A Case Study in Environmental Risk Assessment: The Hazardous Potential to Children of Dietary Exposure to Cholinesterase Inhibiting Pesticides
A Case Study in Environmental Risk Assessment:
The Hazardous Potential to Children of Dietary Exposure
to Cholinesterase Inhibiting Pesticides

By

Judith Regina Ungerleider

B.S. (University of California at Davis) 1985

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

HEALTH AND MEDICAL SCIENCE

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA at BERKELEY

Approved:

Chair: ............................................. 5/16/91  

Date

***************
Acknowledgements

I would like to thank the Dr Richard Jackson for his supervision and suggestions on the cholinesterase risk assessment, and I would like to acknowledge my colleagues at the Pesticides and Environmental Toxics Section of the California Department of Health Services for their technical support on the project.

I would also like to extend my gratitude to James Robinson and Robert Spear who, along with Richard Jackson, formed my thesis review committee. They were invaluable resources and provided excellent advise and editorial comments.

Finally, I would like to thank some of people whose friendship, support, suggestions, and encouragement enabled me to complete this undertaking. Many, many, many thanks to Harrison Alter, Sharon Ungerleider, Jane King, Mark Ripperda, and Jeff Measelle, and, of course, thanks to my UC Berkeley Ultimate Frisbee teammates.
Table of Contents

Introduction ........................................................................................................................................... 1

Risk Assessment ..................................................................................................................................... 6

I. The process of risk assessment ........................................................................................................ 7
II. Carcinogen risk assessment ........................................................................................................... 11
III. Neurotoxic risk assessment ........................................................................................................... 14
IV. Lead exposure and children ........................................................................................................... 19

Hazards of Cumulative Cholinesterase Inhibitors ........................................................................... 25

I. Cholinesterase inhibitors ................................................................................................................... 25
II. Cholinesterase inhibition as a toxic endpoint .................................................................................. 29
III. Increased Susceptibility of Children to Neurotoxic agents .......................................................... 30
IV. EPA food tolerances ....................................................................................................................... 32
V. Methods ............................................................................................................................................ 34
VI. Results ............................................................................................................................................ 42
VII. Discussion ...................................................................................................................................... 44

Analysis and Critique of ChE Risk Assessment ............................................................................... 47

I. Hazard Identification ....................................................................................................................... 47
II. Dose Response ............................................................................................................................... 48
III. Exposure Assessment .................................................................................................................... 57
IV. Risk Characterization .................................................................................................................... 61

Conclusions ......................................................................................................................................... 63

Glossary ................................................................................................................................................ 65

Bibliography ........................................................................................................................................... 67
Chapter 1
Introduction

In recent history, comprehensive environmental legislation has been passed or amended with the objective of protecting human health. Prominent among these are: the Federal Food Drug and Cosmetic Act which requires that all food additives including pesticides be evaluated for toxicity and that health protective tolerances be established. This legislation includes the controversial Delaney Clause which is aimed at protecting the public from carcinogens in food; the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) under which the EPA determines the uses for which each pesticide can be registered considering both the benefits derived from the use of that pesticide and the potential health hazards; the Clean Air Act which requires emissions standards be set for harmful airborne compounds; the Toxic Substances Control Act; the Occupational Safety and Health Act; and the Clean Water Act. Considerable regulatory and technical resources have been dedicated to fulfilling the health protective mandates of these statutes though for many of these pieces of legislation, the goals and mandates outlined are far from being realized. Rather, the regulations have often proven to be a symbolic gesture of congressional responsiveness to the concerns of their constituents rather than practical, enforceable legislation (Dwyer, 1990). The technical and economic difficulties of reducing environmental hazards and the vastly differing opinions about what constitutes a human health hazard, including controversies over what level of human health risk is acceptable and what scientific data are needed to document the safeness or riskiness of a given compound, has led to controversy and regulatory inaction.
There are thousands of hazardous compounds that require study and risk characterization. The characterization of risk using scientific methods can be one of the most important components in the evaluation of hazards and the enactment of environmental controls by regulatory bodies. Frequently, however, sound scientific data about the effects of environmental hazards on human health are not available. Information describing the acute effects of various compounds often exists, but scientific understanding of low dose effects is typically quite limited. Low level exposures to one or a combination of compounds over a prolonged period of time is characteristic of environmental exposure. In addition, determination of the potential level of exposure to a given population is difficult and inexact. Because of the scientific uncertainty that characterizes our general understanding of environmental hazards, regulatory agencies have developed guidelines for the risk assessment process. These guidelines outline the steps necessary to complete a valid quantitative risk analysis and represent an attempt to address areas of debate and uncertainty surrounding the assumptions required in the assessment process. Through the use of guidelines regulators hope to enhance quality, consistency, predictability, and efficiency in the characterization of risks.

Questions about the validity and usefulness of quantitative risk assessment abound, and the field is characterized by uncertainties and disagreement among experts. Enormous amounts of epidemiological, toxicological and exposure data are required for the completion of an assessment of a single compound, and there are innumerable compounds which require evaluation. Further, risk assessment for individual compounds does not explore the interaction and effects of exposure to
multiple compounds. In general exposure to multiple compounds is a much more realistic scenario than is single compound exposure.

Even while the risk assessment process is compromised by great uncertainties, quantitative risk assessment findings are generally required as evidence that an environmental contaminant is truly a hazard. Our dependence on this semi-scientific method for determining risk and deciding what regulatory actions need be taken, warrants that a closer examination of the process of risk assessment be pursued. A look at the scientific uncertainty, the policy decisions forced by such uncertainty, and the controversy surrounding the risk assessment process, is essential to understanding the available regulatory options.

Carcinogens in the environment have received the most extensive study and attention over the past 20 years. Assessing and establishing guidelines for reproductive and developmental hazards have been a more recent focus for study and debate. Even more recently, the process of risk assessment has begun to be outlined for neurotoxins. Some of the same scientific questions and uncertainties, including the use of animal data to estimate effects in humans and the use of exposure modeling, are common to the assessment of all of these different classes of environmental hazard. The experience gained from the study of carcinogens can, therefore, be used in part to develop procedures for the evaluation of noncarcinogenic hazards. Risk assessment for non-carcinogens, however, requires further scrutiny because of differences in such areas as dose response characteristics, endpoint identification, and available data.

The objective of this paper is to examine the process of risk assessment for the human health hazards posed by environmental exposure to neurotoxic compounds, to explore the various regulatory alternatives which
arise from the process depending on the degree of uncertainty within the assessment, and to highlight the scientific policy decisions implicit to the process. To this end, I have undertaken a risk assessment study of a class of neurotoxic pesticides, the cholinesterase inhibiting organophosphate and carbamate compounds. The potential for neurotoxicity based on exposure to these insecticides through food residues was brought to the public's attention in 1989 by the Natural Resources Defense Council (NRDC) in its publication *Intolerable Risk: Pesticides in our Children's Food*. The portion of the publication which described the potential carcinogenic risk of dietary exposure to pesticides and which was integral in publicizing the dangers of the growth regulator daminozide (Alar), received more attention than did the NRDC's assessment of neurotoxicity. However, the consideration of a neurotoxic hazard by an environmental advocacy organization reflects the increasing public concern over neurotoxins in the environment. The study of cholinesterase inhibitors undertaken for this paper is based in general on the NRDC study. This paper expands on the work undertaken by the NRDC through the use of more recent and sophisticated exposure data and modeling made available through Dr. John Wargo at Yale University. Dr. Wargo has been collecting and analyzing data for a comprehensive study by the National Research Council examining dietary pesticide exposure in children and infants. He has provided both data and technical assistance for this project.

The following chapter describes the theory and components of the risk assessment process, and outlines some of the major issues, assumptions and controversies brought up in the assessment of carcinogens as a basis of comparison to those issues for neurotoxic substances. The third chapter contains a risk assessment for cholinesterase inhibitors in children's diets, with the goal of determining the potential risk to children from exposure to
multiple compounds that exert toxicity through a common mechanism of action. The fourth chapter examines the assumptions required for this assessment and attempts to elucidate the amount of uncertainty within the assessment given the state of the art. The regulatory options that arise out of neurotoxic risk assessment and the options available for improving the process are also discussed.
Chapter 2
Risk Assessment

Risk assessment as used in this report is the process of determining and characterizing the hazardous potential of environmental toxins to human health. It is a complex, interdisciplinary process involving toxicology, physiology and medicine, processes of environmental transport related to exposure estimation, and statistical design and modeling. Risk assessors combine available scientific data with extrapolation and assumptions to derive a plausible estimate of the hazard associated with exposure to a given pollutant. The process of risk assessment gained importance as the government became increasingly involved in the regulation of technological processes in the 1970's through agencies such as Occupational Safety and Health Administration (OSHA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). As improved technology enabled the quantification of previously undetectable levels of chemicals in the environment, and the possibility of chronic effects of these compounds became recognized, environmental organizations, health advocates and the public began to demand that the potential adverse effects of industrial pollutants be characterized, controlled and eliminated. Environmental groups and health advocates, however, generally oppose quantitative risk assessment as the means of identifying environmental hazards. These groups contend that the extensive data requirements of this method tend to slow the regulatory process and promote reliance on suspect information supplied by industry.

 Ideally, the role of risk assessment is to characterize environmental hazards, recognize and describe the limits of scientific understanding, to
outline assumptions and uncertainties accordingly, and to inform the regulatory agencies in order to enable the appropriate selection among regulatory options. Early legislation focused on the regulation and reduction of carcinogenic hazards. Consequently, the process of risk assessment has been studied longer and has been more thoroughly debated for carcinogens than for non-carcinogens. The first section of this chapter describes the development of the process of risk assessment and briefly examine the process for carcinogenic compounds. The second section looks at the developing process of risk assessment for neurotoxins. In the final section, lead is examined as a case study and prototype for neurotoxic assessment. Lead has been extensively studied and regulated in recent years, and the history of lead illustrates several important issues for neurotoxins including low dose toxicity and increased susceptibility of certain subpopulations.

I. The process of risk assessment

RISK assessment has been a component of toxics regulation for many years. Limited forms of assessment in the form of dose response studies have been required since the 1950's for licensing of chemicals to be used as food additives, color additives, and drugs for humans and animals as mandated under the Federal Food, Drug, and Cosmetic Act (National Resources Council (NRC), 1983). As scientific assessment became more integral to the regulatory process, emphasis was placed on producing detailed guidelines to outline and unify procedures. The general risk assessment steps outlined by the National Research Council (1983) and adopted as the model for the assessment of carcinogens by the United States Environmental Protection Agency (US EPA, 1986) is divided into four steps: 1. hazard identification 2. dose-response assessment, 3. exposure assessment and 4. risk characterization.
(1) Hazard identification is a qualitative evaluation of the likelihood that a given compound poses either a carcinogenic or non-carcinogenic risk. Identification is based on relevant epidemiologic, animal or other toxicologic indices such as mutagenicity, metabolic and pharmacokinetic properties which suggest that an adverse effect in humans is possible. The EPA recommends that the data be assessed for scientific validity and for the uncertainty associated with the various types of information, and that conclusions be drawn based on a "weight of evidence" determination. This standard considers the quality and adequacy of the data and the kinds and consistency of responses in toxicological screenings.

(2) Dose-Response assessment is the process of determining the relationship between the exposure dose of the agent and the resulting toxic effect in the exposed population. The initial step in this process is the identification of the appropriate toxic endpoint for a given compound. For carcinogens this generally means tumor formation. The endpoints for neurotoxins may vary widely, for example, from obvious and measurable peripheral neuropathy to more subtle cognitive or behavioral changes.

