

CEEH ELSI CORE
Case Study 1

**GENETIC VARIATION IN *PON1* AND PESTICIDE SENSITIVITY:
IMPLICATIONS FOR WORKPLACE SCREENING**

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I. INTRODUCTION

The Ethical, Legal and Social Issues (ELSI) Core of the Center for Ecogenetics and Environmental Health was convened to evaluate when and how ecogenetics research, which focuses on identifying genetic polymorphisms that influence responses to hazardous substances in the environment, can be applied to public health. Specific objectives include the development of educational case studies that illustrate the ethical, legal, social and scientific challenges surrounding use of genetic susceptibility testing for particular gene-environment interactions that adversely impact health; consideration of the research agenda needed to address these implications; and evaluation of other ways in which ecogenetics research can contribute to improvements in public health.

An important potential application of genetic susceptibility testing is in the hazardous workplace. Occupational illness resulting from workplace chemicals and other toxic substances is an important public health problem, and genetic testing holds the promise of mitigating risk for those workers who are predisposed to react adversely to the work environment. However, historical experience with the use of genetic susceptibility testing in the workplace illustrate that this technology can also discriminate, stigmatize and unnecessarily exclude workers (Draper, 1991; 1998; Schill, 2000). In this case study we explore the ethical, legal and social implications of the application of genetic susceptibility testing to prevent or minimize occupational disease from pesticide exposure.

The case study focuses on the interaction between environmental exposure to chlorpyrifos, an organophosphate pesticide (OP) and polymorphisms in the *paraoxonase PON1* gene. The *PON1* gene regulates the activity and expression of PON1, an enzyme involved in the metabolism of certain organophosphate (OP) pesticides, including chlorpyrifos. Chlorpyrifos was selected as the environmental agent of interest because a substantial body of research in genetics, ecogenetics and environmental health suggests that polymorphisms in the *PON1* gene could mediate individual susceptibility to chlorpyrifos toxicity. Because chlorpyrifos is a widely used agricultural pesticide, genetic testing to identify farm workers who are predisposed to react adversely to this particular environmental exposure could play a significant role in efforts to reduce the disease burden associated from pesticide toxicity among agricultural workers

In conjunction with this first case study we designed a template for analysis that can be used with other ecogenetic case studies (Appendix). Consistent with the template, this case study begins by reviewing the environmental risk, the susceptibility genotype and the specific scientific, legal, ethical and social policy parameters that set the stage for a case specific analysis. Next, potential public health interventions are identified, with a

particular focus on genetic susceptibility testing. The case study concludes with an ELSI analysis and recommendations for further research.

II. THE ENVIRONMENTAL RISK

A. Use and Effects of Organophosphate Pesticides

OPs are a class of pesticide widely used to protect agricultural crops, buildings, homes and gardens from insects. OPs, like all pesticides, contribute significantly to the ability of modern agriculture to produce food and fiber in large quantities at low prices (Ecobichon, 1996). Agricultural pesticides as a whole are estimated to prevent a monetary loss of about \$9 billion each year in the U.S; for every \$1 invested in pesticides, the American farmer gets about \$4 in return (EPA, 2000).

First synthesized in the late 1930s in Germany, OP compounds were designed to poison the nervous system of target insects. However, the compounds are not selective. Because of similarities between the central and peripheral nervous systems of insects and mammals, they can also affect humans and other non-target species (Ecobichon, 1996). OP compounds inhibit the enzymes acetylcholinesterase and serum cholinesterase, resulting in the accumulation of acetylcholine, leading to cholinergic overstimulation of nerve fibers.

OPs inhibit the enzyme cholinesterase, leading to a variety of adverse health consequences. Acute toxic symptoms include shortness of breath, confusion, tremor, impaired coordination (ataxia), nausea, diarrhea, muscle weakness and cramping, headache, excessive salivation, increased sweating and bradycardia (Blondell, 1999). Death may occur from respiratory failure due to paralysis of respiratory muscles, increased bronchial secretions and depression of the respiratory center in the brainstem (PIRT, 2000; Blondell, 1999). Individuals with respiratory susceptibilities such as asthma may be particularly susceptible to the respiratory symptoms resulting from acute exposure (PIRT, 2000). Systemic symptoms can occur as a result of inhaled, ingested or transdermal exposure (PIRT, 2000), and local irritation of mucus membranes and conjunctival surfaces can also occur.

Some studies suggest that OPs also cause chronic neurobehavioral symptoms after either acute poisoning or chronic exposure, although the strength of this association is the subject of ongoing debate and study (Rosenstock et al, 1991; Daniell et al, 1992; Keifer et al., 1997). For example, a case control study in termiticide applicators in North Carolina found evidence for diminished performance of some coordination tasks in those exposed compared to non-exposed applicators (Steenland et al, 2000). In addition, the increased incidence of several non-specific symptoms was increased among exposed individuals, including dizziness, difficulties concentrating and problems with memory, depression, and headaches. Other case control studies have also document found chronic neurobehavioral effects resulting from organophosphate pesticide exposure (Savage et al, 1988; Rosenstock et al, 1991; Steenland et al, 1994; Stephens et al, 1995). However, an expert panel convened by Dow Agro Sciences (Dow) concluded that there was insufficient

evidence to document chronic medical symptoms following chlorpyrifos exposure (Gibson et al, 1998). Scientists at the Environmental Protection Agency (EPA) have disputed this conclusion (Blondell, 2000).

B. Chlorpyrifos

Chlorpyrifos is a broad-spectrum chlorinated OP that was first introduced in 1965 for control of foliage- and soil-borne insect pests on a variety of food and feed crops. Currently, chlorpyrifos is used on more than 40 different agricultural crops including: cranberries, strawberries, citrus, apples, figs, pears, nectarines, cherries, peaches, plums, grapes, almonds, pecans, walnuts, nut trees, onions, broccoli, brussel sprouts, cauliflower, asparagus, corn, tomatoes, beans, peas, wheat, peanuts, mint, and bananas. It is one of the most widely used OP insecticides in the U.S., and until recently was one of the major insecticides used in residential, commercial and public settings for pest control. Approximately 21 to 24 million pounds are used annually in the U.S., of which approximately 13 million pounds are applied in agricultural settings (U.S. EPA, June 8, 2000).

Chlorpyrifos is one of several OPs that are organophosphorothioate compounds. These particular OP compounds are inherently weak anticholinesterase agents. However, their oxon analogs are at least three-fold more toxic than the parent compound, posing a significant risk of neurotoxicity (Atterberry et al., 1997). In one published study the oxon exhibited a one-thousand fold greater rate of cholinesterase inhibition than the parent compound (Huff et al., 1994). Chlorpyrifos oxons are found in post-application residue on foliage (Yuknavage et al., 1997) and are also a byproduct of some of the metabolizing processes in humans and certain mammals. The distinction between exogenous and endogenous oxon exposure is an important one because the metabolic pathways available for detoxification appear to vary according to source of exposure, with genetic variation in the *PON1* gene playing the most significant role in susceptibility to exogenous exposures (Li, 2000). Other organophosphorothioate compounds commonly used in pesticides (and their oxon analogs) include parathion (paraoxon) and diazinon (diazoxon).

C. Organophosphates Toxicity as a Public Health Problem

Epidemiologic data on the nature and extent of pesticide poisonings nationwide are sparse to non-existent, making it difficult to estimate the incidence of illness following OP exposure, much less chlorpyrifos exposure (Blondell, 1997). However, the human toxicity of OPs is well established in case and incident reports (Blondell, 1999, Blondell, 2000) and a variety of evidence confirms that OP exposure is an important public health problem. Approximately three million pesticide poisonings occur worldwide, resulting in an estimated 220,000 deaths (World Health Organization, 1990). In the United States,

pesticide use and exposure is regulated at both the federal and state level, including requirements for pesticide registration and reporting requirements regarding use and injury. OPs and carbamates (the other group of widely used pesticides that are cholinergic inhibitors) are the only pesticides for which state law requires biomonitoring of workers (California Code, Title 3, Section 6728), attesting to the degree of concern regarding the public health implications of OP exposure (Wilson et al, 1997).

Data from the State of Washington Pesticide Incident Reporting and Tracking (PIRT) system confirm that OP exposure is an important cause of pesticide-related health problems in this state. PIRT tracks the incidence of pesticide exposure within Washington State, including incidents involving human health, danger to animals or contamination of environmental sites (PIRT, 2000). In 1997 and 1998, 204 complaints relate to pesticide exposure were filed per year with the tracking system, most involving OP exposure. Of these, investigation revealed that 41 in 1998 and 45 in 1997 involved significant exposure to people, livestock or other organisms related to food supply (e.g., bee kills or fish kills). No human deaths directly resulted.

About half the cases of pesticide exposure in Washington State in these two years were attributable to exposure in agricultural settings, 26-30% to residential applications, and the remainder to applications in commercial and other buildings (PIRT, 2000). Most cases involved direct exposure while handling the pesticide or exposure from drift or residues; only 12% were due to accidents.

The best estimate of worker vulnerability comes from the biomonitoring program currently in place among agricultural workers in California. In this monitoring program, cholinesterase levels are monitored as a measure of unsafe exposures to pesticides, because suppression of serum cholinesterase is one of the earliest measurable effects of exposure to high levels of OP and carbamate pesticides. One study found that among workers involved in pesticide application (presumed to be those with highest exposure) 23% had reductions in cholinesterase levels indicating pesticide overexposure, 5% had levels that were below the state's threshold for removal from the workplace and 1.5% had symptoms due to pesticide exposure (Ames et al, 1989). The generalizability of these data is uncertain because rates of enzyme suppression may be highly dependent on a number of local workplace parameters.

However, a clinic-based study comparing migrant farm workers and non-farm workers in North Carolina also suggests that cholinesterase suppression and pesticide exposure with potential health consequences are common among farm workers (Ciesielski et al, 1994). Mean cholinesterase levels were significantly lower in farm workers compared to non-farm workers, and 12% of farm workers had very low levels, compared to 0% of non-farm workers. In addition, half of the farm workers reported being sprayed by pesticides or working in fields with an obvious chemical smell.

D. The Relationship between OP Exposure and Toxicity

1. The Physiological Process and Dose Response

The body's metabolism or disposition of OPs involves the following steps: 1) absorption; 2) distribution; 3) biotransformation; and 4) elimination. OPs are absorbed into the blood stream through the skin, lungs and alimentary canal and distributed throughout the body. The body biotransforms these compounds through a series of chemical reactions designed to detoxify and reconstitute them in a water-soluble form suitable for excretion in the urine. The liver is the most active site for the biotransformation of toxins, although enzymes in the blood contribute significantly to the process of biotransforming many OPs (Rozman, 1996).

Animal studies suggest there are at least three important pathways for detoxifying the oxon analogs of chlorpyrifos and related OPs. First, the oxons can be hydrolyzed by PON1 enzymes in the liver and the blood (Aldridge, 1953; Butler et al., 1985). Second, the oxons can bind to carboxylesterase or butyrylcholinesterase enzymes (Wormhoudt et al., 1999). Third, cytochrome P450 enzymes (CYPP450s) can simultaneously bioactivate and detoxify the oxons (Wormhoudt et al., 1999; Eaton, 2000). Of these, the PON1 mediated hydrolysis reaction is described as the most important route for detoxification of OPs (Mutch et al., 1992). Indeed, the PON1 pathway is thought to be the exclusive primary metabolic pathway for detoxifying environmental oxon exposure that occurs in the field when agricultural workers are exposed to oxidized foliar residue of chlorpyrifos and other OPs (Li, 2000).. The distinction regarding metabolic pathways is relevant to any discussion of interventions to protect workers. It suggests that the greatest risk to workers with abnormal PON1 status occurs from exposures to oxons in the environment as opposed to endogenously bioactivated oxons. In the latter instance, there are multiple pathways for detoxification. All of the enzymes involved in the metabolism of OPs - the CYP450s responsible for bioactivation, PON1 and the other enzymes involved in detoxification - are subject to genetic variation, as well as environmentally regulated expression, particularly in the case of the CYP 450s.

