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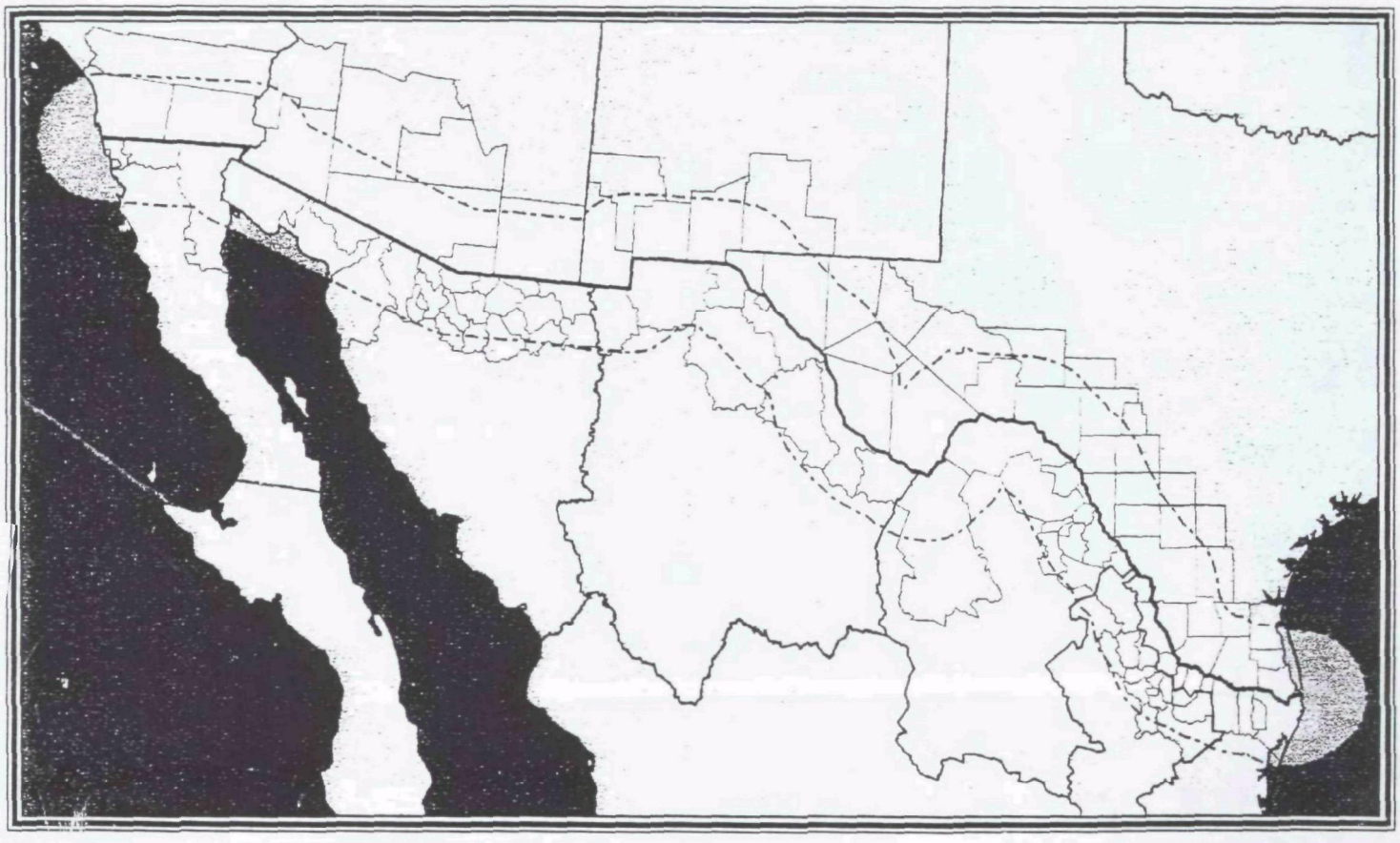

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Proceedings of a Workshop held in El Paso, Texas in December 1997

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PREFACE

The mission of the U.S. Environmental Protection Agency (EPA) is to protect human health and safeguard the natural environment. An area of special concern is the U.S.-Mexican border that stretches nearly 2,000 miles from the Pacific Ocean to the Gulf of Mexico. Mexico and the U.S. have been working together since the 1983 La Paz Agreement to solve the environmental and health problems in the border region. An Environmental Health Workgroup (EHW) was established in 1996 under the auspices of the Border XXI program specifically to address these concerns. The EHW is co-chaired by the EPA Office of Research and Development, the U.S. Department of Health and Human Services, and the Mexican Secretariat of Health.

Much of the border region is devoted to the cultivation of fruits and vegetables. As a consequence, the potential health risks of chronic, low-level pesticide exposure in young children were identified as a high binational priority concern of the EHW. Of special interest are the risks to very young children from persistent, multipathway multipesticide exposures. Recognizing the challenges in studying these risks in young children, a number of projects have been initiated to provide basic tools, methodologies and data to guide the design and conduct of future epidemiologic studies by the EHW and other interested organizations.

To assist in meeting this priority objective, the National Health and Environmental Effects Laboratory of the EPA sponsored a workshop on December 4-6, 1997, in El Paso, Texas, that focused on the identification of health effects associated with exposure to pesticides and how those effects might be measured in very young children. Thirty participants were divided into five working groups to review research methods in neurotoxicity, developmental toxicity, carcinogenicity, immunological effects and respiratory effects. Participants included leading scientists in these five fields, local health care providers, state public health officials and researchers from EPA and the Centers for Disease Control and Prevention. This report contains reviews of state-of-the-art methods currently available in each of these areas for field studies of young children, health effects associated with pesticide exposure in children, and recommendations from each of these working groups for research on the health effects of pesticide exposure in young children. Recommendations will provide guidelines for future EHW projects addressing the assessment of exposure to pesticides and potential health risks to children living in the border region.

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and
Co-Chair, Environmental Health Workgroup

EXECUTIVE SUMMARY

Background

Pesticides are a broad group of chemicals used to kill insects, fungus and undesirable plant species; they are by design, biologically active compounds. Millions of pounds are applied in agricultural, industrial, institutional, commercial, and residential settings within the United States (US) each year. Pesticide exposure is ubiquitous via contamination of food, soil, air, and water, yet the health effects of chronic, low-dose exposure are unknown. There is growing concern that current tolerance levels may not be sufficient to protect the health of children.

Children are at greater risk than adults for increased exposure to pesticides. Hand-to-mouth activity increases the risk of pesticide exposure by the oral route. Children have a proportionally greater surface area and tend to have more dermal contact with soil and indoor floor surfaces than adults. Children consume more food and beverage as a portion of their body weight than adults. Foods such as fruits and fruit juices may form a large part of a young child's diet and represent a major source of pesticide exposure. Children may be particularly vulnerable to the effects of pesticides due to rapidly maturing organ and nervous systems. Children's metabolism of pesticides is not well understood. Differences in biotransformation and elimination may result in children experiencing a greater toxic effect than adults. Children along the US-Mexican border may be at increased risk for pesticide exposure due to the prevalence of year-round agriculture in this region. Research efforts to determine if there are measurable health effects associated with chronic low dose exposure to pesticides will initially be focused in the border region as part of the Border XXI Program.

During December 7-9 1997, the Environmental Health Workgroup sponsored a workshop on the assessment of health effects of pesticide exposure in young children to discuss the current state of the science, and to identify priorities for future research.

Workshop Structure and Goals

Experts from a variety of fields participated in the workshop. The three-day meeting was structured to include discussion of pesticide exposure measurement, potential health effects in the pediatric population, and current research efforts in the US-Mexican border area. Small workgroups were organized to address five health endpoint domains: cancer, neurobehavioral, respiratory, immunologic, and developmental effects. After day one, the respiratory and immunology groups combined to discuss areas of subject overlap. Workgroup members were selected to represent multiple areas of expertise: epidemiology, exposure assessment, clinical medicine, and those with experience conducting research along the US-Mexican Border.

Day One

The first day focused on issues related to the study of children. Dr. Bornschein gave the keynote address: 'Issues in Pediatric Epidemiology' based on his work with lead-exposed children. Dr. David Bellinger discussed potential methods for the neurobehavioral assessment of children at different stages of development. Dr. Anthony Horner's presentation focused on published studies of immunotoxic effects of pesticides in human and animal models. Dr. Antolin Llorente reviewed the neurotoxicant effects of pesticides in adults and the need for well-designed studies of children exposed during periods of rapid neurologic development. Dr. Maria Martinez discussed techniques available to measure pulmonary function in children of various ages. Dr. Jonathan Buckley presented information about the study of children diagnosed with cancer and highlighted the difficulties associated with the evaluation of pesticide exposure assessment in these children.

Workgroup Reports: Day One - Health Effects

The focus of the workgroups on Day One was to identify lists of health endpoints that were relevant and measurable in young children and infants. There is very little documentation of health effects related to low-dose pesticide exposure in young children, so workgroup members extrapolated from their knowledge of occupational exposure studies, acute toxicity reports, and animal studies.

Cancer: In the United States, cancer is the second leading cause of death for children between the ages of one and 14 years. Although overall cancer rates have generally been declining, the rate of childhood cancer has increased in North America. Specific cancer diagnoses such as: acute lymphoid leukemia, tumors of the central nervous system and bone would be of particular interest in the study of environmental exposures.

Developmental: Exposures that may occur during the prenatal period or infancy may have the greatest impact on the developing child. The following developmental health endpoints are listed in priority order as defined by the workgroup: birth defects, stillbirths, and spontaneous abortion; mental, motor, adaptive development; growth; language; birth weight related to gestational age; social development; infant mortality; puberty, age at menarche and development of secondary sex characteristics.

Neurobehavioral: The workgroup identified assessment tools appropriate for evaluation of children in various age groups. The Bayley Scales of Infant Development, Wechsler Preschool and Primary Scales of Intelligence-Revised, the Wide Range Assessment of Visual Motor Abilities, Wide Range Assessment of Memory and Learning, Peabody Developmental Motor Scales, Visual acuity, Wechsler Intelligence Scales for Children, 3rd ed., visual contrast sensitivity, and Neurobehavioral Evaluation System all received priority rankings.

Immunology: The workgroup identified immunologically-associated health endpoints of interest. These included: asthma (reactive airway disease); allergy; primary immunodeficiency; contact dermatitis; lupus erythromatosus; inflammatory bowel disease; infectious diseases; and adverse reproductive outcomes.

Respiratory: The respiratory workgroup discussed both the utility of validated disease endpoints and self-reported symptomatology in assessing overall pulmonary health. · The workgroup discussed four respiratory diseases: upper respiratory infection, acute bronchitis, asthma, and interstitial lung disease.

Day Two - Research issues

The keynote address, 'Health Effects of Pesticides' was delivered by Dr. Donald Ecobichon. He discussed the health effects of acute pesticide poisonings in adult agricultural workers. He emphasized that the research challenge will be to develop methods to measure subtle psychologic, behavioral, and neurologic deficits in children exposed to lower doses of toxic mixtures of the 'inert' and active ingredients in pesticide formulations. Dr. Stephanie Padilla reported that her laboratory is investigating the effects of sub-lethal doses of chlorpyrifos on young rats. Young, postnatal rats are more sensitive to organophosphate pesticides than adults. Differences in levels of detoxification enzymes may account for some of this observed effect.

Dr. Jim Quackenboss discussed the design of a Children's Pesticide Exposure Survey. He discussed that one of the major difficulties of research in the field of health effects of pesticides on children has been the difficulty in selecting 'high' exposure individuals from the general population. Mr. Gary Robertson reported the results of a survey of pesticide use near the US-Mexico border. Methods used to evaluate pesticide usage were different in each state; some states collect actual usage data, in others, usage was estimated from agricultural crop records and acreage under cultivation. Dr. Mary Kay O'Rourke

discussed her current exposure assessment projects. These include: the National Human Exposure Assessment Survey, a survey of residents along the US-Mexican border in Arizona, a Children's Pesticide Survey in Yuma, Arizona, and multiple projects requested by communities along the border, studying health effects such as asthma, diabetes mellitus and lupus erythromatosus. Dr. Jim VanDerslice raised issues related to studying populations along the U.S.-Mexican Border. He stressed that although the Border Region is referred to as a single entity, it is actually a very diverse collection of communities along a 2000 mile long corridor. Dr. Rob McConnell discussed cultural considerations in the conduct of epidemiologic studies along the U.S.-Mexican Border. Dr. James Ellis highlighted potential resources for pediatric research in the Border area. He emphasized the need to build trust between researchers and community members before research is initiated.

Workgroup Reports: Day Two- Development of Strawman Study Proposals

The second day of the workshop was designed to integrate selected health endpoints identified during the Day One workgroup sessions into a collection of potential study designs for implementation along the border region.

Cancer: The workgroup outlined and discussed several possible types of studies including:
1) Use of existing cancer data bases.
2) An ecological study could compare pesticide usage in border and non-border regions and determine if there is a difference in cancer patterns in these areas.
3) A case-control study: cancer cases could be obtained from clinics and hospitals.
4) A prospective cohort study: exposure would be measured with the use of a biomarker and incident cases of cancer recorded.
5) A case control design could identify children with leukemia and determine if they have a higher level of V(D)J recombinase mediated chromosomal rearrangements.

Developmental: Proposed studies were classified as analytic, descriptive or capacity building.

Analytic Studies
1) A prospective prenatal cohort. The study hypothesis would be: pesticide exposure is related to delayed and/or altered development and long term developmental problems.
2) A poisoned children case study. The study hypothesis would be: there are persistent neurobehavioral and neurodevelopmental sequelae of acute pesticide exposure.
3)A prospective closed-cohart study of symptomatic children. The study hypothesis would be: there are no developmental differences between symptomatic children with detectable urinary metabolites of organophosphate pesticides and symptomatic children without detectable urinary metabolites.

Descriptive Studies
1) A cross-sectional study of any correlation between levels of pesticides, anticholinesterase, and related enzymes in maternal and infant biologic samples.
2) A descriptive cross-sectional study using a Geographic Information System approach of infant health status. The main hypothesis is that infant mortality and birth weight are not different in areas with high agricultural pesticide use compared to geographic areas with lower agricultural pesticide use.

Capacity Building
1) Pesticide Dose: a summary of pesticide dose information in young children is needed. 2)Adaptation of neurodevelopmental tests to populations within the border region.

Neurobehavioral: The Neurobehavioral workgroup discussed three study designs--(1) a retrospective cohort design, (2) a cross-sectional study, and (3) a longitudinal cohort study. The basic hypothesis addressed by these studies is that exposure to pesticides produces neurotoxic effects in children.

1) Retrospective Acute, High-exposure Cohort Study. A retrospective cohort study of a group of children with clearly defined, high-level exposure will be selected for an initial study to determine whether or not pesticide exposure produces neurotoxic effects in young children.
2) Cross-sectional Chronic, Low-exposure Study. An exposure questionnaire will be administered to parents of children aged 1.5-2.5 years to select three groups--high, middle and low exposure deciles (10%). The Bayley Test is recommended for neurobehavioral assessment of children. Exposure measures should include house dust and urine samples for biological measures.
3) Longitudinal Cohort Study. 100 children living in a high-risk area could be selected. The Bayley Test would be administered at 3-month intervals for one-two years. Urine samples should be obtained at each testing for measurement of OP levels, metabolites and a-esterases.

Immunology / Respiratory: The group agreed on some study designs to evaluate the association between pesticide exposure and immunological and pulmonary health effects.
1) Cross-sectional study, questionnaire derived exposure combined with self-reported health endpoints. Exposure assessment supplemented by GIS and some environmental sampling. Hypothesis: the prevalence of asthma and other diseases will be higher in individuals with increased pesticide exposure.
2) A cross-sectional study based on exposure status. The study hypothesis: pesticide exposure increases the incidence of and/ or exacerbates pre-existing asthma.
3) A methacholine challenge test to objectively assess airway reactivity would be administered to a group of healthy children. A case - control study would follow with case status assigned to those with airway hyper reactivity. The study hypothesis is that pesticide exposure contributes to airway hyper reactivity.
4) Cross sectional study of children < 1 years of age as a pilot for a longitudinal study of a birth cohort. The study hypothesis: pesticide exposure affects the development of the immune system in infants resulting in altered antibody response to vaccine administration and increased incidence of infectious disease.

Day 3- Group discussion

The following seven research priorities were assembled based on reports from workgroups and individual participants' comments during the day's discussion.
1) The development of efficient methods for screening children for exposure status
2) Questionnaire development and validation in Border communities
3) Targeted environmental sampling to increase efficiency
4) Validation of biochemical measures of exposure
5) The need to establish normal ranges for health endpoints
6) The development of sophisticated modeling techniques to more accurately predict the health effects of exposure to multiple pesticides by multiple exposure routes
7) Studies must have adequate power to detect subtle pesticide-associated health effects

Workshop members stressed that public health officials and health care providers from the border community are requesting better exposure measurements. They would like to know the extent of environmental pesticide contamination, would like to know the potential health effects associated with pesticide exposure, and if current levels of pesticide exposure are causing health problems.

ACKNOWLEDGMENTS

Many people have contributed to the creation of this document. We would particularly like to express our appreciation to Marcia Gardner (SRA Technologies) who assembled the initial draft and Jennifer Hawks, Vickie Worrell, Shanika and Tasha Rogers, Shalaunda Johns and Sully Jaffer who assisted in word processing.

INTRODUCTION

Over the last 30 years, the border region between the U.S. and Mexico has experienced a dramatic increase in population and industrialization. This growth has exceeded the existing infrastructure capabilities of the region, leading to inadequate sewage treatment, insufficient drinking water supplies, and dramatic impact on habitat and biodiversity along the border. In order to address environmental problems associated with growth, the U.S. and Mexican governments signed the Agreement for the Protection and Improvement of the Environment in the Border Area (La Paz Agreement) in 1983. The La Paz Agreement defined the border region as the area lying 100 kilometres north and south of the U.S.-Mexican border. The Integrated Environmental Plan for the Mexican-U.S. Border Area (IBEP) released in 1992 extended the scope of the La Paz Agreement to include environmental health and natural resource issues. Passage of the North American Free Trade Agreement (NAFTA) in 1993 extended U.S.-Mexican activities along the border, including creation of the Commission for Environmental Cooperation. The Border XXI Program, operating under these mandates, is a comprehensive program designed to achieve a clean environment, protect public health and natural resources, and encourage sustainable development.

Much of the border region is devoted to agriculture and aerial pesticide spraying is widespread. A major concern is the risk from repeated, often year-round pesticide exposure. The problem is complicated by exposure to multiple pesticides from different sources (residential as well as agricultural) and multiple pathways (food, water, air), the cumulative impact of which is unknown. Of particular concern are young children (from birth to age five) whose developmental vulnerability puts them uniquely at risk. The Environmental Health Workgroup, part of the Border XXI Program, has identified this concern as a high priority research issue and initiated an extensive project--*Pesticide Exposure and Health Effects in Young Children along the U.S.-Mexican Border*--to assess the nature and extent of this problem.

Phase I of this project was a survey of pesticide usage along the border. Planning of health surveys and a subsequent epidemiological health effects study are also in progress and will be refined on the basis of results obtained in earlier phases of the project. There is a paucity of data on the health effects of pesticide exposure in humans in general and in young children in particular. Most available health effects data are from case reports of accidental or intentional acute poisonings. Little evidence is available on chronic, low-level pesticide exposure in populations living in agricultural areas. Health effect studies of young children are further complicated by language and behavioral limitations. The advice of experts from a variety of disciplines including psychometrics, developmental psychology, immunology, pulmonology and oncology concerning the health endpoints likely to be most sensitive to pesticide exposure in humans and which tests can realistically be administered to young children (five years and younger) will be crucial for planning purposes.

An important objective of this workshop was to review and evaluate appropriate endpoints for use in health effect studies of young children exposed to pesticides. Other objectives included: (1) examination of the existing infrastructure to support such studies in the border region; (2) identification of possible populations for study; and (3) recommendations for possible study designs. Participants in the workshop were assigned to workgroups corresponding to the disciplines considered relevant for pesticide research in children--i.e., psychometrics, developmental psychology, immunology, pulmonology and cancer. The proceedings include papers by invited speakers on health endpoints appropriate for use in studies of young children, issues specific to pediatric research in the border region, and recommendations of the individual break-out groups from the workshop.

PRESENTATIONS

KEYNOTE ADDRESS: Issues in Pediatric Epidemiology*

Robert Bornschein

Department of Environmental Health
University of Cincinnati

The keynote address focused on important issues in determining the potential relation between an environmental exposure and an adverse health effect in young children. Dr. Bornschein drew from his experience investigating health effects associated with pediatric lead exposure. His presentation included a historical perspective on improvements in exposure measurement and the determination of relevant health endpoints.

The first topic addressed was environmental exposure assessment in young children. Standardization of data collection, preparation and analyses was stressed. Sampling should be uniform and based on the child's behavior to achieve the best estimates of dose. For example, environmental sampling should be targeted in areas (e.g., rooms, yard, etc.) where the child frequently spends time. Internal and external quality control including use of standard reference materials, bench-top controls, external reference laboratories and proficiency programs are necessary to optimize exposure assessment. Limiting variability and laboratory error is critical in evaluating low level environmental exposures. National databases such as the National Health and Nutrition Examination Survey can provide general population exposure prevalence estimates for selected compounds. Screening data may also be available from county health departments and through the Centers for Disease Control and Prevention, particularly from the Morbidity and Mortality Weekly Report.

Evaluation of measurements that serve as markers of exposure and effect was discussed. There is a continuum from external dose to the development of exposure-related disease.

External dose -- Internal dose -- Biologically effective dose -- Biologic response -- Disease

It is a big step from external dose to biologic response. Many assumptions are made based on data derived from adjacent steps in the continuum, but it is important not to extrapolate too far. For example, lead in housedust correlates poorly with a child's blood lead level, but hand wipes from the child will be more strongly related to both blood lead and housedust than they are to each other. So, in this case, without knowing the concentration of the intermediate step (hand related exposure) there does not appear to be a relation between housedust lead and blood lead in children. This is an important lesson as we begin investigating the pathways of pesticide exposure in young children.

Exposure models are needed to estimate body burdens in children and pregnant women. These models should address multiple pathways of exposure and multiple exposure sources. Empirical models are the most desirable such as the Integrated Exposure Uptake Biokinetic model for lead exposure and physiologically-based pharmacokinetic exposure models. It is important to realize that typically you can explain very little of the variance associated with body burden of an environmental exposure. For example, an exhaustive model for blood lead in children will typically explain less than 20% of the variance in levels. Analytic variance is

always higher than the explained variance. Unexplained variance may be attributed to poor measurement of diet, air, hobbies or job exposures, prior accumulation of a body burden due to earlier exposures, hematocrit, and other measurement error. Additionally, the relation between the source of the marker (e.g., urine) and the tissue where the compound is deposited or concentrated (e.g., bone, fat) should be known. The clinical sample results should be interpreted based on the physiology and kinetics of the substance in the body. Temporal considerations are also needed. Microenvironments change frequently over time and recent measurements may not be reflective of long term levels. Kinetics can also change over time in young children. Calcium in bone, for example, changes over three times in the first year of life with a much slower calcium exchange rate later in childhood.

Moving from the measurement of exposure and dose to childhood adverse health outcomes, Dr. Bornschein talked about the difficulty in measuring child development. A simple "one exposure leads to one outcome" model may work when there is a large high-dose exposure and the associated outcome is severe. This is typically not the case for many environmental exposures. In a multiple main effects model, each factor is considered an independent contributor to risk (e.g., socio-economic status, parental education, etc.) for poor child development. An interactive model is probably closer to reality, where factors are related to each other as well as having an impact on the outcome under study. For example, socio-economic status is related to maternal IQ, child rearing practices, nutrition, etc.., all of which can interact with each other as well as potentially exert an independent effect on child development.

A variety of study designs are appropriate for investigating the potential health effects in young children associated with an environmental exposure. Dr. Bornschein discussed using cross-sectional and longitudinal studies of child development. Data analyses require particular attention to the treatment of missing data, particularly in the case of exposure measurements below the analytic limit of detection (LOD). Exposure data are not typically normally distributed and decisions about how to handle values below the LOD (e.g., make them zero) can have a major influence on the analyses. Structural equation models can be very useful because they are designed to model complex interactions and a variety of pathways in the analysis.

*** This is a summary of the presentation by Dr. Robert Bornschin**

Assessing Neurobehavioral Effects of Environmental Toxicants on Children: Options and Issues

David C. Bellinger

Children's Hospital (Boston)
Harvard Medical School

Because many environmental toxicants interfere with the development of the central nervous system, a comprehensive assessment of the health effects of such substances in the pediatric population require valid and reliable methods of evaluating neurobehavioral function. Considerable effort has been invested in developing batteries of methods for assessing such endpoints in adults, resulting in options such as the Neurobehavioral Core Test Battery (Anger et al., 1993), the Adult Environmental Neurobehavioral Test Battery developed by the ATSDR (Amler et al., 1995), and the computer-administered Neurobehavioral Evaluation System (Letz, 1991). In contrast, only one battery has been developed specifically for use in studying the neurobehavioral impact of community-level exposures on children, the Pediatric Environmental Neurobehavioral Test Battery (PENTB) (Amler et al., 1996). As a result, the assessment batteries used to investigate neurotoxicant exposures in children have tended to be study-specific, even in the case of the set of prospective lead studies, which were characterized by considerable interaction among the major research groups (Bornschein and Rabinowitz, 1985; Grant, Smith, and Sors, 1989).