It is widely held in the scientific and regulatory community that carcinogens do not exhibit a threshold for effect, meaning that exposure to a carcinogen at any dose represents a risk. Noncarcinogens are believed to exhibit a threshold for effect below which either no adverse effect occurs or below which physiological and biochemical compensatory mechanisms and functional reserve capacity offset potentially toxic changes. Both views are controversial, however, and the following review of carcinogens and neurotoxins will explore the ideas of threshold and toxic endpoint in greater depth.
Animal data are used to establish a dose-response curve when human
data are not available, although the validity of using animal models as a
surrogate for direct human data is debatable. Specifically, the use of animal
data requires that the response of an animal model to a toxic substance be
extrapolated to the human response. The reaction and sensitivity to toxics
exposure may, however, be unrelated because of physiological differences
between the species in the handling of toxic substances. Further, animal
studies often involved exposing the subject to high doses of the compound in
question. These data must then be extrapolated to estimate the response in
humans to low dose, environmental levels of toxin. The differences between
species uptake, metabolism and organ distribution of environmental hazards,
and the need for high to low dose extrapolation complicate the extrapolation
process and increase uncertainty. These problems with animal based data are
a source of controversy for both carcinogens and noncancer risk
assessment. Mathematical and statistical modeling are often used in the
process of assessing dose response. The choice of model and statistical
parameters is determined as much by policy decisions as by scientific
understanding.

(3) Exposure assessment is the process of estimating the dose, the route, the
duration, and the frequency of exposure of a population to an environmental
hazard. This portion of the risk assessment process is complicated by a lack of
information about the amount of compound released into the environment
by various sources, the difficulty in monitoring levels in the environment,
the complexity of modeling chemical disposition in the environment, and
the complexity of modeling human activity and behavior patterns that
influence exposure levels. The exposure determination should include
levels not only for the general population but for potentially susceptible
subpopulations which may be at risk either for higher exposure levels or for potentially greater health risks from the same level of exposure as the general population.

(4) Risk characterization combines the findings of the exposure assessment and the dose response assessment to give a quantitative estimate of risk. The interpretation of findings includes characterization of the assumptions and uncertainties that arose in the foregoing evaluation of biological, statistical, and exposure assessment data.

In the face of uncertainty and gaps in available data, questions about which assumptions and models are appropriate and valid based on current scientific understandings must be addressed. Many analytic choices within a quantitative risk assessment require individual judgements that are based both on scientific and policy considerations. Clearly, the process can not be purely a scientific endeavor. The NRC recommends that the scientific findings and policy judgments embodied in risk assessments be explicitly distinguished from the political, economic and technical considerations that influence the design and choice of regulatory strategies. These considerations along with the directives of regulatory legislation are part of risk management activities. Both the NRC and the EPA strongly recommend that risk management activities and risk assessment processes be kept entirely separate. Risk assessment should be based as much as possible on scientific premises. Scientific policy judgements should be minimized and, where required because of data gaps and uncertainty, must be thoroughly described and justified within the assessment. Risk assessment is used to inform regulatory decision making. The value of an assessment that is biased by undisclosed policy decisions or regulatory mandates becomes greatly diminished.
II. Carcinogen risk assessment

In 1987 the US EPA published the Guidelines for Carcinogen Risk Assessment with the combined goals of informing the public and interested parties about the process used by the EPA to evaluate environmental hazards, promoting quality and consistency in the risk assessment process, and permitting sufficient flexibility for the accommodation of new scientific knowledge and assessment methods (US EPA, 1987). The guidelines describe in detail procedures undertaken within the four risk assessment areas described by the NRC and outlined above.

The guidelines incorporate current understanding of the mechanisms of carcinogenesis with nearly 20 years of study into the process of assessing carcinogenic potential of environmental hazards. The validity of many of the risk assessment assumptions accepted by the EPA in these guidelines have been questioned. Critics, on the one hand, accuse the EPA of recommending assessment options based on very limited and unsubstantiated scientific theories and of consistently choosing overly conservative assumptions, thereby skewing the assessment process such that risks are "monumentally" overstated (US Office of Management and Budget (OMB), 1990). Others, however, suggest that the stringent scientific criteria required by the EPA, considering the substantial data gaps that exist for most chemicals, leads to risk characterizations that could potentially underestimate the true risk from carcinogens (Latin, 1988). Criticism specific to carcinogen risk assessment surrounds the selection of extrapolation models to estimate effects from high dose to low dose exposure and the use of both benign and malignant tumors as toxic endpoints for determination of carcinogenic potential. The choice of exposure assumptions and the use of animal models for human toxicity
evaluation are controversial issues for both carcinogenic and noncarcinogenic compounds.

Dose-response assessment is most frequently based on animal studies in which animals at various exposure levels are assessed for tumorigenesis. Because of the short life span of test animals relative to humans as well as time and economic constraints which limit toxicological testing, animals are generally exposed to much higher doses for relatively short periods of time as compared to the doses that humans are expected to see at environmental levels. In order to extrapolate results from these animal studies to human exposure, the EPA recommends that the linearized multistage model be used. In this model low dose exposures are calculated by linear extrapolation through the origin from a dose response curve generated for tumorigenesis at higher doses. The multistage assessment method based on the 2 stage model for carcinogenesis was outlined by Armitage and Doll in 1957. According to the EPA, linear low dose extrapolation, while not based on the biological understandings of the mechanisms of carcinogenesis, leads to a plausible upper limit risk assessment though it does not necessarily give a realistic prediction of the risk. Critics question the biological plausibility of the multi-stage linearized model and the value of such a conservative risk characterization and suggest that a model which considers the biological mechanisms of carcinogenesis might be more useful (Thorslund et al., 1987). Some critics suggest that the nature of the model does not allow for accurate ranking of risks because the assumption of low dose linearity overestimates the risks for lower risk compounds to a greater degree than for higher risk compounds (Nichols and Zeckhauser, 1988).

Numerous mathematical extrapolation models besides the linearized multistage model have been suggested (Moolgavkar et al., 1988, Thorslund,
The guidelines recommend that other extrapolation models can be used if available data provide evidence for a mechanism of carcinogenesis which is better accounted for by one of the other models. The guidelines advise, however, that the linearized model should also be conducted for comparison and standardization.

The toxic endpoint for carcinogenic chemicals is tumor formation. The identification of this toxic endpoint is readily apparent though not without controversy. The EPA suggests that benign tumors should be considered to have potential malignant behavior and must be considered with malignant tumors when assessing carcinogenicity unless specific data on the benign tumor can demonstrate that no malignant potential exists. Critics suggest that no rationale can be described for excluding benign tumors and that the distinction between benign and malignant tumors described in the guidelines can only lead to increased costs and delays in risk assessment (Latin, 1990). Other critics, however, argue that the EPA protocol leads to an overly conservative estimation of risk especially since the EPA requires that tumor data be collected for the most sensitive species. In some instances these sensitive species have been shown to have a history of spontaneous tumor formation, a characteristic which may predispose these animals to be hyperreactive to chemical insult (US OMB, 1990).

The problems surrounding exposure assessment are similar for carcinogens and non-carcinogens and are explored by specific example within the cholinesterase risk assessment in Chapter III. As described previously, the major issues in the modeling of environmental exposures include the need for adequate compound production data, for monitoring technologies, and for a thorough understanding of the disposition of a compound in the environment. These exposure parameters are characterized by enormous
data gaps for most compounds and regulators are accused of choosing conservative assumptions, including modeling based on worst-case environmental conditions and on the theoretically maximum-exposed individual (US OMB, 1990).

Controversy exists over the use of extrapolation models in the dose-response assessment of carcinogens. The models available, however, are based on a relatively well established and accepted theories of carcinogenesis. The biological basis for neurotoxicity for many compounds is not well understood, and a single unifying theory, given the variety of modes and sites of action of neurotoxins, probably cannot be formulated. The endpoint for carcinogenesis, the formation of a tumor, whether malignant or benign, is relatively obvious. For neurotoxins the issue, similar to the problem with reproductive and developmental toxins, is that end points are often multiple, difficult to assess given current toxicity testing methods and the significance of the changes are not well understood (Pease, Vandenberg and Hooper, 1990). In the following section, the issues surrounding the assessment of neurotoxic risks are described using lead as the primary example.

III. Neurotoxic risk assessment

The number of neurotoxic substances in the environment that pose a significant risk to public health, and the extent of that health risk is not well characterized. The neurotoxicity of only a small number of chemicals has been adequately evaluated, and until recently, toxicological testing for neurotoxicity was not a part of the general toxicology screening for the majority of environmental contaminants (Sette, 1989). The data base on neurotoxicity is, therefore, quite inadequate. Neurotoxicity has, however, become an increasing concern to regulators, environmental advocates and
the public. The designation of the 1990’s as the “Decade of the Brain” by the 101st Congress exemplifies this interest. The U.S. Office of Technology Assessment (US OMB, 1990) published a comprehensive review of the problems of identifying neurotoxins and of assessing regulatory options entitled Neurotoxicity: Identifying and Controlling Poisons of the Nervous System. In that same year, the environmental advocacy group, the Environmental Defense Fund (EDF), published its report Legacy of Lead: America’s Continuing Epidemic of Childhood Lead Poisoning (Florini et al, 1990) calling for the prevention of “intolerable and unnecessary” lead-related nervous system damage to millions of children throughout the United States. As described in the introduction to this paper, the NRDC has also given voice to the escalating public concern about the potential neurotoxic effects of pesticides in foods in their publication Intolerable Risk: Pesticides in Our Children’s Food (1989).

The EPA defines neurotoxicity as an adverse change in the structure or function of the nervous system following exposure to a chemical agent. Adequate risk assessment of neurotoxins, similar to carcinogen assessment, requires quantitative analysis of the dose-response characteristics and exposure data for a compound. The assessment of subtle, subclinical neurological changes indicative of adverse effect requires the use of neuropathological examination in animal models as well as sophisticated behavioral and cognitive testing in both exposed humans and animals. Until recently the only neurotoxicity test required by the EPA under the FIFRA mandate was the testing for organophosphate induced delayed neuropathy (OPIDN). The Toxic Substances Control Act of 1985 established guidelines for the testing of suspected neurotoxins, and the EPA has proposed an expanded approach to neurotoxicity testing for the evaluation of pesticides (Cooper,
Francis and Kimmel, 1989). The OTA, however, estimates that the addition of a complete set of neurotoxicity tests could add from 40 - 240% to the cost of conventional toxicity testing for chemicals currently required by the EPA (US OMB, 1990).

Unlike carcinogens, one of the determinations required in a dose-response assessment for neurotoxins, and for non-carcinogens in general, is whether an effect associated with a chemical exposure is an adverse response or an adaptive or compensatory response that may serve as a marker for exposure but which has no adverse health effect (US EPA, 1987). For example, plasma and blood cholinesterase inhibition occurs following exposure to organophosphate chemicals, but low level inhibition of this enzyme has not yet been correlated to adverse clinical effect. As will be described in the cholinesterase inhibitor risk assessment in the following chapter, scientific risk assessment is hampered by inadequate information about the low dose, subclinical neurotoxicity of environmental contaminants. Further, noncancer effects at low doses, unlike carcinogenic changes, are often considered to be potentially reversible, and this reversibility must be considered and somehow reflected in the assessment of risk.

Another aspect of the dose-response assessment is threshold effect. Carcinogens are thought to exert their toxic effect at all levels of exposure and, therefore, risk models assume that no threshold for carcinogenic effect exists. Noncarcinogens are thought to have a threshold for toxic effect below which exposure to a hazard is not considered to be a risk. Based on this assumption of threshold the EPA recommends the use of the Reference Dose (RfD) model to determine a level of daily exposure that is expected to be acceptable over the lifetime of an individual. Since the RfD model only requires that a single toxicity parameter be described, a dose-response model is recommended to
estimate the likely human response to various exposure level of a particular contaminant (US EPA, 1990).

The RfD model, formerly called the Acceptable Daily Intake (ADI), has been used most frequently in the assessment of noncancerogenic risk. RfD based acceptable levels for exposure are established by determining the appropriate No Observable Adverse Effect Level (NOAEL) for a compound based either on human data when available or on subchronic and chronic animal studies when human data is unavailable or incomplete. The NOAEL is based on observed changes in growth, development, organ function, biochemistry or morphology that do not interfere with normal activity (US EPA, 1990). The observation that a change occurs in response to chemical exposure, or that a NOAEL exists, is not necessarily sufficient evidence to establish that the compound has an adverse effect.