Both experimental data and anecdotal observations confirm the increasing potential for toxicity from pesticide exposure with increasing dose (U.S. EPA Office of Pesticide Programs Health Effects Division, Human Health Risk Assessment: Chlorpyrifos, June 8, 2000). These stem primarily from animal studies. However, variability in dose-response has also been documented both across species and within human populations. (Main, 1956; Brealey, 1980; Costa et al., 1987, Li et al., 1995, Shih et al., 1998, Li et al., 2000).

Additionally, data from two human studies conducted by a leading chlorpyrifos manufacturer suggest that humans may be more susceptible to chlorpyrifos than other mammals and exhibit variable responses to a given dose, measured either by cholinesterase inhibition or clinical signs (EPA, 2000).

2. The Role of the *PON1* Enzyme

The PON 1 enzyme - historically referred to as paraoxonase and/or arylesterase - is predominantly synthesized in the liver and circulates in the serum as a component of high-density lipoprotein (HDL) particles (Furlong et al., 2000). Its role in hydrolyzing the oxon metabolites of several OP pesticides and nerve agents has been extensively studied. The physiologic function of PON1 is still under investigation; recent studies suggest it may play an important role in metabolism of low-density lipoproteins (LDLs) (Furlong et al., 2000).

Animal studies suggest that individual differences in both the rate of PON1 enzyme activity and the level of PON1 enzyme expression influence dose response to chlorpyrifos (see discussion in section IIIA below). Age is an important factor in PON1 activity and susceptibility to OP toxicity. Children under the age of two have lower enzyme activity than adults (Augustinsson et al., 1963; Ecobichon, 1972). An early study of newborns demonstrates that PON synthesis begins prior to 28 weeks gestation and increases until about 12 months of age, at which time PON levels gradually approach those found in the adult (Ecobichon, 1972). A subsequent study of the developmental progression of PON1 and other biotransformation enzymes that play a role in OP detoxification in animals suggests that risk of neurotoxicity from OP exposure is significantly increased in fetuses and infants (Atterberry et al, 1997).

III. CHARACTERIZATION OF THE GENETIC INTERACTION

A. The Contribution of *PON1* to Biotransformation of Pesticides

1. The *PON1* gene

The gene coding for PON1 is located on the long arm of chromosome 7 (7q21-7q22), proximal to the cystic fibrosis gene (Hassett et al., 1991; Humbert et al., 1993). This gene (designated *PON1*) spans approximately 26 kb and its coding sequence is comprised of nine exons, coding for 355 amino acids (Clendenning et al., 1996). Variation in PON1 hydrolytic activity occurs by two mechanisms: 1) a well-characterized polymorphism at position 192 controls catalytic efficiency; and 2) promoter mutations, still under investigation, appear to regulate enzyme expression. The combined effect of the position 192 genotype and level of enzyme expression is referred to as "PON1 status." *PON1* is a member of a multigene family, which includes *PON2* and *PON3* (Primo-Parmo et. al., 1996). The functions of *PON2* and *PON3* are still under study but research thus far suggests neither gene mediates OP metabolism.

2. The position 192 genotype

In the 1980s, a series of *in vitro* studies comparing individual rates of paraoxon hydrolysis in humans suggested the presence of two alleles, one conferring a high rate of hydrolytic activity toward paraoxon and the other a low rate (Geldmacher et al., 1988). The alleles appeared to be autosomal and inherited in a Mendelian co dominant fashion, with three phenotypes among the Caucasian populations studied: low activity (homozygous low), intermediate activity (heterozygous) and high activity (homozygous high) (Furlong et al. 1988, 1989). The molecular basis of the variation in rate of hydrolytic activity is now known to be an amino acid substitution of glutamine for arginine at position 192 of the PON 1 gene (Humbert et al., 1993; Atkins et al., 1993). This substitution, referred to as the Q192 genotype results in reduced paraoxon hydrolytic activity compared to the R192 genotype (Shih et al., 1998; Li and Furlong et al., 2000). The effect of the substitution is reversed for chlorpyrifos oxon and diazoxon (Li et al., 2000).

3. The regulation of enzyme expression

An individual's PON1 expression is stable over time, which is to say the enzyme is constitutive, not inducible (Zech et al., 1974). However, for each of the PON1 position 192 alleles there is at least a 13-fold inter-individual variation in enzyme level (Furlong et al., 1989; Davies et al., 1996; Richter & Furlong, 1999). The level of PON1 expression appears to be under genetic control (Brophy et al., 2000; Leviev & James, 2000; Suehiro et al., 2000). Initially investigators thought expression levels might be a function of a second *PON1* coding polymorphism involving an amino acid substitution of methionine for leucine at position 55. *PON1M55* individuals were found to have lower rates of PON1 activity (Mackness et al., 1998b) and lower levels of PON1 mRNA (Leviev et al., 1997). Subsequent research suggests this effect is due to linkage disequilibrium between the M55 polymorphism and several different promoter mutations that influence levels of PON1 expression (Brophy et al., 2000; Leviev & James, 2000; Suehiro et al., 2000). Efforts to characterize the promoter mutations and their influence on enzyme expression are ongoing and suggest that a polymorphism in the *PON1* regulatory region at position 108 (108C/T) strongly influences PON1 levels, with the 108C allele generating approximately twice the level of plasma PON1 as the 108T allele.

B. The Association Between PON1 Status and Chlorpyrifos Sensitivity

Animal studies support the hypothesis that PON1 status plays an important role in the detoxification of OPs, including chlorpyrifos. These studies have measured the protective effect of pre-treatment with purified rabbit PON1 in rats (Main, 1956; Costa et al, 1990; Li et al, 1995) and mice (Costa et al, 1990; Li et al, 1995). The degree and distribution of protection varies with the OP substrate and its route of exposure. For example, IV injection of rabbit PON1 appears to increase serum PON1 activity in both rats and mice, providing increased protection against toxicity from dermal, oral and IV exposure to chlorpyrifos, with the greatest protection afforded to brain and diaphragm

tissues. . Intraperitoneal injection also provides protection (Li et al., 2000). Achieving the most pronounced and consistent protective effect in brain and diaphragm tissue is important since OP induced neurotoxicity occurs primarily because of accumulation of acetylcholine in the brain and diaphragm (Costa et al, 1990).

Two published studies using *PON1* knock out mice suggest that human PON1 status influences toxicity of chlorpyrifos (Shih et al, 1998; Li et al, 2000). In the first study *PON1* knockout mice (*PON1* *-/-*) had dramatically increased sensitivity to chlorpyrifos, compared to wild type mice (*PON1* *+/+*) at each of three exposure levels (1.5 mg., 3 mg., and 6 mg.) (Shih et al, 1998). The two highest doses produced clinical symptoms in the knockout mice within 1 to 2 hours of exposure and death within 2 to 4 hours. The wild type mice remained asymptomatic with only mild suppression of acetylcholinesterase. The lowest dose inhibited cholinesterase activity in the knockout mice while having no effect in the wild type.

In the second study (Li et al., 2000), *PON1* knockout mice exhibited a dramatic increase in sensitivity to diazoxon and chlorpyrifos oxon compared to wild type controls. Hemizygotes showed intermediate sensitivity. Similarly, doses of diazoxon that did not inhibit brain cholinesterase in wild type controls were lethal to the knockout mice. Furthermore, this study demonstrated that injecting either human PON1192 isoform into wild type mice plasma restores resistance to chlorpyrifos oxon and diazoxon. The degree of resistance depends upon the catalytic efficiency (V_m/K_m) of the specific *PON1*192 isoform for the specific OP substrate. For example, the *R192* isoform offered greater protection against chlorpyrifos oxon. Two of the *Q192* mice developed clinical signs of OP toxicity after exposure whereas no signs were observed in the *R192* mice. In this study, *PON1* knockout mice demonstrated very limited hydrolytic activity in either the plasma or the liver when exposed to chlorpyrifos oxon, and hemizygous mice exhibited 40% of activity found in the wild type mice. When transgenic mice (human *PON1*R-Tg and human *PON1*Q-Tg) were exposed to chlorpyrifos oxon the *R192* genotype exhibited 1.7-fold higher hydrolytic activity and provided greater protection against toxicity. Two of the *Q192* mice developed clinical signs of OP toxicity after exposure whereas no signs were observed in the *R192* mice (Li et al., 2000).

Animal studies thus consistently demonstrate an association between PON1 status and susceptibility to chlorpyrifos toxicity. The combination of the position 192 polymorphism and the variability in expression is estimated to result in a greater than 60-fold inter-individual difference in rates of chlorpyrifos detoxification in humans (Li et al., 1993). However, further epidemiologic studies assessing this association in populations of exposed workers are needed before firm conclusions regarding the strength of this association and the degree of risk for pesticide toxicity can be made. Further characterization of the association between PON1 status and OP toxicity should ideally take into account variation in other metabolic pathways. The body's disposition of hazardous substances involves multiple metabolizing enzymes, all of which are under

some degree of genetic control and many of which are polymorphic. As a result, complex and varied interactions among several genes undoubtedly influence variations in dose response and risk of toxicity (Hirvonen, 1997).

Another factor influencing predictive value of tests for PON1 status is the potential effect of source of oxon exposure has on susceptibility. Oxons are produced in the environment as a result of an oxidation process that transform foliar residue over time, depending upon a variety of environmental and climatic factors (Yuknavage KL et al, 1997) and are also produced endogenously through one of several metabolic pathways for detoxifying chlorpyrifos and other OP parent compounds. PON1 status has the most significant impact on susceptibility to oxons in the environment because it appears to be the exclusive pathway for de-toxifying such residues, whereas it is one of several options for detoxifying endogenous oxons.

C. Measurement of PON 1 Status

Furlong et al. have developed a high-throughput, two-dimensional enzyme analysis that predicts PON1 phenotype (“PON1 status”) and provides an accurate inference of PON1192 genotype (Richter & Furlong, 1999). This enzyme analysis provides a measure of overall PON1 status (Richter & Furlong, 1999). Using a high-throughput microtiter spectrophotometer two-substrate assay, investigators measure the rates of chlorpyrifos hydrolysis and paraoxon hydrolysis and then plot the rates against one another. The resulting population distribution plot clearly resolves PON1 phenotypes and provides an accurate inference of genotype (Brophy et al., 2000). In the study of this assay, samples were also genotyped using PCR. Of the 317 samples, 316 (99.7%) showed agreement between the PCR genotype and the genotype inferred from the enzyme assay (Brophy et al., 2000). Thus, this method provides a means to assess genetic susceptibility to OP pesticide exposure.