The evaluation of neurotoxicant effects in children involves some challenges less germane to adult assessment, due largely to the rapid pace of development over the first few years in the nature and breadth of the response modalities which can be exploited as "windows" onto the status of children's skills in different functional domains. Development is, by definition, a "moving target," requiring the discrimination of changes in behavior that reflect a toxicant effect from changes that reflect normal development. The assessment of children, in contrast to adults, thus requires consideration of the added dimension of time, in that exposure to a toxicant may affect the rate at which a skill emerges as well as the form which that skill takes. As a result, the question, "What is the appropriate age at which to conduct assessments" requires careful weighing of competing considerations. Carrying out assessments within the first year of life has important advantages. Because children's neurobehavior is affected by many factors, isolating outcome variation uniquely attributable to a specific exposure from the variation attributable to other, often correlated (confounding) factors can pose a formidable challenge (Bellinger et al., 1989). Because some of these factors are not strongly associated with children's neurobehavioral status prior to approximately 18 months of age (Golden and Birns, 1982), their impact can be minimized by assessing children in infancy. In addition, the shorter the interval between exposure and assessment, the fewer the opportunities for the occurrence of intervening medical and social events that might change outcome in ways that are independent of the exposure. On the other hand, the ways in which an infant can "tell" us what he or she currently "knows" are limited, impoverishing the empirical bases on which to draw inferences about the likelihood of adverse impact from neurotoxicant exposure. In addition, prior knowledge of the site or mechanism of a toxicant's effects on the neural substrate may lead to the expectation that its primary impact will be on a domain of function that cannot easily be assessed in infants, such as "executive" functions (planning and organization), abstract reasoning, or reading. Thus, although a neurotoxicant may have greatest impact on brain systems undergoing the most rapid

change at the time of exposure, the status of such systems may not yet be clearly reflected in observable behavior. If such systems represent the endpoint domain of greatest interest, it would be necessary to delay follow-up assessments until such time that the response modalities available to a child are sufficiently differentiated that a clear and reliable picture of a child's strengths and weaknesses in these domains can be obtained. Thus, a decision regarding the age at which assessments should be conducted in any particular study involves significant trade-offs.

The specific choices one makes among the large number of assessment instruments available depend on several considerations. One critical factor is the primary goal of the assessment. Is the goal, for instance, to understand the neuropsychological mechanism(s) of neurotoxicant effects, or to estimate the public health or "real world" impact of neurotoxicant exposure? If it is the former, one might select instruments that focus in detail on cognitive "building blocks" (i.e., vigilance, visual-spatial skills, working memory), the component low-level information processing skills that underlie higher-order functions. If the goal is the latter, one might prefer instruments that assess practical skills such as reading, mathematical reasoning, and oral expression. The results of such tests would suggest what "real world" skills may have been affected, but provide relatively little insight as to why. Logistical factors also bear on the selection of instruments, such as the length of time available for assessment of each child, and the level of expertise of study personnel.

To some extent, a discussion of which measures are most suitable for use in neurotoxicant studies can be pursued without specifying a particular neurotoxicant. Neurobehavior represents the highest level of neurological organization, thus depending on the integrity of many more basic processes. As a set of final common pathways, it would be expected to reflect the impact of any neurotoxicant exposure, should the level of exposure exceed the threshold of effect. Although two neurotoxicants may impair different combinations of lower-level neurobehavioral processes, their overall impact might be expressed in the same apical behaviors, particularly in infancy when the response options are so limited. It should not be surprising that certain apical tests, which average performance over many functional domains, have proven to be sensitive to compounds that vary widely in their chemical properties and biological actions (e.g., heavy metals and persistent organic pollutants). Thus, apical neurobehavioral measures appear to be quite sensitive to neurotoxicant exposures but not specific.

The following sections discuss selected approaches to assessing the neurobehavioral function of children of different ages, as well as general assessment issues germane to studies of neurotoxicant exposures.

Infants (0-3 Years)

The two major approaches to the developmental assessment of infants involve sensory-motor and information-processing tests. A third approach, based on informant reports, will be discussed briefly. A fourth option, involving assessments of newborn behaviors using instruments such as the Neonatal Behavioral Assessment Scale (Brazelton, 1984) will not be discussed due to the difficulties in conducting and interpreting such assessments in the context of an epidemiological study of neurotoxicant exposures (Dietrich and Bellinger, 1994).

Sensory-motor *(SM)* Tests

Currently, SM tests are viewed as the "gold standard" for infant assessment. These tests issue from what is referred to as the psychometric tradition. The selection of items is based on a purely statistical criterion, i.e., the ability to discriminate among children of a certain age, rather than on a set of principles or hypotheses about child development. Such tests simply provide for a broad sampling of the behaviors children display at different ages. As a result, they permit inferences about whether a particular child's development is progressing at an age-appropriate pace and in an age-appropriate form. Because children's abilities change rapidly as they age, the items administered to children of different ages differ considerably. For example, items administered to a 3 month old child typically involve orienting and tracking, object manipulation, and awareness of novelty. At age 14 months old, simple visual-spatial skills, language comprehension, and elementary problem-solving skills are assessed. At 2 1/2 years, more complex expressive and receptive language skills and abstract concepts are assessed. The item sets administered to children at different ages essentially constitute qualitatively different tests, which should give pause to one seeking to interpret multiple scores over time as true repeated measures that define a developmental function. To some extent this is true of all childhood assessments, but the differences are greatest during the years of infancy.

Although many SM-based tests are available, the one most commonly used is the Bayley Scales of Infant Development (second edition) (BSID) (Bayley, 1993), which covers the age range of 1 to 42 months. Its primary components are the Mental Development Index (MDI), which assesses cognitive skills (e.g., memory, habituation, problem solving, early number concepts, classification, language, social skills); and the Psychomotor Development Index (PDI), which assesses gross motor planning, balance, ability to imitate postures, visual-motor integration, and fine motor skills. In addition to global MDI and PDI scores, "facet" scores in the following domains are also obtained: cognitive, language, personal-social, motor. The BSID were standardized on a sample of 1700 U.S. children in which the distribution by race/ethnicity mirrored that of the U.S. population.

One disadvantage of all SM tests warrants comment. Although they satisfy most criteria by which the adequacy of psychological tests is evaluated (e.g., test-retest reliability, concurrent validity), scores typically have low predictive validity, even over periods as brief as a few years. For example, the median correlation between 1 year BSID scores and IQ at age 5 to 7 years in low-risk children is approximately 0.1, although it is often considerably higher among infants at medical risk (Kopp and McCall, 1982; Rose and Feldman, 1991). Test scores obtained after age 2 are usually more strongly related to later IQ scores, however, presumably because the skills assessed more closely resemble those assessed by IQ tests. That young infants' scores on SM tests have such low predictive validity is not necessarily a flaw in such tests but most likely reflects the fact that developmental trajectories are dynamic and that the speed with which a child achieves a criterion level of skill within one domain is not necessarily related to how quickly a criterion level will be achieved in a different domain or even a later criterion within the same domain. For example, is it reasonable to expect that the age at which a child masters visually-directed reaching for an object will predict how well that child reads at age 7? Developmental delay does not necessarily reflect developmental deviance and the two may differ in prognostic significance. Scores on SM tests can be viewed as analogous to birth weight (McCall, 1979). Although it does not predict school-age weight, neonatologists find it a useful indicator of a newborn's current health. Similarly, scores on SM tests provide a valid measure of an infant's

current developmental status. Nevertheless, a score on an SM test should not be considered an estimate of "infant IQ."

Information-processing *(IP Tests)*

The poor predictive validity of SM tests has been attributed to dissimilarities in the domains assessed by these tests and by IQ tests. It has been proposed that prediction from infant behaviors would be improved if it were possible to evaluate close analogues of the skills later called upon by IQ tests. Infant abilities considered likely candidates include elements of information processing such as perception, discrimination, storage, retrieval, and classification. Because performance on habituation and expectancy tasks involves such skills, several new assessment procedures based on them have been developed. The best known among these is the visual recognition memory (or novelty preference) procedure called the Fagan Test of Infant Intelligence (FTII) (Fagan et al., 1986). Other methods are based on nonvisual information processing (e.g., cross-modal transfer of information, tactual recognition memory; Rose et al.,1992).

The FTII rests on the principle that an infant will usually prefer to look at something novel rather than something familiar. It consists of 10 trials in which the infant is presented with such a choice. Relying on corneal reflections, an observer records how the infant distributes looking time to a novel and a familiar picture (all photographs of faces). In the last half of the first year, infants allocate an average of 60% of time to a novel picture. In an attempt to combine the best features of SM and IP tests, the most recent revision of the BSID includes several items that assess visual recognition memory in 1 to 3 month olds.

To a limited extent, research bears out the hope that IP tests are more predictive of later IQ than are SM tests (Fagan and Detterman, 1992). A meta-analysis of 31 studies calculated a weighted correlation of 0.36 between novelty preference and preschool IQ (McCall and Carriger, 1993). Scores obtained between the ages of 2 and 8 months appear to be more predictive than scores obtained later and, as with SM tests, preschool IQ scores of children at medical risk can be predicted more accurately than IQ scores of low-risk children. The predictive validity of IP tests does not exceed that of SM tests in samples of at-risk infants, however. Moreover, despite its statistical significance, the prediction afforded by scores on IP tests only accounts for 15% of the variance in preschool age IQ, a level of accuracy that is no higher than the level achieved by relying solely on parental education and socioeconomic status.

An important limitation of the FTII is the narrow age window within which it is normed (up to 12 months). Furthermore, whereas SM tests provide a broad characterization of an infant's developmental status, IP tests provide information about a highly restricted set of behaviors.

Informant-based Methods

Many parent-completed questionnaires on infant and child development are available, developed in large part to help clinicians identify infants who may require additional evaluations. They vary in format and in the breadth of their coverage. One measure of general development is the Child Development Inventory (Ireton, 1992), a 270-item questionnaire applicable to children 15 months to 6 years. It assesses 8 domains: social, self-help, gross motor, fine motor, expressive language, language comprehension, letters, and numbers. The Vineland Adaptive Behavior Scales (Sparrow et al., 1984), administered as a semi-structured interview involving

297 items, provides information on child behaviors in four domains: communication, daily living skills, socialization, and motor skills.

Because of the lack of standardization in parents' experience, observation, and reporting skills, such measures are generally not satisfactory for the purpose of evaluating subtle developmental consequences of toxicant exposure. These factors may be less problematic for questionnaires that focus intensively on salient domains such as language development and behavior problems. If the items are sufficiently specific, parents can be extremely accurate reporters. A set of two instruments, the MacArthur Communicative Development Inventories, take advantage of this and can be used with children 8 to 30 months of age (Fenson et al., 1993). The Vocabulary Production scale, for instance, asks a parent to indicate which words, from a list of 680, a child currently uses. The Syntactic Complexity scale asks the parent to indicate which of two alternative constructions (e.g., "I no do it," "I can't do it") sounds most like the way their child speaks "right now." A widely used questionnaire for assessing behavior problems in 2-to-3 year old children is the Child Behavior Checklist (Achenbach et al., 1987), which asks a parent to indicate the frequency (not true, somewhat or sometimes true, very true or often true) with which a child engages in 99 specific problem behaviors.

Children (Ages 4 and Up)

For children ages 4 and above, the number of instruments for assessing neurobehavioral status is enormous, precluding detailed discussion of individual tests. The key issue is not finding the "correct" or "best" tests. As Bernstein noted, "...there is no single battery for evaluating the potential impact of toxic agents on the developing child. I cannot recommend any specific tests in this endeavor; many are appropriate. Overall strategy, a principled theoretical framework, and adequately specified behavioral domains are what counts, not tests" (1994).

Among the available choices of general intelligence tests rooted in the psychometric tradition are the Wechsler Preschool and Primary Scale of Intelligence-Revised (Wechsler, 1989; ages 3-7 years 3 months), the McCarthy Scales of Children's Abilities (McCarthy, 1972; ages 2 years 7 months-8 years 7 months), the Stanford-Binet, 4th Edition (ages 2 years-adult), the Differential Ability Scales (Elliot, 1990; 2 years 6 months-18 years), and the Kaufman-Assessment Battery for Children (Kaufman and Kaufman, 1983; ages 2 years 6 months-12 years 6 months). The mixes of skills assessed by these instruments overlap considerably, although direct comparison studies reveal that a child's scores on the various tests may vary by several points. This is especially true in clinically-defined samples who present with difficulties performing particular types of tasks, which may be represented more or less prominently on a given test. Several of these tests have proven sensitive to various low-level neurotoxicant exposures, including lead and PCBs.

Several "off the shelf" test batteries are also available, although they have not been widely used in studies of neurotoxicant exposures. Two examples are the Reitan-Indiana Neuropsychological Test Battery (Boll, 1981) and the Luria-Nebraska Children's Battery (Golden, 1981). A recently published test battery, the NEPSY, provides for a developmental neuropsychological assessment of children ages 3 to 12 based on Luria's model. It includes 27 subtests that assess five functional domains: attention/executive functions, language, sensorimotor abilities, visuospatial abilities, and memory/learning. It does not produce a summary IQ-like score. Few published data are available on the NEPSY, although it is the primary endpoint in an NIEHS clinical trial evaluating whether administration of the oral

chelating drug succimer improves the long-term neurobehavioral outcome of lead poisoned children.

As mentioned earlier, the Pediatric Environmental Neurobehavioral Battery (PENTB) was developed specifically to evaluate the behavioral impact of community-level toxicant exposures on children. Its primary goal is to provide a rapid, relatively inexpensive, and reasonably comprehensive evaluation of children from 1 to 16 years of age. Among the factors motivating the selection of tests were the need to limit an evaluation to one hour and to use tests whose administration does not require professional supervision. Because of the expertise required for infant assessment, the battery for children ages 1 to 3 involves only informant-based methods. The 10 performance-based and four-informant-based instruments cover the affective, cognitive, motor, and sensory domains. Affect is assessed using the Vineland Adaptive Behavior Scales and the Personality Inventory for Children. Assessments of cognitive skills include the Kaufman-Brief Intelligence Test, story memory (and delayed recall) from the Wide Range Assessment of Memory and Learning, a divided attention test, the Developmental Test of Visual-Motor Integration, and a verbal cancellation task. Motor skills are assessed using a finger tapping task, the Purdue Pegboard, and the divided attention test. The tests of sensory status are visual acuity, visual contrast sensitivity, and vibration threshold. Field studies demonstrate that this battery of tests is acceptable to parents and children and can be administered reliably. Its ability to detect subclinical impact of neurotoxicants is not yet known, although the battery includes several measures shown in prior studies to be sensitive to such exposures. A useful next step in research on the PENTB would be to determine whether the performance of children with identified developmental or learning problems differs from that of normal controls. If the PENTB does not discriminate such groups, its utility in detecting subtle neurotoxicant effects would be drawn into question.

A major issue in assembling an assessment battery is whether to include a test of general intelligence. On the one hand, such tests are familiar and the construct they measure is valued by policy makers. In addition, because IQ is an endpoint that is easily monetized, it is suitable for use in cost-benefit analyses. On the other hand, some investigators have advocated the strong position that "...the general use of summary scores [e.g., IQ...] is both inappropriate and unscientific" (White et al., 1994, p.513) in part because important exposure-related differences in performance may be obscured when summary scores are employed as the outcome index. This is because such scores, in effect, average an individual's performance over the multiple domains assessed by an apical test. For instance, if exposure to a neurotoxicant impairs only visual-spatial functioning, an exposure-related difference in the full-scale IQ scores might not be apparent because only some of the subtests that contribute to full-scale IQ rely heavily on visual-spatial skills. Similar arguments are often made by investigators who study primarily animal models (Rice, 1993). Although this claim has intuitive appeal (and must necessarily be correct to some extent), it has generally not been supported by the results of human epidemiological studies of neurotoxicants. In lead studies, for instance, the most consistent finding across studies is an inverse association between an exposure biomarker and full-scale IQ, but only limited consistency in the associations between lead and scores on tests that focus on specific neuropsychological domains (National Research Council, 1993). Furthermore, even within specific studies, stronger associations have usually been found on IQ tests than on domain-specific tests. In the Boston prospective study, for instance, whereas blood lead at age 2 and IQ at age 10 were inversely related (Bellinger et al., 1992), only a chance number of significant associations was found between blood lead and scores on tests such as the California Verbal

Learning Test-Children, the Wisconsin Card Sorting Test, the Developmental Test of Visual-Motor Integration, story recall, finger tapping, and grooved pegboard (Stiles and Bellinger, 1993).

The stronger associations found on apical tests than on focused tests could have a toxicologic or methodologic explanation (or both). Whether one type of test is more sensitive than the other may depend on the neuropsychological mechanism of toxicity. The performance decrements associated with increased lead exposure could reflect the joint impact of several independent impairments or impairment of a small set of key functions that underlie many diverse cognitive abilities. If the latter, the result might be slight, nonsignificant decrements in performance across diverse neuropsychological domains. If the decrements are summed in the form of an apical score, such as IQ, a significant exposure-related decrement might be apparent. Moreover, the specific form in which lead's impact on neurobehavior is expressed might differ depending on a variety of host (e.g., age, sex, socioeconomic status) and contextual characteristics (e.g., dosing regimen), what in the animal literature is referred to as the "experimental system." Applied to human studies, this perspective suggests that exposure to a particular neurotoxicant may not produce the same "behavioral signature" under all scenarios (Bellinger, 1995a). If so, exposure-related effects will again be most apparent on tests that average performance over many domains, reducing the impact of cohort-specificity in the expression of toxicity.

Another reason why apical tests more reliably reveal exposure-related differences in performance may be the greater strength of their psychometric properties as compared to domain-specific tests. Unlike IQ tests, domain-specific tests were designed more to identify individuals with clinically-significant deficits in certain types of skills than to discriminate between levels of performance within the normal range. This difference in sensitivity will be especially important when the effects to be characterized are subtle. Domain-specific tests may be most useful for more highly exposed cohorts in which the neurobehavioral effects are substantial and thus detectable using tests that are less sensitive than apical tests (e.g., Bellinger et al., 1994).

Computer-administered Tests

Recently several tests included in the NES2 battery for adults (Letz, 1991) have been adapted for use with children (Winneke et al., 1994; Otto et al., 1996; Dahl et al., 1996). These computer-administered (or computer-assisted) tests are finger tapping, continuous performance test, and hand-eye coordination, which assess aspects of motor response speed, sustained visual attention and response latency, and motor coordination (tracking), respectively. Each of these tests has a long history of use in clinical child neuropsychology. Their cost efficiency and the standardization of administration, data capture, and scoring make these procedures attractive options for use in epidemiologic field studies. In their present form, these tests may be most appropriate for children 8 years and older as substantial numbers of the younger children in the initial studies were unable to complete all tasks. In addition, visual contrast sensitivity may be an important covariate of children's (and adults') performance on these three tasks (Hudnell et al., 1996a), accounting for as much as (or more) of the outcome variance as does low-level neurotoxicant exposures. It appears important, therefore, that visual contrast sensitivity be measured in studies using NES2 tasks (and perhaps conventional, individually-administered tests as well). First, it would reduce error variance in endpoint scores and thus increase the power of hypothesis tests involving neurotoxicant exposure. Second, alteration in visual contrast

sensitivity function may itself be a result of neurotoxicant exposure, representing a mechanism for the exposure-related decrements seen in other functional domains (Hudnell et al., 1996b).

Informant-based methods. As with infants, many parent- or teacher-completed instruments are available for identifying stable socio-emotional/behavioral characteristics that are not readily assessed during a standard neurobehavioral evaluation. These include the Child Behavior Checklist/4-18 (Achenbach, 1991a), the Personality Inventory for Children (Wirt et al., 1990), the Connors' Teacher and Parent Rating Scales (Connors, 1990), and Parent and Teacher Ratings Scales of the Behavior Assessment System for Children (BASC) (Reynolds and Kamphaus, 1992). The CBCL/4-18 (Achenbach, 1991b; Achenbach, 1991c) and BASC families of instruments also include teacher and self-report versions, providing the option to collect data from multiple informants on a given child.

These methods should be considered as adjuncts to, not replacements for, individually-administered assessments of neurobehavioral function.

Additional Issues

Identifying *a* "Behavioral Signature"

Several issues should be considered when attempting to infer the "behavioral signature" of a neurotoxicant on the basis of differences in scores on a battery of tests. First, an individual's score on a neuropsychological test does not reflect the integrity of a single functional domain or region of the brain (Krasnegor et al., 1994). Successful performance on any test depends on multiple skills. For instance, a low score on a test of design copying (e.g., the Developmental Test of Visual-Motor Integration) may be due to problems with graphomotor control, visual perception, planning and organization, motivation, or behavioral modulation (e.g., impulsivity) (Bellinger, 1995b). Second, drawing inferences about the relative sensitivity of neuropsychological domains to a neurotoxicant is problematic unless the tests used to assess different domains are equivalent in their discriminating power, specifically their true-score variance (Chapman and Chapman, 1978). If they are not, differential performance across domains may not be due to differential ability across domains. Deficits may appear to lie in a particular domain simply because the tools used to assess that domain are technically superior to the tools used to assess other domains. One way to address this issue is to employ an instrument that was designed to assess several related aspects of function. One example of such an instrument is the Wide Range Assessment of Memory and Learning (Sheslow and Adams, 1990) for children ages 5 to 17, which includes 9 subtests that assess different types of verbal memory (story, sentence, number/letter strings), different types of nonverbal memory (picture, design, sequence of actions), and different types of learning (verbal, visual, sound-symbol). Another example is the Wide Range Assessment of Visual-Motor Abilities (Adams and Sheslow, 1995) for children ages 3 to 17, which assesses visual-motor integration (design copying), visual-spatial matching, and fine motor function (pegboard). Because the components of such tests are normed on the same population, their reliability coefficients are directly comparable, permitting inferences about the relative magnitudes of toxicant effects on the different aspects of function assessed. The benefits of direct comparisons made possible by such co-norming also extend to sets of instruments. For instance the Wechsler Individual Achievement Test (The Psychological Corporation, 1992), which assesses academic skills such as reading, mathematics, and spelling, was co-normed with the Wechsler intelligence tests (i.e., WPPSI-R, WISC-III, WAIS-R). Because the most frequently employed criterion of a "learning disability" is a significant ability-

achievement discrepancy, the paired use of the WIAT and the age-appropriate IQ test provides solid psychometric grounds for identifying such discrepancies.

Quality Control

Although the need to implement a protocol for maintaining the quality of analytical measurements of biomarkers is universally acknowledged (e.g., Coggon, 1995), implementing analogous procedures for maintaining the quality of neurobehavioral measurements is equally important although less frequently explicitly acknowledged. While this applies to all neurobehavioral measurements, it is especially true with respect to infants. Developmental testing of an infant occurs within the context of an interpersonal interaction that is loosely scripted by prescribed administration procedures (e.g., number of demonstrations, number of trials permitted). However, the examiner is also given latitude to determine "on the fly" the best way in which to engage the infant in the evaluation. Unlike school-age children, infants have no "test-taking set." They do not experience any social pressure whatsoever to cooperate with an assessor, attending only to a task that engages their interest. If a child fails to perform a target behavior, it may be because he or she has not yet developed the underlying skills or simply because of a lack of motivation to perform it (or both). One of the assessor's major tasks is establish a setting that increases the likelihood that a target response will be elicited, should it be in the infant's repertoire. This requires considerable training and practice. Clear procedures must be in place from the outset of a study for thorough training of testers and for periodic evaluation of tester performance. These procedures should also include review of a tester's proficiency in scoring responses and in deriving summary and standard scores from the raw data.

Measurement of Covariates

As final common pathways, neurobehavioral endpoints are multi-determined and responsive to a variety of biological and sociological factors. In most studies, approximately 50% of the variation in IQ is "explained" by such factors. In ascertaining whether an exposure is associated with neurobehavior, it is critical that information about these biological/sociological factors be taken into account. This will serve two purposes. First, it will reduce the outcome error variance, providing for a statistically more powerful test of the hypothesis that the environmental exposure is associated with the endpoint. This is important because a low-level environmental exposure is likely to explain a relatively small percentage of outcome variance. In most lead studies it was less than 5%. Second, it provides the possibility of controlling for confounding, which is present when one of the biological/sociological determinants of the endpoint is also correlated with the exposure. In studies on low-level lead exposure, the factors usually identified as critical confounders included family socioeconomic status, parental IQ, and the quality of the home environment (as assessed by the Home Observation for Measurement of the Environment; Bradley, 1994). The likelihood that confounding is a major issue, and the specific variables that need to be considered as confounders, may be toxicant-specific, depending in particular on the key exposure pathways.