The NOAEL value is multiplied by an uncertainty factor (UF) to derive the RfD. The uncertainty factor is an adjustment of the NOEL (No Observable Effect Level), NOAEL, or LOAEL (Lowest Observable Adverse Effect Level) reported for small populations of humans or experimental animals in order to estimate the comparable NOAEL from chronic contaminant exposure for a large human population (Dourson and Sta, 1983). Uncertainty factors reflect scientific uncertainty in dose-response parameters such as interspecies extrapolation of data from animal to human, variability within the human population, and uncertainty in extrapolating effect from subchronic to chronic chemical exposure. In order to account for human variability a safety factor of 10 is then used. This UF is estimated to protect 80-95% of public though human variation in metabolism of xenobiotics may lead to higher than ten fold variability (Calabrese, 1985). The uncertainty factor also reflects the extent to which data are missing or
inadequate. A ten fold increase in the uncertainty factor is applied for each of
the above areas of uncertainty so, for example, a UF of 10 is applied to data for
which there is one area of uncertainty and a UF of 1000 is applied to data for
which three points of uncertainty exist.

The reference dose model has been the most widely used method for
characterizing risk associated with non-carcinogens. There are, however,
several significant problems with this method. NOAEL levels vary greatly
with experimental design and choice of dosing levels can easily lead to both
over or under estimations of threshold levels. The NOAEL system is further
limited in that it does not make use of all available dose-response data. The
multiplication of NOAEL values by UF's leads to conservative RfD values
which may interfere with risk management decisions (Risk Focus, 1989).
Further, the RfD approach does not estimate the level of response above the
threshold level and, therefore, does not yield a true risk estimate. Finally,
according to one writer, the threshold hypothesis can not be said to be
scientifically substantiated for all or even most systemic toxicants, especially
those producing delayed effects (Wyzga, 1990).

One approach to characterizing dose-response relationships employs
mathematical models to describe the relationship between exposure level and
changes in a given biological system. Dose-response models are based on
experimental data and are, therefore, considered more accurate than the RfD
model. A detailed description of the various mathematical models that have
been used or proposed for use for dose-response estimation is beyond the
scope of this review. Some of the models discussed in the literature include
log-probit models probit models based on bioassay data and a variety of linear
dose-response models (Wyzga, 1989, Gaylor and Slikker, 1990). These models
often require more complete data than are currently available for most
compounds. In addition, understanding of the mechanism of action of the toxicant is important in selecting an appropriate mathematical model. Currently researchers have emphasized selection of models which most accurately mirror the prevalent understanding of the mechanism of action of the neurotoxin being studied. Selection of empirically derived models which are based on the "best-fit" with available data is associated with increased uncertainty, especially when low dose or species to species extrapolation is required. Therefore, when the mechanism of action of a toxicant is not well understood, as is often the case with neurotoxins, the use of several models is recommended in order to compare risk estimations and as a means to explore the level of uncertainty within the risk evaluation.

Environmental lead exposure has been extensively studied and is an instructive example of the evolution of understanding of the neurotoxicity associated with low level exposures to an environmental contaminant. Some of the issues in neurotoxic risk assessment including the need for toxicity testing, reversibility of effect, threshold are illustrated through the following brief case study. In addition, the lead toxicity study is a prototype for the study of cholinesterase inhibitors in the following chapter.

IV. Lead exposure and children

The adverse health effects of lead have been recognized for thousands of years though the toxic potential of low level exposure has only recently been acknowledged. Lead is pervasive in the environment. Sources of exposure to lead include paint, plumbing, gasoline, batteries, and solder. Though the toxicity of lead and the frequency of exposure in industrial areas have not been disputed, regulations aimed at decreasing environmental exposures in the United States were not attempted until about 20 years ago.
(Florini, 1990). In 1970 the Lead Based Paint Poisoning prevention act was passed with the goal of reducing lead levels in housepaint. Lead was totally banned from paint in 1977 by a regulation issued by the Consumer Product Safety Commision. The Clean Air and Clean Water Acts also have provisions requiring the reduction of lead emissions into the environment (US OMB, 1990).

Lead from all sources is absorbed into the bloodstream from the environment mainly through ingestion and inhalation. Fifty to sixty percent of the lead is rapidly eliminated from the body, and the remainder is stored in bone where it can accumulate and from where it can be mobilized in situations of increased bone reabsorption including pregnancy and osteoporosis (Florini, 1990). Lead is toxic to all organ systems; the central nervous system (CNS) appears to be most sensitive. The mechanism by which lead adversely affects the CNS is unclear. Researchers suggest that lead may interfere with ions involved in neurotransmission (Silbergeld, 1990). Specifically, lead has been demonstrated to cause increased uptake of extracellular calcium and a decreased calcium-sodium exchange from intracellular ion stores.

The neurotoxic effects of lead are cumulative and irreversible. High dose lead poisoning causes encephalopathy, convulsions, coma and death. Low dose lead exposure has been linked to decreased intelligence as measured by IQ testing, loss of short term memory, impaired visual-motor function, poor perceptual integration, poor classroom behavior and impaired reaction time (Needleman, 1990a). Children are especially susceptible because of their rapidly growing nervous systems. The acceptable levels for lead exposure according to the Centers for Disease Control, as measured by blood lead level, is currently 25ug/dL. The EPA Science Advisory Board has recommended
that the acceptable level be decreased to 10-15 ug/dL (Florini, 1990). Data suggests, however, that the neurotoxic effects of lead exposure in the central nervous system are without threshold (Silbergeld, 1990). The Environmental Defense Fund estimates that 3-4 million children estimated to have blood lead levels above 15ug/dL and over 200,000 children are estimated to have blood lead levels above 25ug/dL.

Symptoms of low level lead poisoning are nonspecific and may occur in many persons who escape diagnosis. At one time researchers believed that if an individual survived the encephalopathy, convulsions and other systemic toxicities of an acute lead poisoning episode, no permanent neurological sequelae ensued. Sensitive psychometric testing has, however, demonstrated that cognitive and behavioral deficits do occur following an acute poisoning. In the early 1970's, Needleman undertook an epidemiological study of low dose exposure to lead in children. Needleman's research group quantified the lead concentrations in teeth shed by first and second grade children, and correlated dentine lead levels with neurobehavioral function (Needleman, 1990b). In this manner, chronic exposure levels, rather than acute exposure levels, demonstrated by blood lead levels, were quantified. The original study found that children at higher exposure levels demonstrated reduced IQ, impaired hand-eye coordination and developmental delay. These studies have been repeated and confirmed by other researchers. Further a recent follow up study of the children from the 1976 lead study found that, among other deficiencies, young adults who had been found to have high dentine levels of lead as children were seven times more likely to drop out of high school and 5 time more likely to have impaired reading skills than the young adults who were not lead exposed as children (Needleman, 1990a).
A detailed risk assessment of lead is beyond the scope of this paper. Rather, the general data requirements and scientific assumptions used in the four steps of the risk assessment process for this neurotoxic compound are described briefly below:

(1) Hazard Identification - As described above the neurotoxic effects at high dose levels have been well recognized throughout history. The mechanism of action of lead is not well understood, but, because of lead's overt neurotoxic effects, researchers suspected that the developing nervous systems of young children might have an increased susceptibility to lead toxicity.

(2) Dose Response - Researchers once believed that lead was only toxic at the high levels of exposure consistent with lead poisoning. Refined assessment methods using neurobehavioral and cognitive, rather than physical indicators of adverse effects, led to the discovery that neurotoxicity occurs at much lower doses than once suspected. Further, these studies indicate that lead toxicity is best described by a dose-response curve rather than the threshold or NOEL level of effect commonly applied to non-carcinogenic compounds. The use of epidemiological studies eliminates the uncertainty associated with extrapolation of animal data to humans though many of the epidemiological studies are criticized for lack of power or the presence of multiple confounding variables. Metaanalysis of the epidemiological and toxicologic research studies of low dose led exposure, however, strongly supports an overall picture of low dose neurotoxicity (Needleman, 1990b). While individual studies may be criticized for various flaws in study design, the wealth of data on lead decreases the uncertainty about low dose neurotoxicity.

(3) Exposure assessment - Lead is widespread and persistent in the environment. Exposure to lead arises from a number of sources including
lead based paints, gasoline and solder used in the cans used for food-packaging. Biomarkers for both acute and chronic exposure to lead are available for the monitoring of human exposure levels. Blood lead levels provide an accurate measure of current exposure, and bone or dentine samples provide a measure of chronic exposure levels. These biomarkers have been integral to epidemiological study of lead exposure in humans. Humans have no endogenous use for or production of lead, therefore, any lead measured indicates exposure to environmental contaminants.

(4) Risk Characterization - Though the mechanism of action of lead is still not well characterized, sensitive neurotoxicity testing and the availability of biomarkers have enabled researchers to prove that lead has neurotoxic effects at very low levels of exposure. These findings have led to a revised quantification of the risk of lead down to a blood lead level of 10-15\(\mu\)g/dl. Animal research and solid epidemiological evidence minimizes the uncertainty of the risk assessment for lead. The permanence and pervasiveness of lead in the environment and the apparent irreversibility of lead toxicity highlights the significance of the risk incurred by exposure to lead in environmental contaminants, and has provided a strong scientific basis for risk management decisions aimed at reducing public exposure to lead.

The following chapter contains a risk assessment for cholinesterase inhibiting pesticides. Like lead, the acute toxicity of these compounds is widely recognized. Researchers, public health officials and environmental activists have recently begun to explore the possibility of neurological effects associated with chronic low dose exposure to these pesticides. Like lead, concern has, in part, been focused on the possible effects chronic exposure may have on the developing nervous system of a child. The mechanism of
action of these pesticides is known, but a widely agreeable definition of the toxic endpoint for these compounds has not been established. Exposure measurements are also highly uncertain. In the fourth chapter, following the cholinesterase risk assessment, the assumptions and uncertainties of the ChE risk assessment are explored. The preceding discussion of lead risk assessment forms a basis for comparing the risk assessment process of these neurotoxic compounds.
Chapter 3
Hazards of Cumulative Cholinesterase Inhibitors in the Diet of Children

This review examines the possibilities for cumulative neurotoxic effects from pesticides which act through a common toxicologic pathway to inhibit cholinesterases (ChE). The EPA establishes food tolerances for such compounds based on assumptions about exposure and toxicity for individual compounds without formal consideration for the potential additivity of their effect. However, cholinesterase inhibiting effects do appear to be additive. The intake of a combination of these chemicals in an average diet, while perhaps not exceeding established intake limits for a single chemical, may have cumulative effects on cholinesterase enzymes in both the central and peripheral nervous systems. Although such additivity may occur at any age, dietary and physiologic factors are cause for increased concern for infants and children.

The methodology for determining the level of risk due to exposure to multiple compounds in the diet is outlined in this document. Due to the complexities of the computer analysis needed to generate results from the combined residue and exposure data, however, only the preliminary findings are included in this report. With the research design and data collection accomplished, the foundations for further research are laid.

I. Cholinesterase inhibitors

The organophosphate and carbamate compounds are the cholinesterase inhibiting pesticides of primary concern. These insecticides bind with cholinesterases and block their action in the hydrolysis of the acetylcholine (ACh) neurotransmitter. ACh is the principal neurotransmitter at
neuromuscular junctions, at pre- and post-synaptic junctions of the parasympathetic system and in the pre-synaptic junctions of the sympathetic nervous system. ACh is found throughout the central nervous system (CNS) and is found in high concentration in certain areas of the brain including the cerebral cortex and the striatum (Abou-Donia, 1985). ACh has been linked to higher cognitive functions such as learning and memory (Morgan, 1989).

ACh acts as a classic neurotransmitter. Upon excitation of a cholinergic neuron, ACh is released from vesicles at the axon terminal. ACh interacts with ACh receptors leading either to ion channel opening or to activation of second messenger systems depending on the location and activity of the cholinergic neurons. The action of acetylcholine is terminated through hydrolysis by cholinesterases of which there are two types. Acetylcholinesterase, AChE, is found in neural tissue in the ANS and CNS, and at the neuromuscular junction. Butyrl cholinesterase, also known as pseudocholinesterase, is found in the plasma, tissues, liver and other organs. The physiological function of pseudocholinesterase is not well understood. Genetic variation in the rate of pseudocholinesterase activity is observed in surgical patients treated with the muscle relaxant succinyl choline, however, this variation has not been linked to differences in enzyme activity in response to pesticide exposure (Abou-Donia, 1985).