IV. OVERVIEW OF GOVERNMENTAL REGULATION AND LEGAL, ETHICAL AND POLICY ISSUES RELEVANT TO PESTICIDES AND WORKPLACE GENETIC TESTING

A. Existing Federal Regulation of Pesticides

Federal regulation of pesticides is authorized pursuant to several pieces of federal legislation, principally, the Federal Insecticide, Fungicide and Rodenticide Act (FIRFA, 7 U.S.C. secs. 121-136y), the Federal Food, Drug and Cosmetic Act (FFDCA, 21 U.S.C. secs. 301 - 394) and the Occupational Health and Safety Act (OSHA, 29 U.S.C. secs. 651-678). These laws, and the regulations adopted for their implementation, provide a comprehensive framework for promoting public, workplace and environmental health and safety. Primary responsibility for implementation, management and enforcement of these laws falls to the federal Environmental Protection Agency (EPA). The EPA and the

Occupational Health and Safety Administration (OSHA) share responsibility for implementing, overseeing and enforcing laws that promote the health and safety of workers who are occupationally exposed to pesticides.

Pursuant to FIRFA, the EPA is responsible for registering or licensing pesticide products for use in the United States. Pesticide registration decisions, which are based on detailed risk assessments, determine limitations on use. Use limitations, which must be stated on the labels and followed by all applicators, include protective clothing requirements, re-entry levels for treated areas, prohibition of use under certain weather conditions or at certain sites and prohibition of aerial application. Any use not in accordance with label directions and precautions may be subject to civil and/or criminal penalties. In addition to registration requirements, FIRFA requires the EPA to reevaluate older pesticides to ensure their safety and also requires applicators using restricted-use pesticides (i.e. pesticides that are more toxic or otherwise raise concerns about adverse effects if used by untrained personnel) be trained and certified. Since 1978 the states have had primary responsibility for enforcing pesticide use violations and for training and certifying pesticide applicators.

FFDCA governs the establishment of pesticide tolerances -- the maximum level of pesticide residues allowed in or on human food and animal feed. The EPA is responsible for setting tolerance levels and Food and Drug Administration and United States Department of Agriculture inspectors monitor food in interstate commerce to ensure that tolerance levels are not exceeded.

OSHA has responsibility for setting policies regarding handling and exposure to chemicals in the workplace. OSHA regulations, together with EPA's worker protection standards, require that workers who handle or apply pesticides be properly equipped with and properly trained in the use of personal protective equipment. In addition, OSHA, through their Hazard Communication Standard (HCS), requires employers to establish a hazard communication program to keep employees informed on the hazards of chemicals they are working with. HCS requires that pesticide containers be labeled, pesticide hazard information provided in detailed Material Safety Data Sheets, and effective training programs conducted for all potentially exposed employees. Dressing for the job and understanding the potential risks of pesticide exposure are required for anyone who handles, mixes, loads or applies agricultural chemicals.

B. Pesticide Risk Assessment

The process used by the EPA to evaluate the health impact of a pesticide is referred to as risk assessment (NRC, 1983). This process involves four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Additionally, pesticide products currently on the market are open to reevaluation to ensure that regulation reflects current safety information.

1. Hazard Identification

Hazard identification is the process by which potential health effects of pesticide exposure are determined. Generally, hazards are identified through toxicity tests performed in laboratory animals. These tests include evaluation of acute, sub chronic, and chronic exposures (Keifer and Arne, 1997). Acute exposures can produce acute intoxications, dermal irritation, or eye irritation, and in some cases delayed neurotoxicity. Sub chronic exposure studies are normally carried out for 90 days, and can involve dosing via oral, dermal, and inhalation routes. A wide range of toxic endpoints is assayed in these experiments. Chronic exposure studies examine the effects of lifetime exposures, and focus on such health effects as cancer, birth defects, and fertility problems. Pesticide manufacturers conduct these tests with protocols approved by EPA, and EPA scientists evaluate the results. Evaluators also consult published literature and scientific information from other countries as a part of the hazard assessment. In 1998 EPA's Hazard Identification Assessment Review Committee reassessed 40 OP pesticides for neurotoxic, developmental, and reproductive toxic potential using recent toxicology data.

2. Dose-Response Assessment

Dose-response assessment enables the EPA to estimate a safe level of exposure for humans exposed to a particular pesticide. Controlled studies with laboratory animals allow scientists to observe responses associated with particular dose levels, and to establish the lowest observable adverse effect level (LOAEL) and the no observable adverse effect level (NOAEL). The LOAEL is the lowest dose that elicits an adverse response, while the NOAEL is the highest dose that does not elicit such a response. Typically the NOAEL is used as the primary toxicological benchmark for pesticide risk assessments. The NOAEL is determined in the most sensitive species, and for the most sensitive adverse endpoint. In the case of OP pesticides, the inhibition of blood cholinesterase is the endpoint of choice, and significant inhibition (10-20%) is deemed an adverse effect. In most risk assessments the NOAEL is adjusted by two 10-fold-uncertainty factors. The first factor accounts for extrapolation from animal models to humans, while the second factor accounts for variability among humans. Additional uncertainty factors may be included to account for weaknesses in the toxicology data. EPA currently refers to the adjusted NOAEL as the "reference dose". A given pesticide may have several reference doses, depending on routes of exposure and the exposure duration.

3. Exposure Assessment

Exposure assessment attempts to identify the nature and size of the population(s) exposed, along with the magnitude and duration of exposure. Exposure assessment information is not always required for pesticide registration or re-registration, but EPA has the authority to request such information from pesticide manufacturers. EPA most

often asks for information on the decay of pesticide residues on plants and in soil, and in certain cases the Agency will require personal exposure data (e.g., inhalation and skin exposure measurements on workers). All such studies are conducted under protocols approved by EPA scientists. EPA then uses these data to estimate pesticide doses under specific exposure scenarios. EPA will sometimes use so-called “surrogate data” to construct an exposure scenario when chemical-specific exposure data are lacking. For example, if workers have been monitored for exposure to an OP pesticide in an orchard, EPA may use these data to estimate exposures under similar conditions for other OP pesticides. In the case of pesticide applicators, EPA also uses a generic database developed in collaboration with pesticide manufacturers and the Pest Management Regulatory Agency of Canada. The Pesticide Handler Exposure Database contains data from a large number of pesticide applicator exposure studies that have been conducted by pesticide manufacturers. A companion database for agricultural reentry workers is currently under development. EPA has also required exposure data for many common residential uses of pesticides.

4. Risk Characterization

Risk characterization, the final step in the assessment process, combines the principal findings of the hazard identification, dose-response, and exposure assessment steps into an integrated picture of the nature and expected frequency of adverse health effects in exposed populations. Risk characterization involves substantial professional judgment regarding the quality of data and the assumptions employed in the interpretation of data (NRC, 1994). The final risk assessment that emerges from this process is a primary determinant of the risk management strategies that the Agency selects to mitigate pesticide health risks.

In the case of chlorpyrifos, EPA issued a final, revised risk assessment in August of 2000 following an extended comment period (EPA, 2000). The risk assessment found that an estimated 20-24 million pounds of chlorpyrifos are expected to be applied annually - 50% for agricultural purposes and 50% in non-agricultural settings. The mitigation strategies outlined in the risk assessment are expected to reduce the total use of chlorpyrifos by as much as 50% when fully implemented based on available use data from the late 1990's. Worker risks were viewed as moderate. For mixers, loaders, and applicators, risks for some exposure scenarios remain of concern even with maximum personal protective equipment and engineering controls in place.

C. Existing State Regulation of Pesticides

All states are delegated FIRFA enforcement authority, accompanied by EPA funding through a cooperative grant agreement that requires states to investigate all pesticide related complaints and to perform inspections of marketplaces, dealers, producers and users of pesticides. In addition, most states have their own programs requiring 1)

pesticide registration; 2) pesticide-use reporting; 3) registries for chemically sensitive persons; 4) integrated pest management; and 5) pesticide incident reporting requirements. Finally, many states have OSHA approved state agencies that are responsible for implementing, overseeing and enforcing laws regarding the safety and health of workers exposed to pesticides. Not surprisingly, state pesticide programs vary significantly in terms of size, scope of regulatory control and enforcement capabilities.

As an example, several agencies in Washington State have regulatory authority over pesticide use, exposure and monitoring, as determined by state laws (RCW 70.104.030, WAC 16-228-233, WAC 246-100-217). The Washington State Department of Agriculture adopts and administers pesticide regulations, tests and certifies pesticide applicators, administers continuing education requirements for pesticide applicators and investigates complaints of pesticide misuse or misapplication. The Department of Health has responsibility for the medical investigation of suspected human pesticide poisonings and animal poisonings that could relate to human illness. It is also responsible for providing technical assistance and community information regarding pesticide risks and exposure, establishing pesticide illness/reporting mechanisms, and establishing and implementing the pesticide incident reporting and tracking review panel (PIRT 2000). The Department of Ecology is responsible for administering a regulatory and educational program to support proper management and disposal of pesticide wastes, including provision of educational and technical assistance and investigation and enforced mediation of incidents involving spills or other contamination. The Department of Labor and Industry is responsible for safety and health workplace inspections and for measures to promulgate workplace safety, including assurance of compliance with OSHA standards. The Department of Natural Resources has responsibility for permits for pesticide application in forests and for monitoring of permit restrictions. All of these agencies have strict response time mandates to ensure rapid investigation of incidents involving serious pesticide exposure.

Another example of state regulation is the California biomonitoring program that requires employers to provide medical supervision, base-line assessments and ongoing monitoring of blood cholinesterase levels for agricultural employees who are exposed to chlorpyrifos and other cholinesterase inhibiting pesticides for more than 7 days in any 30 day period (California Code, Title 3, Section 6728; California EPA Guidelines for Physicians who Supervise Workers Exposed to Cholinesterase-inhibiting Pesticides, 1995). In addition to the medical supervision and monitoring requirements, employers have strict record keeping and incident reporting requirements. Employers are responsible for obtaining and paying for the required medical supervision and cholinesterase testing. Cholinesterase levels must be tested at a state approved laboratory using testing methods that are approved by the state. Results are to be reported to the supervising physician and the employer. Employees are entitled to learn the results.

This program sets threshold levels of cholinesterase depression that require interventions to minimize workers' risk of illness. If a worker's cholinesterase level falls below 80% of his or her baseline value the employer must review the condition of safety equipment and the employee's work practices, including sanitation, pesticide handling procedures and equipment usage, and to make necessary adjustments in the workplace to reduce exposure. If a worker's plasma cholinesterase level falls to 60% or less of the baseline value (70% for red cell cholinesterase levels), the employer must remove the worker from the job site for the time interval necessary to permit his or her cholinesterase values to return to 80% or more of baseline. Workers who are removed from a job because of depressed cholinesterase levels may be employed at other types of work, if available. If no such work is available the employee is considered occupationally disabled under Workers' Compensation provisions until ready to return to his/her usual job, even though the worker is not symptomatic.

D. Legal Considerations in Workplace Genetic Testing

1. Restrictions on the Use of Genetic Tests in the Workplace

Presently there are no federal or state laws that place an absolute ban on employer use of genetic tests to inform decisions regarding employment, job placement and/or benefits. However federal and state laws impose varying restrictions that arguably act to discourage employers from using this technology to screen workers. Title VII of the Civil Rights Act precludes employers from using any screening tests that, in effect, discriminate on the basis of race, color, religion, sex or national origin. Additionally, federal and state laws prohibiting disability discrimination limit employers' prerogatives with respect to genetic testing. Finally, federal and state genetic non-discrimination laws variably restrict the use of genetic testing in the employment context.