Conclusion

Deciding which tests to use to assess the impact of a toxicant on children's neurobehavior requires consideration of several factors, followed by efforts to reconcile incompatibilities in their implications. Among the most important factors are the following:

(1) the goal of the assessment, e.g., to guide public policy or to clarify basic mechanisms of toxicity,

(2) the design of the study, e.g., cross-sectional versus prospective,

(3) the children's ages at the time of assessment, e.g., infant versus school-age,

(4) the range of children's ages at the time of assessment, e.g., all less than 3 years of age versus a broader span, such as 1 to 15 years,

(5) the length of time available for assessment, e.g., one hour versus three,

(6) the level of training of personnel available to conduct the assessments, e.g., bachelor's degree versus clinical neuropsychologists.

Whatever choices one makes in addressing one of these issues may well constrain the range of options available for addressing others, requiring trade-offs in the breadth and quality of the data that can be obtained.

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Do Pesticides and Other Environmental Exposures Play an Important Role in the Development of Diseases of Immune Dysregulation along the United States Mexican Border?

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Introduction

The immune system has evolved over millions of years to protect self from non-self. Immune competence relies on the rapid activation and replication of clones of antigen specific T and B lymphocytes, and other mononuclear and polymononuclear white blood cells which recognize and respond to infectious agents, foreign proteins, and other small molecular weight molecules in an effort to protect the host. Many immune responses are protective, some are clinically irrelevant, and some are deleterious to the individual. Environmental exposures can cause perturbation of the immune system in a number of ways. Agents can be immunotoxic, act as adjuvants, induce hypersensitivities to themselves, or induce autoimmunity, all of which have unique clinical consequences. Although not covered here, immunocytes, due to their rapid turnover, are also particularly susceptible to malignant transformation. In order to assess whether the U.S. Mexican border is safe from an immunological point of view, it is important to consider these various mechanisms by which immune status can be altered.

To assess the impact of environmental exposures on immunological health in individuals living in target areas, a host of complementary investigations should be utilized. Preliminary studies would include identification of which potentially immuno-modulating materials are present in the environment and a survey of the prevalence and severity of diseases with a potential immunologic etiology. It will be important to compare health information with laboratory data such as: (1) quantitative laboratory studies of antibody including IgE, and cellular constituents of the immune system, (2) qualitative assessment of vaccine specific antibody titers and T-cell proliferation responses, (3) hypersensitivity testing for relevant agents, either serologically or by skin tests, (4) markers of auto-immunity, and (5) in the case of immunologic diseases of the respiratory tract, pulmonary function testing and chest x-rays. A philosophical approach to the assessment of immunological health and its clinical consequences in individuals living on the U.S. Mexican border will be outlined.

Immunological Principles

Immunotoxicity

Cytotoxic compounds generally affect rapidly dividing and metabolically active cells selectively. Malignant cells, hair follicles, bowel mucosa, and bone marrow derived red and white blood cells are particularly sensitive to cytotoxic agents because they fall into this category. Immunotoxin is a term which is occasionally used to describe agents which stimulate the immune system. I will reserve the term immunotoxin to describe agents which cause a deterioration in immune parameters. Agents which promote immune responses are called adjuvants in most of the immunology literature. I will use the term immuno-adjuvant to describe

those agents which promote immune responses. Therefore, by my narrow definition, an immunotoxin would be expected to lead to a fall in the numbers and health of immunocytes over time leading to poor immunity and susceptibility to infectious disease. Malignant and auto-immune disease can also be seen as a consequence of immunodeficiency.

The immunotoxic potential of pesticides and other manmade products is generally assessed in animal models before their use is approved (Ladics et al., 1994; Vohr, 1995; Madsen et al., 1996). One major limitation of animal models, however, is that they may not accurately reflect the consequences of exposure in humans. For example, the pesticide pentachlorophenol (PCP) has been shown to have a number of immuno-inhibitory effects in animals. However, a recently published study demonstrated that factory workers, some exposed to high levels of PCP (up to 1442mg/l in plasma) for greater then 10 years had essentially no differences in immunologic parameters nor health when compared to age matched controls (Colosio et al., 1993). In the case of the insecticides deltametrin and α-cypermetrin, published animal studies have shown immunostimulatory, immuno-inhibitory and neither effect (Masden et al., 1996). The route of exposure has further immunologic consequences. For example in the case of carbaryl, inhalation leads to a fall in antibody titers while dermal and oral exposure has no effect (Ladics et al., 1994). These cited examples reflect the limitations of experimental animal models in the identification of immunotoxins and other immuno-modulators of clinical relevance. Agents with high immuno-modulatory potential should be easy to identify, with consistent results across species, and identification as a health risk in the laboratory before their use is approved. Therefore, those agents that make it to market and into common use are likely to have relatively weak immuno-modulatory potential. Epidemiological studies play an important role in monitoring the ongoing health consequences of the environment people live in. Given the limitations of animal studies, population based research needs to be conducted to identify immunotoxins and other immune modifying agents in the environment which might not be identified in animal studies.

Immuno-adjuvants

Some compounds promote immune reactivity to other compounds without necessarily inducing an antigen specific immune response themselves. This phenomenon has been termed an adjuvant effect in immunology. In the laboratory, adjuvants are used to induce allergy, auto-immunity, and other immunologically reactive states. Allergic immune responses (Th2) are seen in experimental animal models with the co-injection of antigen with alum, pertussis toxin, or cholera toxin (Snider et al., 1994; Oettgen et al., 1994). Immune responses more characteristic of viral or mycobacterial infections (Th1 responses) are seen with the co-injection of Freund's adjuvant (Dvorak and Dvorak, 1974), and more recently with immuno-stimulatory sequence DNA(ISS) (Raz et al., 1996). Although the adjuvant effect has been well described in animal models, less is known about the possible adjuvant effect of environmental exposures on the immune status and health of people. However, recently, Diaz-Sanchez et al. (1997) have demonstrated that diesel exhaust promotes IgE synthesis in *in vitro* systems, and increases ragweed specific IgE in nasal secretions of ragweed allergic individuals exposed to diesel exhaust and allergen. This type of phenomenon may well occur with exposure to other manmade and organic compounds whose adjuvant properties have yet to be identified. Given that allergic disease is more common in developed then in primitive populations, there is concern that exposure to diesel exhaust and other forms of manmade pollution may be responsible for this excess disease burden.

Antigen/allergen Specific Hypersensitivities

Exposure to pollens, molds, chemicals, and other agents in the air can lead to hypersensitivity, and clinical disease. Since the majority of these exposures is via the respiratory tract, skin, and gastro-intestinal tract, clinical disease is likely to manifest itself in one of these organ systems. These immunologically mediated hypersensitivities can take many clinical forms including: (1) classic IgE mediated allergic diseases such as asthma and allergic rhinitis, (2) hypersensitivity pneumonitis and contact dermatitis which are thought to be due to cell mediated hypersensitivities, and (3) diseases due to multiple immune mechanisms such as allergic broncho-pulmonary aspergillosis (ABPA). Hypersensitivity mediated illness has been well described in humans exposed to high levels of pesticides and other industrial products and byproducts in the workplace. Examples include isocyanate induced asthma and specific IgE production, and IgG mediated hypersensitivity pneumonitis (Baur et al., 1994), and chromate induced asthma and contact dermatitis (Bright et al., 1997; Fisher, 1983). Rural environments are also rich in unique organic allergens of animal and vegetable origin, which can provoke asthma and other classic allergic diseases (Berstein and Berstein, 1993). In addition, more complex immunologic diseases such as ABPA and hypersensitivity pneumonitis have been well described in agricultural workers exposed to high concentrations of aspergillosis and other molds in green houses and other settings (Yoshida et al., 1993). Clearly, a comprehensive assessment of the prevalence and severity of common allergic diseases, and the spectrum of allergen sensitivities will be an important part of the entire immunologic profile for the people living in the U.S. Mexican border region.

Auto-immunity

The role of the environment in the development of autoimmune disease has not been well studied. However, based on our current immunologic understanding, there is reason to believe that auto-immune disease can be induced by the environment. We know that some medications can induce a lupus-like disease and hemolytic anemia, with serological evidence of auto-immunity (Hess, 1995). In this vein, it has been published that: (1) breast augmentation using silicone implants increases the risk of scleroderma (Hess, 1995), (2) some cases of systemic lupus erythematosus might be caused by industrial pollution (Koeger et al., 1997), and (3) that Raynaud's phenomenon, sclerodermatous skin changes and acroosteolysis can be induced by exposure to vinyl chloride. Certainly, a potential exists that some of the micro-environments within the U.S. Mexican Border region promote the development of auto-immune phenomenon.

Assessment of Immune Status and Clinical Consequences at the U.S. Mexican Border

Clearly, pesticides and many other environmental exposures can affect the immune and many other organ systems in animals and humans. Immunologic pathology can obviously lead to clinically evident disease or may be so minor as to not be of concern. In order to carry out an investigation of the clinical impact of environmental exposures on the development and perpetuation of disease along the U.S. Mexican border two important issues need to be addressed initially, as they will aid in the development of an enlightened and therefore more productive and focused secondary investigation. The first issue to be addressed is, to what extent and to what agents are the inhabitants of the U.S. Mexican border region exposed? The second is, what are the active health problems of the people living in the various communities of the border region? Is there an excess disease burden? And what potential influence could relevant exposures play in the development of the diseases identified?

Given our present state of knowledge, we can make some educated guesses about some of the immunologically mediated health problems we might identify in the area under study. The environment clearly plays an important role in the pathogenesis of respiratory disease. In the case of asthma, there is a large body of information which suggests that poor communities with poor air quality are at particular risk for experiencing an excess disease burden. Therefore, asthma is likely to have a high prevalence along the U.S. Mexican border, and other immunologically mediated pulmonary diseases may also be common. Survey data on the incidence and severity of respiratory illness should be sought in the target communities, and this should be collected in conjunction with air sampling studies to identify potential agents of disease. Spirometric and CXR evaluation of the population should be pursued along with allergy testing to provide more objective measures of disease and hypersensitivity. Finally, pulmonary hypersensitivity testing, utilizing environmental agents identified in the ambient air should be used to establish a cause and effect relationship between exposure and respiratory disease.

A link between asthma and other pulmonary and cutaneous hypersensitivity mediated diseases and the environment has been clearly established. However, the role of the environment in the development of other immunologically mediated conditions including those of the gastro-intestinal tract is much less well understood. Survey information of individuals, their health care providers, and hospitals will be important, to establish whether the immune status and health of the population living along the U.S. Mexican border is in fact compromised compared to control populations. The general immunological health of the population should be assessed by focusing on the frequency and severity of community acquired viral and bacterial disease, as well as more atypical and invasive infections. In addition, information regarding auto-immune manifestations should be sought. Screening immunologic evaluations should include complete blood counts. T-cell and B-cell immunity should be assessed both quantitatively and qualitatively to get an accurate assessment of function. Quantitative T-cell and B-cell studies would include T/B cell subsets and total immunoglobulin and IgG subclass levels. Qualitative assessment of T-cell and B-cell function should include delayed type hypersensitivity skin testing to agents such as candida antigen which does not require previous vaccination or disease for positivity, and antigen specific antibody titers which might require vaccination for reliability. Hypersensitivity testing using skin or serum should be conducted to identify potential agents of disease. Provocation testing may also be appropriate to see if exposure induces symptoms of disease. In addition, physical examinations and hematologic markers of auto-immunity may be needed to objectively assess for auto-immune disease should it be identified as a potential health problem in the population under study.

Conclusion

Not much is known about the health risks for people living along the U.S. Mexican border. However, this is a region of rapid change. With the opportunities and consequences of NAFTA yet to be realized, the EPA has a unique opportunity to identify potential environmental health hazards and to incorporate their findings into the policies of NAFTA that will govern the border region in the years to come. However, study of the border and the health of the people living along it is complicated by the simple fact that the U.S.-Mexican border is not homogeneous. Agricultural, industrial, urban, and rural areas with undoubtedly unique environmental conditions exist along the border. However, one consistent theme along the border is duplicity. There exist two governments establishing two sets of environmental

standards for two sets of industries, and two populations. In addition, there is great heterogeneity in the ethnic, socio-economic, and cultural backgrounds of the people in this area. These issues will be important considerations in developing a plan to study the health consequences of living along the U.S. Mexican border.

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Evaluation of Developmental Neurocognitive and Neurobehavioral Changes Associated with Pesticide Exposure: Recommendations for the U.S. Environmental Protection Agency Workshop on the Assessment of Health Effects of Pesticide Exposure in Infants and Young Children

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Abstract

This manuscript provides recommendations for the assessment of chronic low-level pesticide exposure-related cognitive changes in infants and young children. In addition to a review of critical issues associated with the detection of neurocognitive and neurobehavioral alterations, a list of tests and procedures is presented from which a test battery could be devised to optimally assess the effects of neurotoxins in this population. The domains assessed by these instruments include attention and concentration, overall developmental or intellectual functioning, language, learning and memory (verbal and visual), motor skills, neurobehavioral functioning (adaptation, behavior, etc.), and visual processing. The use of repeated observations, in conjunction with a set of instruments encompassing a broad assessment scope with enough sensitivity, should be capable of detecting subtle neurocognitive changes subsequent to neurotoxic exposure.

Introduction

Although the neurodevelopmental effects of certain neurotoxicants on the Central Nervous System (CNS) have been well documented, including the impact of lead (Pb) (Beattie et al., 1975; David et al., 1982; Needleman, 1993; Needleman et al., 1990; Rutter, 1980; Yule et al., 1981), polychlorinated biphenyls (PCB's) (Jacobson, Jacobson, and Humphrey, 1990; Rice, 1997; Rogan and Gladden, 1991, 1992), and acute pesticide poisonings (Kaplan et al., 1993; Zwiener and Ginsburg, 1988), the effects of chronic low-level pesticide exposure on neurocognitive and neurobehavioral outcomes in infancy and childhood have received limited attention. This is unfortunate as these agents have been shown to cause significant neuropsychological (NP) impairments in adults (Ecobichon and Joy, 1994; Hartman, 1995), through acute poisonings (Dean et al., 1984; Muldoon and Hodgson, 1992; Ratner, Oren, and Vigder, 1983) or chronic exposure to these substances (Metcalf and Holmes, 1969; Rosenstock et al., 1990). The fact that the effects of chronic low-level pesticide exposure on the developing brain have received increased attention recently is also timely as the use and production of these compounds for commercial and domestic purposes have spiraled in recent years (Ecobichon and Joy, 1994; Lang, 1993).

Therefore, the study of developmental cognitive and behavioral effects due to chronic low-level exposure to pesticides warrant special consideration. The study of the CNS sequelae of these compounds in individuals along the U.S.-Mexico border, a primary focus of this workshop, also merits resources and time allocation as a substantial number of infants and young children are at risk

in this frontier due to the extensive use of agricultural pesticides in this region. However, prior to embarking on a review and tabulation of assessment procedures useful in measuring developmental neurocognitive and neurobehavioral changes due to chronic low-level pesticide exposure through multiple pathways, critical developmental, methodological, strategic, and theoretical issues associated with the evaluation of these variables are addressed.

Review of Selected Child Development and Maturation Variables

A brief review of developmental issues merits consideration since they are intrinsically related to the assessment of cognitive changes subsequent to neurotoxicant exposure. Brain weight is a marker capable of elucidating the rapid developmental changes occurring in the CNS of infants and young children. During the first year of life, an infant's brain more than doubles its weight (Lemire et al., 1975). In addition to substantial increases in brain weight, there are considerable maturational changes taking place in the developing brain. Schadé and Ford (1965) showed that a fourfold increase in the number of branching points from dendrites in layer III of the middle frontal gyrus occurred during the first six months post-birth. These investigators also reported substantial increments in the total length of these dendrites in the same brain region (Schadé and Ford, 1965). Accompanying these surges in dendrite development, extensive increments in synaptic growth, dendrite pruning, and myelination occur postnatally partly responsible for the increase in the complexity of the developing CNS (Huttenlocker, 1984, 1990; Kolb and Fantie, 1997).

Although other developmental markers are worthy of consideration (e.g., glial cell development, head circumference), the indices presented above underscore the staggering and rapid maturational changes that a developmental neurocognitive and neurobehavioral assessment must be able to gauge when evaluating the effects of chronic low-level exposure to pesticides in the developing CNS. The assessment of neurocognitive and neurobehavioral changes must also be capable of disentangling the effects of pesticides and similar neurotoxins from these maturational changes. A thorough understanding of these developmental issues is additionally important since domains in critical periods of development, or those undergoing the greatest amount of change, tend to be the ones at greatest risk (Scott, 1962) to the effects of neurotoxins.

Neurodevelopmental and Neurophysiological Factors Associated with Increased Neurotoxic Susceptibility

It is critical to review factors that make the developing CNS of infants and young children highly susceptible to neurotoxicants. According to Hartman (1995), the developing brain is especially vulnerable to the effects of these substances. The added vulnerability is partially the result of the substrate composition of the CNS (e.g., 50% of the total dry weight of the brain is lipide), making it a preferred site of accumulation for neurotoxins as these substances tend to be lipophilic (Hartman, 1995). The CNS of the young child may also be more susceptible to neurotoxins due to the disruptive effects of these substances during critical and active periods of brain development, quite capable of interrupting or hampering cell division or other developmental processes. Hartman (1995) also notes that the young brain is especially sensitive to hypoxia, a frequent mechanism of neurotoxic action. Finally, the effects of neurotoxic compounds, including pesticides, may be more pronounced in the rapidly maturing brain secondary to the underdeveloped status of the blood-brain barrier of infants and young children (Chusid, 1982; Claudio, 1992).

Theoretical, Methodological, and Strategic Issues in the Assessment of Cognitive Change Secondary to Neurotoxicant Exposure

As with any area of scientific inquiry, it is critical that the intensity, chronicity, frequency, and routes of exposure be clearly defined. Although exposure to pesticides, particularly low-level chronic exposure, may be difficult to measure and quantify, it is imperative that its measurement and definition be as specific and well defined as possible, thus allowing for later comparison with other investigations assessing the effects of these substances. In addition to well-defined exposure, the definition of neurodevelopmental or neurobehavioral impairment should be clearly demarcated with impairment cut-off scores clearly stated for the same reasons. Albeit studies with adults suggest that pesticides may be capable of infringing upon NP performance (Ecobichon and Joy, 1994), investigators should not assume that the effects of exposure to pesticides in infants and youths cause the same type or magnitude of sequelae as those observed in adults. Researchers should also recognize that their conceptualizations of exposure simply represent hypotheses requiring flexibility and the ability to evolve as new information emerges modifying data-based findings of the neurodevelopmental effects of low-level contamination through multiple pathways.

Although cross-sectional designs may be initially required as an exploratory method to elucidate the effects of various types of exposures or other parameters, as these designs or methods may aid understanding this phenomenon, an argument is presented for the use of longitudinal or repeated measure designs to assess subtle alterations in neurocognition and neurobehavior associated with chronic low-level pesticide exposure. Longitudinal designs are best qualified to assess the additive and chronic impact of exposure to neurotoxins. Longitudinal designs are superior relative to other designs in assessing rates of changes in development (Achenbach, 1978) coupled with superimposed alterations in cognitive functioning associated with neurotoxins. In addition, they allow experimental subjects to serve as their own controls, permitting an idiographic rather than a nomothetic approach to the evaluation of cognitive change. The latter is an important issue to be addressed as a large proportion of NP measures necessary to assess alterations in cognitive functioning may not have available normative data for ethnic minority populations living along the U.S.-Mexico border. Finally, longitudinal relative to cross-sectional designs are superior in detecting delayed-onset impairments that may occur as a result of chronic low-level pesticide exposure.

Validity and reliability are critical issues worthy of significant consideration. It should be noted that the issue of validity not only relates to the populations under study, but also addresses the applications under investigation (pesticides and similar neurotoxins) (see Franzen, 1989). In other words, although neurodevelopmental procedures may have been shown capable of assessing delays as a result of prematurity, developmental delays, or similar syndromes, the majority of instruments used to assess changes in cognition in infants and young children have not been validated for the specific application at hand (assessment of pesticide-related effects).

Aside from issues related to sample size capable of infringing upon an investigation evaluating the effects of pesticides on neurodevelopment, other issues associated with subject selection strategies should be strongly considered when evaluating NP functioning. Subject selection biases may occur as a result of idiosyncratic characteristics specific to the desired study and border populations. Study populations selected as a mere result of experimental convenience may also reflect biases associated with these populations. For example, the selection of participants referred from clinics near agricultural areas with significant pesticide exposure may exhibit greater

symptomatic levels relative to levels exhibited by infants and young children selected from non-referred agricultural regions with elevated pesticide exposure. Lack of multi-site participant selection may also fail to capture differences in border populations or exposures because certain areas within the U.S.-Mexico border may vary substantially in their predominant industrial versus agricultural utilization and their subsequent environmental conditions.

Although exclusion criteria play a major role in any investigation as some subjects must be excluded from a study because their participation could adversely influence an investigation (e.g., history of child abuse or head trauma), some variables which may meet criterium for exclusion may interact with neurotoxins. In other words, studies investigating the effects of neurotoxicants including pesticides should not discard specific variables simply as a matter of convenience since many of these variables may interact with the toxins under investigation (e.g., maternal alcohol or drug use). A large number of exclusionary variables may also render a study ecologically invalid as it becomes unrepresentative of the population under investigation. A posture making use of formal experimental designs rather than quasi-experimental designs with multiple control groups (e.g., infants and children with high and low exposures, infants and children exposed to other neurotoxicants, unexposed children) is critical to the generalization of the findings from such an investigation. Although this may seem a moot point, a number of investigations with adults assessing the toxic impact of solvents on neurocognition employed designs without the use of controls leaving their findings to much speculation (see Juntunen et al., 1980).

A balance in examination scope versus length is another issue which should be given special consideration, particularly in investigations employing infants as participants. With regard to breadth, the evaluation of neurotoxicant-related cognitive changes should have a broad range of domains in its assessment aims. It should further comprise breadth in the assessment of participants, namely the infant and the caretaker as respondent for the infant's functioning. This assessment approach has been shown to be significantly helpful in the assessment of infants and young children for obvious reasons (c.f., Edelbrock et al., 1985). An evaluation that includes the child and the caretaker may be productive in the assessment of toxicants should early impairments associated with these substances become evident in the behavioral repertoire of youths noticeable to caretakers prior to the emergence of subtle alterations in cognition detectable during NP assessments. The length of a developmental neurocognitive assessment should also be regarded as critical when assessing this population as individual variables such as frustration, motivation, and stamina may play key assessment roles. Finally, the length and scope of a test battery have time allocation implications associated with economic factors that must be taken into account.

Whenever longitudinal assessment or similar designs employing repeated measures to investigate cognitive changes require sequential examination or repeated exposure of test materials, the issue of practice effect associated with multiple test administration should be given due weight (Sattler, 1988). This is especially important when assessing the effects of factors whose variance may be small (e.g., pesticides) relative to the variance due to the developmental changes taking place or the effects associated with the repeated presentation of the procedures used in an investigation. In this regard, it should also be noted that the sensitivity of certain psychological instruments to detect neurotoxic-related cognitive changes depends on the novelty of the test or procedure as well as the frequency of administration. Therefore, repeated NP task administrations to the same subject diminishes its novelty and consequently its sensitivity to detect changes in NP functioning. One approach to reduce practice effects is to use measures resistant to the gains associated with repeated

administration. Another approach is to use alternative equivalent measures of the same NP procedures when available. Alternatively, an assessment stance which employs a monitoring battery nestled within a comprehensive battery (comprising the monitoring battery), whereby the procedures comprising the comprehensive battery are repeated with less frequency (due to their diminished reticent to test-retest effects) relative to the procedures in the monitoring battery, may be of aid in dealing with the issue of frequency of test exposure.

Assessment Considerations with Ethnic Minorities and Populations Along the U.S.-Mexico Border

Since the majority of individuals will come from the frontier between the U.S. and Mexico, or from regions within the U.S. with predominant ethnic minority representation, it is critical to address issues associated with the cognitive evaluation of these persons. A large portion of immigrants from Mexico and its border with the U.S. tend to be from low educational, socioeconomic, and under-served backgrounds (Portes and Rumbaut, 1990; U.S. Bureau of the Census, 1984). This is an important factor to consider in NP evaluation as it directly impacts performance on most developmental neurocognitive procedures used to detect neurocognitive and neurobehavioral alterations (Adams et al., 1982; Ardila, Roselli, and Rosas, 1989; Laosa, 1984; Perez-Arce, 1984).

Language and its impact on neuropsychological performance (Gordon, 1980; Paradis, 1978) and the limited availability of suitable instruments in Spanish from which valid inferences can be drawn are two factors deserving careful consideration since a large proportion of individuals living along the U.S.-Mexico frontier are monolingual (Spanish) while others are bilingual (English/Spanish). The use of interpreters, as it relates to the assessment of cognitive functions, should be avoided as there is precedent in the NP literature to indicate that such an assessment posture is capable of biasing results (c.f., LaCalle, 1987).

The availability of normative data for these populations is also scarce (Ardila, Roselli, and Puente, 1994) precluding the appropriate use of a nomothetic approach to the interpretation of test results. This limitation is a strong argument supporting the use of experimental designs employing longitudinal or repeated measures allowing subjects to serve as their own controls as noted earlier. Issues associated with the standardized administration of developmental neuropsychological procedures, examiner competence, and migrational variables associated with these populations (c.f., Llorente, 1997; Llorente et al., in-press; U.S. Immigration and Naturalization Service, 1991; Warner, 1992), play a major role in these types of investigations. These influences, left unaccounted, are likely to bias the assessment of neurodevelopmental variables.