Organophosphate compounds, such as acephate, chlorpyrifos, malathion and parathion, bind and irreversibly phosphorylate the active site of cholinesterase thereby inactivating the enzyme. Carbamates, including aldicarb, lannate, methomyl, propoxur and carbaryl, interact with the cholinesterase (ChE) receptor by reversible carbamylation of the phosphate moiety at the active site of the enzyme (Murphy, 1986).
The organophosphate and carbamate insecticides have high acute toxicities and are the pesticides most frequently involved in human poisonings. Acute poisoning with ChE inhibiting compounds may lead to cholinergic crisis, with symptoms of headache, nausea and vomiting, cramps, weakness, blurred vision, pinpoint pupils, chest tightness, muscle spasm and coma (California Department of Health Services (DHS), 1988). Low level exposure to these compounds may cause behavioral changes and may alter neurological function. Accumulation of ACh in the CNS due to ChE inhibition (ChEI) can lead to tension, anxiety, restlessness, insomnia, headache, emotional instability, nightmares, apathy and confusion (Murphy, 1986). Repeated subacute exposures may also lead to persistent anorexia, weakness and malaise. Clinically, symptomatology is difficult to recognize, both because the complaints are non-specific and because most physicians have limited familiarity with the signs and symptoms of pesticide poisoning.

Most ChE inhibitors degrade relatively rapidly in the environment and do not appear to accumulate or concentrate in the food chain (in contrast to the organochlorine pesticides). In addition, accumulation of the pesticide in the body does not occur since the ChE inhibitors are rapidly biotransformed and excreted. Accumulation of the cholinesterase inhibitory effect upon repeated pesticide exposure, however, can occur, and may lead to signs and symptoms of poisoning following small repeated doses (Murphy, 1986). Though the long term effects of acute pesticide poisoning and subacute exposure are uncertain, studies have found chronic, subtle neurologic sequelae to acute organophosphate poisoning (Savage et al, 1988). Animal studies have demonstrated an association between chronic exposure to low dose carbamates and changes in motor and learning behavior in rats as well as increases in ACh levels in certain areas of the rat brain (Boyd, Weiler, and
Porter, 1990). Review of the epidemiologic literature by the Office of Technology Assessment (OTA), including case reports and studies of agricultural workers with and without histories of acute poisoning, found evidence of delayed, persistent or latent effects in humans (US OTA, 1990).

The organophosphates and carbamates may undergo conversion in the environment or in vivo to form metabolites of potentially greater toxicity than the parent compounds. Synergism between organophosphate compounds, such as that demonstrated between malathion, EPN, and other organophosphates, may also be an important variable when considering the potential toxicity of exposure to multiple compounds. In addition, some of the organophosphates and carbamates have toxicities not related to their cholinesterase inhibitory effects. For example, organophosphate induced delayed neuropathy (OPIDN) is a neurotoxic effect demonstrated to occur follow OP poisoning with phosphate or phosphonate compounds such as leptophos, and EPN. Damage to nerve axons in the peripheral and central nervous systems, which presents in OPIDN as a distal sensorimotor neuropathy, is associated with the inhibition of "neurotoxic esterase" (NTE) rather than with the OP effect on the ChE enzyme (Abou-Donia, 1986). Other organophosphates such as demeton and disulfoton are associated with optic nerve degeneration. Some cholinesterase inhibitors, such as acephate, are classified as possible carcinogens and exposure to others including methidathion and methomyl is associated with non-neurotoxic effects such as hepatic and liver changes. These are all important aspects of the toxicology of the cholinesterase inhibiting pesticides requiring further assessment. This review, however, will focus only on the cholinesterase effects of these compounds. Simplified in this manner, the assessment strives to determine the potential risk from exposure to multiple compounds with a common mechanism of toxicity.
II. Cholinesterase inhibition as a toxic endpoint

The correlation between laboratory findings of cholinesterase inhibition in plasma and red blood cells and clinically recognizable neurotoxic effects is a subject of debate. Neurotoxicity is defined by the EPA as an adverse change in the structure or function of the nervous system following exposure to a chemical agent (EPA 50FR188). Whether ChE inhibition itself represents an adverse change or whether a given level of inhibition is associated with adverse change is unclear. Some authors question the validity of measuring cholinesterase inhibition in peripheral tissues, i.e. in plasma and blood as a surrogate for measuring central cholinesterase inhibition (US EPA, 1990a). In 1988 a US EPA risk assessment technical panel concluded that cholinesterase inhibition should be considered a potentially toxic effect (US EPA, 1988). The panel admitted, however, that overt, clinically recognizable responses to ChE inhibiton are difficult to predict and difficult to correlate with actual levels of cholinesterase inhibition. In May 1990, the EPA Science Advisory Board (SAB) "expressed doubt about the validity of plasma and red blood cell cholinesterase inhibition as indicators of toxicity" (US EPA, 1990a). The SAB report explained that the relationship between degree of ChEI and toxicity is unclear, experimental correlations between exposure indices and neurotoxic manifestations tend to be weak, and consensus about what level of cholinesterase inhibiton and which types of cholinesterase inhibition (red blood cell, plasma, or brain) are associated with overt toxicity does not exist. Further, the effects of in utero ChEI on nervous system development and postnatal function have received only minimal study, and the research
evidence for enhanced developmental susceptibility to cholinesterase inhibition is ambiguous (US EPA, 1990a). The SAB committee suggested that ChE inhibition may not be an adverse effect because AChE has a very high turnover rate and because of a large functional reserve of ChE throughout the brain.

Further study is required to correlate ChEI with identifiable changes in the central and peripheral nervous systems. Techniques for measuring neurotoxic effects include nerve conduction studies, sensory studies, evoked brain responses, electrocardiogram, and biochemical assays (US OTA, 1990). In addition, neurobehavioral and neuropsychological testing is being used to explore subtle changes in behavior, learning perception and emotion (Savage et al. 1988). These techniques have not yet been widely applied in the study of cholinesterase effects but, as described above, early evidence suggests that subtle neurologic changes do occur following exposure to ChE inhibiting pesticides. The lack of solid evidence and acceptable data necessitate that, for the purposes of this report, a policy-based decision rather than a strictly scientific determination establish whether or not ChE inhibition is a toxic effect. This study was undertaken in cooperation with the California Department of Health Services which has as its mandate the protection of public health. The duty to protect public health leads to the acceptance of two central assumptions within this risk assessment; that peripheral cholinesterase effects are reflected centrally, and that cholinesterase inhibition is a significant and unacceptable toxicologic event.

III. Increased Susceptibility of Children to Neurotoxic agents

Children appear to be at an increased risk from the exposure to neurotoxic chemicals because of dietary and physiological differences from adults.
Newborns and very young children (under two years) have different gastrointestinal absorption than adults. They are less capable of binding such agents as plasma proteins so that xenobiotics have a greater opportunity to reach the toxic site of action. Further, metabolic routes of excretion including glucuronidation, sulfation and acetylation are developmentally immature. The nervous system is still developing and the blood brain barrier is not completed (Calabrese, 1988). In some instances, the physiological differences of children may actually be protective. For example, the toxicity of certain compounds is the result of metabolic activation, a function which may be decreased in children relative to adults. Further, the young nervous system has greater plasticity than the mature nervous system and may be better able to respond to chemical insults. Research strongly suggests, however, that the young are at the greatest risk from neurotoxic agents such as heavy metals, lead, anesthetics, analgesics and numerous organophosphates (Calabrese, 1988).

Children are also at increased risk because their eating habits are different from adults. This risk assessment focuses on the dietary exposure of children ages 1-6 years. Within this age range the diet is not as broad as that of older children or adults, but it is also not as limited as the diet of infants. Children in this age range eat considerably more fruits and vegetables per body weight than do adults. The NRDC estimated that, based on dietary intake studies conducted in 1985-1986 by the United States Department of Agriculture, by weight children consume, for example, 11 times more bananas, 10 times more peanut butter, and 6 times more apples than do adults (NRDC, 1989). This significantly increased intake of fresh fruits and vegetables combined with the documented increased susceptibility of children to neurotoxins, warrants the identification of children as a highly susceptible subpopulation.
IV. EPA food tolerances

Originally under an amendment to the Federal Food, Drug and Cosmetic Act in 1952, food tolerances were established based on the amount of pesticide necessary for the greatest efficacy in the field and were contingent on the pesticide use not having "unreasonable adverse effects" on human health or the environment (NRC, 1987). FFDCA requires the EPA to establish maximum legal limits of pesticide residues, known as tolerances, that may be present in raw and processed foods. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requires that the EPA consider the economic, social and environmental costs and benefits, as well as the protection of public health, in the use of any pesticide.

The tolerance principle, outlined in sections 408 and 409 of the Food, Drug and Cosmetics Act, bases allowable levels of pesticide residues on food crops on two primary criteria: (1) acceptable daily intake levels based on toxicologic data establishing no observable effect levels (NOEL) and lowest observed effect levels (LOEL) for each pesticide; and (2) approximate daily exposure levels based on dietary intake estimates (NRC, 1987). The complexities of establishing acceptable daily intake levels and of estimating dietary exposures are described in detail in other sections of this paper. That tolerance levels do not reflect the potential susceptibility of the developing nervous system or the dietary differences between adults and children (likely to increase the level of residue exposure in children) will also be discussed elsewhere.

Tolerance levels must be set for each food crop on which a given pesticide is used. In theory, the maximum allowable residue level for each compound, derived by adding up the tolerance levels across all the food uses for that compound, must not exceed the EPA established acceptable daily intake level.
of that particular pesticide. In practice, tolerances frequently exceed the ADI level by substantial margins (US EPA, 1988b). Many argue, however, that tolerances greatly overestimate true pesticide exposure levels because the tolerance values are determined using the assumption that the maximum allowable amount of a pesticide will be sprayed on 100% of the crop. Tolerance levels, therefore, neither delineate safe use levels nor do they provide accurate measures of exposure when estimating risk.

The section of the EPA regulatory code that addresses additivity of effect states that pesticide chemicals with related pharmacologic effects should be regarded as having an additive deleterious action. Where residues of two or more chemicals in the same class are present on an agricultural commodity, the tolerance for the total is considered the same as the chemical having the lowest numerical tolerance in that class unless otherwise specified (EPA 40 CFR 180.3). There is no specific regulation for the establishment of tolerances for chemicals of the same class which, while not found on a single product, may be present on a combination agricultural products likely to be consumed at the same time.

As part of their 1989 report, Intolerable Risk: Pesticides in our Children's Food, the Natural Resources Defense Council (NRDC) outlined a risk assessment method which recognizes the different food consumption patterns of children, the potential for daily exposure to multiple cholinesterase inhibiting pesticides in an average diet, and the potential for additive adverse effect due to the common toxicologic pathway of these compounds (Sewell and Whyatt, 1989). The calculations in the following section of this report are aimed at addressing these same issues and are based on refinements of the NRDC risk assessment methods, using the more recent
and complete exposure data available through Dr John Wargo at Yale University (personal communications, Jan-Apr, 1991).

V. Methods

The mixture assessed in this review is comprised of various organophosphate pesticide residues that may be found in combination on the food products, especially fresh fruits and vegetables, consumed in the average diet of children 1-6 years of age. Although there are over forty organophosphates and carbamates registered for use on foods, only twenty are considered in this study. The criteria for inclusion included; documented use of the pesticide on food, the ability of the FDA and California Department of Food and Agriculture (CDFA) multiresidue pesticide screenings to detect the residue in sampled foods, and the availability of adequate toxicology data, including a NOEL or LOEL based on cholinesterase inhibition. The compounds of interest and the above inclusion parameters are illustrated in Table I.

Although organophosphates and carbamates both act through cholinesterase inhibition, carbamates were excluded from the preliminary analysis completed within this document. The validity of adding the cholinesterase inhibitory effect of the carbamates to that of the organophosphates is questionable because of the rapid reversibility of carbamate ChEI activity. Rather than unnecessarily elevating the level of uncertainty of the preliminary analysis, the carbamates were excluded although these compounds, especially aldicarb, appear to contribute significantly to the overall daily exposure of children to cholinesterase inhibitors.
The Hazard Index

The first risk assessment technique applied to the determination of cumulative ChEI exposure is the EPA Hazard Index method for the analysis of health hazards from chemical mixtures (US EPA, 1986b). The Hazard Index is a numeric indication of proximity to acceptable limits of exposure, or the degree to which acceptable exposure limits are exceeded. As the Hazard Index approaches unity, the probability of cumulative exposure becomes comparable to that of individual chemical exposure as that exposure approaches or exceeds its legally acceptable level. This method assumes dose additivity for compounds that induce the same effect by similar modes of action and requires the consideration of pharmacokinetic and toxicologic characteristics including bioavailability, half-life and environmental persistance.