Title VII of the Civil Rights Act prohibits discrimination on the basis of race, ethnicity, gender or religion. At least one court has held that this federal law prohibits nonconsensual genetic testing that has a disparate impact on a protected group (*Norman-Bloodsaw v. Lawrence Berkeley Laboratory* 135 F.3d 1260 (9th Cir. 1998)). In this case the Ninth Circuit Court of Appeals ruled that black employees who were subjected to nonconsensual genetic screening for sickle cell trait during a pre-placement medical evaluation had a legitimate Title VII claim against their employer because the testing program had a disparate impact these employees. To successfully defend a Title VII claim an employer must show that its genetic testing program is job related and a business necessity. This is a difficult defense to make in the context of a genetic screening program related to an environmental exposure because a less restrictive means for accomplishing protection of workers exists - reducing the hazardous exposure for all employees. Moreover, the courts have clearly set a high threshold for business necessity. Saving money is not an adequate justification. *Chrapliwy v. Uniroyal, Inc.* 458 F. Supp. 252, 259-72 (N.D. Ind. 1977) held that an employer may be forced to incur large expenses in

order to comply with Title VII. Even avoiding fetal injury is an insufficient business necessity to overcome a claim of disparate impact by a protected class. (*United Automobile Workers v. Johnson Controls, Inc.* 111 S.Ct. 1196 (1991).)

The principal source of protection for workers from disability discrimination is the Americans with Disabilities Act (“ADA”, 42 U.S.C. secs. 12010-12213). Additionally there is the Rehabilitation Act of 1973 (29 USC secs. 701-796) protecting federal workers from disability discrimination, and numerous state laws similar in scope and effect to the ADA. The ADA prohibits employers of 15 or more employees from discriminating against a “qualified individual with a disability” in hiring, promotion, discharge, compensation and other terms/conditions of employment. To be “qualified” an individual either has, has had or is regarded as having one or more physical impairments that substantially limit a major life activity. Employers must make reasonable accommodations in the workplace for qualified individuals if such accommodations are needed and do not impose an undue hardship on the employer. The Act does permit employers to discriminate if they can establish that the adverse employment action is job related, a business necessity or required because of a direct threat to the health or safety of other individuals in the workplace (Rothstein, 2000; Rothstein, 1992).

The ADA regulates employers’ ability to use genetic tests by dividing the employment process into three stages: 1) application (“pre-employment”); 2) conditional offer (“pre-placement”); and 3) employed. At the application stage employers cannot make any inquiries regarding a prospective employee’s health or medical history and cannot require any medical screening. Once an offer of employment is extended, employers can require medical screening. The scope of the screening process can be as comprehensive as the employer desires - it need not be limited to job related conditions - and can include genetic testing. However, the information obtained during this process cannot be used to discriminate in job placement or benefits unless the discriminatory action is job related or dictated by business necessity (*Norman Bloodsaw v. Lawrence Berkeley Laboratories*, 135 F.2d 1260 (9th Cir., 1998)). The ADA does not permit genetic testing of existing employees unless the testing is directly related to their qualifications to complete the job or otherwise necessary for employee safety.

To the extent it precludes or limits an employer’s ability to use genetic information in decisions regarding employment and/or benefits the ADA indirectly impacts occupational use of genetic screening tests. The ADA generally prohibits employers from discriminating against disabled individuals in decisions regarding hiring, termination, compensation and other terms/conditions of employment. To qualify a person must have, have had or be regarded as having one or more physical impairments that substantially limit a major life activity. Thus, the ADA clearly precludes employer use of genetic information to discriminate against individuals with a symptomatically genetically related illness or history of the same and therefore argues against using genetic testing as part of an employee screening process. However, it does not directly preclude

an employer from basing employment related decisions on asymptomatic, predictive genetic information, such as a predisposition to pesticide toxicity., on their genetic information. The Equal Employment Opportunity Commission (EEOC) has interpreted the ADA as prohibiting employers from discriminating against employees on the basis of predictive genetics tests (EEOC Compliance Manual, secs. 902-45 (March 14, 1995)) However, the EEOC's interpretation of the Act has yet to be tested in court and the nuances of related court opinions provide less than clear guidance regarding whether and how the court might apply the ADA in the context of adverse employment decisions based upon genetic screening for increased susceptibility to workplace toxins (Miller, 2000; Kaufman, 1999).

More than half of the states and the federal government have enacted genetic non-discrimination laws that vary in their impact on the employer's ability to use genetic tests to screen potential and/or current employees. The principal focus of these laws is to prevent employers from discriminating against currently healthy individuals who are able to meet job demands because of a genetic pre-disposition or susceptibility to future illness (Yesley, 1999; Rothstein, 2000). The concern is that employers will exclude these individuals to avoid the potential for increased health care costs, worker compensation claims and expenses arising from accommodating the absenteeism and potential disability needs of sick workers.

Genetic non-discrimination laws in Iowa, New Hampshire, New York, Oregon, Rhode Island, Texas and Wisconsin prohibit employers from soliciting, requiring or administering a genetic test in either the application process or as a condition of employment. However, in Iowa, New Hampshire, New York and Wisconsin an exception exists for consensual genetic testing for the purposes of investigating workers' compensation claims or for determining and monitoring worker susceptibility to workplace toxins. New York laws permit employers to require genetic testing for traits that indicate increased risk of disease as a result of workplace exposures.

Beyond specifically limiting the use of genetic tests, state laws provide varying levels of restriction on an employer's ability to use genetic test results - or genetic information generally - in employment decisions. About half of the states have enacted laws that prohibit genetic discrimination in employment, although some make exceptions when the discrimination is related to the employee's ability to perform the job. Whether and/or to what extent genetic traits that increase susceptibility to workplace exposures fall into this exception is, as in the ADA context, an open question and ongoing source of debate. To date, only California appears to have addressed this question directly. In 1998 the state legislature amended its Fair Employment and Housing Act (FEHA) to prohibit employment discrimination based on asymptomatic genetic characteristics, in companies with five or more employees (Draper, 1999). The statutory definition of genetic characteristics includes "any scientifically or medically identifiable gene or chromosome, or combination or alternation thereof, that is ... determined to be associated with a

statistically increased risk of development of a disease or disorder that is presently not associated with any symptoms of any disease or disorder” (Cal. Stat. ch. 99). Although California does not specifically prohibit employers from using genetic screening tests for workers, the 1998 FEHA amendments provide greater express limitations on the extent to which employers can use test results than the ADA or most other state non-discrimination statutes.

At the federal level Federal Executive Order 13145 to Prohibit Discrimination in Federal Employment Based on Genetic Information (“Executive Order”)(65 Fed. Reg. 6,877 (2000)) prohibits federal employers from requiring or requesting genetic tests as a condition of being hired or receiving benefits. Moreover, it precludes federal departments and agencies from requesting or requiring employees to undergo genetic tests to evaluate their ability to perform a job. However, federal employers may request or require family medical history from employees who have received a conditional offer of employment, to the extent necessary to evaluate whether further medical evaluation is needed to diagnose a current disease, medical condition or disorder that could prevent the employee from performing an essential function of the job (EEOC, 2000). The employer must subject all employees who have received a conditional offer of employment in the same job category to the same assessment and may not follow up by requiring, requesting or using genetic tests or the results of genetic tests. The Executive Order mandates a similar approach to current employees. Whether an employee is qualified to perform essential job functions must be decided on the basis of present ability to safely perform (EEOC, 2000). The scope of the Executive Order has yet to be tested in the courts; however from the perspective of the EEOC it is a clear and unequivocal statement that use of genetic information to make employment decisions based upon a worker’s predisposition to a disease, medical condition or disorder which has no bearing on his or her present ability to do the job is inappropriate (Miller, 2000).

In addition to state and federal statutory law, the United. States. Supreme Court’s opinion in *United Automobile Workers v. Johnson Controls, Inc.*, 111 S.Ct. 1196 (1991) provides a disincentive to use genetic screening tests in an effort to exclude high-risk workers. The court overturned a fetal protection policy that resulted in the exclusion of fertile women from relatively high-paying jobs involving lead exposure. The justification for the policy was the belief that exposing women to lead might damage fetuses and also expose the employer to lawsuits seeking compensation for fetal injuries. The court’s decision rested on two considerations. First, employers should not discriminate against one of many possible high-risk groups. In this case “fertile women” was but one of the subgroups of workers who were at risk from lead exposure. Instead of excluding fertile women, the employer should undertake workplace protections that benefit all exposed workers. Second, employers should not unilaterally decide that fetal health takes priority over women’s right to equal employment opportunity. In other words, the decision whether to take on the health risks associated with lead exposure should be left up to the worker. Although the excluded workers in this case were members of a class that Title

VII of the Civil Rights Act protects from employment discrimination (i.e. women) the court's reasoning arguably limits an employer's ability to use individual worker safety as a grounds for excluding genetically susceptible workers in situations where the workplace exposure at issue is potentially harmful to all workers (Draper, 1992).

2. Legal Incentives to Use Genetic Screening Tests

Experts have debated whether OSHA guidelines can be construed as requiring employers to use genetic susceptibility screening (Rothstein, 2000; Kaufman, 1999). Some argue that OSHA's mandate to provide safe and healthy workplaces obligates employers to use genetic testing for increased susceptibility to workplace exposures. Others contend that genetic testing to identify and exclude workers with increased susceptibility runs counter to OSHA's goal of providing safe workplaces that are free from recognized hazards that threaten serious physical harm. Providing a mechanism for removing susceptible workers encourages employers to continue operating hazardous work sites while at the same time creating a class of unemployable workers. In addition to the consideration of these questions, any use of workplace genetic testing would be subject to federal and state regulation prohibiting employment discrimination, as well as scrutiny under federal and state constitutional provisions protecting individual rights to privacy (Miller, 2001; Kaufman, 2000; Rothstein, 1983).

State laws governing workers' compensation plans arguably provide a powerful incentive for employers to use genetic screening tests. Workers' compensation is a state mandated program in which employers are assumed to be responsible for their workers' job related injuries. Pursuant to these programs employers are required to provide compensation and medical coverage to workers who suffer injuries or illness resulting from their job. The claimant must prove that his/her injury or illness is work related and many states permit the current employer to apportion liability among all of the companies that could have contributed to the employee's current condition (Rothstein, 1983). The results of genetic tests could be used to refute the claim that workplace exposures caused an injury or illness. Alternatively, genetic screening tests could be used to exclude high-risk workers, reducing the potential for workers compensation claims, or to advise employees of their risk and potentially shift responsibility for adverse health reactions to employees who knowingly assume the risk and go forward with work.

Although the *Johnson Controls* case discussed above supports the employer who permits informed workers to make choices regarding risks, it does not speak to the effect on worker compensation rights should the employee decide to accept a workplace risk and become ill following workplace exposure. Many believe limited job opportunities and economic imperatives prevent workers in this situation from making truly autonomous choices and would argue that a choice to work should not deprive the genetically susceptible worker from employer paid compensation for workplace injuries (Draper, 1991). Rothstein and others argue that disqualifying individuals at genetically

increased risk of occupational disease from workers' compensation would violate important public policies underlying these programs, would eliminate all incentives for employers to clean up hazardous workplaces and would treat genetically at-risk workers less favorably than others workers with occupational illnesses - essentially permitting employers to accomplish indirectly what state genetic non-discrimination laws seek to prevent (Rothstein, 2000). At least one state has addressed this issue in the context of pesticide workers who are exposed to chlorpyrifos. The California mandatory biomonitoring program expressly provides that workers shall receive worker compensation benefits while unable to work because of depressed cholinesterase levels.