Other Potential Confounding Factors Associated with Cognitive Assessment

Other potential confounds capable of infringing upon the assessment of cognitive changes as a result of exposure to neurotoxins or pesticides in youths deserve special merit. Maternal drug use during gestation and nursing periods demand proper attention as they have direct bearing on child development outcome (Coles et al., 1992; Jacobson et al., 1994). Issues associated with the treatment of childhood illnesses through the use of multiple health care delivery systems readily available near both borders should also be addressed (Warner, 1992). Nutritional variables and family history of medical and psychiatric illnesses (c.f., Brockman and Ricciuti, 1971; Cravioto and DeLicardie, 1966) are potential confounds worthy of exploration as they may also account for a

portion of the variance in neurodevelopmental performance which otherwise may be inadvertently attributed to neurotoxins.

Tests and Procedures to Evaluate Developmental Neurocognitive and Neurobehavioral Alterations Subsequent to Neurotoxicity

Certain health and research organizations and individual investigators have made recommendations with regard to the assessment of neurotoxicity in humans (see Hartman [1995] for a comprehensive review). The World Health Organization (WHO) Neurobehavioral Core Test Battery was developed for adolescents and adults for this purpose. This battery assesses several domains including motor skills (steadiness and fine motor coordination), attention and concentration (response speed), perceptual speed, visual processing, learning and memory (visual and auditory), and affect (Baker and Letz, 1986). With regard to children, Winneke and Collet (1985), as part of an effort spearheaded by the WHO, also recommended assessing a broad range of domains when evaluating cognitive changes associated with exposure to neurotoxins including overall intellect, attention and concentration (e.g., reaction time), visual processing and visuo-motor abilities, and motor skills. Similarly, a relatively recent attempt at developing a comprehensive battery of tests and procedures for youths was conducted by the U.S. Government, Agency for Toxic Substances and Disease Registry (ATSDR). The outcome of that effort was the Pediatric Environmental Neurobehavioral Test Battery (PENTB) (ATSDR, 1997). This battery of tests and procedures assesses a broad range of abilities and skills through the use of instruments administered to the child and the caretaker. Unfortunately, the assessment of infants as part of this effort was left to the sole use of rating scales to be completed by the caretaker failing to take advantage of individually administered procedures available for infants and young children useful in detecting changes in neurocognition. Nevertheless, these efforts represent great strides in the assessment of neurobehavioral effects of neurotoxins in infancy and childhood. Table 1 below shows a list of recommended developmental neurocognitive and neurobehavioral tests and procedures capable of detecting cognitive changes subsequent to chronic low-level exposure to pesticides and other neurotoxicants through multiple pathways from infancy to late childhood. The domains assessed by these instruments include overall developmental or intellectual functioning, attention and concentration, language, learning and memory (verbal and visual), motor skills, neurobehavioral functioning including adaptation, and visual processing. The overall aim of such a list of instruments is the development of a battery of tests with enough sensitivity, which in conjunction with the use of repeated observations, will be capable of detecting subtle neurocognitive changes subsequent to pesticide exposure.

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TABLE 1

Recommended Developmental Neuropsychological and Neurobehavioral Tests and Procedures by Age and Domain[1]

I. Developmental Tests and Procedures (Ages 4 months-42 months)[2]

Instrument	Recommended Age Range[3]
Bayley Scales of Infant Development	≥ 4 months-42 months
Child Development Inventory	≥ 15 months
Minnesota Infant Development Inventory	4-15 months
Vineland Adaptive Behavior Scales	≥ 4 months

II. Procedures and Tests for Pre-school and School-age Children (Ages 48 months and older)[4]

Domain/Instrument	Recommended Age Range[3]
Intellectual	
Woodcock-Johnson Tests of Cognitive Abilities (WJ-R; Subtests 1-14)	≥ 4 years
Attention/Concentration	
Children's Color Trails 1 and 2	> 6 years
Numbers Reversed (WJ-R, Subtest 9)	≥ 3 years
Reaction Time (e.g., Gordon Diagnostic)	> 6 years
Trailmaking Test A and B (Children's Version)	> 9 years
Language	
Verbal Fluency	≥ 6 years
Clinical Evaluation of Language Fundamentals (CELF-Third Edition, expressive and receptive tests only)	≥ 6 years
Learning and Memory	
WJ-R Memory Scales (Subtests 1, 2, 8, 9, 15, 16)	≥ 3 years
Stanford-Binet-4th ed. (Bead Memory)	≥ 3 years
Rey-Osterrieth Complex Figure	≥ 6 years
Motor	
Bruininsks-Oseretsky Motor Proficiency Tests	> 5.5 years
Grip Strength Test (Child Version)	> 6 years

Table 1. Recommended Developmental Neuropsychological and Neurobehavioral Tests and Procedures by Age and Domain[1] (continued)

Grooved Pegboard Test (Child Administration)	> 5 years
Peabody Motor Scales	> 1 year-6 years
Visual Processing	
Developmental Test of Visual-Motor Integration	≥ 3 years
Stanford-Binet-4th ed. (Pattern Analysis)	≥ 3 years
Rey-Osterrieth Complex Figure (Copy)	≥ 6 years
Adaptive/Behavioral/Emotional (Administered or Completed by the Caretaker)	
Child Behavior Checklist	≥ 4 years
Conners' Rating Scales	≥ 3 years
Vineland Adaptive Behavior Scales	≥ 4 months

Notes:

[1]The test selection shown above attempted to take into consideration as much as possible the use of tests and procedures that have been adapted (not translated) in Spanish so they can be used with populations along the U.S.-Mexico border.

[2]The battery of tests for infants should not surpass an hour's time in its administration.

[3]Age-range shown does not necessarily represent the lower age-bound of the test as published by the test developer.

[4]A battery from this list of tests and procedures should be selected to be administered to children at specific age levels. The battery selected should not surpass more than four hours in administration time (including test breaks) for children 48 months of age or older.

[5]The reader is referred to Lezak (1995), Sattler (1988), and Spreen and Strauss (1991) for references for the tests and procedures presented above.

Pesticides and Children: Pulmonary Outcome Measures

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Air pollution and pesticides are two important health concerns for communities along the U.S.-Mexico border. Abundant literature exists regarding the association of air pollution and pesticides with pulmonary complications, but not much is known about the potential effects of chronic pesticide exposure on lung function. To begin with air pollution, Ware et al. (1986) found that higher particulate levels were associated with increases in cough frequency, bronchitis, and a composite measure of lower respiratory illness. In addition, a review of several studies of ambient air pollution and respiratory disease concluded that pollution, while not a causal factor in its development, aggravates preexisting respiratory disease (Abramson and Voigt, 1991). Air pollution has been associated with bronchoconstriction both in healthy individuals as well as in asthmatics. Turning to pesticides, there is extensive literature available regarding acute pesticide poisoning, but, little has been published describing lung function abnormalities and chronic pesticide exposure. Both bronchorrhea and bronchoconstriction have been observed in acute pesticide poisonings, while, interstitial lung disease has been described in association with paraquat exposure (Reigart, 1995).

Thus, the literature reveals little about chronic pesticide exposure and its potential effects on lung function. In planning a study to identify pulmonary sequelae of such exposure, there are several outcome measures which might be used. These include clinical data, questionnaires, and physiologic measures.

The first outcome measure to consider is clinical data. Advantages of utilizing clinical data include such features as economy and availability, as well as a contemporaneous record. Being a contemporaneous record, recall bias is nearly eliminated since the data were obtained at the time of the visit. Health care utilization patterns, on the other hand, may bias the results. Subjects of lower socioeconomic status, though they may actually have worse lung disease, may not frequent a physician on a regular basis. This population may wait till their acute symptoms are severe before going to an emergency department or urgent care facility, or may not go at all. In this setting, continuity of care and, therefore, records are less likely.

Questionnaires are another relevant aspect of studying lung function abnormalities in different populations. Before getting into questionnaires I will briefly explain the Tucson Children's Respiratory Study (CRS) since I will be using it as an example in the remainder of this presentation. This study is an ongoing, longitudinal study of respiratory health in 1,246 children which utilizes a combination of physician reporting, questionnaires, home visits, and physiologic studies to examine potential risk factors and their association with the development of respiratory disease (Taussig et al., 1989). Questionnaires provide more detailed and in-depth information about the targeted population than clinical charts do since questionnaires can be geared to the specific study group. In addition, they can be self-administered as well as coded and stored in a computerized data base. When administering a questionnaire, both linguistic and cultural compatibility with the

targeted population must be considered. If not self-administered, trained personnel are required. In contrast to what we observed with the clinical data, recall bias can be introduced with questionnaires.

Other challenges to be considered when preparing a questionnaire include labeling of symptoms, translation, and cultural differences. Regarding labeling of symptoms, one must know what terms subjects in the study area may use to define the outcome variables of interest. After knowing the local idioms and local definitions of respiratory outcome variables, as well as of their symptoms, one can translate the questionnaire. In doing so, one can aim to get a better understanding of what is happening in the study populations.

Some examples of questionnaires used to assess respiratory outcomes include: the CRS questionnaire, the American Thoracic Society/NIH Division of Lung Diseases (ATS/DLD) questionnaire, The International Study of Asthma and Allergies in Childhood (ISAAC), and the American Thoracic Society questionnaire. The CRS questionnaire has been useful in categorizing children with respect to distinct asthma phenotypes which has resulted in the ability to correlate physiologic measures with clinical disease expression. In older children one can use the ISAAC which consists of a video tape with five different vignettes. The video tape is shown in conjunction with the administration of a questionnaire to which the parents respond. No words are spoken in the tape: rather images and audio of different symptom complexes are provided. Presently there is an international effort by ATS and NIH to create a new standardized questionnaire that contains different modules (i.e., a module for respiratory core questions for children and another for adults).

In addition to clinical data and questionnaires, physiologic outcome measures can be entertained. Pulmonary physiologic measures can correlate exposure with disease, are relatively simple to perform, are noninvasive, and are reproducible. Challenges include the requirement for special equipment and trained personnel resulting in increased cost and subject cooperation.

The pulmonary physiologic parameters that can be measured depend on the child's age. In young children, four to five years old, one can obtain partial expiratory flow volume curves (PEFV). An example of a PEFV curve can be seen in Figure 1. These curves are reproducible, correlate with disease, and allow tracking over time. Of the original 1246 children in the CRS, PEFV curves were obtained in approximately 600 (males: n=279, females: n=298). Seventy-five percent of these 600 also completed a cold dry air challenge. The mean age was 6.04 years with 40% being younger than six years of age. These curves allow for measurement of maximal flow at functional residual capacity, a good measure of airway function. In assessing the effectiveness of this measure, the Tucson group found that intersubject variability was 29.5%. While this may appear to be high, variability was actually lower than that found in the adult population using comparable measures. The intrasubject variability was only 7.9%.

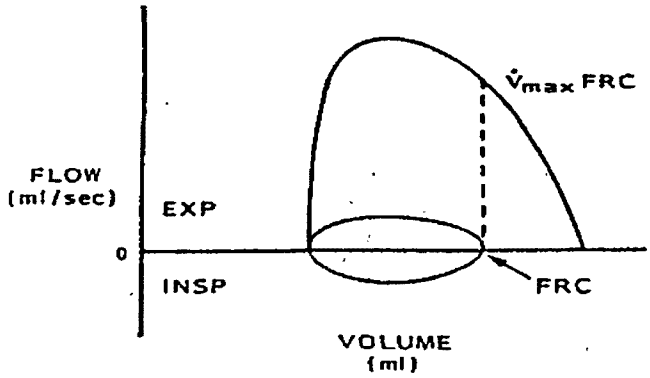
One can standardize these measures by indexing V'max FRC by lung volume (V'max FRC/FRC). When V'max FRC/FRC is plotted against the four different groups of wheezing obtained from the questionnaire, one can see how this measure can assess lung function at a given point in time. The four different wheezing groups consist of: (1) children who had not wheezed by age six, (2) transient early wheezers who wheezed early in life, but not at six years of age, (3) late wheezers who did not wheeze early in life, but did wheeze by six years old, and (4) persistent wheezers who wheezed both in early life as well as at six years of age (Martinez et al., 1995).

It is important to keep in mind factors other than disease or exposure, such as height, which may be important in interpreting physiologic measures. The taller the subject, the larger the lung volume (FRC). PEFV curves can be used to assess lung function at a point in time, as well as for long-term follow up. In addition, PEFV curves can also be used in conjunction with cold dry air (CDA) and methacholine challenges to assess bronchial reactivity.

When assessing lung function in older children one can use standard spirometry, since greater subject cooperation is obtainable. These two measures (spirometry and PEFV) are comparable in utility. However, spirometry is a more complete assessment of pulmonary function. Another measure that can be used in both older and a younger child is peak expiratory flow rate (PEFR). Though there is wide intersubject variability, the intrasubject reproducibility is good. This measure is very good for trending. If a restrictive abnormality of lung function is found, one can further test the older child by doing a diffusing capacity of the lung for carbon monoxide (D_LCO). The D_LCO is a measure of gas exchange at the level of the alveolar membrane. Though it is a good indicator of interstitial lung disease, it requires extensive subject cooperation and additional equipment.

In summary, it is clear that pollutants aggravate lung disease in children, but little is known about chronic effects of pesticide exposure on the lung health of children. We have also seen that while clinical data is inexpensive and reliable, it provides only non-directed information and may not be available for all potential subjects. Questionnaires provide more in-depth information, but require consideration of cultural and linguistic features of the population. Lastly, physiologic outcome measures provide correlates to clinical/questionnaire data, correlates with disease, and allow longitudinal tracking, but also add complexity and expense.

Figure 1



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Pesticides and Childhood Cancer - An Overview

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Background

The epidemiological literature contains numerous studies that implicate pesticides as a risk factor for increased cancer risk, in both adults and children. However much of that literature reports modest increases in risk, does not establish that these associations are causal, and commonly does not implicate specific agents. Appreciation of the limitations of the epidemiological approach, and of the fact that many reported exposure-disease associations that have enjoyed brief notoriety have faded under closer scrutiny, suggests that we need to be cautious about the link between pesticides and cancer. Perhaps the most persuasive argument in support of the association is its specificity: in study after study, where increases in cancer risk have been reported the malignancy arises from the lymphoid or hematopoietic system. This is apparent in both case-control studies in adults, where lymphomas are the malignancies most frequently linked to pesticide exposure, and in children where studies of acute lymphoblastic leukemia (ALL), acute myoblastic leukemia (AML), and lymphoma have all yielded positive results.

Methodological Considerations

In children, a **cohort approach** is infeasible because of the rarity of the endpoint—each year, approximately 3 new cases of the most common cancer (ALL) occur per 100,000 children under the age of 15. Occasionally, **cancer clusters** come under investigation, and some of these are in locales where above average pesticide exposure naturally leads to suspicion of these chemicals, but the study of clusters is generally a frustrating and unrewarding business and rarely yields useful insights into the basis for the cluster. **Ecological studies**, showing spacial (and/or temporal) patterns of cancers that correlate with documented patterns of pesticide use can provide indirect evidence for an association, but such studies will be limited in the U.S. until a national population-based registry for pediatric cancers is available.

In practice, **case-control** designs, with cases necessarily drawn from a very large population, have provided most of the data on cancer risk associated with pesticide use.

Published Data on Pesticides and Cancer

Zahm and Blair (1992) recently summarized the data concerning pesticides and non-Hodgkins lymphoma (NHL). They compiled reports from 21 cohort studies of farmers and found 11 with odds ratios (OR) greater than 1, 3 significantly so, with OR ranging from 0.6 to 2.6. In 19 case-control and cross-sectional studies, which might be expected to provide better data, 12 gave OR greater than 1 with 8 significant. Zahm and Blair conclude that these data are equivocal, possibly due to the fact that exposure is inferred from a broad occupational category "farming."

Studies based on more specific exposure data have generally shown higher risk estimates. Hardell et al. (1981) reported a 6-fold risk of lymphoma for persons with exposure to phenoxyacetic

acid herbicides (a class including 2,4-D and 2,4,5-T) or chlorophenols. Hoar et al. (1986) found an OR of 2.2 for NHL in farmers who used phenoxy herbicides, with OR over 7 for those reporting more than 20 days use of 2,4-D per year. Risk was also increased in those not reporting use of protective equipment. In a similar study in Nebraska the risk was 3.3 for farmers handling 2,4-D for more than 20 day per year. LaVecchia et al. (1989) report a significant trend with duration of exposure to herbicides and Persson et al. (1989) report an OR of 4.9 for NHL for occupations exposed to phenoxy acids. Pearce et al. (1987) reported a significant OR of 3.7 for orchard workers. Ollson and Brandt (1981) found a greatly elevated risk of cutaneous NHL (OR=10.0) with exposure to phenoxy acids, but a much smaller OR (1.3) overall. Other pesticides have been studied less extensively, but significant positive results have been reported for atrazine, chlorophenols, and fungicides in general.

Similar associations have been reported for leukemia and multiple myeloma in adults (Viel and Richardson, 1991; Burmeister, 1990; Brown et al., 1990; Cantor and Booze, 1989) and in children (Buckley et al., 1989; Buckley et al., 1994; Lowengart et al., 1987; Savitz et al., 1995).

Childhood Cancer - Results from the Children's Cancer Group

The Children's Cancer Group (CCG) is a cooperative clinical trials group that includes over 100 hospitals in the U.S. and Canada. It has had an active epidemiological research program for many years, and has conducted case-control studies on most of the major cancers in children (Robison et al., 1995). Although the focus of these studies has varied, most have included questions about exposure of the child (and parents) to pesticides.

The first generation of studies tended to cover a wide range of exposures and associations of possible relevance, and consequently most topics received relatively superficial attention - in the case of pesticide exposure, the questionnaire typically asked about pesticides as a single entity; in some studies we asked about home, garden, and occupational exposures separately. Significant associations were found for both acute myeloblastic leukemia (AML - Study CCG-E05, Table 1, Buckley et al., 1989) and NHL (Study CCG-E08, Table 2).

As interesting as these results were, the lack of detail concerning the types of pesticides, the pests being treated, periods of use, protective measures employed, and who was exposed, made interpretation difficult. In the next generation of studies (CCG-E14 for AML, CCG-E15 for ALL, and a proposed study of NHL) we attempted to obtain this detail, through a much more comprehensive interview and, for CCG-E15, through in-home measurements. Analysis of these data is underway.

Methods

As outlined earlier, ecologic studies and investigation of clusters have a role to play, but it is likely that case-control studies will be continue to be the method of choice. However, the investigator hoping to confirm and better delineate a link between pesticide exposure and childhood cancer risk faces some formidable obstacles. These may include issues relating to the diagnosis, to case and control selection, to control for sociodemographic confounders—as for any case-control study—but the overriding problem is EXPOSURE ASSESSMENT.

Table 1: Odds Ratios (OR) for Childhood AML Associated with Pesticide Exposures. (The person/group exposed is given in parenthesis. p-values are tests of trend.)

Exposure	Category	Cases/Controls	OR	p-value
Household pesticides (child)	None	128/148	1.00	
	<1/wk	46/33	1.77	
	1-2/wk	13/9	2.02	
	most days	8/3	3.48	0.04
Occupational pesticides (parent)				
All ages	None	164/182	1.00	
	1-1,000 days	16/15	1.50	
	>1,000 days	24/7	4.26	0.001
Age 5 and under	None	75/89	1.00	
	1-1,000 days	8/5	1.91	
	>1,000 days	12/1	12.79	0.001
Monocytic/myelomonocytic	None	49/59	1.00	
	1-1,000 days	7/2	8.88	
	>1,000 days	6/1	16.17	0.002

Table 2: Odds Ratios (OR.) for Childhood NHL Associated with Pesticide Exposures. (The person exposed is given in parenthesis. p-values are tests of trend.)

Exposure	Category	Cases/Controls	OR	p-value
Household insecticides (mother)	Never	185/199	1.00	
	<1/wk	46/51	0.97	
	1-2/wk	18/10	1.94	
	most days	6/1	6.45	0.06
Garden sprays (mother)	Never	237/253	1.00	
	<1/mo	9/6	1.60	
	>1/mo	14/8	1.87	0.13
Exterminate around home (mother)	No	238/257	1.00	
	Yes	31/12	2.78	0.004
Herbicides or pesticides (child)	No	215/244	1.00	
	Yes	50/23	2.47	0.0009
Occupational pesticides (parent)	No	248/256	1.00	
	Yes	21/13	1.67	0.21

Exposure Assessment

The simplest, cheapest, and most direct method is to ask the parents about exposures. While most parents can provide information about general patterns of pesticide use, this approach in unlikely to yield useful detail regarding the chemicals used, and generally gives only a crude estimate of the amount the child will have been exposed to over his/her lifetime. For older children, the questionnaire must elicit details of use going back many years. In addition there will always be concerns that differential response rates of case and control parents will introduce a systematic bias. Further complicating the issue is uncertainty about the critical period of exposure—pre-conception, *in utero*, or postnatal?

Similarly, determining parental occupational exposure by questionnaire is problematic. Again, the specific chemicals may be unknown and the quantities impossible to estimate reliably, but in addition the pathway from exposure to effect in the child is not known. If it is via a genotoxic effect to the testes/ovaries, pre-conception exposure is what counts; if the effect is due to exposure of the child, the model must include a means of transferring the chemical from the workplace to the home. Quantitating exposure, based on questionnaire data, under such a model is clearly impossible.

In CCG studies we have tried a number of approaches to determining pesticide exposures for children in our studies. One is to concentrate on the products used (e.g., insect sprays); an alternative is to concentrate on the pests (e.g., termites, weeds). Another approach that relies less on memory, but may give a limited snapshot of pesticide use, is to take an inventory of products in the home at the time of interview.

An alternative to the questionnaire-based approach is to sample from the child, parent, or the home environment. While this seems to be an appealing way of avoiding the limitations of a questionnaire-based approach, it is not without its own difficulties.

Environmental/Sampling

Direct measurement of residues of pesticides inside or around the home(s) that the child has lived in is certainly possible under some circumstances. I will focus on housedust sampling, since this is the approach used in a recent CCG study, but similar considerations will apply to other sampling methods (e.g., air, water, or soil sampling).

Advantages to this approach are obvious there: such assays provide a **quantitative** and **objective** measure of **specific** pesticide residues in proximity to the child.

While this is very appealing, the **limitations** and difficulties need also to be carefully considered:

(1) Proper sampling requires specialized sampling equipment (essentially a purpose-built vacuum cleaner), a clearly defined sampling protocol that covers such things as the area(s) to be sampled, the vacuuming procedure, and cleaning procedures between samples. Clearly, it also requires home access, which could be problematic for many reasons, especially for national studies.

(2) The specificity of the assays is a double-edged sword, since there is no way of knowing whether the important residue(s) have been included in the list of substances to measure.

(3) Assays are expensive, which can limit the number of subjects being tested, and/or the number of samples per household.

(4) There can be technical problems with the assays due in part to the fact that the sample (dust) contains many organic contaminants. In the CCG study, there was substantial batch-to-batch variation for many analytes.

(5) This approach measures only one source of exposure. Thus housedust sampling ignores the contributions from water and food.

(6) The effective (internal) dose to the child is not known.

(7) The measurement is of the current concentrations of the analytes. This reflects not only patterns of recent use, but also stability of the residue in housedust—which will vary greatly for different residues—and patterns of past use, including use by previous occupants. Since we know little about the most important period of exposure for these children, the extent to which the measurement captures relevant data is not clear.

It is useful to compare housedust measurements with questionnaire data. Questionnaire data can potentially be targeted to specific time periods, such as when the child was *in utero*, or can cover an interval of many years and may include both the current and previous homes. However, the quality of the data is questionable. Since a recent CCG case-control study of childhood ALL included both questionnaire and housedust measurements, it is possible to determine the extent to which these approaches agree, at least for some types of pesticides. Presented in Tables 3a and 3b are preliminary results from this study. These tables show odds ratios for any versus no self-reported use of pesticides to treat selected pests, based on questionnaire data alone, classifying individuals according to the amount of pesticide (grouped according to the common use(s) of the pesticide) found in the housedust of the child's home. In general, it can be seen that the self-report is consistent with measurements made on the dust in that parents reporting that they treated fleas or ticks, say, tended to fall into the upper tertile with respect to measured quantities of products used to treat fleas and ticks. The associations are not particularly strong, but are remarkable none the less. There are a host of reasons why associations might be expected to be weak and difficult to detect. (1) Products classified under a particular use may have many other uses. (2) Products classified under a particular use represent only a portion of all products available for that purpose and thus may not include the product used by the parent. (3) The measurement reflects recent use plus a (variable) contribution from past use in the home. The sample is from a single site, that may not be ideal for any given type of pesticide. In contrast the reported use is either during a specific time period (the pregnancy)—commonly many years previously—or averaged over most of the child's life. This use may actually have been in a previous home. Usage could have occurred in any room, or outdoors. (4) Misclassification is likely in the self-reported use due to difficulties in recalling events long in the past. (5) Technical difficulties with the assays will create misclassifications in the measured values.