The Hazard Index (HI) is described by the following formula:

\[ HI = \frac{E_1}{AL_1} + \frac{E_2}{AL_2} + \ldots + \frac{E_n}{AL_n} \]

where \( E = \) level of exposure and \( AL = \) acceptable level (acceptable daily intake level or reference dose).

Toxicity Equivalence Factors

The second method for examining cumulative exposures to ChEI compounds uses toxicity parameters which are derived based on the EPA guidelines for the study of mixtures containing dioxins and dibenzofurans (US EPA, 1987). This method establishes toxicity equivalence factors (TEFs) for estimating the hazard of a complex mixture. The TEF is a ratio comparing toxicity of a compound which has been established as the equivalence and a second compound of interest. Multiplication of exposure values for each compound by its TEF allows exposure to be expressed as an equivalence to the...
baseline compound. In the case of the ChE inhibiting pesticides, this method enables a comparison of the total dietary exposure to multiple ChE inhibitors to the acceptable daily intake level of a single pesticide of this class. The EPA suggests that this is an interim procedure for use in a situation in which the direct biological assessment of the components of a mixture is not possible. The EPA Science Advisory Board, however, cautions that this approach is not based on a thoroughly established scientific foundation and discretion is advised in the application of this risk assessment methodology (US EPA, 1987).

Chlorpyrifos (Dursban®), one of the most widely used organophosphate insecticides, was selected as the equivalence compound. Chlorpyrifos is used extensively on fruits, vegetables, nuts and field crops as well as in pest control in the home. Chlorpyrifos is an organophosphate of intermediate toxicity and is one of the few pesticides for which complete toxicological data is available. The TEFs are determined by comparing the appropriate toxicologic parameter, NOEL or LOEL for cholinesterase inhibition, for each pesticide of concern to that same parameter for chlorpyrifos (Table II). The ratio derived in this manner demonstrates the relative toxicities of each compound as compared to chlorpyrifos. This ratio is used to adjust the laboratory-detected residue values for each ChE inhibitor to chlorpyrifos equivalents. The adjusted residue values are used in combination with dietary intake information to determine the cumulative ChEI exposure for each intake scenario examined. The cumulative exposure levels are compared to the NOEL and reference dose values for chlorpyrifos.

The total pesticide exposure as determined by the TEF method is described by the following formula:
Total Exposure = (TEF₁)(E₁) + (TEF₂)(E₂) + . . . . . . (TEFₙ)(Eₙ)

where TEF = Chlorpyrifos NOEL/Compound NOEL (values in Table II) and E= level of exposure

The NOEL and LOEL values for the various pesticides are established based on differing criteria. NOEL/LOEL may be based on blood, plasma, or brain cholinesterase inhibition, and the studies may be done in humans, rats or dogs. NOEL values were available for chlorpyrifos in all three species for cholinesterase inhibition in blood or plasma. TEF values were determined by comparing the chlorpyrifos NOEL to the most toxicologically similar NOEL available for each compound of interest. Table II outlines the TEF value as derived from the ratio of toxicity information for each compound, the toxicity parameter used and the species, and also, for comparison, gives the ratio of chlorpyrifos LD₅₀ to the LD₅₀ for each compound. The LD₅₀ ratio is not used in the calculations; rather it is included in Table II for comparison to the TEF ratio. The acute toxicity, represented by the LD₅₀, would be expected to reflect, to a limited extent, the ChE inhibitory potential of a given compound.

Exposure Scenarios

In the preliminary analysis, designed to test the ChEI summation methods and to provide an overview for exposure, pesticide exposure was determined across all fruits and vegetables consumed by children ages 1-6 years. In this portion of the analysis 90th percentile consumption was assumed for all foods.

The final dimension of the analysis was designed to provide a more accurate assessment of daily exposure base on three theoretical diets for children ages 1-6 years. Diet A is based on the 5 fruits and vegetables most commonly consumed by children. As previously described these foods are
consumed at significantly higher levels by children than by adults and include orange juice, apples, potatoes, tomatoes, and bananas. Diets B and C based on California Department of Health Services Dietary Guidelines which recommends between 5-9 servings of fruits and vegetables per day including 1 serving of a Vit A rich food (containing at least 2000IU of vitamin A) and at least 1 serving of Vit C rich food (containing at least 30mg of vitamin C) (DHS, 1990) are outlined below:

Diet B: 5 servings including orange juice (6oz), sweet potato (1/2 cup cooked), 1 med apple, 1 med banana and green beans (1/2 cup cooked). For ages 1-3 assume 1/2 serving size

Diet C: 7 servings including apple juice (6oz), carrots (1 small), strawberries (1/2 cooked), tomatoe (paste or raw), potatoe (1 med), pear, and spinach (1/2 cooked)

Exposure was determined by computer analysis of actual residue data available through the FDA and dietary intake information gathered from the 1985-1986 USDA Food Consumption Survey. Computer analysis was done at Yale University by Dr. John Wargo. For all analyses residue values were assumed to be at the mean. The residue level for samples on which no residues were detected were presumed to be zero for the preliminary analysis. In further analyses, non-detect samples will be analyzed using three assumptions; that the residue level on non-detect samples is 0, that residue level on non detect sample is half of the level of quantification (LOQ = .5) of the residue detection method, and that the residue level on non-detect samples is equal to the level of quantification (LOQ = 1). For the preliminary analysis, the consumption level was assumed to be 90th percentile across all foods. In the final analysis exposure will be assessed at both 50th and 90th percentile consumption levels.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Major food uses and total food use</th>
<th>NOEL for ChE inhibition (if NOEL unavailable NOAEL or LOEL given)</th>
<th>Reference dose (Rfd) mg/kg based on ChE inhibition</th>
<th>Residue detection limit (RDL) in food and TMRC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>acephate (Orthene)</td>
<td>beans, caulif, bell peppers, celery, lettuce, mint</td>
<td>NOAEL (no observable adverse effect level): .120 LOEL: .25mg/kg (5ppm) brain,rbc,plasma ChEI - in rats</td>
<td>EPA: .54 WHO: .53</td>
<td>not detected on multires screen?</td>
</tr>
<tr>
<td>aldicarb (Temik)-carbamate-</td>
<td>potatoes, citrus, soybeans, peanuts, pecans...</td>
<td>LOEL: .02mg/kg (1ppm) in dogs 1988</td>
<td>EPA: .0002 WHO: .003</td>
<td>RDL 2ppm TMRC: .112mg/kg</td>
</tr>
<tr>
<td>azinphos-methyl (Guthion)</td>
<td>widely used on tree nuts and fruits, melon, tomato, potato</td>
<td>NOEL: .1250 mg/kg (5ppm) in dogs LOEL: 1.96mg/kg (39.20ppm)</td>
<td>EPA: 0 WHO: 0.0025</td>
<td>RDL 2.0ppm TMRC: .668mg/kg</td>
</tr>
<tr>
<td>carbaryl (Sevin)-carbamate-</td>
<td>over 120 crop uses -citrus, fruits, nuts, veggies, grains</td>
<td>NOEL: 10mg/kg (200 ppm) in rats LOEL: 20mg/kg (400ppm)</td>
<td>EPA: 0 WHO: .01</td>
<td>RDL 2ppm TMRC: 5.48mg/kg</td>
</tr>
<tr>
<td>carbofuran (Furadan)</td>
<td>potatoes, sweet corn, grapes, cucurbits, bananas</td>
<td>NOEL: .5mg/kg (20ppm) dog LOEL: 12.5mg/kg (100ppm) brain,plasma,rbc Chel in rats</td>
<td>EPA: .005 WHO: .01</td>
<td>RDL 4ppm TMRC: .3415mg/kg</td>
</tr>
<tr>
<td>chlorpyrifos (Dursban)</td>
<td>corn, field crops, nuts, fruits, vegetable</td>
<td>NOEL: .03mg/kg plasma ChEI in human. -.1mg/kg/day plasma ChEI rats 1989 -.01mg/kg (1ppm)pls Chel in dogs. (1mg/kg for brain ChEI) LOEL: .10 mg/kg RBC. human -3mg/kg brain Chel rat -.10mg/kg plasma Chel in dog</td>
<td>EPA: 003 WHO: .01</td>
<td>RDL: .1ppm TMRC: .5637mg/kg</td>
</tr>
<tr>
<td>demeton</td>
<td></td>
<td></td>
<td>EPA: based on tox data for disulfoton</td>
<td></td>
</tr>
<tr>
<td>dimethoate</td>
<td>widely used on fruits and vegetables</td>
<td>NOEL: .05mg/kg rbc ChEI in humans (note: LOEL for optic nerve degen. = .04mg/kg in rat)</td>
<td>EPA: .0002 WHO: .01</td>
<td>RDL: .1ppm</td>
</tr>
<tr>
<td>Compounds</td>
<td>Major food uses and total food use</td>
<td>NOEL for ChE inhibition (if NOEL unavailable LOEL given)</td>
<td>Reference dose (Rfd) mg/kg based on ChE inhibition</td>
<td>Residue detection limit (RDL) in food</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------</td>
<td>------------------------------------------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>disulfoton</td>
<td>fruits, vegetables</td>
<td>NOEL: 0.05mg/kg rbc ChEi in rats</td>
<td>EPA: 0.0004 WHO: 0.002</td>
<td></td>
</tr>
<tr>
<td>ethion</td>
<td>fruits, nuts</td>
<td>NOEL: 0.05mg/kg human .06mg/kg dog brain ChEi LOEL: .075mg/kg</td>
<td>EPA: 0.0005 WHO: 0.006</td>
<td>RDL: .1ppm</td>
</tr>
<tr>
<td>fenitrothion (Sumithion)</td>
<td>orchard fruits, vegetables and rice</td>
<td>NOEL: .125mg/kg (5ppm) dog plasma ChEi LOEL: .25mg/kg 10ppm brain 4ppm 1988</td>
<td>EPA: 0.0040 WHO: 0.005</td>
<td>RDL: .1ppm</td>
</tr>
<tr>
<td>malathion</td>
<td>dates, lettuce, citrus avocado, beans</td>
<td>NOEL: 23 mg/kg human ChEi 100ppm (brain) LOEL: .34 mg/kg human 1000ppm (brain) rat 1988. Dogs 62.5mg/kg rbc/plasmal</td>
<td>EPA: .02 WHO: .02</td>
<td>RDL: .1ppm TMRC: .016mg/kg</td>
</tr>
<tr>
<td>methamidaphos (Monitor)</td>
<td>vegetables</td>
<td>LOEL: .05 mg/kg/day (2ppm) in dogs plasma and rbc ChEi</td>
<td>EPA: 0.00005 WHO: 0.0006</td>
<td></td>
</tr>
<tr>
<td>methidathion</td>
<td></td>
<td>NOEL: .3mg/kg for plasma, rbc , and brain ChEi in rats</td>
<td>EPA: 0.001 WHO: 0.005</td>
<td></td>
</tr>
<tr>
<td>methyl parathion</td>
<td></td>
<td>NOEL: .025mg/kg rat RBC LOEL: .25mg/kg (5ppm)</td>
<td>EPA: 0.0025 WHO: .02</td>
<td>RDL: .1ppm</td>
</tr>
<tr>
<td>mevinphos (Phosdrin)</td>
<td>vegetables, lettuce, tomatoes, strawberries</td>
<td>NOEL: .025mg/kg/day plasma/rbc ChEi in dogs LOEL: .075</td>
<td>EPA: 0 WHO: .0015</td>
<td>RDL: .1ppm</td>
</tr>
<tr>
<td>monocrotophos (Azodrin)</td>
<td>use withdrawn ??</td>
<td>NOEL: .0045mg/kg (.09ppm) plasma/rbc brain rat 1988 .16ppm 1987 LOEL: .045 (.9ppm) rat</td>
<td>EPA: 0 WHO: .0006</td>
<td>not on multi-residue screen?</td>
</tr>
<tr>
<td>parathion</td>
<td>widely used</td>
<td>LOEL: .01mg/kg dog for rbc/plasma/brain ChEi 2ppm in rats 1990</td>
<td>EPA: 0 WHO: .005</td>
<td>RDL: .1ppm</td>
</tr>
<tr>
<td>phosalone</td>
<td>widely used on fruits, tree nuts, potatoe</td>
<td>LOEL: 25ppm in rats (pls and rbc) LOEL: .25mg/kg (10ppm) plasma and rbc in dogs</td>
<td>EPA: 0.0025 (provisional) WHO: .006</td>
<td>RDL: 2.0ppm</td>
</tr>
<tr>
<td>Phosmet</td>
<td>fruits and nuts</td>
<td>NOEL: 2.0 mg/kg (40ppm) plasma ChEi in rats</td>
<td>EPA: 0.02 WHO: .02</td>
<td></td>
</tr>
</tbody>
</table>

*TMRC, the theoretical maximum residue concentration, assumes maximum exposure at tolerance levels for all legal food uses.