Prior to the advent of worker compensation programs, employers had common law duties to provide for the protection of employees. Those duties included providing a safe place to work, providing safe tools and equipment, warning of dangers about which the employee might reasonably be expected not to know, providing a sufficient number of suitable coworkers to ensure the safety of each worker and promulgating and enforcing rules that would make the work safe (OTA, 1983). However, employees who suffered injuries or illnesses at work had to sue their employers to recover compensation. Although these common law duties continue, most state laws provide that workers compensation is the exclusive remedy for workplace injuries and illnesses. In some states, such as Washington State, an employee can elect to waive his or her worker's compensation and sue the employer directly for negligence. In this instance, a defense based upon genetic screening test results and the worker's assumption of the risk could prove beneficial to the employer in offsetting liability for occupationally induced illnesses or injuries.

3. Privacy and Confidentiality

Federal and state constitutional provisions recognizing and protecting individual privacy rights from government interference provide a barrier to mandatory, nonconsensual testing programs, in most instances. However, individual privacy rights are not absolute and must be balanced against the government's interests in protecting public health and safety. Thus, concerns regarding public health or safety could provide a justification for mandatory genetic screening, in circumstances where a test is highly predictive of risk of serious illness following specific workplace exposures.

The ADA, the Rehabilitation Act of 1972, the Executive Order and the various state genetic privacy and non-discrimination laws contain provisions intended to protect the confidentiality of employee's genetic information. Generally, these provisions require that any genetic information obtained by employers be maintained with the employee's medical information and that release of that information requires written employee consent.

E. Ethical Considerations in Workplace Genetic Testing

A concern for worker autonomy - the right of their employees to make independent, informed decisions about their working circumstances - is at the forefront of any discussion of genetic testing in the workplace (Omenn, 1995). The employer's established obligation to recognize and respect worker autonomy argues against mandatory genetic testing programs that yield highly personal and potentially stigmatizing information about the individual worker and his or her pre-dispositions toward disease. However, employers also have ethical and legal duties to act beneficently, protecting and promoting the health and safety of their employees. It is these duties that prompt consideration of mandatory genetic screening programs as a tool for protecting workers from injury. When the workplace involves hazardous exposures should autonomy or beneficence be the prevailing guidepost?

Historical experience with workplace genetic testing provides some insights regarding how to resolve this dilemma. Significant interest in workplace genetic screening began in the early 1960s when scientist Herbert Stockinger, then at the National Institute for Occupational Safety and Health (NIOSH) published a series of articles suggesting that certain genetic traits that could be tested for might predispose exposed workers to increased risk of adverse health outcomes (Stockinger et al., 1973). Over the next 20 years a number of companies employed genetic tests to screen workers for sickle cell trait, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, alpha1-antitrypsin deficiency and other traits thought to increase risks for anemia, hemolytic crisis, emphysema or other health problems in response to exposure to certain chemicals (OTA, 1983). In the 1980s the use of these tests was challenged on the grounds that they failed to meet established scientific criteria for predictive value and were therefore potentially labeling and excluding workers inappropriately (Omenn, 1982; OTA, 1983). In spite of these challenges, and ongoing uncertainties about the predictive value of most available genetic susceptibility tests (Vineis et al, 1994; Schill, 2000) genetic susceptibility testing continues to be used by some employers (OTA, 1990; American Management Association, 1999 and 2001).

Screening for sickle cell trait provides one of the most glaring examples of the misapplication of susceptibility screening in the occupational setting. (King, 1992; Kaufman, 1999; Rothstein, 1984). In the early 1970s many employers excluded African American employees who were carriers of sickle cell trait from certain jobs, believing they were genetically susceptible to workplace toxins such as benzene, lead, carbon monoxide and cyanide, even though available evidence failed to establish a meaningful association. Indeed, sickle trait status has no impact on individual health, susceptibility or ability to perform job tasks. Because this trait is distributed primarily along racial lines, the application of the sickle trait testing compounded other social forces limiting employment opportunities of black workers.

Numerous surveys suggest the public is concerned that employers will use genetic screening tests to discriminate against workers, in an effort to avoid long term medical expenses, workers' compensation expenses and losses in productivity, even when the association of genetic traits that with job capability or vulnerability to work place exposures is uncertain (Billings, 1992; Geller, 1996; Lapham, 1996). Indeed, a recently settled lawsuit filed by the Equal Employment Opportunity Commission (EEOC) against Burlington Northern Railroad (BNRR) involved a situation where an employer allegedly used highly speculative genetic testing in an effort to avoid paying worker compensation claims to employees who were suffering from carpal tunnel syndrome (EEOC, April 18, 2001). Carpal tunnel syndrome is a form of peripheral neuropathy that is associated with pain and numbness. In the vast majority of work-related cases it is the result of repetitive stress injuries. The test used by BNRR assessed the presence of mutations in the PMP-22 gene that increase individual susceptibility to peripheral neuropathies, including carpal tunnel syndrome (Bird, 2001); however, the prevalence of these mutations is exceedingly rare - 2 to 5/100,000 cases - and they are unlikely to be a cause of work-related carpal tunnel syndrome.

This lawsuit raises important questions regarding the extent of legal protections against genetically based employment discrimination. Furthermore, it underscores worker concerns regarding autonomy, informed consent and confidentiality of medical information. BNRR, the employer, allegedly performed the tests for PMP22 mutations without the knowledge or consent of its employees and intended to disclose the test results to dispute workers' compensation claims (EEOC, April 18, 2001). One of the provisions of the settlement of the case is that any future genetic screening programs must be voluntary and cannot be undertaken without first obtaining the consent of the union (EEOC, April 18, 2001).

Indeed, the union consent provision of the BNRR settlement agreement reflects what ethical analysis would suggest as the appropriate resolution of the autonomy vs. beneficence debate. The fact that it leaves the door open to the use of voluntary screening programs is a tacit recognition that there may be circumstances where using genetic technology to identify workers with genetically increased susceptibility to workplace hazards would be of greater benefit than harm. However, it recognizes that employers have conflicting interests when it comes to worker protection and places responsibility for the ultimate decision whether to undergo testing with the workers and their agent, the union.

Additionally, in recognizing the need for employers and employees to agree on the benefit of a particular screening program, the BNRR settlement recognizes the importance of fully informed consent. In order to provide a benefit and minimize the risk of harm workers need to be carefully and accurately counseled regarding the range of implications that flow from a genetic susceptibility test and, in particular, the implications test results may have on both worker safety and present and future employability. How this

informed consent obligation can be met in the context of susceptibility testing remains an open and pressing ethical and policy question, given the predictive ambiguities that inhere in most if not all genetic susceptibility tests (Olden, 2000; Welch & Burke 1998).

The BNRR case illustrates another important ethical consideration involved in workplace genetic testing: confidentiality. Historically, and in this particular case, employees are often not informed regarding their employer's possession and/or use of genetic information obtained during the course of occupational medical examinations, which typically include medical history questionnaires, physical examinations, blood and urine tests and x-rays (Draper, 1991). Employers often have direct access to employee medical information, either through the company physician whose loyalties to the employer abridge traditional obligations to respect and protect patient confidences or through the process of reviewing medical claims if the employer is self insured (Rothstein M, 1983; Draper E, 1991). Employers disclose employee medical information in management disputes, worker compensation proceedings and lawsuits.

In addition to concerns regarding autonomy and beneficence, workplace genetic screening programs raise questions regarding justice. The potential for discrimination, discussed above is one aspect of the justice issue. An additional concern is who should bear the costs associated with identifying and protecting high-risk workers? If protection means exclusion from the workplace, whose responsibility is it to find alternative work, provide retraining or provide an alternative source of income and benefits? Finally, an emotionally charged aspect of the justice issue centers on the impact genetic screening programs could have on employers' efforts to clean up the workplace (Omenn, 1982). Critics are concerned that screening programs invite employers to focus on workers as the problem, diverting needed attention and resources from efforts to clean up hazardous work sites (Draper, 1991).

F. Policy Considerations in Workplace Genetic Testing

1. Stakeholder Perspectives

Policies related to workplace testing must take into account the rights and interests of all parties. Employers and workers are the obvious stakeholders in questions concerning genetic testing in the workplace and their viewpoints may differ regarding the promise of genetic technology for disease prevention in the occupational setting. In addition physicians, researchers, occupational medicine experts and environmental activists have stated vested interests in the outcome of this debate (Draper, 1991).

Employers may be inclined to favor genetic screening because it promises a mechanism for identifying at-risk workers and reducing disease. Employers may see genetic susceptibility screening as a more cost effective method of prevention because removal of susceptible workers may reduce occupational disease without extensive

modifications to the workplace or to production practices. In essence genetic screening programs operate from the presumption that the workplace conditions are generally safe and focus on the innate characteristics of the worker as the cause of occupational illness.

Workers may be inclined to oppose genetic screening programs because the likely outcome is to exclude workers from jobs, often unnecessarily given the limited predictive value of available screening tests. Workers and environmental activists share the concern that screening programs may divert scarce resources and attention from occupational health practices that more effectively protect all workers and the environment, and may reduce motivation to improve current practices and conditions.

Other interested parties - occupational medicine experts, researchers, public health officials - may have varied views of workplace genetic screening, influenced by their perception both of testing as a means to achieve gains in disease prevention and of the personal or social costs of such testing. Society as a whole has a vested interest in choosing occupational illness prevention measures that achieve the appropriate balance among competing needs in the most cost effective and efficient manner. Society's competing needs include the need for the goods and services that are produced using hazardous substances, the need to protect and promote worker safety and well-being and the need to protect and promote the environment.

2. Existing Policy Statements on Workplace Genetic Testing

In 1984 the American Public Health Association issued a policy statement that urged industry to cease genetic testing in the workplace setting and recommended the formation of a multidisciplinary panel to develop ethical and scientific guidelines for the utilization of genetic testing in the workplace. These recommendations were based upon the following concerns: 1) genetic susceptibility testing in the workplace was occurring; 2) information suggested that some of the testing being done by industry was being conducted primarily to benefit the company rather than the individual worker; 3) there were many unresolved ethical principles involved in workplace genetic testing including how the tests were done, how the information was used, including issues of justice in matters of compensation to genetically susceptible workers; 4) there was risk that genetic testing would be used to exclude "susceptible" workers rather reducing hazardous exposures for all workers through cleanup of the workplace; 5) workplace genetic testing had been and could be used to discriminate against individuals; and 6) none of the current genetic tests met established scientific criteria for routine use in an occupational setting (APHA, 1984).

Subsequently, a number of professional organizations have reviewed the issues surrounding workplace genetic screening and compiled recommendations to guide policy development in this area. Policy statements and/or guidelines have been issued by the following professional societies and government agencies: U.S. Department of Labor,

Department of Health and Human Services, Equal Employment Opportunity Commission and the Department of Justice (Joint Memorandum, January 20, 1998); The Hereditary Susceptibility Working Group of the National Action Plan on Breast Cancer (NAPBC) and the National Institutes of Health, Department of Energy Working Group on Ethical, Legal and Social Implications of Human Genome Research (NIH-DOE ELSI Working Group, Recommendations on Genetic Information in the Workplace, 1997); American College of Occupational and Environmental Medicine (ACOEM Position Statement on Genetic Screening in the Workplace, October 24, 1994); and American Medical Association (AMA Council on Ethical and Judicial Affairs, Opinion 2.131: Genetic Testing by Employers, 1991).