Biological Sampling

Biological sampling may circumvent a number of the problems associated with environmental sampling (e.g., internal dose, exposure from multiple sources) but may not solve others (e.g., choice of relevant analytes, cost). Drawing samples directly from the case (or control) may be undertaken for essentially two reasons:

(1) to measure **pesticide load** in the body—for example the concentration of DDT residues in fat.

(2) to measure possible **biological effects** of pesticide exposure. Such effects could be sufficiently specific as to implicate a single compound (e.g., adduct formation) or may be quite non-specific and only be tied to pesticide exposure through ancillary data, such as self-reported pesticide use. One biological marker of interest is the frequency of translocations mediated by VDJ-recombinase. This enzyme system is responsible for splicing of genomic DNA in maturing lymphocytes to produce diversity in antibody and T-cell receptor proteins. It also appears to act aberrantly, at low frequency in normal lymphocytes to produce translocations at a variety of sites (Cortopassi and Arnheim, 1992; Fuscoe et al., 1992), and some of these translocations are important in the pathogenesis of leukemia (Gu et al., 1992) and lymphoma (Kirsch and Lista, 1996). Completing this model is a study which shows that agricultural workers had an elevated frequency of aberrant VDJ recombination during the summer months, when they were most exposed to pesticides (Lipkowitz et al., 1992). CCG currently has a proposal under review to obtain questionnaire data on pesticide use from approximately 600 children with lymphoma and an equal number of matched controls, and to examine the frequency of aberrant VDJ recombinase translocations in the cases.

Conclusions

While there is ample reason to suspect that exposure to some pesticides may increase the risk of lymphoma and leukemia in children, confirming the link in a convincing fashion and determining what the agent(s) and mechanism(s) are, is a formidable challenge. Questionnaires can be improved to obtain the most reliable information possible, but it is unlikely that questionnaire data alone will suffice. Quantitative assessment of exposure and/or biological markers associated with pesticide exposure, combined with interview data, may be necessary to make substantial progress.

Table 3a: Comparison of Pests Treated (By Questionnaire) and Detection of Pesticide Residues in Housedust. The period (for self-report) is during the index pregnancy only. (p-values are for trend.)

Pesticide Group		Pests Treated (According to Questionnaire)		
		Fleas/ticks	Termites	Weeds
	Tertile			
Flea/tick products	1	1.00	-	1.00
	2	1.62		1.67
	3	2.60		1.04
		p=0.009		p=0.39
Termite control	1	1.00	1.00	1.00
	2	1.06	1.83	1.38
	3	1.61	3.02	1.05
		p=0.06	p=0.23	p=0.62
Herbicides	1	1.00	1.00	1.00
	2	1.43	1.81	2.27
	3	0.63	0.87	1.47
		p=0.93	p=0.74	p=0.01

Table 3b: Comparison of Pests Treated (By Questionnaire) and Detection of Pesticide Residues in Housedust. The period (for self-report) is from the index pregnancy to a year prior to diagnosis. (p-values are for trend.)

Pesticide Group		Pests Treated (According to Questionnaire)		
		Fleas/ticks	Termites	Weeds
	Tertile			
Flea/tick products	1	1.00	1.00	1.00
	2	4.32	0.88	0.63
	3	5.16	1.32	0.78
		p<0.0001	p=0.59	p=0.23
Termite control	1	1.00	1.00	1.00
	2	1.69	0.61	0.83
	3	3.61	2.06	0.84
		p<0.0001	p=0.07	p=0.44
	Median			
Herbicides	Below	1.00	1.00	1.00
	Above	1.00	0.85	1.58
		p=0.99	p=0.93	p=0.08

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Health Effects of Pesticides

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While the efficacy of pesticides for the protection/preservation of health, food, and fiber cannot be disputed, there are costs for these benefits, one being an estimated, annual, 3 million cases of acute, life-threatening intoxications worldwide, perhaps as many or more unreported cases and some 220,000 deaths (Hayes, 1992). There is a long history of pesticide poisonings among workers in agriculture, in residential treatment and in public health (Hayes, 1992). However, none of the numbers quoted take into account persistent and/or delayed effects arising from either acute, high-level or from prolonged, low-level exposures, including adverse reproductive outcomes (fertility, abortions, teratology), neurological and neurobehavioral development. Such endpoints of toxicity are poorly studied or go unreported except as "interesting" anecdotal cases in the literature. The incidence of acute poisonings in emerging nations is some 13-fold higher than in industrialized, agricultural nations. One can only suspect that there might be an association of similar magnitude for other endpoints of toxicity.

I am not going to address pesticide-induced, acute toxicity since this aspect has been well described by many authors, myself included (Ecobichon, 1996; Ecobichon, 1995). My interest lies with the immediate and later-developing neurotoxicity as a consequence of acute and prolonged exposure to pesticides. By way of example, look at the scenarios presented in Figure 1. The afflicted individuals all showed similar neurological sequelae upon presentation and during later progression of their conditions. Identification of the causative agent would have required extensive chemical analysis. Neurological assessment would not have been conclusive. To simplify the cases, the first was caused by manzidan (Maneb-zineb, a fungicidal mixture), the second by diazinon, an insecticide, and the third by the herbicide, 2,4-D. Similar signs/symptoms can be elicited by different chemicals (Ecobichon and Joy, 1994).

Exposure may involve concentrated products or diluted, prepared-for-use formulations, either by dermal and/or inhalation: (1) during preparation and application; (2) as bystanders in sprayed areas (fields, greenhouses, homes); or (3) via environmentally deposited agents in water, food, or soil. As is shown in Figure 2, one is not only dealing with the active ingredients but also with "inerts," both petrochemical-based or completely synthetic compounds, many having the potential to elicit neurotoxicity themselves, without even contemplating possible interactions with the toxicity elicited by the pesticides. Perhaps, the best example is that of the herbicide glyphosate (N-phosphonomethylglycine) where the toxicity (gastrointestinal, respiratory, cardiovascular, CNS) can be attributed largely to the surfactant poly-oxyethyleneamine (POEA) used in formulations to promote wetting of plant surfaces and rapid penetration (Sawada et al., 1988; Adam et al., 1997).

My experience in Central America and southeast Asia with agricultural pesticide usage is that, once the crop is planted, the husband usually migrates to an urban area in search of work, leaving the care of the crop, including pesticide applications, to his wife and the older, pre- and post-

pubertal children. When spraying occurs, everyone goes to the fields or paddies, the younger children tending the toddlers on site, frequently on ground areas where chemicals are stored, spilled from concentrates during dilution or dumped at the day's end when tanks are rinsed. With little in the way of protective clothing, those spraying (mothers, sons, daughters) receive extensive dermal exposure. The younger family members, staying at the storage/mixing/loading site, will receive oral as well as dermal exposure from the contaminated soil.

Do such agricultural practices elicit adverse health effects? In southeast Asia, there is an exceptionally high incidence of birth defects in agricultural (rice growing) regions. With rice, there is extensive use of herbicides, insecticides, and fungicides from preplanting through to harvesting. A colleague in Thailand just shakes his head when questioned about the obvious association. He has been unable to amass a suitable data base through regional hospitals. In the same agricultural region, there were an estimated 8,268 pesticide poisonings/100,000 workers reported for 1983 (Boon-Long et al., 1986).

Returning to neurotoxicity, early data from Washington state reveals just how much material (DDT, parathion) was being applied to apple orchards (Table 1) (Batchelor, 1953). In 1951-52, DDT was rapidly becoming the panacea for all insect problems while parathion was making its debut as an alternative insecticide. Not surprisingly, acute toxicity was observed! One of my colleagues claims that if it had not been for parathion, it might have taken much longer to learn about this class of insecticides. The paper of Grob et al. in 1950 provided conclusive evidence of the methanism(s) of organophosphorus ester (parathion) toxicity (Grob et al., 1950). The paper of Summerford et al. in 1953 and the landmark paper of Batchelor and Walker in 1954 demonstrated both the toxicity and the estimated occupational exposure to parathion (Summerford et al., 1953; Batchelor and Walker, 1954).

By the mid-1950s and early 1960s, there was ample evidence in the literature of the persistent neurological effects following organophosphorus ester poisoning in agricultural workers. However, much of this data was ignored or was treated as anecdotal or irrelevant. In Canada, Davignon and her colleagues, in a study of apple growers, demonstrated higher (and persistent) incidence of neurological manifestations and anomalies among insecticide handlers which correlated well with the number of years of exposure to both organochlorine and organophosphorus insecticides (Davignon et al., 1965). The 1983 study by Wharton and Obrinsky was, in my opinion, a watershed publication for those interested in long-term effects, the study demonstrating the persistence of blurred vision, muscle weakness, headaches, and nausea for up to four months after poisoning (Whorton and Obrinsky, 1983). It would not be surprising to learn that, even in the present decade, there are studies (and highly sophisticated ones) still being conducted on orchard workers in Washington state (Rosenstock et al., 1990).

TABLE 1
Ground Application of DDT and Parathion to Fruit Orchards in North Central Washington State in 1951 and 1952

Formulations	Material Applied (lb/acre)					
	DDT			Parathion		
	H	L	M	H	L	M
Liquid (suspension or emulsion)	22.5	1.0	15.1	5.0	0.3	2.3 *
	25.0	1.8	9.4	9.8	0.2	1.6 **
Dusts	12.0	10.7	10.3	1.6	1.2	1.4 **

* Hand-spray equipmen

** Portable, power-driven (truck, tractor drawn)

My personal involvement in the persistent neurological problems of pesticide poisonings began in 1976 with a case of acute intoxication of an adult, female technician by the organophosphorus ester, fenetrothion (Ecobichon et al., 1997). The patient developed an interesting set of neurological and psychiatric sequelae approximately three weeks after the subsidence of the characteristic acute signs and symptoms, these persisting to some degree for almost a year after the event (Table 2). These signs/symptoms were common in the nerve gas literature but not for insecticides except in "anecdotal" published papers. Intrigued, a colleague and I reviewed the literature pertaining to pesticides and neurological diseases, these efforts culminating in a book of the same title, in 1982 and a revised, second edition in 1994 (Ecobichon and Joy, 1982;1994). By 1994, there was a lot more relevant material in the literature, as people began to recognize subtle neurological changes. To make a long story much shorter, in addition to the neuromuscular effects (fasciculations, tremors, persistent muscular weakness), there are a large number of neurobehavioral changes associated with pesticide exposure (Table 3). How does one begin to test those parameters?

TABLE 2
PERSISTENT SYMPTOMS ASSOCIATED WITH ACUTE FENITROTHION POISONING*

- Anxiety
- Frequent headaches
- Nausea
- Inability to concentrate
- Trembling, muscle spasms
- Muscle cramps, especially in legs
- Generalized muscle weakness
- Fatigue and lethargy
- Mental depression

* From Ecobichon et al. Can. Med. Assoc. J. 116, 377-380 (1977)

Over the years, a variety of test batteries have been developed, the significant characteristic being the frustrating mix-and-match approach as investigators select some tests and reject others in attempts to fine-tune measurements in the hope of improving the results in a meaningful manner. This has been only partially successful. One early test battery is that developed by Feldman et al. (Feldman et al., 1980) (Table 4). The WHO-UNDP core test battery has proven useful but, as can be seen in Table 5, it is specific only for anti-cholinesterase insecticides (Maroni, 1986). WHO has also described a behavioral test battery (Table 6). The most recent assessment system has been that of Stephens and his colleagues, developed and tested in the "sheep dip" problem in the U.K., involving diazinon, propetamphos, or a mixture of diazinon and chlorfenvinphos (Stephens et al., 1995; Stephens et al., 1996) (Table 7). While we have not achieved "nirvana" in this field, the fine-tuning is producing more sophisticated endpoints of toxicity, as Stephens' results show.

TABLE 3
NEUROBEHAVIORAL SEQUELAE RELATED TO PESTICIDE POISONINGS

FEATURES	EFFECTS
Cognitive Disturbances	Reduced vigilance and alertness Attention deficits Slowed information processing Psychomotor retardation Impaired memory functions Reduced comprehension
Expressive Language Deficits	Speech difficulties Speech slurring, reduced enunciation Difficulties in saying what is intended formulating thoughts repetition
Psychopathological Sequelae	Depression Restlessness, insomnia Excessive dreaming Emotional lability Weeping spells Schizophrenic reactions Irritability Phobias Outbursts of temper (rage) Belligerent behavior Obsessive-compulsive behavior

The current legal matters in the southern U.S.A. associated with the indoor use of chlorpyrifos and effects on children bear examination. Given the now-familiar scenario that children are not "little

adults", body size/surface area differences and the ease of penetration of the skin, it is not surprising that toxicity has been observed with this rather potent organophosphorus ester. As is shown in Table 8, the number of children and adults "poisoned" by homeowner use of one agent, chlorpyrifos, is impressive to say the least (Blondell, 1997). To my knowledge, no one has addressed the possibilities of delayed and/or persistent effects. In a recent paper studying acute poisonings by two rather potent carbamate insecticides (methomyl, aldicarb), it would appear that children showed predominantly CNS effects (depression, seizures, hypotonia), while adults showed mostly PNS symptoms (miosis, fasciculations, bradycardia, bronchorrhea) (Lifshitz et al., 1997) (Table 9). These results suggest differences in susceptibility as well as in target sites.

TABLE 4
Common Tests Used to Detect Behavioral Effects of Neurotoxins*

TEST TYPE	TEST FUNCTION
Memory	Wechsler Memory Scale
Overall Intelligence	Wechsler Adult Intelligence Scale (WAIS)
Sustained Attention	Continuous Performance Test Bourdon-Wiersma Vigilance Neisser Letter Search
Dexterity and Eye-Hand Coordination	Santa Ana Dexterity Test Flanagan Coordination Test Michigan Eye-Hand Coordination Test Finger-tapping Test
Reaction Time	Simple reaction time test Choice reaction time test
Psychomotor Function	Mira Test Digit-symbol Substitution Task
Personality (Mood)	Eysenck personality Inventory Rorschach Test Feeling-tone Checklist

*Feldman et al. Am. J. Indus. Med. 1, 211-227 (1980).

How will we test subtle, neurotoxicological, psychological, and/or behavioral changes/adaptations in children? Most of the test batteries in use are focused on adults, are computer-driven, and have complex instructions and paradigms developed to isolate and identify specific endpoints/deficits. Reading some of the instructions for conducting these tests is a challenge in itself.

We have gone so far in the sophistication of these tests, from what used to be done with paper and pencil to keyboards and VDTs, that these have become impractical as simple tests. There are test batteries, e.g., the acquired cognitive test (ACT) by Stollery, which have seen use in heavy metal poisoning and solvent exposure (Stollery, 1996a; Stollery, 1996b; Stollery, 1996c). There are neurological assessment tests for children exposed to heavy metals. Can these be simplified and adapted for children's exposure to pesticides, co-solvents, emulsifiers, and surfactants? It would be a challenge for pediatric neurologists. While many young children, aged 7 years or older are computer literate, how can younger children (aged 5 or less) be tested?

TABLE 5
WHO-UNDP Core Methods for Health Assessment Following Pesticide Exposure*

Assessment	Data Collected
Exposure/Absorption	Type of agent, amount Metabolites in urine Plasma and erythrocytic cholinesterase activities
Health Effects	Background (health and Occupational) Symptoms/signs (based on VBC 82/1 and WHO/NIOSH questionnaire Neurological examination (semiquantitative) Neurobehavioral examination Nerve conduction (sural, ulnar and peroneal nerves). Neuromuscular junction function Behavioral tests (WHO battery)

*Maroni et al. Toxicol. Letters 33, 115-123 (1986)

The question that comes to mind is: why are we observing persistent neurological and neurobehavioral effects in adults and children as a consequence of exposure to pesticides? Are there any testable hypotheses? The anticholinesterase-induced neuropathic sequelae are poorly understood, and the search for other mechanisms of action continues (Figure 3). Firstly, there is considerable evidence of a direct interaction between organophosphorus esters and secondary targets such as muscarinic (mAChR) and nicotinic (nAChR) acetylcholine receptors, the results showing a competitive block (antagonism) at mAChR and an induced desensitization of nAChR following a partial agonistic effect (Eldefrawi et al., 1992). Evidence suggests that organophosphates may cause a hyperpolarization of nAChR with an inhibition of ACh binding (Bartels and Nachmansohn, 1969). Some of this work goes back to the extensive studies by Dettbarn where injections of small doses of paraoxon into the regions caused necrotic damage that now appears NOT to be artifact (Wecker and Dettbarn, 1977). At micromolar levels in the circulation, certainly attainable in poisonings, organophosphorus esters (or oxon metabolites) may directly induce toxicity at mAChR and nAChR whereas, at nanomolar levels, toxicity is due to the inhibition of nervous tissue AChE and the effects of ACh (Bakry, 1988). However, in many

studies, the agents tested (nerve gases, DFP, echothiophate) are not really representative of the insecticide molecule beyond sharing the same chemical classification.

TABLE 6
The WHO Behavioral Test Battery*

	Test Objectives/Purposes
Reaction Times	Auditory and visual
Santa Ana Test	Timed perceptual-motor coordination
Wechsler Digit-Symbol Test	Measuring perceptual and motor speed
Profile of Mood States	Measuring mood and affective states
Aiming Test	Measuring hand steadiness
Digit Span Test	Immediate auditory memory
Benton Visual Retention Test	Visual memory
Digit-Symbol Test	Measuring perceptual organization, motor dexterity, attention, speed of performance
Helsinki Subjective Symptom Questionnaire	Investigates psychological, neuro-vegetative, gastrointestinal and neurological symptoms

*Maroni et al Toxicol. Letters 33, 115-123 (1986)

Secondly, there is also considerable experimental evidence of a loss of acetylcholine (ACh) receptors during severe poisonings by organophosphorus ester insecticides AND prevention of their recovery (or re-synthesis) by localized damage and necrosis. This aspect was reviewed in a number of chapters in Chambers and Levi (1992). As is shown in Figure 4, the superabundance of ACh at nerve endings during the acute phase of the poisoning can have two effects: (1) stimulation, with a subsequent depolarizing blockade which is reversible; or (2) overstimulation, with a desensitization (reduced binding and affinity) of the receptors and a down-regulation of the receptor population as a built-in safety measure, with possible damage (necrosis?) to the postsynaptic membrane and an inability to synthesize new receptors. This has been shown to occur with both mAChR and nAChR in the peripheral and central nervous systems.

TABLE 7
Test Batteries Used in the "Sheep Dip" Studies in the United Kingdom*

Questionnaires	Demographic, lifestyles pesticide exposure history general health
Cognitive Tests	Automated Cognitive Test short-term memory visual spatial memory
	Sustained Attention-reaction time
	Information Processing symbol-digit test
	Syntactic Reasoning truth-falsehood of statements
	Long-Term Memory Test
	Serial Word Learning Test

*Stephens et al - Lancet 345, 1135-1139 (1995)
- Neurotox. Teratol. 18, 449-453 (1996)

Can either of these hypotheses, admittedly focussed on the organophosphorus esters, explain the persistent effects seen following anticholinesterase-type insecticide poisoning? In my opinion they can and are testable! Some years ago, carbaryl, administered subchronically to swine, was found to produce CNS edema and fragmentation of myelin tracts along with neuromuscular lesions showing necrosis (ischemic myodegeneration, acute hyaline and vacuolar degeneration, dystrophic calcification) (Smalley et al., 1969). More recently, carbaryl has been associated with persistent neuromuscular weakness and CNS effects in at least one individual. This and other incidents known to me have involved exceptionally high-level, exposure (Ecobichon and Joy, 1994). Is the active ingredient always the culprit? Could it be something else in the formulation (organic co-solvents, emulsifiers, surfactants, wetting or stabilizing agents) that causes the neurotoxicity? This takes me back to Figure 1 and scenarios #1 and #3 where the neurotoxicities were due to the fungicide manzidan and 2,4-D, respectively. There were other "ingredients" in those formulations, exposure to solvents and/or emulsifiers being excessive. Can these neurotoxicological test batteries differentiate between solvent-induced neuropathic changes and a pesticide-related toxicity? This is a definite challenge for future research!

TABLE 8
Adult and Child Chlorpyrifos-Related Cases Seen or Referred to Health Care Facilities (HCF) and Hospitalized-U.S. Poison Control Centers, 1985-1992.

Age Group	Health Care Facility		Hospitals	
	Homeowner	PCO	Homeowner	PCO
Non-occupat. adults	877	393	70	34
Children 0-5 yrs	828	150	84	23

Homeowner - 44 products used in home
PCO - 10 products used by pest control operators

TABLE 9
Symptoms Observed in Children and Adults With Methomyl and Aldicarb Intoxication*

	Children		Adults	
Symptoms	N	(%)	N	(%)
Coma/Stupor	36	100	0	
Hypotonia	36	100	0	
Seizures	3	8	0	
Miosis	20	55	22	91.7
Fasciculations	2	5.5	20	83.3
Bradycardia	6	16	8	33.3
Diarrhea	12	33	0	
Salivation	0		2	8.3
Bronchorrhea	0		4	16.6

*Lifshitz et al Clin Toxicol. 35, 25-27 (1997)

POISONING SCENARIOS

#1	MANZIDAN™	(Maneb, zineb) Ethylene bis-dithiocarbamates	FUNGICIDE

Dizziness, tiredness, muscle weakness, headache, nausea, slurred spech, disorientation, tonic and cloinic convulsions, loss of consciousness

#2	DIAZINON	organophosphorus ester	INSECTICIDE

Flu-like signs (headache, nausea, vomiting), physical weakness, slurred speech, muscle spasms, disorientation, memory loss, mental confusion

#3	2,4,-D	chlorophenoxy acetic acid	HERBICIDE

Nausea, vomiting, headache, dizziness, fatigue, muscle pain, myotonia

Fig. 1 Three poisoning scenarios involving pesticides, the signs and symptoms being similar, although caused by different pesticides - a fungicide, an insecticide and an herbicide.

PESTICIDE EXPOSURES

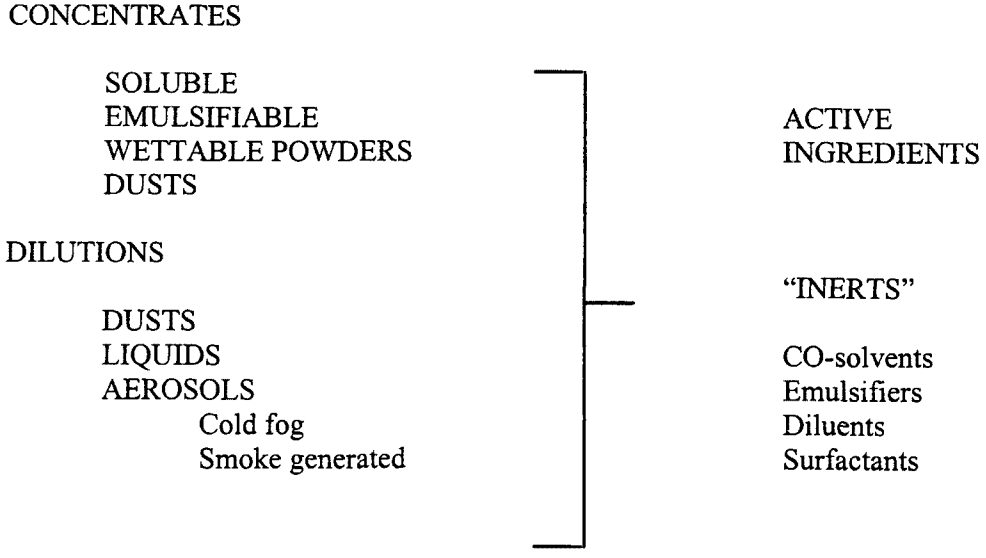

Fig. 2 Effects resulting from exposure to pesticides should be considered in the light of not only the formulation active ingredients but also the "inert" ingredients, agents required in the final end use product to maintain stability during spraying.

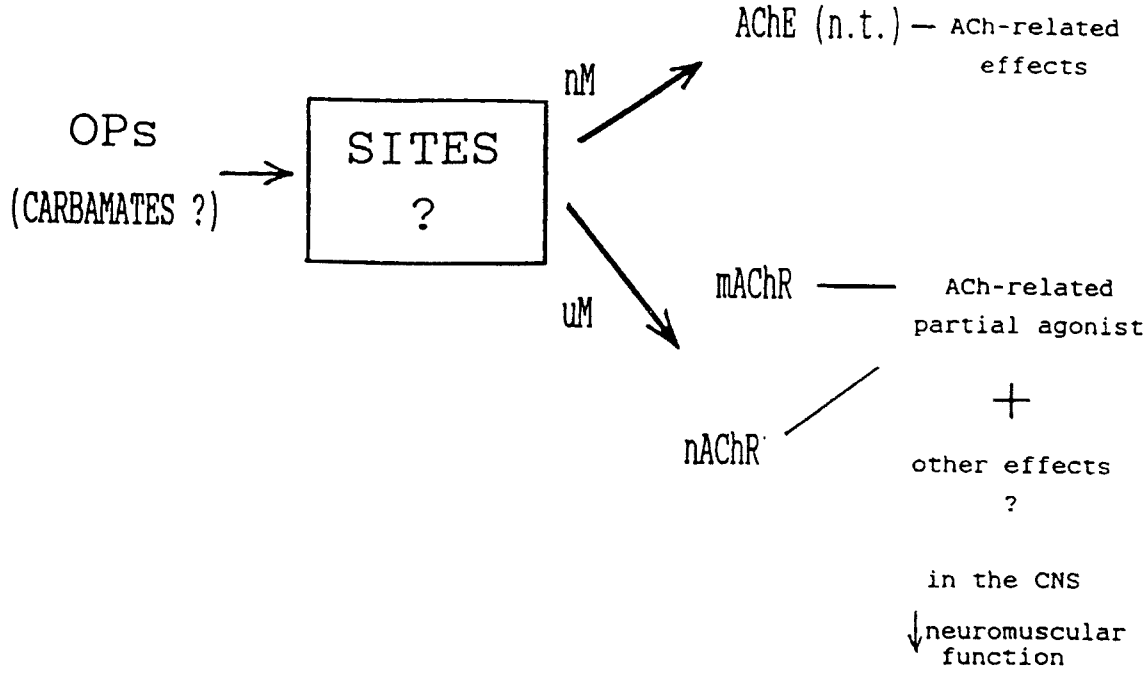

Fig. 3 A schematic diagram of sites of action of organophosphorus and possibly of carbamate ester insecticides in neuronal tissue. At nanomolar (nM) concentrations, inhibition of nervous tissue acetylcholinesterase (chE) would occur, with acetylcholine-related signs/symptoms. At micromolar (uM) levels, frequently seen in poisonings, direct action at muscarinic receptors (MAChR) and/or nicotinic receptors (nAChR) may result in a competitive blockade, hyper-polarization and/or desensitization of the receptors.