1. NOEL information from CDFA "Super Summaries" and EPA Pesticide Fact Sheets unless otherwise noted.
3. Major food use information from California Department of Health Services Food Protection Program Pesticide Priority List and from Farm Chemicals Handbook, '87.
4. Residue detection information from Department of Food and Agriculture Chemical Lab Services Branch 1988 - Multiresidue Pesticide Screen.

Table II: Ratio of NOEL (LOEL) animal-specific data for establishment of chlorpyrifos reference toxicity (LD50 and acute toxicity ratio included for comparison to subacute toxicity values)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ratio Chlorpyrifos NOEL (LOEL) / Compound NOEL (LOEL) mg/kg/day</th>
<th>Toxicologic Data Used to Establish Ratio</th>
<th>Acute LD50 mg/kg - Oral Rat</th>
<th>Ratio LD50 Chlorpyrifos / Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>acephate</td>
<td>.1/.12 = .83</td>
<td>LOEL rat</td>
<td>945 (male rat)</td>
<td>.17</td>
</tr>
<tr>
<td>aldicarb</td>
<td>.1/.02 = 5.0</td>
<td>LOEL dog</td>
<td>9</td>
<td>181</td>
</tr>
<tr>
<td>azinphos-methyl</td>
<td>.01/.125 = .08</td>
<td>NOEL dog</td>
<td>4.4</td>
<td>37</td>
</tr>
<tr>
<td>carbofuran</td>
<td>.01/.5 = .02</td>
<td>NOEL dog</td>
<td>11</td>
<td>14.8</td>
</tr>
<tr>
<td>carbaryl</td>
<td>.1/10 = .01</td>
<td>NOEL rat</td>
<td>255</td>
<td>.64</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>demeton</td>
<td>.03/.05 = .6</td>
<td>NOEL human</td>
<td>4</td>
<td>41</td>
</tr>
<tr>
<td>dimethoate</td>
<td>.1/.05 = 2.0</td>
<td>NOEL rat</td>
<td>215</td>
<td>.75</td>
</tr>
<tr>
<td>disulfoton</td>
<td>.1/.05 = 2.0</td>
<td>NOEL rat</td>
<td>12.5 (male rat)</td>
<td>13.1</td>
</tr>
<tr>
<td>ethion</td>
<td>.03/.05 = .6</td>
<td>NOEL human</td>
<td>96 (tech grade)</td>
<td>1.7</td>
</tr>
<tr>
<td>fenitrothion</td>
<td>.01/.125 = .08</td>
<td>NOEL dog</td>
<td>800</td>
<td>.2</td>
</tr>
<tr>
<td>malathion</td>
<td>.03/.23 = .13</td>
<td>NOEL human</td>
<td>1375 (male rat)</td>
<td>.11</td>
</tr>
<tr>
<td>methamidaphos</td>
<td>.1/.05 = 2.0</td>
<td>LOEL dog</td>
<td>21</td>
<td>7.8</td>
</tr>
<tr>
<td>methidathion</td>
<td>.1/3 = .33</td>
<td>NOEL rat</td>
<td>44</td>
<td>3.7</td>
</tr>
<tr>
<td>methyl parathion</td>
<td>.1/.025 = 4.0</td>
<td>NOEL rat</td>
<td>25</td>
<td>6.5</td>
</tr>
<tr>
<td>mevinphos</td>
<td>.01/.025 = .4</td>
<td>NOEL dog</td>
<td>12</td>
<td>13.5</td>
</tr>
<tr>
<td>monocrotophos</td>
<td>.1/.0045 = 22.2</td>
<td>NOEL rat</td>
<td>23</td>
<td>7.08</td>
</tr>
<tr>
<td>parathion</td>
<td>.1/.01 = 10.0</td>
<td>LOEL dog</td>
<td>13</td>
<td>12.5</td>
</tr>
<tr>
<td>phosmet</td>
<td>.1/2.0 = .05</td>
<td>NOEL rat</td>
<td>230</td>
<td>.7</td>
</tr>
<tr>
<td>pirimiphos-methyl</td>
<td>.03/.25 = .12</td>
<td>NOEL human</td>
<td>2000</td>
<td>.08</td>
</tr>
</tbody>
</table>
VI. Results

In the preliminary analysis, the total cholinesterase exposure to 3 year old children was determined using 1988-1989 mean residue data from the FDA and 90th percentile food consumption rates for 3 year olds calculated from the 1986 National Food Consumption Survey (USDA CSF III, 1986). Using the TEF and Hazard Index methodologies described above, cumulative exposure to 5 organophosphate chemicals was determined. In this initial study pesticide exposure over all foods was considered. The 5 organophosphates which were believed to contribute most significantly to overall exposure based on toxicity, residue levels and use were selected for this portion of the study.

The results of the preliminary analysis using the TEF methodology are outlined in Table III and illustrated in Figure I. The cumulative daily exposure to cholinesterase inhibitors was equivalent to .005mg/kg of chlorpyrifos. This rate of exposure exceeds the EPA reference dose for chlorpyrifos which is .003mg/kg/day.

The results of the analysis using the Hazard Index methodology for determining cumulative exposure to cholinesterase inhibiting compounds is outlined in Table IV. The Hazard Index, as described in the methods section above, indicates that cumulative exposure exceeds acceptable limits if the calculated value is greater than unity. The calculated value of the Hazard Index for the 5 ChEI compounds was 3.36.

The risk analyses of the three dietary scenarios outlined in the methods have not yet been completed. These analyses are scheduled for completion by July 1991. Analysis of the dose-response relationship between chlorpyrifos exposure and degree of cholinesterase inhibition is also planned.
Table III: Toxic equivalent factor (TEF) method for identifying the risk of cumulative exposure to 5 cholinesterase inhibiting compounds through pesticide residues on foods for children age 3 yrs.

<table>
<thead>
<tr>
<th>ChE Inhibiting Pesticide</th>
<th>mean residue exposure* (mg/kg/day)</th>
<th>TEF value (from Table II)</th>
<th>TEF modified exposure level (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acephate</td>
<td>.000684</td>
<td>.83</td>
<td>.000567</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>.000417</td>
<td>1.0</td>
<td>.000417</td>
</tr>
<tr>
<td>dimethoate</td>
<td>.0002732</td>
<td>2.0</td>
<td>.000546</td>
</tr>
<tr>
<td>monocrotophos</td>
<td>.0009945</td>
<td>22.0</td>
<td>.002209</td>
</tr>
<tr>
<td>parathion</td>
<td>.00012771</td>
<td>10.0</td>
<td>.001277</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td>.00501</td>
</tr>
</tbody>
</table>

* mean residue exposure was determined from FDA 1988-1989 actual residue data. In this analysis the level of quantification (LOQ) was 0 for samples in which no residue was detected. Exposure estimates were based on 90th percentile consumption rates.

Table IV: Hazard Index (HI) estimates characterizing the risk of exposure to 5 cholinesterase inhibiting compounds through pesticide residues on foods for children ages 3 years.

<table>
<thead>
<tr>
<th>ChE Inhibiting Pesticide</th>
<th>mean residue exposure* (mg/kg/day)</th>
<th>Reference Dose (from Table I) mg/kg/day</th>
<th>Hazard Index (mean exp/RfD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acephate</td>
<td>.000684</td>
<td>.004</td>
<td>.171</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>.000417</td>
<td>.003</td>
<td>.139</td>
</tr>
<tr>
<td>dimethoate</td>
<td>.0002732</td>
<td>.0002</td>
<td>1.366</td>
</tr>
<tr>
<td>monocrotophos</td>
<td>.0009945</td>
<td>.0006 (WHO)</td>
<td>1.658</td>
</tr>
<tr>
<td>parathion</td>
<td>.00012771</td>
<td>.005 (WHO)</td>
<td>.026</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td>3.36</td>
</tr>
</tbody>
</table>

* in this analysis the level of quantification (LOQ) was 0 for samples in which no residue was detected. Exposure estimates were based on 90th percentile consumption rates.
VII. Discussion

As discussed elsewhere in this report, diet is only one of many routes through which children may be exposed to neurotoxic chemicals in their environment. Cholinesterase inhibiting pesticides are widely used for household insect control, for lawn care, and for agricultural pest control. In the case of the Medfly control campaign in California, control of an agricultural pest has led to widespread spraying of malathion far beyond the agriculture sector.
In the regulation of pesticide food uses where dietary exposure is of concern, the cholinesterase inhibiting compounds are regulated individually despite their common toxicologic endpoint. Our initial analysis demonstrates that, when considered as a class, dietary exposure levels to the cholinesterase inhibiting compounds may approach or exceed levels of acceptable daily exposure based on current toxicologic indices. The Natural Resources Defense Council in their 1989 report *Intolerable Risk: Pesticides in our Children's Food*, demonstrated that at tolerance levels, the theoretical maximum exposure level based on legal limits, the allowable exposure levels to children are exceedingly high. Uncertainty in assessing risks using animal data has led to the accepted practice of multiplying NOEL values by an Uncertainty Factor to establish an acceptable margin of safety. In this report, we have calculated that cumulative exposure to cholinesterase inhibitors in the diet, based on actual residue values, not only exceeds acceptable levels by greater than three fold for children ages 1-3 years, but encroaches on the No Observable Effect Level established by animal studies. By comparison exposure levels for adults, while too high when considering legal tolerances limits, approaches, but does not exceed the acceptable daily intake levels established by toxicologists for cholinesterase inhibition when using exposure levels based on actual residue data.

The Federal Food Drug and Cosmetic Act requires that tolerances be established for every pesticide used on foods. These are intended to reflect safe exposure levels and should provide an accurate measure of allowable residue levels. The use of tolerances to determine potential exposure, however, is considered unacceptably conservative by many toxicologists and regulators because tolerances often overestimate actual residue exposure. The uniform objection to the use of these legal limits leads to questions about the
value of tolerances which are so out of line with reality that they do not reflect actual agricultural practice nor do they incorporate health safety considerations.

The regulatory process for cholinesterase inhibiting pesticide use must be reevaluated in order to insure the safety of children from the potential neurotoxic risks posed by these compounds. The criteria by which tolerances are established must be improved for all pesticides. For the cholinesterase inhibitors, the regulatory system must also consider the multiplicity of exposure pathways and the possibility of cumulative toxicity when establishing the allowable levels of pesticide use.
Chapter 4
Analysis and Critique of ChE Risk Assessment

In this chapter, the scientific assumptions chosen for the preceding risk assessment of cholinesterase inhibiting compounds are described. The aim of this chapter is to provide adequate information for determining whether the assumptions and uncertainty in the preceding risk assessment enable a plausible estimate of risk, and to provide information for determining the significance of that risk. To this end, this chapter describes areas in which policy decisions influenced the scientific data, and the reasoning by which these policy choices were made. Alternatives to the scientific assumptions chosen are also discussed. The areas of uncertainty within the assessment are reviewed and the degree of uncertainty assessed. Improved analytic methods and additional data requirements are recommended where needed to reduce the level of uncertainty within the assessment.

