and only two handlers had "AChE depression" at the work practice evaluation threshold of >20% inhibition (ChESAC, 2006). Finally, high variability in AChE measurements was observed in analyses of data from the state monitoring program in 2007 (16.7% CV) (Kalman and van Belle, 2007); this would likely have obscured any associations between PON1 status and AChE inhibition in our study.

Our study approach has several strengths. By recruiting participants from the recently implemented Washington State ChE monitoring program, we have established a cohort of agricultural pesticide handlers with confirmed recent OP exposure. Because pesticide handlers (i.e., mixer/loader/applicators) are considered to be more highly exposed to pesticides than agricultural workers who perform other activities, this population is ideal for evaluating PON1-mediated susceptibility to OP exposure. Previous studies of PON1-related susceptibility among individuals with occupational OP exposure have relied on self-reported health outcomes such as chronic ill health (Mackness et al. 2003) or symptoms of chronic toxicity (Lee et al. 2003). In this study, we used BuChE inhibition as a quantitative biomarker of OP-related effects as our primary outcome. Finally, some previous studies have relied exclusively on *PON1* genotype. For some OPs (i.e., chlorpyrifos), the Q192R polymorphism is important as it affects the catalytic efficiency of hydrolysis. However, plasma PON1 activity level is important for all OPs that are metabolized by PON1 at physiologically relevant rates (Li et al. 2000). Plasma PON1 activity has been shown to vary widely among individuals with the same Q192R genotype (Costa et al. 2005; Eckerson et al. 1983; Furlong et al. 2006). Determination of both *PON1* genotype and level of plasma PON1 activity in this study allows for a better characterization of overall PON1 status than Q192R genotype alone (Richter and Furlong, 1999).

However, there are also several limitations to our study methods. The cross-sectional design limits our ability to infer a causal relationship between plasma PON1 activity and BuChE inhibition, because PON1 activity may be modified to some extent by certain medications (e.g., statins), dietary habits (e.g., vitamin C and E intake), and environmental exposures (e.g., smoking) (Durrington et al. 2002; Jarvik et al. 2002). However, most previous studies suggest that plasma PON1 activity tends to be relatively stable over time and is mostly regulated by genetic factors, particularly the C-108T promoter polymorphism (Brophy et al. 2001a, b; Durrington et al. 2002; Ferre et al. 2003; Furlong et al. 2000; Jarvik et al. 2002; Leviev and James, 2000; Suehiro et al. 2000; Zech and Zurcher, 1974). Nonetheless,

it is possible that some exposures that modulate plasma PON1 activity may also affect BuChE activity or recovery following OP/CB exposure. Future studies with prospective collection of blood specimens for determination of PON1 status are needed.

Other studies may also benefit from recent work evaluating new biomarkers of OP exposure. Several previous studies have found that acylpeptide hydrolase (APH) is particularly sensitive to inhibition by some OPs, in that biological effects can be observed at levels of OP exposure that do not inhibit traditional biomarkers such as AChE (Quistad et al. 2005; Richards et al. 1999). Moreover, there are several other advantages of using APH as a biomarker of OP exposure. Whereas BuChE is relatively short-lived in serum, APH is present in red blood cells and therefore has a longer life span. Consequently, it may be possible to detect exposures that occurred during the preceding several months. Using mass spectrometry (MS), it should be possible in some cases to identify which particular OP compound resulted in APH inhibition. MS analyses should also provide a more accurate measure of exposure when used with appropriate heavy isotope-labeled internal standard biomarker proteins spiked into the starting samples.

8 Conclusions

Based on evidence from extensive research in animal models and some epidemiologic studies, regulatory risk assessments should take differences in PON1-related sensitivity to OP insecticides into consideration when characterizing inter-individual variability in risk related to OP exposure. At some point in the future, biologic monitoring for PON1 status among pesticide handlers may be warranted to identify individuals who are at particularly high risk of OP-related health effects. However, issues of test validity as well as the ethical and legal aspects of genetic testing in the workplace need to be addressed before such a program could be implemented (Battuello et al. 2004).

Acknowledgments We would like to thank all of the workers who participated in this study. We also acknowledge the following individuals who contributed to this study: Zahra Afsharinejad for her work on the PON1 genotyping assays; Kelly Fryer-Edwards for her assistance with outreach to study participants; Pam Ernst and Joe Cozzetto from Central Washington Occupational Medicine for their assistance with our recruitment efforts; and Maria Negrete and Pablo Palmandez from the Pacific Northwest Agricultural Safety and Health Center for their assistance with field data collection. Financial support for this project was provided by CDC/NIOSH grants U50OH07544 and T42OH008433, and NIEHS grants P30ES07033, T32ES07262, P42ES04696, and ES009883.