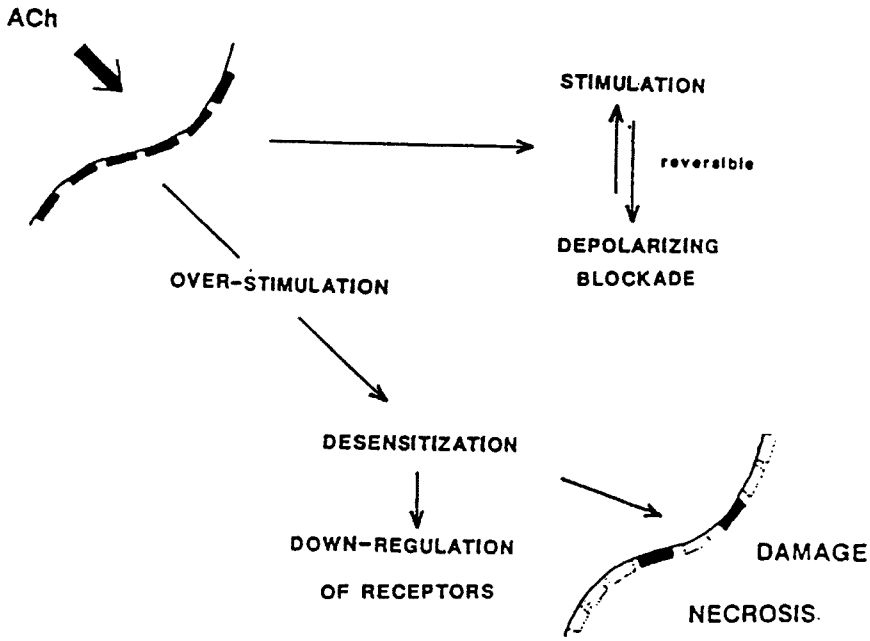

Fig. 4 A diagramatic representation of acetylcholine-induced effects of exposure to anticholinesterase-type insecticides. Low levels of agent might produce the classical events of stimulation with a reversible depolarizing blockade. High levels of accetylcholine (Ach) might elicit over-stimulation followed by desensitization and down-regulation of receptor numbers, with necrotic damage to postsynaptic and neuromuscular membranes and no resynthesis of the normal receptor population.

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Increased Sensitivity to Pesticides in the Young: Possible Explanations

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Our laboratory is especially interested in determining if children may be more sensitive than adults to the effects of anticholinesterase pesticides. Our investigations have centered around characterizing the mechanism(s) for this postulated age-related sensitivity in a standard laboratory animal, Long-Evans rats.. We have chosen the most commonly used organophosphorus pesticide, chlorpyrifos [Dursban® or Lorsban®, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothionate], as our first pesticide to investigate in depth.

It has been known for over 30 years that young postnatal animals may be more sensitive than mature animals to the lethal effects of organophosphorus pesticides, but little work has been done comparing the biochemical and behavioral endpoints in young and adult animals at less-than-lethal dosages, or to explore the mechanisms for this differential sensitivity. Acutely administered chlorpyrifos is approximately 5 times more toxic to young rats (postnatal day 17, PND17) as compared to adult rats, measured by changes in clinical signs and motor activity (1,4). The present group of experiments was designed to determine the basis for this increased sensitivity in the young. Our research plan was to look at two general factors: (1) whether the target enzyme, cholinesterase, was more sensitive to chlorpyrifos inhibition in the young and (2) whether the young were less able to detoxify the pesticide and its metabolites.

Our research to date has shown that the brain cholinesterase in very young rats (*i.e.*, PND4) and adults is equally sensitive to inhibition by various organophosphorus or carbamate pesticides (2,3). These data would not support target enzyme sensitivity as an explanation for age-related sensitivity to the acute effects of pesticides.

In other experiments which were designed to assess some of the toxicokinetic factors of chlorpyrifos toxicity, we delineated the developmental profiles of two detoxification enzymes: A-esterase and carboxylesterase activity. A-esterases detoxify by hydrolyzing the active metabolite of chlorpyrifos, chlorpyrifos oxon, and carboxylesterases detoxify by binding up and deactivating chlorpyrifos oxon. We found that young animals are severely deficient in these enzymes (2-4). As the animals mature, their A-esterase and carboxylesterase activities generally increase and their sensitivity to acute chlorpyrifos toxicity concurrently decreases.

The above data indicate that one explanation for increased sensitivity of the young to chlorpyrifos toxicity may be their inability to detoxify chlorpyrifos as efficiently and rapidly as adults. Given this explanation, what evidence is there that children may also be deficient in these detoxification enzymes? A search of the literature revealed that a few studies (5,6) using human serum/plasma indicate that young humans (i.e. below 2 to 8 years of age) are also

deficient in these detoxification enzymes--a fact which should cause some concern when considering the potential effects of anticholinesterase pesticides in children.

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Screening Design for a Children's Pesticide Exposure Study

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Introduction

The Minnesota Children's Pesticide Exposure Study (MNCEPS) was conducted as a demonstration/scoping project for a NHEXAS (National Human Exposure Assessment Survey) "Phase-III" study, *"to determine the causes of exposure for high risk groups, including those at the high end of the exposure distribution and those who are more biologically susceptible* (Sexton et al., 1995a; 1995b)." This report describes the survey design and questionnaires, which were developed to identify and screen households and individuals from the "high end" of the exposure distribution for selected pesticides, and from a "susceptible" population sub-group (i.e., children). Children have been identified as a susceptible population sub-group in terms of the potential for exposure to environmental contaminants and the likelihood of adverse response to these exposures (NRC, 1993). Behavioral patterns and diets may result in greater exposures to contaminants in the environments where children live and play, and in the foods they consume. Small body size, and developing organ systems and immune systems may put children at greater risk from these exposures.

Households were selected based on the usage of pesticides reported in questionnaires, and on the presence of products with target compounds identified through a product inventory. Questionnaires are frequently used to obtain exposure-related information, and to classify population groups based on their likelihood of exposures. These classification groups may be used to stratify and select individuals for more detailed monitoring, as described in an accompanying manuscript (Quackenboss et al., 1999), or for follow-up on health status or disease outcomes (for epidemiological studies). In both cases, there is an interest in the ability of the questionnaire items to distinguish more highly "exposed" individuals. Comparisons between the questionnaires used in each screening phase, and with the measurements obtained in the follow-up exposure monitoring phase, may provide important quantitative evidence regarding the utility of questionnaire and household inventory data for this purpose.

Urban and suburban application of pesticides to lawns, golf courses, roadsides, public buildings, homes, and apartment buildings may contribute to human population exposures, even though they are a smaller proportion of total pesticide use than are agricultural applications. The

1992 "National Home and Garden Pesticide Use Survey" collected data from 2078 randomly-selected households in 29 states (Whitmore et al., 1993). This survey collected information on the frequency and types of pesticide use in and around homes, both by household members and professional applicators. Approximately 35% of households reported treating the primary living area with insecticides at least once per year; 10% reported frequent insecticide use, on average, more than once per month. In addition, nearly 20% of households reported using a pest control service to treat homes for fleas, roaches, or ants during the past year. The Minnesota Department of Health identified urban (structural) and suburban (turf) applications as being of concern due to the numbers of complaints received (by the Minnesota Department of Agriculture), especially for multi-unit and renter-occupied dwellings.

The follow-up exposure monitoring component of the study, described by Quackenboss et al. (1999), evaluated the feasibility of making multi-media exposure measurements for a sample of children.

Methods

The screening survey design is described below, including a description of the sample population and questionnaires used. The hypothesis addressed here is that a screening design and questionnaires are useful in identifying (stratifying) households and individuals (i.e., classification groups) with higher exposures. The survey design assigned a higher probability of selection to children considered "more likely" to be exposed to pesticides in and around their homes, based on screening questionnaire data and a household inventory of pesticide products. The first phase of the survey was to "identify" households with age-eligible children and with more frequent pesticide use. The second phase was to "screen" these households for more detailed and specific information about pesticide use and characteristics (in-home screening questionnaire and product inventory). The third phase was to conduct "follow-up" monitoring on those more likely to be "exposed," and a sample of other households.

Due to limitations in the staff available for both in-home screening and the follow-up monitoring phases, samples of between 125 and 150 telephone numbers were selected for each of 17 "team-weeks" in Phase 1. The larger number of telephone numbers was selected for non-urban areas, in order to provide a sufficient number of households on private wells. These were contacted to identify and select about 18-20 households for MDH in-home screening (questionnaire and product inventory), which was conducted over a 10-to-12 day period (Phase 2). These results were then used to select six monitoring participants for each field-team and week (Phase 3).

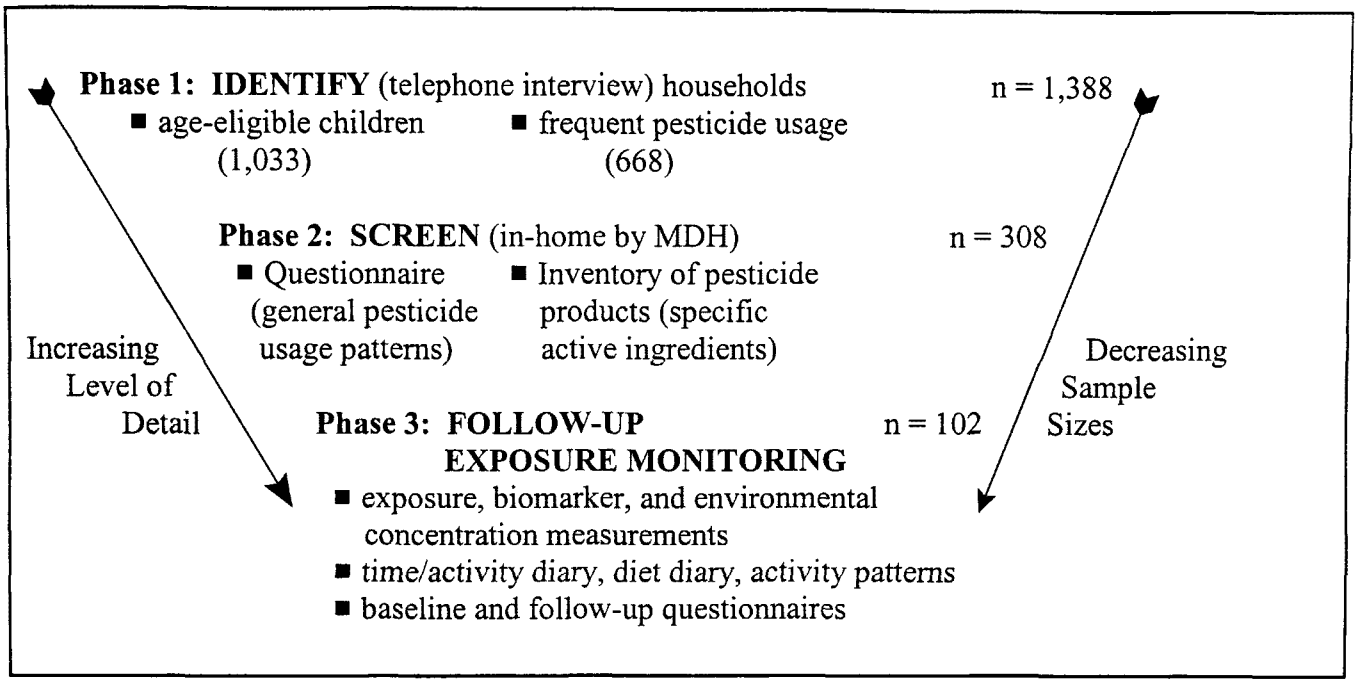

Figure 1. Summary of Three-Phases in Survey Design collecting minimum level of detail on larger number of subjects (Phase 1) and intensive monitoring (Phase 3) with sub-sample of subjects.

Phase 1: Identification of Households

A sample of 2,303 telephone numbers was selected from a list obtained from Genysis Systems, Inc. (Fort Washington, PA) that was predicted to have age-eligible children (ages 3-12) in the cities of Minneapolis and St. Paul, or in Rice or Goodhue counties in Minnesota. Eligibility for participation was limited to children between the ages of 3 and 12 (inclusive) to maximize the potential for collecting the first morning void urine samples, while still covering a portion of the age ranges identified for children ("younger" ages 1-6; "older" 7-12) and pesticide exposures (NRC, 1993). As a partial adjustment for under-representation in the sample list, which is based on households with listed telephone numbers, telephone numbers from lower socioeconomic (inner city) Census Tracts in Minneapolis and St. Paul were sampled in proportion to population size in the 1990 Census.

Telephone Screening. Given the frequency of households with children between the ages of 3 and 12, which was estimated using 1990 Census data to be less than 20%, a telephone-based interview was conducted by RTI to identify potential study participants. The call was proceeded by a letter which notified household residents of the call, and indicated that the study was being conducted in cooperation with the Minnesota Department of Health. The telephone survey was conducted using a Computer-Assisted Telephone Interview (CATI) approach. An adult member of the household was interviewed to confirm eligibility (within city limits of Minneapolis or St. Paul; or in Rice or Goodhue counties), and to obtain a roster of household members who were full-time residents (i.e., those who lived in the residence year round). General information about the frequency of pesticide use for indoor and outdoor insect control was also requested.

Questions were based on the NOPES (Whitmore et al., 1994) and the Home and Garden survey (Whitmore et al., 1993) questionnaires. These were adapted to identify specific types of pest problems likely to be treated in Minnesota homes, and included applications by the respondent, other household members, and pest control services. The following four "yes/no" questions were used for over-sampling the frequent pesticide users.

(Q4) During the summer months, that is from June to September, do you or any member of your household apply pesticides *inside* your home or apartment more than once to control insect pests, such as fleas, ants, roaches, or silverfish?

(Q5) During the summer months, that is from June to September, do you or any member of your household apply pesticides in the yard *outside* your home or apartment more than once to control insect pests, such as fleas, ants, mites, aphids, or webworms?

(Q6) During the summer months, that is from June to September, do you use a pest control service to treat the yard *outside* your home or apartment to control insects such as ants?

(Q7) During the past year, have you used a pest control service to treat the *inside* of your home or apartment more than once for insect pests, such as fleas, ants, roaches, or silverfish?

All households that responded "Yes" to either Q4 or Q7 were selected as "frequent" insecticide users for Phase 2 (in-home screening); 50% of the remaining households were randomly sampled for screening. Those with only one age-eligible child were sub-sampled (50%) to reduce the survey design effect which would result from the unequal probability of selection relative to multi-child households.

Selection of the Sample Frame. Children were over-sampled in this module because they are a potentially sensitive sub-population for exposures to pesticides. In order to select a sample of children for this study, four alternative sampling frames were considered:

1) an area household sampling frame (like the NHEXAS Region V field study);
2) a random-digit-dialing (RDD) telephone number frame;
3) all telephone numbers listed in the current telephone directories serving the target areas; and
4) all households with telephone numbers listed in the current telephone directories serving the target areas that are predicted by the vendor to contain children in the target age range.

Costs for the first two approaches, area household frame and RDD, were increased by the large proportion of households without age-eligible children and of non-residential phone numbers. The third approach might provide better coverage of the target population, but at the cost of contacting a large proportion of households without age-eligible children. The minimum survey cost, which provided adequate representativeness to support the objectives for this study (i.e., to evaluate feasibility and to make comparisons between exposures, environmental concentrations, and questionnaires), was associated with option 4.

Thus, the sampling frame selected for the Children's Pesticide Study was the list of households in Minneapolis and St. Paul (urban and sub-urban areas), or in Goodhue and Rice counties (rural areas), which are predicted by Genesys Sampling Systems, Inc. to have age-eligible children. These households are essentially the households listed in the current telephone directories that are predicted to contain children aged 3 to 12 based on birth records and other publicly-available data used in marketing research. We recognized, however, that the list was not

complete. It excluded households not in the current telephone directories; 75% coverage was estimated for Minneapolis and St. Paul. More importantly, it includes only that portion of households with children in the target age range for whom marketing information is available on the ages of household members. Since selection of households from a commercial list might bias the income level upward, proportionately more urban households were selected from areas identified as "inner city neighborhoods" based on census characteristics. Limited inferences regarding the central tendency in exposures for the population covered by these listings are possible, although these are limited by the sample size and unequal sampling weights (effective sample size).

Phase 2: Household Screening

The second phase was for MDH staff to "screen" the households to identify those with recent (or routine) pesticide applications. During this visit, a household screening questionnaire was completed, which included

a roster of all household members, including age, gender, race, ethnicity, whether employed outside the home, educational level, smoking status, and occupational exposures;

demographic information and housing characteristics, including household income, home ownership, type of home, ground cover on the area around the house, and whether the property was used as a farm;

insecticide usage *inside* and on the *exterior/foundation* of building, including who and where applied, the frequency by user, and when used;

regular *lawn or yard* treatments (who, type, frequency, and last use); and

activity information for the selected child.

Non-leading, scripted probes were used to ensure that questions were answered as completely as possible.

Pesticide use questions. These questions were based on the NHEXAS questionnaires, with some items added or modified to meet specific objectives for the MDH and University of Minnesota components of the study, and to identify specific insect pests and treatments that were likely to be encountered in Minnesota. Some of the pesticide usage questions are listed below.

(S18) In the *past 6 months*, were any chemicals for the control of fleas, roaches, ants or other insects used inside this (house/apartment)?
{If YES, the interviewer continued with S19}

(S19) What room(s) in your home were treated?
{the rooms were listed, with a Yes/No response recorded for each}

(S20) Which areas within the room(s) were treated?
{the areas were listed, with a Yes/No response recorded for each; if "Other" was selected, the respondent was asked to specify a location}

Questions were used to record the frequency of applications inside this (house/apartment) during the past six months by 1) a household resident, 2) a professional exterminator, or 3) someone else. The last month that insect control products were applied indoors was recorded, together with information about how the product was prepared for application, and who and where the product was mixed. A similar series of questions was asked about "chemicals for the control of fleas, roaches, ants or other insects" that were used during the past six months "on the exterior or

foundation of this (house/apartment)." Regular treatments to the lawn or yard outside the respondent's house or apartment were identified, together with information about who applied, the type of application (wet, dry), the number of treatments for weed control and insect control, and the month of last treatment.

Product Inventory. Respondents were asked "Do you have any pesticide products used to control for insects or weeds in or around your home?" They were also shown a card listing different types of pesticide products in order to obtain a complete inventory of all pesticide products (Figure 2). Disinfectants were excluded from the inventory. Agricultural pesticides were also excluded unless they had been used in or around the home. Interviewers recorded the name and EPA registration numbers of each pesticide product. In a few instances, an EPA registration number could not be located on the product. Interviewers noted the presence of specific, commonly used pesticide ingredients as indicated in the list of active ingredients for each product. These ingredients were those which had been selected for analysis in the subsequent sampling phase. Neither duplicate products within a household nor product volume was recorded. As each product was inventoried, interviewers asked whether that product had been used during the past year. Interviewers prompted respondents to identify all areas inside and outside the home where pesticides might be stored. After all products had been inventoried, respondents were asked to identify the last product used. In order to limit the time required to complete the interview, detailed usage information was not obtained for each product.

The EPA registration numbers were entered into a database to create an electronic list of products by household. Based on the EPA registration number products which contained any of the target pesticides (chlorpyrifos, diazinon, malathion, atrazine, and 2,4-D) were identified. Products determined not to be pesticides were eliminated. Active ingredients of inventoried products were identified using PEST-BANK software and an internet site maintained by the California Department of Pesticide Regulation (DPR) of the California Environmental Protection Agency, in affiliation with the EPA, Office of Pesticide Programs (CalEPA, 1997). For several products that were not listed in the database, some information about active ingredients was derived from the product name. Additional details regarding the inventory and analyses of these data are described by Adgate et al. (1999).

Screening Score. A "screening score" was assigned and used to select a sample of about 10-to-12 households per monitoring team and week. This score assigned a higher ranking to households having and using a target pesticide, than for other insecticides, or to having products containing target or non-target pesticides stored in the home but not used in the past year (Figure 3). Another factor included was occupational contact with pesticides by one or more family members. For each team-week, about 11 homes were selected to complete the baseline study questionnaire and for recruitment into the Phase 3 exposure monitoring. The top five scores were selected "with certainty"and a simple random sample of six of the remaining households was selected from those screened by MDH in that week. Six of those completing the baseline questionnaire, and who agreed to participate in the monitoring phase, were assigned to a field-team for monitoring during a specified one-week period (team-week).

Types of Controls*	Types of Pests
Insect sprays Baits and traps Insect repellents, lotions Pet collars Shampoos Bombs and room foggers Fly and insect strips Mouse and rat bait Lawn chemicals Weed and other plant sprays Flower and shrub insect or mold control Vegetable garden insect or mold control Slug controls *Any product used to control pests, such as weeds, insects, or rodents.	**Insects** Ants, spiders, mosquitoes, ticks, chiggers, fleas, cockroaches, bees, hornets, wasps, moths, lice, flies, soil-dwelling insects (nematodes), plant-chewing insects, plant-sucking insects (aphids). **Microorganisms** Mildew, mold, wood decay or rot, plant diseases **Plants** Algae or moss, brush, grass-like weeds, broad-leaf weeds **Animals** Slugs and snails, mice and rats, birds, bats, other mammals

Figure 2. Show card used for MDH Product Inventory.

	Score Assigned
Target pesticide used in past year [I]	Highest
Indoor insect treatment in past 6 month [Q]	↕
Outdoor insect treatment in past 6 months [Q]	↕
Routine insecticide exposure at work [R]	↕
Target pesticide stored (not used) [I]	↕
Non-target pesticide used in past year [I]	↕
Non-target pesticide, not used [I]	Lowest

Figure 3. Screening score assigned based on Inventory [I], in-home screening Questionniare [Q], or Roster [R].

Phase 3: Follow-up Exposure Measurements

The third phase was to "follow-up" on those expected to have higher exposures (based on their screening data) by monitoring of environmental, exposure, and biological media concentrations for specific pesticide compounds for 102 households and children. Environmental and personal exposure sample collection methods were selected to measure the extent of an individual's exposure to specific pesticide compounds. Personal sample collection methods (air, diet, dermal) were used to assess the total (or aggregate) exposure to selected pesticides and polyaromatic hydrocarbons (PAHs). Selection of pesticides was based on information about both the ranges of likely population exposures and on the hazard of the chemicals. A set of "primary" pesticides were used for development of the survey design, to define quality assurance (QA) goals for selection of sampling and analytical methods, and were collected for most sample types and media (Table 1). Secondary pesticides were reported from the analyses of selected samples. Samples of vacuum dust were collected as part of special study for the MDH.

Environmental sample collection methods were selected to provide information about the source of the chemicals and the exposures and the relative importance of the media and location to total exposure, dose, and risk. Some of the samples were collected by the participants (dietary, urine), while the remainder were collected or setup by field technicians (personal, indoor, and outdoor air monitors; tap water, surface wipe and press, dermal rinse, soil). The types of samples collected included:

Air -- Indoor, Outdoor (10% of urban homes), Personal (6-day-integrated);
Surface Wipes and Press -- at two indoor locations (main play area; inside family room);
Water Sample -- Tap during the week (10% of urban homes);
Foods and beverages-- Duplicate diet, 4 day composite;
Activity Observations (video-tape) -- four hours (approximately 20 homes);
Dermal Rinse for adhesion -- one day;
Urine -- first morning void on days 3, 5, and 7; and
Baseline and follow-up questionnaires, and activity diaries.

This combination of environmental, exposure, human activity, and biological measurements was selected to evaluate the relative contribution of multiple media, pathways, and routes to "aggregate" (or total) exposure and to body burden measurements. This includes an evaluation of the importance of understanding detailed activity patterns (e.g., surface contact and mouthing activities) in children in interpreting the relationship between environmental media concentrations and exposure. More detailed descriptions of the methods used are provided in Quackenboss et al. (1999).