The position of the American Medical Association (AMA) is that the use of genetic testing to exclude workers with genetic risks of disease from the workplace is generally inappropriate because the limited predictive value of these tests would result in unfair discrimination against individuals who have abnormal test results and because there are other ways for employers to protect workers and maintain cost effective production goals, such as biomonitoring, routine testing for actual capacity to perform the job and employee education regarding the risks of injury posed by workplace exposures. The use of genetic testing to exclude workers who have a genetic susceptibility to occupational illness would be appropriate in those limited instances where the disease develops so rapidly that serious and irreversible illness would occur before monitoring programs could be effective in preventing harm, the genetic test is highly accurate, empirical data demonstrate that the genetic abnormality results in an unusually elevated susceptibility to occupational illness and the costs of lowering the level of exposure for all employees are extraordinary relative to both the costs of production and the costs of using genetic testing. In no instance should genetic testing be performed without the informed consent of the employee or applicant (Council on Ethical and Judicial Affairs, AMA, 1991). Of note, not genetic tests meeting these criteria were identified.

The American College of Occupational and Environmental Medicine (ACOEM) opposes workplace genetic testing unless it is used to identify a trait that directly affects job performance or predisposes a worker to a significant, consistent adverse outcome following an otherwise acceptable workplace exposure (ACOEM, 1994). Moreover, it recommends that any testing should be voluntary and that the employee be provided both informed consent and a guarantee that test results will not be disclosed to others, including the employer, without his or her consent. Employers would be entitled to learn only what impact test results have on the employee's fitness to perform a particular job. Finally, ACOEM recommends that insurability decisions of employees by employers or others should not be based on genetic status, including genetic test results or employability information derived from those test results (ACOEM, 1994).

The Hereditary Susceptibility Working Group of the National Action Plan on Breast Cancer (NAPBC) and the NIH-DOE ELSI Working Group recommended that employers

be prohibited from using genetic information to affect decisions regarding hiring, termination or the terms, conditions, privileges and benefits of employment unless this information is “job related and consistent with business necessity”; that employers be prohibited from requesting or requiring the collection or disclosure of genetic information from employees or applicants for employment unless this information is “job related and consistent with business necessity”; that employers should be restricted from accessing genetic information contained in medical records released by individuals and other sources; and employers should be prohibited from releasing genetic information without prior written authorization of the employee directing to whom the disclosure will be made (Rothenberg et al., 1997). Additionally, these guidelines recommend enforcement measures, including a private right of action against violators.

In 1998 a joint commission representing the U.S. Departments of Labor, Health and Human Services and Justice, and the EEOC published “guiding principles” for federal legislation to protect against misuse of genetic information in the workplace. These recommendations generally oppose the use of genetic testing in the workplace, stating: 1) employers should not require or request that employees or applicants either provide genetic information or undergo genetic testing as a condition of employment or benefits; 2) employers should not use genetic information to discriminate against, limit, segregate or classify employees in a way that would deprive them of employment opportunities; and 3) employers should not obtain or disclose genetic information about employees or applicants under most circumstances. There are two exceptions to these general recommendations. The first would permit biomonitoring of employees for the effects of a particular substance found in the workplace that could cause genetic damage should the employees experience ongoing exposure. Biomonitoring could occur only with the informed consent of employees, and the employer’s options for responding to positive results would be limited to identification and control of adverse conditions in the workplace and “actions necessary to prevent significant risk of substantial harm to the employee or others” (U.S. DOL, 1998). The second would permit the use of genetic testing to enforce workplace safety and health laws or conduct occupational or other health research. Employers would not be able to disclose employee genetic information without written, informed consent of the individual employee.

In addition to the professional societies referenced above, leading scholars in the fields of occupational medicine, environmental health, epidemiology, law and bioethics have published criteria for occupational screening tests (Omenn, 1982; Lappe, 1983; Vineis and Schulte, 1994; Rothstein, 2000). Omenn’s guidelines in particular emphasize the need to consider both the state of scientific evidence and the ethical, legal and social implications of testing: 1) the prevalence of the predisposing trait must be high enough to be detected in the usual size of worker population, taking into account ethnic and geographic differences in prevalence; 2) the relative risk for a significant adverse outcome must be at least three and preferably should exceed 10 so as to enhance the likelihood that the particular tested predisposition is, indeed, putting those workers at a significantly higher

risk than workers with other non-testable predispositions; 3) the test must be reliable, inexpensive and well characterized with respect to sensitivity, specificity, and predictive value; 4) there must be a clear understanding between management and unions and their workers about how test results are to be utilized, what actions might be taken on the basis of the results and who would have access to the information (Omenn, 1982, 1995).

Lappe's examination of the ethical aspects of genetic screening concluded with the following criteria for a workplace genetic screening program: 1) an attainable purpose; 2) workforce participation; 3) equal access and/or random participation; 4) adequate testing procedures; 5) absence of compulsion; 6) informed consent; 7) protection of subjects; 8) worker access to information; 9) provision of counseling/follow-up; 10) understandable relationship between tests and therapy, if any therapy results; and 11) protection of the worker's right to privacy (Lappe, 1983).

Rothstein's recommendations focus on important issues surrounding employee autonomy, consent and confidentiality. He proposes that employers have a duty to inform applicants and employees regarding genetic markers of increased susceptibility to workplace exposures and to provide them the option of undergoing genetic testing, at the employer's expense, administered by a physician of their choosing, with the results available only to the worker. A positive test result should be accompanied by genetic counseling regarding the risks of employment, and the choice of whether to accept or continue in the job would be the employee's. An exception permitting employer directed testing and exclusion of genetically susceptible workers would arise only in the rare situation where employment of the genetically susceptible worker would create a "direct, immediate and severe risk of harm to self, others or property" (Rothstein, 2000).

Taken together, these guidelines suggest consensus on several points. First, employers' use of genetic tests and genetic information for workplace screening or monitoring should be limited to those few instances in which the need for genetic information is job related, of business necessity and would assist in preventing significant risks for the employee or for public safety. Second, the assessment of whether the genetic information qualifies should be based upon the state of the science surrounding the predictive value of the test and the ability to use positive test results to prevent or minimize occupational illness. Third, genetic screening programs should be voluntary, tests should not be performed without the employee's informed consent, there should be strict procedures in place for protecting confidentiality of test results and employers should not disclose employee's genetic information without the employee's written informed consent. Finally, there is consensus about the need for protections beyond those provided by current federal and state regulations, to prevent discrimination from workplace genetic testing and to ensure the privacy and confidentiality of genetic information obtained in the workplace.

These areas of consensus point to the importance of defining procedures for evaluating new opportunities for workplace genetic testing. The procedures need to include methods to determine whether a new testing opportunity meets the agreed-upon criteria and, if so, how testing should be implemented. The evaluation process should be well-defined and acceptable to all stakeholders. Because policy statements have suggested that workplace genetic screening programs could be justified by “job relatedness” and “business necessity”, the application of these vague terms will require careful assessment in the context of a specific testing opportunity, utilizing accepted standards for the testing process, the disease prevention measures, and the protections to be put into place against discrimination and breach of privacy.

3. Health Policy: Test Parameters for Effective Screening Programs

The potential for a genetic screening program to assist in identifying and protecting workers depends in large part upon the analytic and clinical validity of the screening test and the clinical utility of the screening program (Newill et al, 1986; OTA, 1983, 1990; Van Damme et al., 1995; Holtzman and Watson, 1997). These test properties were identified by both the NIH-DOE Task Force on Genetic Testing (Holtzman NA et al., 1997) and the Secretary’s Advisory Committee on Genetic Testing (SACGT) (NIH, 2000) as central in assessing the medical and public health benefit of genetic tests. Analytic validity is the accuracy with which the test assay identifies the genetic trait under consideration. Clinical validity is the accuracy with which the test results predict a clinical outcome. Clinical utility is the health benefit provided by the testing process.

The analytic validity of a test determines its acceptability as a method to identify people with a genetic condition or predisposition, whereas the test’s clinical validity determines whether genetic screening is a feasible method for identifying people at risk. Clinical utility depends primarily on the availability of interventions to reduce risk in people who test positive for the genetic condition or predisposition. Key parameters involved in determining analytic and clinical validity include: 1) population frequency of the genetic marker; 2) cumulative incidence of the disease outcome of interest; 3) strength of the association between the marker and the disease; and 4) based on these, the predictive value of the screening test (Holtzman and Watson, 2000; Newill et al., 1986; Van Damme et al., 1995). In the case of PON1, analytic validity refers to the accuracy of the assay for PON1 status in identifying people with low PON1 enzyme activity. Clinical validity refers to the accuracy with which low PON1 status predicts an adverse outcome after a defined OP pesticide exposure.

In assessing analytic validity of a test it is important to keep in mind that analytic predictive value is significantly influenced by of gene frequency, i.e. the prevalence of the genetic trait in question among the population under study. As shown in the following table (from Van Damme et al, 1995), the analytic predictive value of a reliable test with 90% sensitivity and 90% specificity for identifying a genetic trait that has with a

frequency of 5% is only 32%. This means that out of a group of workers who receive a positive test result only 32% will truly carry the genetic trait in question. When the trait is more frequent, the test's analytic predictive value increases. Note that this use of the term predictive value refers only to the accuracy with which a test identifies a genetic trait; the more relevant use of the term in public health is in predicting clinical outcome (see discussion of clinical validity below) If this group is excluded from work to minimize disease risk, that intervention will be unnecessary for 68% of the workers in the group.

Table 1: Predictive value in relation to different prevalences of a factor and different sensitivities and specificities of tests
(based on Van Damme et al., 1995)

Prevalence (%)	Sensitivity and Specificity (%)			
	99	95	90	80
20	96.1	82.1	69.2	50.0
10	91.7	67.9	50.0	30.8
5	83.9	50.0	32.1	17.4
1	50.0	16.1	8.3	3.9
0.1	9.0	8.7	4.3	2.0

Analytic validity determines the acceptability of particular laboratory assay as a means to identify people with a genetic condition or predisposition. It is clinical validity, however, that determines the feasibility of a genetic test as a means to identify people at risk, who may be candidates for special preventive measures. Clinical validity refers to the accuracy with which a test result predicts health outcomes. It is derived from an empirical assessment of the strength of the association between a positive test result (i.e. the individual tested has the susceptibility trait) and the risk of developing the disease or other adverse health effect. This association can be calculated from epidemiologic studies that measure and compare the incidence of disease among exposed workers with and without the specific genetic trait, yielding two important measures of clinical validity: 1) relative risk (RR); and 2) clinical predictive value. Accurate measurement of the exposure is an important factor in these measurements.

Relative risk (RR) is a quantitative measure of the extent to which a particular risk factor – e.g., a genetic trait - increases the likelihood of a particular health outcome: if a genetic trait confers a RR of 10 for an adverse reaction to a workplace exposure, a person who has the trait is 10 times more likely to experience an adverse reaction than a person without the trait. RR is a useful indicator of the potential for a genetic testing program to prevent disease. The higher the RR, the more likely that the genetic test will identify the population most at risk from exposure, and thus most like to benefit from preventive measures.

Clinical predictive value is another measure of a genetic screening test's clinical validity. In this use of predictive value, the likelihood of clinical outcome is calculated for positive and negative genetic test results. The positive predictive value (PPV) measures the likelihood that a carrier will suffer an adverse outcome with a given exposure when the genetic test result is positive; the negative predictive value (NPV) measures the likelihood that a non-carrier will not suffer an adverse outcome with the same exposure. PPV and NPV are a function of the sensitivity and specificity of the screening test and the prevalence of adverse events following exposure. The prevalence of adverse events, in turn, are partially dependent on the prevalence of the genetic trait. The following examples using a population sample of 100 exposed workers illustrate the interplay of these parameters.

In the first example the frequency of the genetic trait conferring increased susceptibility to workplace exposure is 10% and the rate of adverse events is 8%. Among those with adverse events, empiric observation determines that 5 (63%) carry the genetic trait. This observation defines the sensitivity of the test and allows other test parameters to be calculated. In the second example the frequency of the genetic trait remains at 10% but the rate of adverse events increases to 20%. The number of people with adverse events who carry the genetic trait in this example is found to be 7 (35%). These observations permit the calculation of PPV and NPV, as summarized in the following tables.