I. Hazard Identification

The common mechanism of action of the cholinesterase inhibiting pesticides is well understood. However, the significance of low level cholinesterase inhibition is not clear. Because these insecticides are widely used on multiple agricultural products, environmental, public health, and consumer groups have identified these pesticides as potential hazards to children through dietary exposure. Occupational exposure to organophosphate and carbamate compounds is certainly more significant than dietary exposure. Exposure to pesticides through the manufacturing process, through field work, and through environmental exposures to people living in agricultural areas are relatively high dose and frequent in
comparison to pesticide exposure through the diet. Yet, public concern about pesticides in the diet needs to be addressed in a reasonable manner to alleviate unfounded anxiety, and, if a measurable risk to the general public is demonstrated, to support appropriate regulatory measures.

The acute toxic effects of organophosphates and carbamates are well described. Many of these compounds have very low LD50s, and pesticides of this class are among the most common causes of pesticide poisonings throughout the world. The preceding assessment focused on the potential for cumulative effects from multiple compound exposure in part because environmental exposure to multiple compounds seems a more realistic scenario than does single compound exposure. The assessment was also aimed at addressing a perceived gap in regulatory legislation which, in the past, did not address the problem of multiple exposure to compounds of common toxicological pathway. As will be described in the following discussions of dose-response and exposure, studying multiple compounds complicates and increases the uncertainty of the risk assessment process.

II. Dose Response

The ChEI risk assessment was plagued by many of the controversial aspects of dose response assessment for environmental hazards described earlier in this paper including the extrapolation of animal data generated from high dose exposures to human response and the reliance on NOEL levels and uncertainty factors. In addition, this assessment was complicated by disagreement over toxic endpoint identification and by the selection of a complex mixture of compounds as the hazard of interest.
Biochemistry and toxicity of a hazardous mixture

Although the mechanism of action, of the compounds selected for the previous study is essentially the same for all of the pesticides studied, individual compounds have different biochemical properties which may influence the overall hazardous potential of the mixture. For example, the organophosphates irreversibly phosphorylate the cholinesterase enzymes while the enzyme inhibition of carbamates is rapidly reversible. Although not directly demonstrated, the reversibility of carbamate ChE inhibition implies that chronic neurotoxic effects from these compounds may be of less concern than for the organophosphates. Chronic toxicity, however, has been documented to occur following sustained, repeat exposures to certain carbamates, and further study is needed to determine the contribution of these chemicals to the overall toxicity measured in the risk assessment of cholinesterase inhibitors (Baker, 1990).

The absorption, distribution and metabolism of individual compounds vary based on factors such as molecular size, lipophilic properties, and formulation leading to different toxicity profiles for individual chemicals. In addition, impurities and multiple compound exposure can, in some cases, enhance the toxicity of a given compound. Malathion toxicity has been shown, for example, to be increased both by the impurities that are frequently found in commercial formulations, and by previous or concurrent exposure to other organophosphate compounds (Casseret and Doull, 1985). In order to simplify the risk assessment process the individual chemical properties of the compounds in this study, other than cholinesterase inhibiting potential as determined by the NOEL and the LD50, were not thoroughly explored. Cholinesterase inhibition was assumed to be strictly additive, and peripheral ChE inhibition was assumed to reflect central ChE inhibition uniformly for
all compounds. These assumptions obviously contribute to the uncertainty of the calculations. However, I could not find evidence that these assumptions lead to a strictly conservative bias. Given the current state of knowledge combined exposures appeared equally as likely to increase as to decrease toxicity. Further, the toxicity equivalence factor (TEF) method outlined by the US EPA and used in the ChEI risk assessment for estimating the hazard of a mixture of OP and carbamates was selected and modified in order to minimize the uncertainty within the risk estimate for a mixture of compounds.

Chlorpyrifos was selected as the reference compound for the toxicity equivalence factor method because of its intermediate toxicity, as reflected by the LD50 value of 163 mg/kg, and its moderate cholinesterase inhibiting activity. The dose at which cholinesterase inhibition was measured for each compound was compared to the dose at which inhibition occurred following chlorpyrifos exposure. Toxicity data were matched for common endpoint (ChEI in brain, rbc or plasma) and for species (data obtained for human, dog or rat). In establishing TEFs a variety of toxicologic data were required. Ideally, human data would have been used whenever possible. Faced with the lack of human data, animal data for the same species and same endpoint (NOEL, NOAEL, or LOEL) for all of the compounds would have been compared if those data were available. These data, however, were not available for all the chemicals examined in this study. Therefore, for many compounds different toxicity dimensions were used, though these were matched by species and endpoint with like dimensions for chlorpyrifos. For example, the endpoint and species for malathion was the NOEL in humans which was compared to the human NOEL for chlorpyrifos to arrive at the TEF. By contrast, the TEF for acephate was derived by comparing LOELs in rats with those values for
chlorpyrifos, and the TEF for mevinphos was derived from the ratio of the NOEL in dogs (chlorpyrifos NOEL/ mevinphos NOEL).

Assuming that the NOEL, NOAEL and LOEL values for each of the compounds was derived from experiments with common standards and requirements, the use of different endpoints would be made insignificant through use of equivalence ratios. By carefully matching the experimental data, the problem of differing biochemical properties of compounds in a mixture was partially addressed. However, experimental data is highly variable so the use of different species and different endpoints in this risk assessment increases the imprecision and uncertainty of the results. Furthermore, issues such as synergism and central nervous system exposure were not directly explored.

As suggested by the EPA in the guidelines describing the use of TEF, the scientific validity of this interim assessment procedure has not been thoroughly explored. EPA experts agree that in the absence of data on the chemical properties and biological effects of mixtures the TEF method is useful in assessing chemical mixtures. The level of uncertainty within the assessment, however, is greatly increased by employing this unverified method.

ChEI as a toxic endpoint

The disagreement over the significance of cholinesterase inhibition as a toxic endpoint was partially described within the risk assessment chapter. In 1988 a US EPA risk assessment technical panel concluded that cholinesterase inhibition should be considered a potentially toxic effect. The panel admitted, however, that overt, clinically recognizable responses to ChE inhibition are difficult to predict and difficult to correlate with actual levels of cholinesterase
inhibition. In May 1990, the EPA Science Advisory Board (SAB) "expressed
doubt about the validity of plasma and red blood cell cholinesterase
inhibition as indicators of toxicity" (US EPA, 1990). The SAB report explains
that the relationship between degree of ChEI and toxicity is unclear,
experimental correlations between exposure indices and neurotoxic
manifestations tend to be weak, and consensus about what level of
cholinesterase inhibiton and which types of cholinesterase inhibition (red
blood cell, plasma, or brain) are associated with overt toxicity does not exist.
The SAB committee suggests that ChE inhibition may not be an adverse effect
because AChE has a very high turnover rate and because of a large functional
reserve of ChE throughout the brain.

For the purposes of the preceding risk assessment, I selected ChEI as a
measure of toxicity despite the controversy about this endpoint. The SAB
recommends defining toxicity on the basis of function or morphological
indices and then determining what degree of CheI predicts the neurotoxic
response. Pesticides have not, however, been screened for neurotoxicity other
than through the measurement of cholinesterase inhibition and, in the case
of the organophosphates, through testing for the overt neurological changes
associated with OPIDN. The use of more subtle measures of neurotoxicity
including histopathological and morphological changes, and neurobehavioral
and cognitive testing have not yet been systematically undertaken. As with
the lead experience described in the first chapter of this paper, researchers run
the risk of overlooking subtle but significant toxicity by dismissing the
importance of changes measured following low dose exposures.

Increasingly, the neurobehavioral and histopathological evidence from
studies of cholinesterase inhibiting pesticides has indicated toxicity at low
level exposure (Katz and Marquis, 1989, Boyd et al, 1990). At this time,
cholinesterase inhibition is the only endpoint for measuring low dose responses for which data are available. Studies aimed at elucidating the significance of ChEI will need to be undertaken in order to clarify the issue. In the meantime, the ChEI risk assessment presents some recent data supporting the idea that ChEI is a toxic endpoint and explores the extent of cholinesterase inhibition from dietary exposure to insecticides. The use of this controversial endpoint, however, increases the uncertainty of the assessment and contributes to the conservative, health-protective bias of the study.

*Animal data vs human data*

Unlike the lead risk assessment in which basic animal research findings have been strongly supported by quantitative epidemiological evidence of lead toxicity, animal data are the major source of toxicity information for the cholinesterase inhibition risk assessment. The validity of using data from animal experimentation to describe the potential toxicity to humans from low dose exposure to cholinesterase inhibiting pesticides, as for most environmental hazards, is highly controversial. Aspects of this controversy were discussed in chapter one (pg 11). The reliance on animal data and the insufficiency of quantitative human data is another aspect of the ChEI risk assessment which increases the uncertainty level of the study.

Human data and epidemiological studies of ChEI compounds are predominately qualitative in nature. As described in the earlier discussion of lead, human exposure to lead is easily calculated since the body has no endogenous lead stores. Changes in blood cholinesterase levels, however, are difficult to assess because levels vary between individuals (studies have found approximately a 13 to 15% variability in erythrocyte cholinesterase...
activity), and may vary within an individual for reasons other than exposure to pesticides. Though variability within an individual is well documented, the physiological basis for this variability is not understood (Hayes and Laws, 1990).

Pre-exposure, baseline cholinesterase levels are needed in order to accurately determine the degree of cholinesterase inhibition following exposure. Human studies in which volunteers were fed capsules containing incremental doses of various cholinesterase inhibitors were conducted by several investigators during the late 1950's and 1960's (Rider et al). These studies, however, only considered overt signs of inhibition as a response to the cholinesterase inhibitors. In the case of methyl parathion, for example, signs such as miosis, nausea and vomiting, and sweating did not occur until volunteers ingested about 22mg/kg of the chemical. Cholinesterase inhibition averaged about 20-25% at that dose. Doses in this range far exceed potential exposure levels from the diet. The possibility of more subtle changes associated with the cholinesterase inhibition at lower dose, however, were not considered in these early studies and still have not been adequately explored.

The application of neurobehavioral and cognitive testing of ChEI exposed individuals is still in its infancy. Researchers do not agree on which testing is most accurate or most reliable. EPA guidelines for neurotoxicity testing have been outlined but not yet implemented. A considerable amount of work aimed at establishing baselines, controls and validity of methods for neurobehavioral and cognitive testing will be needed to increase the usefulness of these methods in the study of neurotoxic compounds.
NOEL and Uncertainty Factors vs Dose-response curve

Traditionally, the acceptable daily exposure level for non-carcinogens has been defined based on the No Observable Effect Level (NOEL) or No Observable Adverse Effect Level (NOAEL) and Uncertainty Factor (UF) method described in Chapter 1. This approach assumes that a threshold level, represented by the NOEL, exists below which most individuals in the exposed population are free of risk. The threshold level is multiplied by a UF (also called a safety factor) to account for animal to human extrapolation, human variation, and data uncertainty. Despite the incertitude of the NOEL/UF approach, the most recent EPA guidelines for non-carcinogens recommends the use of this risk assessment method (US EPA, 1988 -draft).

The NOEL/UF approach described in the first chapter has increasingly come under attack. Critics charge that by selecting a single, quantal, data point to describe the toxicity of a compound, the method ignores sample size, does not measure dose-response and does not take into account statistical power, dose spacing, variability or trends in the data (Kimmel, 1990, US EPA SAB/SAP, 1990). The NOEL statistically depends heavily on the number of animals used in a given study. Smaller experiments tend to provide higher NOEL values and may, therefore, be rewarded for poor experimentation by leading to higher reference dose levels for man. In addition, the dose response curve which could most accurately relate exposure to incidence and severity of neurotoxicity and which may have steep or shallow slopes throughout the range of dose levels is not considered (Krewski and Brown, 1984). The size of the safety factor should reflect the relationship between the experimental response and the risk associated with chemical exposure. Uncertainty factors, however, are often perceived as leading to very conservative reference dose values. In the case of cholinesterase inhibitors,
the US EPA Science Advisory Board suggests that large UF s seem generally unwarranted because of inherent margins of safety between ChEI and detectable neurotoxicity (EPA-SAB-EC-90-014, 1990).