Sample Size. The target study sample sizes were as follows: 72 for Minneapolis and St. Paul (36 in each city, and in each of the urban and suburban domains within those cities combined), and 30 in the rural area (Goodhue and Rice counties). The survey design effect due to unequal probabilities of selection reduces the effective sample size for population inferences relative to a simple random sample of population members. For the MNCPES, the survey design effect was expected to be approximately 1.31 for most statistics. The factors contributing to this design effect were: oversampling for MDH monitoring of households that appear to be regular pesticide users based on the telephone screening data ($deff_I = 1.11$); selection of one age-eligible child at random

from the age-eligible children within sample households ($deff_2$ = 1.09; reduced from about 1.30 selecting only half the households with one age-eligible child); and oversampling children who appear to be most likely to have been exposed based on the MDH screener and inventory of household pesticide products ($deff_3$ = 1.08).

Therefore, the effective sample sizes in these areas were expected to be the following: 55 in Minneapolis and St. Paul; 27 in each city and in each of the urban and suburban domains; and 23 in the rural area. The standard errors of population proportions (e.g., proportion of children age 3 to 12 in the target area with measurable exposure to chlorpyrifos) are adequate to support accurate estimates when the true proportion is larger than about 10 percent for Minneapolis and St. Paul or greater than about 20 percent for the other, smaller domains. Several objectives for the MNCPES involved establishing relationships or correlations between study observations (e.g., pesticide exposures and concentrations of pesticide metabolites in the urine). These sample sizes provide sufficient power to detect correlations (i.e., significantly different from zero) of about 0.50 or greater for the urban, suburban, and rural analysis domains for which the effective sample size will be about 23 to 27, as discussed above. For the larger sample available for the entire Minneapolis and St. Paul area, correlations of about 0.40 or greater will be significantly different from zero. Thus, these sample sizes were considered adequate because inferences are limited to making comparisons (i.e., among environmental, exposure, and biomarker concentrations), evaluating exposure assessment models, and summarizing central tendency in children's pesticide exposures.

Table 1. Identification of target pesticides for the Minnesota Children's Pesticide exposure Study.

Emphasis	Pesticides	
Primary	Atrazine Chlorpyrifos	Diazinon Malathion
Secondary	Alachlor Dichlorvos Dieldrin *trans*-Chlordane *cis*-Clordane 4,4'-DDE 4,4'-DDD 4,4'-DDT	Endosulfan 1 Heptachlor Metolachlor *cis*-Permethrin *trans*-Permethrin Pyrethrums Simazine
In Vacuum Dust only (Special study for MDH)	2,4-D Dinoseb MCPP MCPA	Methyl Parathion Silvex 2,4,5-T

RESULTS

The Children's Pesticide exposure Study was conducted during the summer of 1997. The "identification" phase started in May, 1997; the "follow-up" field monitoring was completed by the end of September. This report summarizes the results of the Phase 1 telephone "Identification" survey, and of the Phase 2 in-home "Screening" using the household questionnaire and pesticide product inventory. Results from the Phase 3 sampling of the Minnesota Children's Pesticide Study are not yet complete and are not included. These data provide a picture of pesticide storage and use patterns in the surveyed homes.

Survey

Identification phase. Telephone interviews were completed for 1,388 households (67% of those determined as eligible). Of these, 1,030 had at least one age-eligible child and were asked to complete the pesticide related questions. The overall proportion of those identified as "frequent users," based on "yes" responses to questions Q4 and Q7, was 27.8%. This rate was slightly higher for inner city Census Tracts (33.3% and 31.8% in Minneapolis and St. Paul, respectively) than in the other urban areas (28.8% and 27.8%). The rate was higher in Rice county (30.3%) than in Goodhue county (17.6%), the two rural areas. Households responding to the telephone survey were more likely to use pesticides inside the home than in the yard, and were far more likely to apply pesticides themselves than to hire a professional applicator. In order to improve the likelihood of obtaining measurable insecticide concentrations in the monitoring phase, 602 households were selected based on those reporting frequent pesticide use and a 50% sample of the others. Those households with only one age-eligible child (32.8%) were sub-sampled to reduce the variance inflation (survey design effect) that results from randomly selecting one child from all age-eligible children in a household, resulting in a sample of 477 homes. Of these, 348 homes (73%) agreed to set an appointment for a "screening" visit by MDH staff.

Household Screening phase. The MDH was able to complete in-home screening in 294 homes (88% of 335 attempted) within the time required (10 days) to use this data to select households into the follow-up (monitoring) portion of the study; an additional 14 homes were competed after this time. Of these 308 households, 225 (73%) were in the urban area (Minneapolis and St. Paul) and 83 (27%) were located in Goodhue and Rice Counties. Although most homes in Goodhue and Rice Counties were in rural areas, some were within population centers, such as Red Wing and Faribault, Minnesota. Thirty-four percent of households in Goodhue and Rice Counties indicated that their homes were located on "working farms."

The "screening scores," based on results of the telephone and household questionnaires/inventory, were used to select 183 households for follow-up. Baseline (NHEXAS) questionnaires were completed for 174 (96%) of these cases; 159 (91%) were available for monitoring during their selected sample-week. Of these, 109 households were selected to set monitoring appointments, and 102 households (94%) participated in follow-up visits ("core" components).

Follow-up Monitoring phase. Participation rates in the optional components of the monitoring phase were very good. Of the 102 participants, 75 participated in the personal air monitoring and

89 provided urine samples. In addition, 88 children provided hair samples and 61 provided blood samples (archived). The overall response rate, calculated as the product of responses to each item identified above, was 37% (Quackenboss et al., 1999), which is comparable with other human exposure surveys (Callahan et al., 1995). The largest contributions to losses were in the "identification" phase, with 47% response rate. One factor may have been the use of a telephone call as the initial contact, since telephone surveys traditionally have lower response rates than personal contact in a door-to-door survey, which was not feasible to apply in this study. Another factor which may have contributed to this rate was the limited time available (10 days) to complete the telephone interviews for a "cohort" of households, so that the results could be transferred to MDH for them to begin the "screening" phase. Close coordination of between these efforts was needed to ensure that an adequate number of households was available at the same time that the monitoring teams were ready to begin the next cohort.

Once the "identification" phase was completed, participation rates in the household "screening" and "follow-up" monitoring phases were excellent, with response rates of 88% and 82.1%, respectively. In part, this might be due to the active participation and support of the state health department (MDH). An additional factor may have been the high level of interest among participating families in a study that addressed an issue of concern to parents--the potential exposure of their children to pesticides in foods and from other sources.

Household Screening Questionnaire

Frequency and Locations of Applications. Sixty-nine percent of all homes indicated some pesticide use during the six months preceding the survey. Pesticide products were used inside the house more frequently than they were used outside: 52% of all respondents indicated that they had used pesticide products for insect control inside their homes within the past 6 months; 21% had insect control treatments to the foundation or exterior of the house; and 38% had regular treatments to the lawn or yard (Table 2). Of the 115 households with regular lawn treatments, 60 (52%) reported one treatment and 35 (30%) reported two or more treatments for weed control in the past six months. In contrast, only 8 households (7%) reported lawn or yard treatments with insecticides. No difference was identified between urban and rural households with respect to lawn treatments. Nearly a quarter of all households used pesticides in flower or vegetable gardens or on fruit trees.

Within the home, the kitchen was the room most likely to have been treated: approximately 80% of households that had used pesticides inside the home, and nearly 42% of all homes, had applied pesticides in the kitchen. The floor and baseboard were the most common sites of application within rooms: nearly two-thirds of households that had used pesticides inside the home, and just over one-third of all households had applied pesticides to the floor. Of households applying pesticides inside the home, 11% had applied pesticides to cupboards in which food was stored and/or to cupboards in which dishes, pots, and pans were stored, and 24% had applied the products to counter tops. Since this latter response was not an alternative offered by the interviewer, but had to be elicited in response to a general query about "other" areas, the actual percentage may have been even higher. The combination of greater frequency of use in the kitchen, and self-reported use on counter-tops (associated with the kitchen as the room treated) may be a significant finding in terms of the relationship between patterns of insecticide use and

human exposures, especially in terms of the potential for contamination of foods during storage and preparation in the home and the contribution of this to dietary exposures.

Professional applications. Few households had employed professional pesticide applicators for pest problems associated with their house itself. Within the past 6 months, professional exterminators had applied pesticides inside less than 2% of all homes and on the exterior or foundation of less than 3% of all households. Employment of a professional to apply pesticides to the lawn or yard was somewhat more common. Over 7% of all households, and just over 24% of households that had treated the lawn and yard with pesticides, indicated that application had been performed by a professional.

Table 2. Rooms and Surface Areas Treated for Insect Control in the Past Six Months for all households and for those reporting indoor insecticide use ("users" = 52.3% of total).

Rooms Treated	#	(%) total	% users	**Surface Areas Treated**	#	(%) total	% users
1. Living Room	41	(13.3)	25.5	1. Floors	109	(35.4)	67.7
2. Family Room	24	(7.8)	14.9	2. Baseboards	58	(18.8)	36.0
3. Dining Room	30	(9.7)	18.6	3. Lower half walls	16	(5.2)	9.9
4. Kitchen	129	(41.9)	80.1	4. Upper half walls	14	(4.5)	8.7
5. Bathroom(s)	42	(13.6)	26.1	5. Ceilings	13	(4.2)	8.1
6. Bedroom(s)	34	(11.0)	21.1	6. Cupboards with dishes, pots, pans	18	(5.8)	11.2
7. Basement	52	(16.9)	32.3	7. Cupboards with food	18	(5.8)	11.2
8. Other Room(s)	30	(9.7)	18.6	8. Storage cabinets	24	(7.8)	14.9
				9. Closets	15	(4.9)	9.3
				10. Window sills	34	(11.0)	21.1
Don't Know	2	(0.6)		11. Other Areas	66	(21.4)	41.0
[total=308 respondents] [users=161 households; 52.3%]							

Pets. Because of the potential for frequent, close contact between pets and children, use of pesticide products on pets (e.g., to control fleas and ticks) is of particular concern to those investigating children's exposure to pesticides. Over 71% of households had pets such as dogs, cats, gerbils, hamsters, rabbits, guinea pigs, birds, or horses. Respondents in nearly one quarter of these homes (or approximately 17% of all homes) gave an affirmative response to the question

"Are any chemicals or collars used on any of these pets to control fleas or ticks?" Rural households were more likely than urban households both to have pets (80% compared to 68%), and to use pesticide products on pets (24% compared to14%).

Inventory of Pesticide Products

Pesticide products were found in all but 9 households (97.1%). A total of 2,058 pesticides products were inventoried. Respondents indicated that 1,083 products, or slightly over half of those inventoried, had been used within the past year. Two hundred seventy-eight households (90.3%) reported having used pesticide products at least once during the past year. The mean number of products inventoried per household was 6.7(±5.4); the mean number of products used was 3.5 (±2.8). No more than 26 products were inventoried in any other household. The maximum number of inventoried products used in any household during the past year was 16. While, in the screening questionnaire, 69% of households reported using pesticides within the past six months, 90% of households reported use of specific inventoried pesticide products within the past year. The 21% difference may reflect, in part, the timing of the survey. For those households surveyed early in the process, the preceding six months would have been approximately mid-November through mid-May; the six month period applicable to households surveyed later in the process would have encompassed more of the summer season in Minnesota.

Active ingredients. Nearly 170 active ingredients were identified in inventoried products. DEET (diethyl-meta-toluamide and other isomers), an ingredient of many insect repellents, was the most common ingredient both found (64% of households) and used (53% of households). Products containing piperonyl butoxide, a synergist, were inventoried in 49% of homes and used in 30% of homes surveyed. Pyrethrins, which are derived from plants, were found and used in 48% and 29% of homes surveyed, respectively. Of homes surveyed, 30% had products containing permethrin, a synthetic pyrethroid compound, and 21% had used a product containing permethrin within the past year. Chlorpyrifos, an organophosphate insecticide, was found in 27% of households and used by 18% of households. The last of the most frequently used pesticide ingredients listed above, MCPP, DMA salt, is a chlorophenoxy herbicide. MCPP, DMA salt was found in 35% of surveyed homes and used in 18% of surveyed homes.

Types of pesticides. Approximately 90% of all pesticide products found and used were insecticides, insect repellents, or herbicides. Insecticides alone accounted for nearly 50% of all products found or used. Together, insecticides and insect repellents accounted for more than 75% of product use. Use of pesticides to control for non-insect pests (e.g., weeds or rodents) was far less common. The graph below shows the breakdown of major types of products used (Figure 4). Types of products included in the category "Other" are acaricides, growth regulators, molluscicides, and vertebrate repellents. Products that could not be associated with any type of pest are also included in this category.

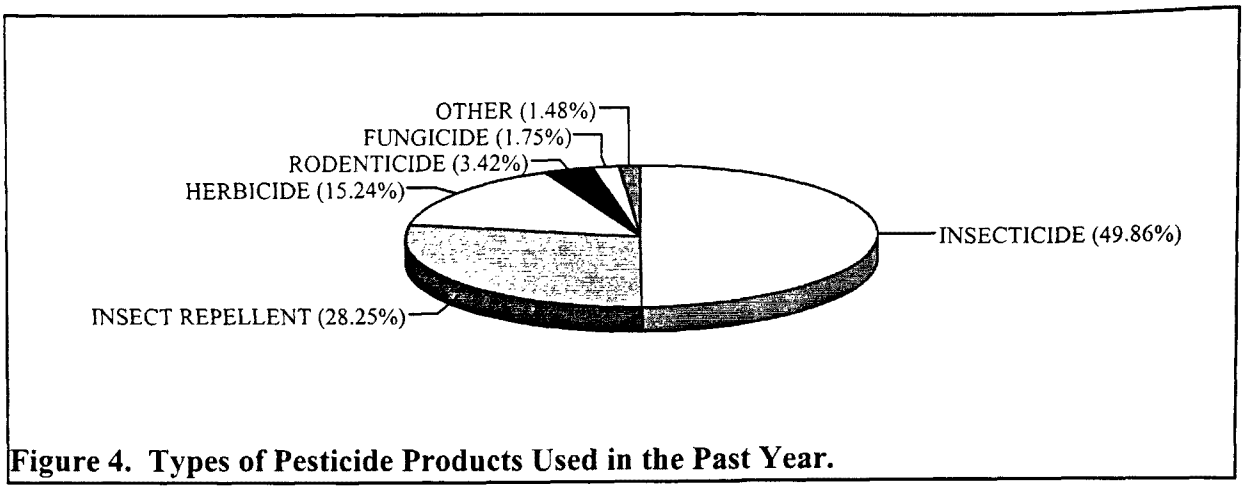

Figure 4. Types of Pesticide Products Used in the Past Year.

Comparisons

Between Questionnaires. Comparisons were first made between the telephone- ("identification") and household- ("screening") based classification groups for pesticide usage: "frequent users," defined from the telephone survey as those responding "yes" to either Q4 or Q7, and "others." The proportions of households reporting insecticide usage on the household screening questionnaires (MDH) are shown in Table 3 for two groups, as defined by their telephone survey responses. More than 75% of the 139 "frequent users," who indicated pesticide use during the summer months (June-September) on the telephone survey, also reported using products for insect control indoors within the past six months on the household screener (MDH). This compares with less than 32% of the 162 "others." The corresponding rates for using insecticides in the kitchen were 61% and 25%, for the "frequent users" and "others." Although this classification was only based on indoor use, reported pesticide usage by the "frequent" group was also slightly greater for exterior insect control (28% vs. 16%), regular lawn or yard treatments (44% vs. 32%), and for application of chemicals to control weeds or insects in a flower, vegetable, or fruit garden (27% vs. 19%). The identification of specific insecticides (active ingredients) in the household product inventory is compared with the telephone-based classification in Table 4. There was an increase in chlorpyrifos use among the "frequent users" households (20% vs. 12%), although the proportion of households having chlorpyrifos-containing products was similar (30% vs. 24%).

The use of pesticides for indoor insect control during the past six months, reported in the household questionnaire, was compared with the total number of products found and reported to be used during the past year in the product inventory. Distributions for the number of products found and used in each home were skewed to the right and there were several outliers. Thus, non-parametric analyses (Wilcoxon rank-sum test and Median test) were used to compare these classification groups (Table 5). The number of products present was only slightly higher for those reporting indoor insecticide use. This difference was greater, and consistently significant using both tests, when use of these products during the past year was considered. There was considerable overlap between these groups, which might be reduced when considering specific types of pesticides and their likely uses. Applications to indoors, exterior/foundation, lawn/yard, and garden were combined to form a general index of pesticide use for comparison with the

product inventory. The number of products found and used were both higher for the combined pesticide use group (Table 6).

With other studies. Two national surveys of household pesticide storage have been conducted (US EPA, 1980; RTI, 1992). In addition, a number of regional studies have included pesticide inventories and there have been local surveys that reflect on pesticide use in the Minneapolis and St. Paul areas (Kamble, 1982; US EPA, 1990; Davis, 1992; Bradman, 1997). Although some results of this study can be compared to the results of the earlier studies, caution should be exercised in drawing any conclusions, given the different geographical areas, pests, and climates; the fact that some of these studies are now nearly 20 years old; and the differences between the underlying purposes and resultant methodologies of the studies.

The mean number of pesticide products inventoried in this study (6.7) is considerably higher than means estimated for the 1976-1977 National Household Pesticide Usage Study (1.7) and found in the National Home and Garden Pesticide Usage Study (3.8), conducted in 1988-1989 (USEPA, 1980; RTI, 1992). However, in neither of these earlier studies were frequent pesticide users over-sampled as they were in the current study. Part of the study population in the Non-occupational Pesticide Exposure Study was chosen to represent higher pesticide use; in this study, the mean number of pesticides products for frequent pesticide users was 5.3 (USEPA, 1990). However, even within this group of frequent pesticide users, the maximum number of products in any home was 23, substantially lower than the maximum number of 45 found in this study. Notwithstanding the differences in sample selection, with one exception, all national and regional studies, including this study, reported that approximately 90% of participating households used pesticide products.

Generally, a comparison of the most commonly found active ingredients in these studies shows increasing use of pyrethins and pyrethroids. This is consistent with a trend toward using pesticides that are considered less hazardous and reflects the fact that pyrethroids entered the market only as recently as 1980. Other noticeable differences can be explained by the banning of certain popular ingredients.

DISCUSSION

The simple set of yes/no questions used for the telephone "screening" phase were useful to identify or classify households by general patterns of pesticide use, when compared to the in-home screening questions and the product inventory, given differences in time frame and level of detail.

The household screening phase indicated that health department interviewers could successfully inventory and identify pesticide products which were stored in the home. Nearly all households in the survey (97%) had pesticide products. Approximately 90% of households reported having used pesticides during the year preceding the survey. Although households determined to be "frequent pesticide users" were over-sampled, this percentage is similar to that found in other studies. The mean number of pesticide products inventoried per household was 6.7. This is higher than the number of products reported in other studies. This may reflect geographic variations or temporal shifts in pesticide usage patterns, or may be an artifact of different survey objectives and strategies. Approximately 50% of all pesticide products inventoried and used were insecticides. Insect repellents comprised approximately 23% of

products inventoried and 28% of products used. Herbicides constituted the third most common type of pesticide product, accounting for approximately 18% of products inventoried and 15% of products used. Insect repellents were more likely to be used than either insecticides or herbicides.

Just over half of respondents reported that insecticide products had been used inside their homes within the past six months. The room in which insecticides were most often applied was the kitchen. In nearly 42% of the households in which the kitchen had been treated, at least one of the areas treated was the counter tops. Overall, floors and baseboards were the most frequently treated surface areas within rooms. The most frequently reported "other" surface area treated was the counter-top (associated with kitchen as the room treated).

Demographic analysis indicates that selection of the sampling frame, which was based on listed telephone numbers and commercial marketing data, may introduce some bias into the results of this survey. Despite efforts to increase the proportion of lower income participants by over-sampling inner city areas, the median household income of the study population (approximately $56,250), was high relative to a two year moving average of the median income in Minnesota ($40,022). Therefore, results may not accurately reflect pesticide practices in lower income groups. In addition, the homes in this study were almost exclusively (95%) single family unattached homes. Therefore, no conclusions can be made regarding pesticide storage or use in other housing types, such as apartments, town homes, or mobile homes. A large proportion of these homes were owner-occupied (94%). Both the high income level and the high proportion of single family homes in this study may have resulted from the use of a commercial telephone list. Higher income families may have been more likely to have engaged in activities that would result in their inclusion on the initial telephone list. Restriction of selection to a phone list also excluded families without telephones.

Another aspect of selection bias is self-selection. Selection was a multi-step process and attrition occurred at each step. The response rate to the telephone survey was low, due in part to calls that were not answered, or were answered only by a machine. No information regarding these households is available, precluding any meaningful evaluation of the potential non-response bias. Overall, the completion rate for the telephone survey was low, less than 70%. Response rates for the in-home interviews and inventories were better; interviews and inventories were completed for 308 of 335 (92%) households visited. Higher response rates may have been due to having the local health department (MDH) contact, or to elimination of "nonresponders" at an earlier stage.

Several issues relating to the sampling design affect the extent to which conclusions derived from this survey can be generalized beyond the survey population. Although the sample was population based, certain households were assigned a higher probability of selection in order to ensure that particular groups, e.g. frequent pesticide users, were represented in the final sample. In order to generalize the survey results beyond the survey population, data must be weighted (by the inverse of a household's probability of selection) to offset this unequal probability of selection. Weighted results will be available in later publications based on data from this study.

Table 3. Comparison of Telephone "Identification" and MDH Household "Screening" Questionnaires for Reported Pesticide Use*

Household Screening		Telephone Identification User Group	
Location Treated (in Past 6 Months)	**Total (%)**	"Indoor Users" in Summer (%)	"Others" (%)
Inside (insect control)	51.8	75.5	31.5
-> in the Kitchen	41.5	61.2	24.7
Exterior (insect control)	21.6	28.1	16.1
Regular Lawn or Yard	37.5	43.9	32.1
Garden (insect control)	22.6	26.2	19.1
Number+ in each "Group"	301	139	162

* % of households from each Telephone Identication "User Group" who also reported using pesticides in the MDH Household Screening Questionnaire

+ total=301 (missing 7 cases for telephone or questionnaire records)

Table 4. Comparison of Telephone "Identification" and MDH Household "Screening" for Insecticides Present and Used In Past Year*

Household "Screening"			Telephone "Identification" User Group	
Insecticide on Product Inventory	**Product**	**Total (%)**	"Indoor Users" in the Summer (%)	"Others" (%)
Chlorpyrifos	Present	26.9	30.2	24.1
	Used	16.0	20.1	12.4
Diazinon	Present	20.9	21.6	20.4
	Used	12.3	12.2	12.4
Malathion	Present	9.0	10.8	7.4
	Used	4.0	5.0	3.1
Number in each "Group"+		301	162	139

*% of households from each Telephone Identication "Group" with target pesticides identified on MDH Screening Product Inventory and their use in the past year

+ total=301 (missing 7 cases for telephone or inventory records)

Table 5. Comparison of the product inventory with the household screening questionnaire: number of pesticides found and used in each home, by reported indoor insecticide use.

Product Inventory	Indoor Insecticides Used in Past 6 months		Wilcoxon rank-sum	Median test
# Products Found	**No**	**Yes**		
Mean	6.2	7.2	$p < .05$	n.s.
Median	5	5		
25th - 75th percentiles	3 - 8	4 - 10		
# Products Used in past year				
Mean	3.0	4.0	$p < .01$	$p < .01$
Median	2	3		
25th - 75th percentiles	1 - 4	2 - 5		
Number of Homes	145	161		

Table 6. Comparison of the product inventory with the household screening questionnaire: number of pesticides found and used in each home, by combined pesticide use (indoors, exterior/foundation, lawn/yard, or garden)

Product Inventory	Combined Pesticide Use		Wilcoxon rank-sum	Median test
# Products Found	**No**	**Yes**		
Mean	5.2	7.3	$p < .01$	$p < .01$
Median	3	6		
25th - 75th percentiles	2 - 7	4 - 10		
# Products Used in past year				
Mean	2.2	4.0	$p < .01$	$p < .01$
Median	1	3		
25th - 75th percentiles	1 - 3	2 - 5		
Number of Homes	89	219		

The need to restrict the interview and inventory to a reasonable amount of time limited questions regarding use of specific pesticides. For example, the only product specific information collected was whether a product was present and whether it was used during the past year. No

information was collected as to where each product was used, how frequently it was used, and what quantity was used.

These data provide a rare, inside view of household pesticide storage and use practices in a sample of Minnesota homes. From a public health perspective, some of the data are reassuring: the most common active ingredients are considered to be among those pesticide ingredients less hazardous to health. While the percentage of households that used pesticides was similar to results in other studies, the numbers of products stored and used were much higher. This leads to questions about the rate of application. Does the larger average number of products reflect a higher rate of application or simply more varied applications? Common use in the kitchen leads to concerns about the safety of such applications, which occur near food and food preparation areas. This study appears to have under-represented lower income households and renters. Do lower income households and/or renters have unique pesticide practices? This study did not address other questions directly related to the potential for exposure. For example, the questionnaire and inventory did not attempt to identify how products were used, patterns of use (e.g., following label directions), or accessability of storage locations to children.