Example 1

	Adverse Events	No Adverse Events	Totals
Test Positive	5	5	10
Test Negative	3	87	90
Totals	8	92	100

Sensitivity - $5/80 = 63\%$
 Specificity - $87/92 = 95\%$
 PPV = $5/10 = 50\%$, NPV = $87/90 = 97\%$

Example 2

	Adverse Events	No Adverse Events	Totals
Test Positive	7	3	10
Test Negative	13	77	90
Totals	20	80	100

Sensitivity = $7/20 = 35\%$
 Specificity = $77/80 = 96\%$
 PPV = $7/10 = 70\%$, NPV = $77/90=86\%$

These examples demonstrate that the prevalence of adverse events changes the clinical predictive value of a genetic screening test. In the first example, 50% of workers with a positive test were likely to develop disease whereas only 3% of those with a negative test were at increased risk. In the second example, where the prevalence of adverse events was higher, positive predictive value increased to 70%, but the number of workers with adverse events among those testing negative also increased to 14%. These results underscore the importance of understanding precisely how adverse events are defined before beginning an assessment of clinical validity. For example, a liberal definition of adverse event, one focused on a biochemical measure of exposure instead of a clinical symptom, will increase the number of events that occur in response to a specific exposure and may reduce the specificity and NPV of a test. It is also important to verify that exposure levels are constant for measurements used to calculate these test parameters.

Unless a genetic screening program employs a test with very high analytic and clinical validity, significant numbers of false negative and false positive results can be expected. Results that incorrectly label workers as having increased susceptibility or provide false reassurance that they are not at increased risk undermine the ability of the screening test to provide a health benefit and increase the likelihood of ethical, legal and social costs associated with genetic screening programs. For this reason, any plan to use genetic susceptibility screening programs to reduce occupational illness and injury must include a careful assessment of the analytic and clinical validity of the genetic test to be employed, as well as an assessment of the measures to be used to protect susceptible workers. These policy considerations can be applied to testing workers for PON1 status as a means to reduce adverse events from pesticide exposure. If the protective measures for susceptible workers involve limiting their pesticide exposure through job assignments or working schedules, it is important to know the accuracy with which a measurement of PON1 status predicts adverse reactions. Low PPV will result in changes in work status for many workers unlikely to suffer adverse reactions, and low NPV will result in the failure to protect many susceptible workers.

V. POTENTIAL INTERVENTIONS TO REDUCE TOXICITY IN PEOPLE WITH GENETIC SUSCEPTIBILITY TO OP PESTICIDES

With this background, our case study focuses on agricultural workers who are exposed to OPs, and, in particular, to chlorpyrifos. Data recording the health effects of agricultural pesticide exposure and the sites where pesticide exposure is most likely to occur suggest that pesticide applicators are among the individuals most at risk. Thus, our analysis will consider the legal, ethical and policy implications of a workplace genetic screening program to identify pesticide applicators whose low PON1 status could render them susceptible to adverse health effects from occupational exposure to chlorpyrifos and certain other OPs. It will compare the implications of a genetic screening program with two alternative interventions - amending exposure standards and cholinesterase biomonitoring – which are described below. A description of the genetic screening intervention is included in our analysis (Section VI).

A. Amending existing exposure standards

The option of amending exposure standards arises from well-established occupational health policy that requires employers to provide safe and healthy workplaces and relies on federal and state agencies to set and enforce safe exposure standards. As outlined above in the review of federal and state regulations related to pesticides, the amount of pesticide that can be used in a given application, methods of handling and storage are determined by EPA and OSHA regulation, and are intended to result in exposures that are sufficiently low to prevent adverse health effects. State oversight mechanisms help to ensure that safe standards are maintained and to monitor incidents of excess exposure or adverse health effects.

Despite these measures, pesticides continue to be a cause of health hazard to agricultural workers (PIRT, 2000, Blondell, 2000). Adverse events from EPA determined “safe” exposure levels might occur because of incident specific alterations in exposure levels caused by weather conditions, drift or accidents. However, PON1 data raise the possibility that adverse events following presumed safe levels of exposure may occur as a result of genetic variation. People who have low PON1 status may be highly susceptible to chlorpyrifos or other OP exposure. For this group of workers, lower exposure standards - or even zero exposure standards - may be necessary to protect them from occupational disease.

B. Biomonitoring Workers to Measure the Effect of Ongoing Exposure.

Biomonitoring involves periodically examining a group of workers by collecting blood or other body fluids to assess the effect of the exposure of interest. Evidence of a significant effect typically triggers an investigation of the workplace and an effort to reduce exposure for all workers. It may also lead to the temporary exclusion of some

workers from the workplace. Advocates of biomonitoring believe this approach is more beneficial for the workplace and all workers than a genetic screening approach focused only on targeting and removing susceptible individuals. Biomonitoring programs are criticized for inherent difficulties in interpreting the significance of results and predicting disease risk from specific physiological measurements (OTA, 1983).

The California program to monitor agricultural workers exposed to chlorpyrifos and other cholinesterase inhibiting pesticides is an example of a government mandated biomonitoring program that attempts to assess the effect of exposure to chlorpyrifos by measuring cholinesterase levels for evidence of depression (California Code, Title 3, Section 6728). If a worker's blood cholinesterase level falls below statutorily defined high risk thresholds steps are taken to protect the worker, including temporarily removing the worker from the job site (see description above in section IV.B) Adopting this program at the national level under the auspices of OSHA and FIRFA is one potential intervention to protect workers from OP toxicity.

However, available data document adverse events following pesticide exposure despite biomonitoring (Ames et al, 1989). Such events are consistent with the possibility that workers who experience reduced cholinesterase levels under conditions of normal workplace exposure represent a genetically susceptible group. If so, a genetic testing program might provide a less expensive and more efficient way of identifying susceptible workers and preventing their exposure to chlorpyrifos-containing pesticides.

VI. ANALYSIS AND CONCLUSIONS

A. Characteristics of an Acceptable Genetic Testing Program

Legal, ethical and policy considerations inform us that the key components of a workplace genetic screening program are a genetic test that is scientifically valid, has high predictive value, and identifies workers for whom appropriate preventive measures can be taken; and a test administration process that respects worker autonomy, providing informed consent, appropriate counseling and protection of confidentiality (OTA, 1983, 1990). A genetic testing program to assess PON1 status in pesticide applicators should, therefore, be a voluntary genetic screening program in which employees are informed regarding the risks attending pesticide exposure, the availability of genetic testing to identify genetic markers for increased susceptibility and the predictive value, risks and benefits of the test. Employees (either current employees or those who have received conditional offers of employment) would be given the option of obtaining employer funded testing through a physician/lab provided by the employer. Those with positive test results would receive additional counseling regarding the implications of their PON1 status for themselves and their family if they proceeded to work. They would be given the option of continuing with their current or planned employment or, if available, moving into a different job that involved less risk of exposure. Employees whose genetic

susceptibility resulted in exclusion from the workplace would be eligible for workers compensation pending job relocation or retraining. Similarly, susceptible employees who continued to work and developed exposure related illnesses would not be disqualified from workers compensation on the grounds that they knowingly assumed the risk. The program would be employer funded and would apply to workers who are hired for jobs that involve regular handling (mixing, loading, or applying for more than 6 days in any 30-day period) of pesticides containing chlorpyrifos.

In this analysis, we assess whether these criteria can be met by a program based on testing for PON1 status, and whether a PON1 testing program with these characteristics is a feasible approach to reducing risk of adverse reactions to chlorpyrifos among pesticide applicators.

B. Is the proposed testing program scientifically valid?

The state of the science forms the foundation for a meaningful ELSI analysis of any genetic screening program. Thus, the first issue for consideration is whether testing to determine PON1 status (or genotype once the relevant promoter mutations are defined and correlated with Q/R 192 genotype) is an accurate and relevant method for identifying workers with genetically increased susceptibility to chlorpyrifos and other OPs. Furlong et al. have developed a high-throughput, two-dimensional enzyme analysis that measures PON1 status and provides an accurate inference of PON1192 genotype (Richter & Furlong, 1999). With respect to *PON1 192Q/R* genotype this testing method demonstrates acceptable analytic validity in the academic research setting where sensitivity, specificity and predictive value approach 100%. When study samples underwent follow-up genotyping using PCR 316 of 317 samples (99.7%) showed agreement between the PCR genotype and the genotype inferred from the enzyme assay (Brophy et al., 2000). Moreover, the PON1 assay measures enzyme expression directly, thus taking into account other genetic variants that in the promoter region that contribute to chlorpyrifos sensitivity. Thus, this method provides a means to identify individuals with genetic susceptibility to OP pesticide exposure. However, the assay has yet to be tested outside of the research setting, and the prevalence of low PON1 status in the population is unknown, making it difficult to assess analytic validity. .

Turning to clinical validity, the key parameters are relative risk and clinical predictive value, both of which depend upon epidemiologic data on the association between low PON1 status and chlorpyrifos toxicity. Although we have data regarding *PON1 192Q/R* genotype prevalence, frequencies for low, intermediate and high PON1 status are unknown. Moreover, epidemiologic studies measuring PON1 status among exposed agricultural workers and comparing outcomes still need to be done. However, by extrapolating from animal data, gene frequencies in human populations for the relevant *R192* genotype and chlorpyrifos toxicity incidence data from the California biomonitoring program we can roughly estimate the parameters necessary to estimate clinical validity.

These estimates, summarized in the following tables, are derived from the following assumptions. First, we assume that the frequency of low PON1 status among agricultural workers is 10%. This is based upon on the prevalence of the *R192* genotype (25% to 45%) and estimates that 20% to 40% of *R192* carriers have reduced PON1 levels (Richter et al., 1999; Furlong et al., 2000). Second, we assume the rate of disease among exposed workers is 1.5% for clinical events (acute symptomatic episodes), 5% for severe cholinesterase suppression and 23% for moderate cholinesterase suppression, based on data from the California biomonitoring program (Ames et al., 1989). Using these assumptions, we calculate predictive values for PON1 testing across a range of relative risk (RR) thresholds: RRs of 3 and 10 (reflecting minimum thresholds recommended by Omenn (Omenn, 1982)), 40 and 80 (reflecting the maximum differences in susceptibility seen for animals with low PON1 and normal PON1 in laboratory studies of OP exposure (Costa et al., 1995; Shih et al., 1998; Li et al, 2000)).

These calculations suggest that a PON1 screening program would have limited clinical validity even at high relative risk (Table 2). At RR of 3, the sensitivity and positive predictive value of the test are very low. At RR of 10, the sensitivity of the test is 7.7% for adverse clinical events and rises to 68.7% for moderate cholinesterase suppression; these numbers increase for RRs of 40 and 80. At both RR of 40 and RR of 80, PON1 testing can detect about 75% of those at risk for either severe cholinesterase suppression or clinical events, but will also detect and label a larger number of workers who will not develop these adverse effects. Most of those with low PON1 status detected at these high levels of RR will be at risk for moderate cholinesterase suppression, but an even larger number of people with normal PON1 status will be at risk for this complication.

This exercise underscores the importance of additional data and policy debate. First, the outcome of testing for PON1 status cannot be accurately predicted until additional studies are done to determine the RR for adverse responses to pesticide exposure among exposed workers with low PON1 status. The RRs of 40-80 predicted by animal studies need to be either confirmed or disproved for human exposures to OP pesticides. Second, if testing is contemplated as a means to protect workers, the threshold for test use needs to be debated. At what level of clinical validity is the test acceptable? Specifically, what proportion of false positive and false negative test results is acceptable in a workplace genetic testing program?