References

Alavanja MC, Hoppin JA, Kamel F. Health effects of chronic pesticide exposure: cancer and neurotoxicity. Annu Rev Public Health 2004;25:155–197.

Amitai G, Moorad D, Adani R, Doctor BP. Inhibition of acetylcholinesterase and butyrylcholinesterase by chlorpyrifos-oxon. Biochem Pharmacol 1998;56(3):293–299.

Battuello K, Furlong CE, Fenske R, Austin MA, Burke W. Paraoxonase polymorphisms and susceptibility to organophosphate pesticides. In: Khoury MJ, Little J, Burke W (eds.). Human Genome Epidemiology: A Scientific Foundation for Using Genetic Information to Improve Health and Prevent Disease. New York: Oxford University Press; 2004.

Brophy VH, Hastings MD, Clendenning JB, Richter RJ, Jarvik GP, Furlong CE. Polymorphisms in the human paraoxonase (PON1) promoter. Pharmacogenetics 2001;11(1):77–84.

Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet 2001;68(6):1428–1436.

CalEPA. Report for the application and ambient air monitoring of chlorpyrifos (and the oxon analogue) in Tulare County during spring/summer, 1996. Sacramento, CA: California Environmental Protection Agency; 1998.

Calvert GM, Karnik J, Mehler L, Beckman J, Morrissey B, Sievert J, et al. Acute pesticide poisoning among agricultural workers in the United States, 1998–2005. Am J Ind Med 2008;51(12):883–898

ChESAC. Cholinesterase Monitoring of Pesticide Handlers in Agriculture: 2004–2006. Olympia, WA: Scientific Advisory Committee for Cholinesterase Monitoring; 2006 November 13.

Cholinesterase Monitoring. In: Washington Administrative Code, Chapter 296–307, Part J-1; 2004.

Cole TB, Walter BJ, Shih DM, Tward AD, Lusis AJ, Timchalk C, et al. Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism. Pharmacogenet Genomics 2005;15(8):589–598.

Costa LG, Cole TB, Vitalone A, Furlong CE. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. Clin Chim Acta 2005;352 (1–2):37–47.

Durrington PN, Mackness B, Mackness MI. The hunt for nutritional and pharmacological modulators of paraoxonase. Arterioscler Thromb Vasc Biol 2002;22(8):1248–1250.

Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet 1983;35(6):1126–1138.

Ecobichon DJ. Toxic effects of pesticides. In: Klaassen CD (ed.). Casarett and Doull's Toxicology: The Basic Science of Poisons. 6th ed. New York: McGraw-Hill; 2001. 763–810.

Ferre N, Camps J, Fernandez-Ballart J, Arija V, Murphy MM, Ceruelo S, et al. Regulation of serum paraoxonase activity by genetic, nutritional, and lifestyle factors in the general population. Clin Chem 2003;49(9):1491–1497.

Furlong CE, Costa LG, Hassett C, Richter RJ, Sundstrom JA, Adler DA, et al. Human and rabbit paraoxonases: purification, cloning, sequencing, mapping and role of polymorphism in organophosphate detoxification. Chemico-Biological Interactions 1993;87(1–3): 35–48.

Furlong CE, Holland N, Richter RJ, Bradman A, Ho A, Eskenazi B. PON1 status of farmworker mothers and children as a predictor of organophosphate sensitivity. Pharmacogenetics and Genomics 2006;16(3):183–190.

Furlong CE, Li WF, Richter RJ, Shih DM, Lusis AJ, Alleva E, et al. Genetic and temporal determinants of pesticide sensitivity: role of paraoxonase (PON1). Neurotoxicology 2000;21(1–2):91–100.

Furlong CE, Richter RJ, Seidel SL, Costa LG, Motulsky AG. Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. Anal Biochem 1989;180(2):242–247.

Hofmann JN, Keifer MC, Furlong CE, De Roos AJ, Farin FM, Fenske RA, et al. Serum cholinesterase inhibition in relation to paraoxonase-1 (PON1) status among organophosphate-exposed agricultural pesticide handlers. Environ Health Perspect 2009;117(9)1402–1408.

Jarvik GP, Jampsa R, Richter RJ, Carlson CS, Rieder MJ, Nickerson DA, et al. Novel paraoxonase (PON1) nonsense and missense mutations predicted by functional genomic assay of PON1 status. Pharmacogenetics 2003;13(5):291–295.

Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD, et al. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1(192) or PON1(55) genotype. Arterioscler Thromb Vasc Biol 2000;20(11):2441–2447.

Jarvik GP, Tsai NT, McKinstry LA, Wani R, Brophy VH, Richter RJ, et al. Vitamin C and E intake is associated with increased paraoxonase activity. Arterioscler Thromb Vasc Biol 2002;22(8):1329–1333.

Kalman D, van Belle G. 2007 Cholinesterase Analysis. URL: http://www.lni.wa.gov/Safety/Topics/AtoZ/Cholinesterase/files/report120307.pdf. Letter. Seattle, WA: University of Washington; 2007.

Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MC, Sandler DP. Neurologic symptoms in licensed private pesticide applicators in the agricultural health study. Environ Health Perspect 2005;113(7):877–882.

Kamel F, Hoppin JA. Association of pesticide exposure with neurologic dysfunction and disease. Environ Health Perspect 2004;112(9):950–958.

Lee BW, London L, Paulauskis J, Myers J, Christiani DC. Association between human paraoxonase gene polymorphism and chronic symptoms in pesticide-exposed workers. J Occup Environ Med 2003;45(2):118–122.

Leviev I, James RW. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. Arterioscler Thromb Vasc Biol 2000;20(2):516–521.

Li WF, Costa LG, Richter RJ, Hagen T, Shih DM, Tward A, et al. Catalytic efficiency determines the in-vivo efficacy of PON1 for detoxifying organophosphorus compounds. Pharmacogenetics 2000;10(9):767–779.

Lotti M. Cholinesterase inhibition: complexities in interpretation. Clin Chem 1995;41(12 Pt 2):1814–1818.

Lotti M. Clinical toxicology of anticholinesterase agents in humans. In: Krieger R (ed.). Handbook of Pesticide Toxicology. 2nd ed. San Diego, CA: Academic Press; 2001. 1043–1085.

Mackness B, Durrington P, Povey A, Thomson S, Dippnall M, Mackness M, et al. Paraoxonase and susceptibility to organophosphorus poisoning in farmers dipping sheep. Pharmacogenetics 2003;13(2):81–88.

McCauley LA, Anger WK, Keifer M, Langley R, Robson MG, Rohlman D. Studying health outcomes in farmworker populations exposed to pesticides. Environ Health Perspect 2006;114(6):953–960.

Nolan RJ, Rick DL, Freshour NL, Saunders JH. Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol 1984;73(1):8–15.

Quistad GB, Klintenberg R, Casida JE. Blood acylpeptide hydrolase activity is a sensitive marker for exposure to some organophosphate toxicants. Toxicol Sci 2005;86(2):291–299.

Reigart J, Roberts J. Recognition and Management of Pesticide Poisonings. 5th ed. Washington, DC: Environmental Protection Agency; 1999.

Richards P, Johnson M, Ray D, Walker C. Novel protein targets for organophosphorus compounds. Chem Biol Interact 1999;119–120:503–511.

Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status requires more than genotyping. Pharmacogenetics 1999;9(6):745–753.

Richter RJ, Jampsa R, Jarvik GP, Costa L, Furlong CE. Determination of paraoxonase 1 (PON1) status and genotypes at specific polymorphic sites. In: Mains M, Costa L, Reed D, Hodgson E (ed.). Current Protocols in Toxicology. New York, NY: John Wiley and Sons; 2004.

Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and non-agricultural Hispanic workers. Environ Health Perspect 2006;114(5):691–696.

Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, et al. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 1998;394(6690):284–287.

Smith T. 2006 Crop Protection Guide for Tree Fruits in Washington. Pullman, WA: Washington State University Extension; 2006. Report No.: EB0419.

Sozmen EY, Mackness B, Sozmen B, Durrington P, Girgin FK, Aslan L, et al. Effect of organophosphate intoxication on human serum paraoxonase. Hum Exp Toxicol 2002;21(5):247–252.

Suehiro T, Nakamura T, Inoue M, Shiinoki T, Ikeda Y, Kumon Y, et al. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. Atherosclerosis 2000;150(2):295–298.

WASS. Washington's agricultural fruit chemical usage, 2007: Apples. Olympia, WA: Washington Agricultural Statistics Service; 2008.

Wilson B. Cholinesterases. In: Krieger RI (ed.). Handbook of Pesticide Toxicology. San Diego: Academic Press; 2001. 967–985.

Yuknavage KL, Fenske RA, Kalman DA, Keifer MC, Furlong CE. Simulated dermal contamination with capillary samples and field cholinesterase biomonitoring. J Toxicol Environ Health 1997;51(1):35–55.

Zech R, Zurcher K. Organophosphate splitting serum enzymes in different mammals. Comp Biochem Physiol B 1974;48(3):427–433.

WSUCE. Crop Profile for Hops in Washington. Richland, WA: Washington State University Cooperative Extension; 2001. Report No.: MISC0353E.

WASS. Washington Ag Chemical Use. URL: http://www.nass.usda.gov/Statistics_by_State/Washington/Publications/Ag_Chemical_Use/index.asp. In. Olympia, WA: Washington Agricultural Statistics Service; Accessed December 2008.

USEPA. The use of data on cholinesterase inhibition for risk assessments of organophosphorous and carbamate pesticides. Washington, DC: Office of Pesticide Programs; 2000.