The risk assessment for cholinesterase inhibiting compounds employs a variation of the NOEL/UF approach. NOEL values were gathered from EPA and California Department of Food and Agriculture (CDFA) summary reports of subchronic and chronic cholinesterase inhibition for each pesticide. The CDFA reviews the toxicology data and judges whether the study designs are sufficient for an accurate determination of NOEL. The use of summary data in the ChEI risk assessment did not allow for the development of dose-response curves for individual compounds, and the uncertainty issues arising from the use of NOELs remain because of these data limitations. Toxicologists suggest that the inconsistency of the research leads to approximately a 10 fold variability in the NOEL values. In other words, the actual no observable effects level may be 10 time higher or 10 times lower than the experimentally derived NOELs used within the risk assessment (Dourson and Stara, 1983).

In determining toxicity equivalence factors NOEL values from animal and human data were used directly. The use of NOELs contrasts the approach taken by the National Resources Defense Council (NRDC) in its study of ChE inhibiting pesticides. The NRDC used the reference dose values (Rfd) which are the NOELs adjusted with uncertainty factors. The use of Rfds was criticized for leading to an overly conservative risk estimate. By using NOELs directly assumptions about the uncertainty and variability of the data were removed from the body of the assessment. Exposure data were compared to NOEL levels, and the interpretation of the risk associated with these raw data were left to risk managers and to the public.
III. Exposure Assessment

The potential pesticide exposure levels from dietary intake were estimated using a sophisticated computer analysis which was developed at Yale University for use by the National Academy of Sciences committee studying pesticide in children's food (John Wargo, 1991). Pesticide exposure was estimated using a complex matrix in which the most current food residue data available from the FDA were compared with dietary intake estimates derived from the USDA National Food Consumption Surveys of 1985-1986 (USDA, 1987). Statistical modeling, described within the risk assessment document, was developed to estimate exposure levels for various intake scenarios. The ChEI assessment represents one of the first studies to use actual residue data rather than tolerance levels to estimate dietary exposure. In addition, the dietary intake data provided the most complete picture of food consumption patterns for different age groups, especially children, available.

Even though the exposure analysis provides the most accurate and detailed information about pesticide exposure in the diet available to date, the limitations of this portion of the assessment must still be addressed. These limitations include the variability of residue determinations and the lack of residue information for processed foods, the lack of accurate pesticide use data, incomplete food consumption information, and the complexity of establishing an inclusive statistical model for exposure.

To begin, the risk assessment considered exposure to ChEI pesticides in a limited number of foods. Specifically, residue levels in fresh fruits and vegetables were explored. The potential for exposure through other sources such as ground water, meat, poultry and dairy products, and through non-
dietary sources such as household and lawn insect control were not examined. The cholinesterase inhibitors are widely used and exposure through these other sources likely contributes significantly to total exposure.

One of the commonest responses of regulators to public concerns about pesticide exposures in food is increased monitoring of the food supply for the presence of pesticide residues. Theoretically, increased data about pesticides in foods would enable risk assessors to better determine the actual risk to the public from pesticides in food. The variability of the residue data, however, fosters uncertainty within the risk assessment. The data analysis for the assessment was slowed considerably because of the difficulty in determining which of the available residue data most accurately reflected true exposure levels. Whether actual residue data are more reflective of exposure than are residue estimates put forth by experts using modeling techniques remains unclear (Wargo, 1991).

The EPA, FDA, CDFA and industry all monitor food pesticide residues. The incentives and goals of residue monitoring vary between agencies as do sampling techniques, residue detection capabilities and methods of chemical analysis. A detailed description of the process of residue monitoring and its limitations are described in the OTA publication Pesticide Residues in Food: Technologies for Detection (OTA, 1988) and in The Invisible Diet: Gaps in California's Pesticide Residue Detection Program (Price, 1988). The results of residue analyses not only vary widely between agencies but also within the different labs used by a given agency. Residue values reported by the different agencies are so disparate and inconsistent that use of data from different sources for exposure analysis was nearly impossible. The risk analysis, therefore, relied primarily on FDA data which were the most extensive and the most complete.
Accurate pesticide use estimates for different crops and for different regions of the country are not available. This information would assist agencies in determining the type of residue testing needed and, in addition, information about the type and the quantity of chemicals used on various crops would be an important supplement to the residue data. For example, if risk assessors knew that compound X was used on 70 percent of all green beans grown in California, and residues were discovered on 5 percent of all green beans sampled in market basket surveys, estimates about dietary exposure could be made more efficiently and accurately than if the only data available showed that 5 percent of a limited sample of beans had pesticide residue. Of equal importance in the study of exposures to multiple cholinesterase inhibiting pesticides is the question of whether the use of one ChEI compound precludes the use of a second ChEI pesticide on the same crop or if several of these compounds may be found on a given food item. California is the only state which requires detailed use information, and this reporting requirement was only recently implemented. Currently data are only available for a 6 month period. These limited data do not provide enough information to enable accurate exposure estimates.

Another problem of pesticide residue estimation is that researchers and regulators are unclear about what happens to pesticide residues during commercial food processing, or through the washing and preparation of fruits and vegetables in the home. Studies conducted by Kubacki and Lipowska found that the highest reduction in organophosphate residues on apples followed scalding (40% reduction), and straining (20% reduction). Washing, steaming and peeling apples reduced residue levels to a lesser degree (Hayes, 1991b). The degree to which market basket estimates of residue on foods reflects the actual dietary intake of those compounds is essentially unknown.
The Food and Drug Branch of the California Department of Health Services has been charged with monitoring pesticide residues in foods processed in California. Budget constraints, however, have slowed the development of effective monitoring programs. The project is still in its beginning stages and little data are available.

Inexact information in the food consumption data contributed to uncertainty in the exposure analysis. In the 1985-86 CSFII food consumption survey, women were questioned about their daily dietary intake and that of their children ages 1-6 years on six occasions over a one year period through 24 hour dietary recall interviews. Studies of dietary intake patterns suggest that people tend to eat a lot of a given food for a short period of time (acutely) though they may not eat a lot of this particular food when averaged over a long period of time (chronically). The repeated 24 hour recall format of the USDA study did not allow consumption patterns to be fully elucidated especially since less than 50% of the respondents provided dietary information for all of the six days of the study. This type of intake information probably led to overestimation of the consumption of certain foods and underestimation of others.

Finally, the question of exposure to multiple compounds led to statistically difficulties in estimating residue exposures and in estimating the rate of consumption for different fruits and vegetables eaten in combination. For the preliminary analysis completed for this paper, mean residue values were used. The number of samples on which no residues were detected was very high. In the preliminary analysis these non-detects were presumed to have zero residue on them. It is likely, however, that residues at levels below the level of detection of the residue monitoring method may be found on
some samples. An assumption of zero residue on non-detects leads to an
underestimation of the total residue exposure.

90th percentile consumption levels were used in the first analysis, and
cumulative exposure was calculated by adding residue levels on all foods
rather than for a selected diet. While these lead to an overestimation of
exposure in the preliminary analysis, the assumption of 90th percentile
consumption levels for a limited number of foods, as in the diets outlined in
chapter 3, would not be overly conservative and would demonstrate the
potential residue exposure levels for a significant segment of the population.

IV. Risk Characterization

The role of risk characterization is to summarize the findings of the
dose-response and exposure portions of the assessment, and to review the
areas of uncertainty. This final summary of the data enables the
determination of whether or not the level of uncertainty within the
assessment is acceptable. In some instances the uncertainty of the assessment
requires that quantitative findings be qualified or, in some cases, the
uncertainty is so great that the risk assessment process can only yield a
qualitative rather than a quantitative description of the risk.

The quantitative risk assessment of cholinesterase inhibiting pesticides
demonstrates that food residue levels of cumulative ChEI's leads to possible
dietary exposure levels in children which lie well above the reference dose
level or acceptable daily intake level for the equivalence compound
chlorpyrifos. It is important to note that the reference dose for chlorpyrifos is
derived using a relative small Uncertainty Factor of 10. The hazard index is
derived using reference dose values and, therefore, incorporates into the
toxicity equation the UF's of all compounds in the mixture of interest. The
calculated Hazard Index value suggests that the dietary exposure of children is three times greater than acceptable level.

The preliminary findings of the quantitative risk assessment for cholinesterase inhibiting pesticides indicate that the cumulative exposure to ChEI residues in food may lead to dietary exposure levels in children which lie within the range of the NOEL for the equivalence compound chlorpyrifos. While the numbers generated in the risk assessment suggest that children's exposure to cholinesterase inhibiting pesticides is considerably higher than the level that is conventionally considered safe by the regulatory community, the significance of these numbers must be considered in light of the factors which contribute to the uncertainty described in this chapter. Further analysis of exposure using the theoretical diets outlined in chapter 3 must still be undertaken to obtain a more accurate picture of daily exposure levels.
Chapter 5
Conclusions

Two important standards for quantitative risk assessment are discussed in this paper. One is the use of the best available scientific information to determine the risk to human beings from exposure to environmental health hazards. The second is that the political, economic and technical considerations be separated from the scientific aspects of the study. In this paper, I have attempted to apply these criteria to the assessment of the risk to children from pesticides residues in foods. Gaps in data and in the current understanding of the neurotoxic potential of these pesticides, however, required that I make numerous assumptions and decisions about the data that were not strictly scientific. Rather, these were essentially policy choices. The decisions made about the significance of cholinesterase inhibition as a toxic endpoint, the degree to which residue data reflected dietary exposure, the usefulness of NOEL information, and the validity of the method for studying a hazardous mixture, for example, are assumptions based on the limited information available and do not represent a consensus by the scientific community. These assumptions, however, affected the outcome of the risk analysis. Another researcher selecting other alternatives would likely obtain very different results.

In the case of cholinesterase inhibiting pesticides, the conclusions that could be drawn from the risk assessment were limited by the extent to which the many assumptions increased the uncertainty of the results. Better toxicity data, improved residue monitoring, and more information about the correlation between cholinesterase inhibition and neurotoxic effect are
needed to increase the reliability of the findings. Several regulatory alternatives do, however, arise from the preliminary results. Consideration of the common toxicologic endpoint of this group of chemicals should lead to reform of the registration requirements and allowable tolerance levels of individual pesticides. Manufacturers should be required to provide more thorough toxicology data for each compound, and priority should be given to further research and to improved neuropsychologic and behavioral testing to identify subclinical indicators of neurotoxicity. Finally, since exposure of subpopulations, such as infants and children, exceeds that of the general population, the potential risks to these groups should receive additional attention.
Glossary

The following is a list of the abbreviations used throughout this paper:

ACh = acetylcholine
ADI = acceptable daily intake
ANS = autonomic nervous system
CDFA = California Department of Food and Agriculture
CDHS = California Department of Health Services
ChE = cholinesterase
ChEI = cholinesterase inhibitor
CNS = central nervous system
EDF = Environmental Defense Fund
FDA = Food and Drug Administration
FFDCA = Federal Food, Drug, and Cosmetic Act
FIFRA = Federal Insecticide, Fungicide and Rodenticide Act
HI = hazard index
LD50 = lethal dose to 50 percent of study population
LOAEL = lowest observable adverse effect level
LOEL = lowest observable effect level
LOQ = level of quantification
NOAEL = no observable adverse effect level
NOEL = no observable effect level
NRC = National Research Council
NRDC = Natural Resources Defense Council
NTE = neurotoxic esterase
OPIDN = Organophosphosphate induced delayed neuropathy
RBC = red blood cell
RDL = residue detection limit
Rfd = reference dose
TEF = toxicity equivalence factor
TMRC = theoretical maximum residue concentration
UF = uncertainty factor
USEPA = United States Environmental Protection Agency
USOMB = United States Office of Management and Budget
WHO = World Health Organization
Bibliography


37. Sette WF. Adoption of New Guidelines and Data Requirements for more extensive neurotoxicity testing under FIFRA. Toxicology and Industrial Health. 5(2): 181-94 1989

39. Sielken R.L. Assessing Age Dependent Exposures including exposures which decrease from childhood to adulthood: Substantial conservatism in EPA's cancer risk assessment methodology and a fundamental mathematical error in NRDC's methodology. 1989


45. US EPA Fact book 1988 (b)

46. US EPA 40 Code of Federal Regulation 180.3

47. US EPA 50 Code of Federal Regulation 188


51. US EPA. General Quantitative Risk Assessment Guidelines for Noncancer Health Effects - DRAFT. 1988(b)