Table 2
Clinical Validity of PON1 Testing
at Different Relative Risk Levels

Relative Risk(RR) Test Properties	Clinical events	Severe cholinesterase suppression	Moderate cholinesterase suppression
RR= 3 Sensitivity Specificity PPV NPV	3.7% 98.7% 24.7% 90.2%	12.0% 95.9% 24.0% 90.7%	44.0% 79.3% 19.1% 92.7%
RR= 10 Sensitivity Specificity PPV NPV	7.7% 99.2% 51.0% 90.6%	23.4% 97.0% 46.8% 91.9%	68.7% 82.1% 29.9% 95.9%
RR= 40 Sensitivity Specificity PPV NPV	12.0% 99.7% 79.7% 91.1%	37.0% 98.6% 74.0% 93.4%	88.2% 84.2% 38.4% 98.5%
RR= 80 Sensitivity Specificity PPV NPV	13.3% 99.8% 88.5% 91.2%	42.0% 99.1% 83.9% 93.9%	93.5% 84.8% 40.6% 99.2%

C. Is the proposed testing program feasible from an ELSI perspective?

From an ethical standpoint, the predictive uncertainties of the screening test challenge the promise that genetic screening will benefit workers, employers and society. A test with limited or uncertain predictive value will likely result in misclassification of many workers and could unnecessarily increase worker concerns or perceptions regarding risk. Absent robust epidemiologic data regarding the degree of risk associated with low PON1 status, it is questionable whether even accurate labeling offers workers or employers protection from the risks associated with pesticide toxicity.

Finally, the predictive uncertainties make it difficult to justify the expense to workers, employers and society for worker education, testing, genetic counseling, workers compensation, unemployment benefits, delays in production and lost opportunities to focus on overall reduction of workplace hazards that will attend an occupational genetic screening program. The expense government programs will incur as a result of job loss should not be overlooked, particularly in the case of agricultural pesticide workers where comparable alternative jobs are essentially non-existent. Pesticide applicators have more training and earn more money than fieldworkers whose responsibilities include thinning, moving irrigation, propping trees. Other job categories include fruit testing and integrated

pest management. However both of these require additional training beyond what is generally provided to pesticide workers. Moreover, field research experience of one of the authors (RF) suggests that with the exception of major agricultural companies like Dole, most employers represent small scale operations that do not hire a large number of employees and often expect employees to handle more than one category of responsibility.

In addition to ethical concerns arising from the predictive uncertainties associated with current PON1 testing capabilities, there are reasons to question the practical feasibility of a genetic screening program that meets the competing ethical, legal and social criteria regarding worker autonomy, informed consent, privacy, worker safety and business necessity. Because the proposed program would be voluntary, is preceded by informed consent and genetic counseling, and because it would allow the worker to make the final decision to decide regarding whether to continue or leave the job, the kind of testing program we propose satisfies most of the ethical and legal concerns regarding worker autonomy, privacy and confidentiality and discrimination. However, it does little to address the business, liability and worker safety concerns of employers. If employers are responsible for funding the program, yet are unable to use test results to remove high risk workers or avoid liability for workers compensation payments, the program might be difficult to implement. Employers would undoubtedly prefer a program that limits their responsibility to educating employees and placing them on notice of their risk, but leaves the testing option and follow-up genetic counseling to the employee to pursue and fund. Under this scenario, the employer would be relieved of any liability for failing to warn an employee of a workplace hazard or steps to minimize their risk, continue with the same obligations for worker compensation and acquire no additional expense for genetic testing programs that have limited predictive value and could result in liability for misclassification.

Even if a voluntary genetic screening program were successfully implemented, however, the predictive uncertainties, the expense and the lack of employer control over job placement would provide powerful incentives to disregard the program or structure employment practices so that the program does not apply. For example, employers could avoid the program by limiting the number of employees engaged in pesticide application or limiting the days of exposure per employee to fall below program applicability criteria. It is worth noting that this effort on the part of employers to circumvent a proposed genetic testing program would not represent a bad outcome from a public health perspective. In fact, such measures would be the preferred approach to prevention.

Some of these issues have been explored with respect to chronic beryllium testing (Bartell et al, 2000). They indicate that a genetic testing program faces many challenges and is unlikely to be undertaken unless compelling evidence of benefit can be provided. Without question, employers are going to be more interested in pursuing mandatory

screening programs that provide them the prerogative to exclude high risk and potentially costly workers and offer an option to expensive programs designed to reduce overall exposure for all workers. However, these programs are going to be an option only when a genetic test is highly predictive for occupational illness, with persuasive arguments that exposure-related illness could not be averted in the absence of genetic testing - that is, in circumstances where “job relatedness” and “business necessity” can be argued strongly. Given the multifactorial complexities of most gene-environment interactions such tests are likely to be vanishingly rare. Our review of current data concerning PON1 and OP pesticide exposure indicates that testing for PON1 status is unlikely to meet this standard, although there are insufficient data to be certain about this conclusion.

D. Feasibility of Alternative Interventions

1. Amending existing exposure standards.

Although OSHA generally requires that occupational exposure standards assure, to the extent feasible, that “no employee” will suffer material impairment of health, even if exposed for his or her entire working life, the courts have limited the scope of this requirement. Standards need to be reasonably necessary or appropriate to remedy a significant risk of material health impairment and must be both technologically and economically feasible (Rothstein, 1983). It is unlikely that exceedingly low or zero exposure standards intended to protect small groups of highly sensitive workers will be enforceable (Rothstein, 1983; OTA, 1983). This is especially true with workplace exposures like pesticides, which are integral to the production of quality produce in large quantities and at relatively low prices. For this reason, employers and occupational health experts may be particularly interested in the potential for biomonitoring and/or genetic testing programs to prevent and/or minimize the risk of occupation illness from toxic workplace exposures.

2. Biomonitoring Program

A program structured along the lines of the California biomonitoring program raises similar issues regarding scientific relevancy and accuracy validity and attendant ethical, legal and social implications. Employers tend to oppose and employees tend to support biomonitoring programs for the same reason: these programs focus on the issue of exposure and require employers to respond to evidence of an adverse effect by intervening in the workplace to reduce exposure rather than focusing on and encouraging exclusion of susceptible workers.

From the standpoint of the science, a biomonitoring program identifies evidence of pesticide effects prior to the development of symptoms and calls for corrective action. Theoretically, therefore, it allows for worker protection and assurance of safe work

standards. However, available outcome data indicate that the existing biomonitoring program established in California does not prevent all symptomatic events of pesticide exposure (Ames et al., 1989). A retrospective review of case studies and monthly monitoring practices suggest poor monitoring compliance by the companies and employees (Fillmore et al, 1993), making it difficult to accurately assess the program's potential impact on disease incidence if fully implemented. Perhaps more important, this type of program does not protect against long-term health effects resulting from chronic "safe" levels of exposure. Nor does it protect the highly susceptible worker whose initial exposure to chlorpyrifos or environmentally induced chlorpyrifos oxon is sufficiently toxic to produce profound adverse health effects. Finally, because the degree of disease risk associated with the state defined threshold effect levels has not been scientifically tested the program could be requiring costly monitoring and interventions that, in reality, have little impact on the incidence of OP related illness.

From an ELSI standpoint, mandatory biomonitoring programs raise issues regarding worker autonomy, confidentiality and privacy as do genetic screening programs. However the test results do not carry the same potential for stigma and discrimination that attends genetic labeling. The predictive uncertainties raise concerns regarding the true public health benefit of these programs and data from California regarding employer compliance suggest feasibility issues. From the standpoint of employees and society, the fact that these programs focus on the issue of exposure and require employers to respond to adverse outcomes by reducing exposure for all workers, is a benefit not available with genetic screening programs.

E. Appropriate Future Steps

Any discussion of genetic testing to address diseases related to gene-environment interactions must take into account their multifactorial nature (Bartell et al, 2000; Bates, 1994). Multifactorial diseases involving interactions between genotypes and a variety of environmental triggers pose monumental challenges in disease attribution and apportionment of causal contribution, making it difficult if not impossible to precisely characterize dose-response relationships and cost out the value of proposed interventions to reduce adverse health outcomes (Bates, 1994). Further challenges are posed by the limited availability of data on human response to hazardous substances in the workplace or the environment generally, forcing researchers and analysts to derive estimates of human response from animal studies (Bates, 1994).

Our review of PON1 status and OP pesticide exposure indicates that there are considerable uncertainties about the effects of genetic testing for PON1 status as a means to identify pesticide susceptible workers who may be exposed to OP pesticides. The goal of such testing would be to protect susceptible workers from unsafe exposures, through job modification or re-assignment. Uncertainties about the clinical validity of testing and the uptake of testing in a voluntary program make it difficult to assess the

health benefits to be derived. There are also uncertainties about the social and legal consequences of testing, related to worker and employee liability for adverse outcomes of pesticide exposure, worker compensation, and unemployment rates. As a result, it is difficult to compare the outcomes of a genetic testing program to its two alternatives: biomonitoring to detect early evidence of excess pesticide exposure; and stricter standards for safe exposure levels.

Additional research studies would help address these questions. However, large-scale studies to address the epidemiology of pesticide exposure, relationships between PON1 status and health outcomes in exposed workers, and cost and feasibility of the different methods to monitor and reduce pesticide exposure would be costly. Analytic methods to identify the most critical research questions can help to focus research efforts. Tools such as risk assessment and decision analysis, developed to assist policy makers, can also be used by researchers by providing a model for assessing the effect of variability of key parameters on predicted costs and benefits (Bartell et al, 2000). For example, studies assessing the practical value of genetic based interventions to minimize adverse health outcomes from occupational exposure to beryllium and benzene provide information about the effect of differences in sensitivity, specificity and predictive value of the tests, the strength of the genotype-disease association, participation rate in screening and/or biomonitoring programs and cost factors (Nicas et al., 1999; Bartell et al., 2000). These studies help to pinpoint the variables that have the greatest impact on testing outcomes. In the case of PON1 status, these variables include definitive measurement of the prevalence of low PON1 status and of the association between PON1 status and health outcomes in exposed workers.

Decision analysis traditionally emphasizes measurable health outcomes and economic costs of testing programs. However, our analysis indicates the importance of additional ethical, legal and social factors, some of which must be assessed qualitatively – eg., perceptions about the meaning of positive genetic test results. Others represent social outcomes rarely studied in clinical trials, such as employment and insurance coverage. If we accept that the primacy of public health and safety over considerations of cost and feasibility is essentially a moral imperative (Moreno and Bayer, 1985; Bates, 1994), these factors, which inform the risk of workplace genetic testing, must also be incorporated into future studies. With appropriate estimates, outcomes related to these qualitative and social factors can also be incorporated into a decision model.

F. Conclusion

In this first case study we have used the example of *PON1* mediated pesticide sensitivity to both illustrate and assess the ethical, legal, social and scientific challenges attending application of genetic susceptibility testing to mitigate the adverse public health implications of gene-environment interactions in the occupational setting. In the case of PON1 additional empiric data are required to determine the outcomes of a genetic testing

program. However preliminary calculations based on available data suggest that the ultimate public health benefit of applying current genetic screening techniques to identify and protect genetically susceptible pesticide applicators would be limited, both because the predictive value of the test appears to be limited and because genetic testing would expose workers to additional potential harms.

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