The Minnesota Children's Pesticide Study will make a significant contribution toward quantifying (1) concentrations of pesticides in environmental media in and around the home and (2) children's exposure levels for multiple pathways. The survey and monitoring results will assist in a determining whether household pesticide practices are associated with body burden. Ultimately, these studies will be important to evaluating policies affecting residential pesticide use and children's exposures to pesticides.

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Pesticide Usage Along the U.S.-Mexican Border*

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Introduction

The passage of the North American Free Trade Agreement (NAFTA) and the accompanying environmental side agreements commit the U.S. government and the U.S. Environmental Protection Agency (EPA) to insure a safe environment as industrialization, trade, and population growth occur along the U.S./Mexico border. Even before the passage of NAFTA, many communities along the border were beset by infrastructure deficiencies, e.g., lack of public drinking water, sewage system, and garbage disposal. These infrastructure deficiencies are a direct result of the rapid growth along the border, especially over the past 15 years. During this period, the population of the border region has doubled to more than six million people. Economic growth has been accompanied by increased potential for water and air quality degradation. Residents of these communities have strong concerns about their possible exposures to environmental contaminants, including those which may be coming from across the border (transboundary pollution), or from local sources, including traffic, refuse burning, and extensive pesticide use throughout the agricultural areas.

Understanding and evaluating the nature of pesticide exposure to the pediatric population (i.e., multiple pathway/multiple pesticide exposure) and potential health implications are of national interest and certainly relevant to border residents. As a result of discussions with the state agencies and border communities, this project was identified as one of the priority areas for the Environmental Health Workgroup of the Border XXI program. In a general way, this project is the first step of a multi-phase program to assess the effects (if any) of multiple pathway, multiple pesticide exposures on children's health.

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*This paper is abridged from a report entitled "Pesticide Exposure and Health Effects in Young Children. Part I: Pesticide Data" prepared by RTI for the U.S. Environmental Protection Agency. The complete report (#EPA/600/R-99/008) is available from the National Technical Information Service, 5285 Port Royal Rd, Springfield, VA 22161. It has been peer reviewed by the EPA and approved for publication. Mention of trade names does not constitute endorsement or recommendation for use.

The goal of this first phase of the program was to inventory and gather the data available in each of the four border states, namely, Arizona, California, New Mexico, and Texas. Data were obtained related to existing agricultural and pesticide usage practices in counties within 100 kilometers of the U.S./Mexico border. These data are summarized and examined to provide descriptive analyses of simple comparisons (e.g., counties of highest pesticide usage, pesticides used most heavily in these counties, and major pesticide usage by crop) among counties within each state. Implementation of the project began with a meeting with officials in the State Health Department, State Department of Agriculture, and other interested parties in each of the four states: Arizona, California, New Mexico, and Texas. During the meetings, the project was introduced and the need for pesticide and health information was discussed together with identifying the appropriate state and local contacts who could provide the local, county, and state data. The type of pesticide information of interest was discussed, including the common names and chemical formulae of pesticides; when and where they were typically applied; and the frequency and typical rates of application. Based on discussions with the state officials in California and Arizona, all of the border counties within 100 km of the U.S./Mexico border were to be included in the study area. These counties included Imperial and San Diego counties in California and Cochise, Pima, Santa Cruz, and Yuma counties in Arizona. Only two counties in New Mexico (i.e., Doña Ana and Luna counties) and four counties in Texas (i.e., Cameron, El Paso, Hildago, and Webb counties) were to be included in this phase of the project because state officials felt that they would adequately represent both the major agricultural areas within the state and the majority (~90%) of the population living along the border.

The availability of pesticide information data varied by state depending upon the existence of state-specific regulations which permitted collection of the information either from sales or actual usage reports submitted by the licensed distributor or pesticide applicator. In Arizona and California, state regulations require all commercial applicators to file reports indicating pesticide usage by location, crop, year, and quantity applied in pounds of active ingredient. These records are collected and maintained in computerized databases. Therefore, data presented in this report for Arizona and California are from actual pesticide application records.

In contrast, Texas and New Mexico do not have state regulations which require pesticide data submission to the state authorities, so actual pesticide usage data are not available. Thus, pesticide usage in New Mexico and Texas was estimated using crop-specific pesticide usage information obtained from the Arizona data and adjusting these estimates by the actual crop acreages grown in New Mexico and Texas. Average pesticide usage rates, in pounds of active ingredient per acre by crop category (e.g., small grains, vegetables, orchards, etc.), in Cochise, Pima, and Yuma counties, Arizona, were calculated to provide an estimate of typical pesticide usage rates in the U.S./Mexico border region. The number of acres, by crop category, in each border county for the study area in New Mexico and Texas were obtained from both states Departments of Agriculture for the years 1992-1995, where available. After discussions with local county agricultural extension agents in New Mexico and Texas to determine similarities in agricultural practices and pesticide usage between their state and Arizona, the average pesticide usage rates for the Doña Ana and Luna counties in New Mexico and for El Paso and Webb counties in Texas were derived from the average rates of pesticide usage in Cochise and Pima counties, Arizona. Yuma county, Arizona, pesticide application rates were used to characterize pesticide usage for Hidalgo and Cameron counties, Texas. To obtain the final estimated pesticide usage figures presented in this report, the appropriate county's average usage rate of active ingredients per acre by crop categories was multiplied by the number of acres in that county.

For each agriculturally active county within the four states bordering Mexico, a summary of the acres of cropland, crops grown, the most heavily-used pesticides (in pounds of active ingredient applied per year), and pesticide use on major crop types are presented for the study period 1992-1995 in Arizona, New Mexico, and Texas while California data are presented for the years 1991 through 1994.

The most agriculturally active counties (as indicated by the most acres harvested) are Imperial County, California; Hidalgo and Cameron Counties, Texas; and Yuma County, Arizona, respectively, for calendar year 1992. These 4 counties comprised 78.8% of the total harvested acres associated with all 12 agriculturally active counties examined in this report. The most commonly grown crops in these four counties are cotton, orchards (predominantly citrus), and vegetables. Other major crops grown throughout the border region include hay and small grains.

In general, across all counties in the four border states, insecticides were the most frequently applied pesticides and accounted for greater than 50% of all the pesticides used. Herbicides, accounting for about 30% to 40% of all pesticides used, were the second most frequently applied pesticides. The use of fungicides ranked third (about 15% or less of the total pesticides used) in the U.S./Mexico border region and were generally associated with orchard and vegetable crops. The least used (<5% of total applied pesticides) of the major classes of pesticides were the soil fumigants (except in California) and defoliants.

The heaviest usage of pesticides on a per acre basis was associated with growing orchard crops. Vegetable and cotton crops required lesser yet still substantial quantities of pesticides to be applied when compared to "other crops" (hay, grain, etc.) which required the least amount of pesticides. As a result of this general relationship, the counties where the greatest total pounds of active pesticide ingredient were applied are Imperial and San Diego Counties, California; Hidalgo County, Texas; Yuma County, Arizona; and Cameron County, Texas, in order of decreasing pesticide usage rates. Selected tables from the complete report are presented below including the most heavily used pesticides in Imperial County, California (Table 1), Yuma County, Arizona (Table 2), and the most frequently sold pesticides in Cameron, Hildago, and Willacy Counties, Texas (Table 3).

Table 1. Most Heavily-Used Pesticides in Imperial County, California: Pounds of Active Ingredient Applied by Year.

		Pounds of A.I. Applied by year			
Pesticide	Primary Use	1991	1992	1993	1994
Metam Sodium	Fungicide	983422	1755879	1115498	1906805
Sulfur	Fungicide	2489597	3520508	2121163	1724271
Trifluralin	Herbicide	303811	132552	149661	217380
Malathion	Insecticide	257352	182039	169643	214289
EPTC	Herbicide	259598	186045	143652	157259
Methomyl	Insecticide	138544	76394	100638	135844
Dacthal	Herbicide	220471	340050	157516	129842
Chlorpyrifos	Insecticide	141170	97062	94151	107145
Dimethoate	Insecticide	144548	X*	X	79083
Endosulfan	Insecticide	143117	X	X	X
Linuron	Herbicide	X	78985	159428	X
Maneb	Fungicide	X	X	X	473622
Methyl Bromide	Fumigant	X	137626	X	199586
TOTAL		5081630	6507140	4211350	5345126
		(77.7)**	(85.7)	(68.2)	(81.3 est.)

* X = less than lowest number shown in column.
** ( ) = % of the sum of tabled entries in column to overall total pesticides used [14].

Table 2. Yuma County: Most Heavily-Used Pesticides by Year in Pounds of Active Ingredient.

Pesticide	Primary Use	Year			
		1992	1993	1994	1995
Guthion	Insecticide	111194.0	<*	<	<
Lorsban	Insecticide	43808.2	49228.5	32553.5	54753.0
Orthene	Insecticide	23493.8	23447.1	36595.5	44919.6
Sulfur	Fungicide	142158.4	60029	113073	95522.0
Diazinon	Insecticide	10864	12375	<	19497.5
Dimethoate	Insecticide	18644.3	34891.1	12484.4	45931.9
Lannate	Insecticide	108559.1	110768.1	20056.5	139597.6
Kerb	Herbicide	20990.5	<	<	<
Phosdrin	Insecticide	27196.0	33200.0	30796.0	17560.0
Thiodan	Insecticide	29215.0	59220.0	29771.0	54337.5
Balan	Insecticide	19494.6	24010.8	13761.0	33856.2
Dacthal	Herbicide	24120.8	29779.5	<	22847.2
Permethrin	Insecticide	14649.2	16171.9	<	24551.8
Malathion	Insecticide	10341.6	32029.0	42808.0	37929.6
Treflan	Herbicide	25775.7	15585.6	20390.9	17163.2
Alliette	Fungicide	<	64264.0	<	93392.0
Carzol	Insecticide	<	16663.5	<	11929.6
Diuron	Herbicide	<	27856.5	<	29154.5
DiSyston	Insecticide	12442.8	<	<	<
Ridomil	Fungicide	<	20721.7	<	<
Maneb	Fungicide	<	76382.6	10400.8	26230.1
Prowl	Herbicide	<	<	10128.8	<
Prometryn	Herbicide	<	<	12232.0	<
Prefar	Herbicide	<	<	<	25940.0
EPTC	Herbicide	<	<	<	15388.0
TOTAL		542,948	706624	385,052	810,501
	Insecticide	310408 (57.2%)**	387954 (54.9%)	205064.9 (53.2%)	451008.1 (55.6%)
	Herbicide	90381.6 (16.6%)	97232.4 (13.8%)	56512.7 (14.7%)	144349.1 (17.8%)
	Fungicide	142158.4 (26.2%)	221397.3 (31.3%)	123473.8 (32.1%)	215144.1 (26.5%)

* < = less than 500 pounds active ingredient applied.
** ( ) = % of total used within pesticide type.

Table 3. Pesticide Sales Data (1992) of the 20 Most Sold Pesticide Products from Cameron, Hidalgo, and Willacy Counties, Texas*.

Formulation (active ingredient)	Quantity Sold (in lbs or lbs/gal)	# A.I.*
Temik 15G (aldicarb)	184842	27726.3
Lorsban 15G (chlorpyrifos)	124890	18733.5
Ridomil/Bravo 81W SP (metalaxyl & chlorothalonil)	116366	94256.5
Furadan 15G (carbofuran)	112950	16942.5
Dacthal 75% WP (DCPA)	95280	71460.
Terrachlor Super X (PCNB)	50000	5000.
Iron Sulphate (Iron Sulphate)	47870	47870.
Javelin WG (Bacillus thuriglensis var. kurstaki)	47689	**
Bravo 90 DF (chlorothalonil)	48809	43928.1
Orthene 90S (acephate)	43280	38952.
Atrazine 4L (atrazine)	40665	162660.
Guthion 2L (azinphos-methyl)	30975	61950.
Roundup (glyphosate)	28980	101430.
Karmex DF (diuron)	28104	22483.2
Methyl Parathion (MP)	26806	107224.
Treflan TR-10 (trifluralin)	23900	2390.
DiSyston 15G (disulfoton)	22560	3384.
Dropp 50 WP (thidiazuron)	22218	11109.
Solicam (norflurazon)	22100	17370.6
Ridomil MZ 58 (mancozeb & metalaxyl)	21756	12618.5
	Total	819,618.2

* - data compiled by Texas Department of Agriculture.
** - only chemically-based pesticides included in total pounds of A.I.

Pesticide Use and Assessment along the U.S.-Mexico Border

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Introduction

This paper addresses three topics related to pesticide use and assessment along the U.S.-Mexico border. They are: (1) a brief synopsis of the projects underway at the University of Arizona and the practical problems/issues encountered, (2) a summary of questionnaire information gleaned about potential exposure of children to pesticides, and (3) a synopsis of preliminary environmental pesticide data collected from homes in the NHEXAS Survey. The information provided in my lecture was delivered to stimulate workshop exercises.

The presentation utilizes information collected by the Arizona NHEXAS Consortium under the direction of Michael D. Lebowitz. The paper is written from the perspective of project design, field collection and data assembly. None of the laboratory or modeling issues are addressed here. Further, the presentation is based on analyses of incomplete data sets. Final numbers will differ from those contained in this report, although the data probably represents the dominant trend.

Current Projects Evaluating Pesticide Exposure

The University of Arizona is collaborating with Battelle Memorial Institute and Illinois Institute of Technology in two surveys: NHEXAS (The National Human Exposure Assessment Survey) and a special "Border" Survey (Total Human Exposure in Arizona: A comparison of border communities with the rest of the state). A third study has been undertaken by the University of Arizona and the Western Arizona Health Education Center (WAHEC) to evaluate the exposure of children to pesticides in Yuma County. Lay health workers from the community (*promotores*) serve as interviewers, field technicians and health educators.

NHEXAS: The objectives of this field study are to determine the distributions of total human exposures to multi-media pollutants in the classes of metals, pesticides and volatile organic compounds

(VOCs) by studying a proportionate-based sample of the total population (with a nested design for the different stages of sampling). Specific aims are: 1) document the occurrence, distribution and determinants of total exposures in the general population; 2) characterize the 90th percentiles

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of total exposures to each of the pollutants; 3) evaluate the different media, personal-time and activity factors that contribute to current total exposure; 4) evaluate biomarkers in blood and urine for the target pollutants; 5) to perform evaluations of relationships between exposure reports, environmental measurements, and biomarkers of the target pollutants; and 6) to predict total exposures for individuals and populations including Hispanics. The proportional based population sampling of households within blocks occurs in stage 1.

The target is 1200 such households, interviewed utilizing the NHEXAS questionnaires. In stage 2, additional questionnaires will be completed and environmental sampling will take place in about 450 households, representatively selected from the respondents. Environmental sampling will include: metals in dust, soil, outside air and some tap water; pesticides in dust, soil, and some tap water, and total VOCs in air. In stage 3, a representative selected subset of households will be reevaluated for metals, pesticides and VOCs using methods with greater resolution and reliability. Subjects in the households will be asked to complete questionnaires and provide biological samples.

Total exposure models to pollutants sampled during the study (VOCs, metals, pesticides) will be developed. These models will be associated with multi-media contact. Probabilistic exposure models have been developed for the NHEXAS AZ study population and projected upward to assess risk in the state.

Border Survey: There are concerns among border communities that exposures are high relative to other parts of the country. These communities believe they encounter elevated exposure related to their proximity with Mexico. Associated with increased exposure, is a community-wide fear of increased health effects. Currently, there are no data available to validate this perception of elevated exposure among the border communities. However, EPA has funded a project to characterize the total exposure of residents in the state of Arizona (NHEXAS AZ). In NHEXAS AZ, multiple media (air, soil, house dust, skin, food and beverages, water, blood and urine) will be evaluated to determine contributions to exposure through various pathways (inhalation, absorption, ingestion). We have been funded to conduct a special, complementary exposure study along the Arizona-Mexico border, so for the first time, border exposures can be compared with those from an adjacent non-border area (NHEXAS AZ).

Like NHEXAS AZ the "Border Study" will determine the distribution function of exposure to selected metals, pesticides and volatile organic compounds (VOCs). Most of the target contaminants will be the same for the two studies. However, we have added selected pesticides (organochlorines) and polycyclic aromatic hydrocarbons (PAHs) to the analyte list since we expect to find greater concentrations of these along the border.

Along the border, 300 households will be contacted with a minimum enrollment of 225 homes. Preliminary work will be performed in all 225 homes. Detailed, intensive sampling will be performed in a 100 home subset (of the 225 homes). This will provide enough homes to test differences in the geometric means of contaminant concentrations between the Border and State. These homes will be selected using the same population based probability research design as NHEXAS AZ. All census divisions have already been randomized for the NHEXAS study. We will select the next blocks in sequence. Thus the populations of the two studies will be independent and non-overlapping. An exhaustive quality assurance plan (QSIP) complete with standard operating procedures (SOPs) was developed for every aspect of the NHEXAS study, and will be employed in the Border Study. (Additional SOPs will be added for PAHs.)

In the Arizona-Mexico border study, exposure information will be gathered directly from subjects, from environments frequented by subjects (primarily subject home environments) and from public records. Questionnaires will be employed to characterize the study population, evaluate common practices believed to contribute to exposures and evaluate potential bias in the study due to non-participation. Blood and urine samples will be collected directly from subjects and concentrations of target pollutants will be measured. Additional concentrations of target pollutants will be measured from the air, dust, soil and water of home environments. Duplicate diets (regardless of food and beverage source) will be collected. Public records containing usable information on target pollutants (soil, air, and water) will be used where available. Exposure assessment models will be generated using direct and surrogate measures varying in the intensity of detail.

Total exposure models to pollutants sampled during the study (VOCs, metals, pesticides and PAHs) will be developed. These models will be associated with multi-media contact. Probabilistic exposure models developed for NHEXAS AZ will be applied to this proposed border population study. These models will be fine-tuned to reflect the differences between the two study populations as needed. The precision and accuracy of the previously developed models will be tested with the independent data obtained from the border population. The objectives of these models are to estimate the multi-media pollutant exposures to the subject and determine the sources of inter-individual variability.

Children's Pesticide Survey: Yuma County is responsible for growing much of the nation's fresh fruit and vegetable supply during the winter months. These crops are tended by seasonal and migrant laborers who frequently live near the edge of fields with their families. Many of the pesticides used are pyrethroids, some are dinitroanilines. Further, organophosphates (OPs) like diazinon and chlorpyrifos are used in fields against pests, and in homes to combat termites and roaches. As a result, children living in these homes are at great risk for routine exposure to pesticides. Health effects in response to OP exposure have been observed. For instance, OPs inhibit acetylcholinesterase causing the accumulation of acetylcholine. In turn, this affects the central nervous system (sympathetic and parasympathetic) and elicits symptoms of sweating, diarrhea and others. Poisoning is usually associated with occupational exposure associated with agriculture. Mortality rates are high among the poisoned and are usually caused by respiratory insufficiency. Some investigators report cardiac complications associated with OP poisoning.

The association with agriculture is particularly worrisome for the citizens of the Yuma area. Many of them work in the orchards and vegetable fields and are concerned with associated health risks. Investigators have documented different cholinesterase levels between farm workers (30.28 U/g hemoglobin) and others living in the community (32.3 U/g hemoglobin). Very low levels of cholinesterase were found among farm workers who actually report being sprayed while in the fields. Spraying of OPs is also a common practice for flower growers and OP poisoning has been reported among some florists.

Since many of the fields in the Yuma area are sprayed from the air, residents with no occupational exposure are also concerned. Housing adjacent to fields can be contaminated. In other cases, people intentionally spray the inside of their homes to reduce pests. Misapplication can result in heavy exposures and toxic responses particularly for children.

Ingestion is a major path for exposure to some OPs. Low income farm workers are frequently given produce and fruit from the fields. They may not have a suitable running water supply to

appropriately clean the produce. In some cases they may lack the knowledge of how to handle this additional risk.

Toxic pesticide exposures are a community concern, but cancer is a wide-spread community fear. Numerous studies have been conducted and suggest an association. One Canadian study examined a population based tumor registry's records and found an association between lung cancer tumors and farming in Saskatchewan; further brain cancer associations were reported by researchers in Rome.

Although there are currently no data available to validate the perception of elevated exposure in Yuma County, community-based organizations are actively educating the local population to reduce exposure. One group, WAHEC, has been a pioneer in using lay-educators (*promotores*) to educate farm-workers about exposure in the fields and possible secondary exposures to their families.

We propose a study of 300 children living in the agricultural areas of Yuma County, formerly served by the Valley Health Clinic, and recruited primarily through Head Start Programs. Most participants will be low income Hispanics. The study will be undertaken with local participation from WAHEC (Western Arizona Area Health Education Center) and employ *promotores* as interviewers and field technicians. Screening samples (urine and house dust) will be collected from parents at the Head Start Program or by a *promotore* (a) at the home. In 100-150 homes, screening collections will be made. 30 homes will be selected for multi-media sampling to evaluate OP concentrations in homes. All 300 children will be evaluated for urinary metabolites indicative of pesticide exposure. We expect to sample households of the upper 20% for pesticides. We will sample air, dust and other media as funding permits. We will seek separate funding to evaluate other media (food and beverage). To model "total" exposure, we will supplement these databases with regional information garnered while sampling for the NHEXAS project.

We expect to find that children from low SES households have greater exposure than those of the rest of the state as determined by the NHEXAS evaluation. We expect that children living in homes where parents have knowledge of pesticide exposure mitigation will have lower exposures than homes where little is known about pesticides. Even so, since more pesticides are used in the Yuma area, we expect to find greater pesticide exposure in Yuma than elsewhere along the U.S.-Mexico border of Arizona.

Community Requested Projects: Researchers write proposals and enter communities to evaluate issues they consider to be priorities. Frequently, the communities have concerns not addressed by these studies. Our group has become involved in several projects at the request of the communities. For instance, in both Nogales and Douglas, Arizona, the communities are interested in the potential for elevated asthma prevalence among children. We have undertaken a study in each city in conjunction with the Arizona Department of Health Services. The Hispanic community in Douglas is also interested in whether they are at elevated risk for diabetes. Dr. Lebowitz is working with the Rural Health Office on this study. CDC and ADHS are pursuing an evaluation of Lupus in Nogales at the request of residents, while Rural Health and the University of Arizona Epidemiology Unit re-examine the original questionnaire results for validity. In Sommerton, Gadsden and San Luis, substance abuse is an issue.

Description of Selected Field Implementation Issues

Selection of Target Analytes: Literally thousands of pesticides are in use in the country. Many are used for more than one insect and in more than one environment. Some are used in agriculture and the same pesticide may be used safely for residential treatment. A good example is chlorpyrifos. It is used on selected crops as an insecticide and used to treat foundations of homes as a termiticide. The pesticide is not used inside the house, but under the slab. In some cases, misapplication of agricultural pesticides inside residences can prove fatal (i.e., methyl paraquat). Selection of pesticides for our studies was based on: (1) common usage as determined by sales records and information from the state agricultural inspector, (2) potential exposure for many people throughout the state, (3) established collection and analysis protocols for all media and biomarkers, and (4) reasonable sample stability under storage and shipment conditions to insure sample integrity. A great study design can be developed but if few people are exposed, or sample collection, analysis and stability are questionable, then little can be done to understand the nature and extent of exposure or associated health effect.

The Researcher's Commitment to the Community: Fortunately for the well-being of science, most researchers believe in the projects they propose. They believe their proposed project is in the best interest of the community. Sometimes communities see these research projects as a benefit for the community, but they often believe that only the researcher or the institution will benefit from the project. Once a researcher is involved with a community, the commitment cannot be taken lightly. Carpet-bag research is rarely welcomed by a community. Researchers should enter the relationship with a long-term community commitment in mind, the commitment must be there after the project ends.

Initial Contact: Prior to entering a community it is useful to release general project information to the newspaper, radio and television. Notify local agencies that concerned residents can call, including the public relations department of the police or sheriff's office and Border Patrol. Many people will still miss the announcement, so mail to people living on the specific blocks to be sampled is essential. Attempts to contact potential study participants should be made during the day and evening and on weekend and weekdays. Special calling cards can be left announcing the intent to contact and inviting the participant to call the office. When going door to door, identity badges, a project vehicle and a project handout should be used. Provide business cards and phone numbers to all contacted. Employ mixed gender, mixed age, mixed race and mixed ethnicity field teams appropriate to the region being sampled. In the southwest each field team should have at least one bilingual speaker at all times.

Culturally Appropriate Staff and Materials: Border communities present an interesting challenge when performing survey work. Major issues include gender, language, culture and degree of acculturation. Some issues are common: women are unlikely to answer the door to a man if there is no man in the house. Many people speak Spanish; this does not mean they can read Spanish as well or better than English. Many people speak Spanish and read English. There may be a great diversity of language and reading skills within a single home crossing several languages. Approach the home with a mixed field team and be prepared for any outcome. We have had some cases where Anglos refused to participate because of efforts to include those who do not speak or read English. Be prepared for illiteracy among all groups. Some study participants cannot read or write in any language.

Field Truthing: From 1991-1995, US Census data indicate that Arizona was the second fastest growing state in the Union. As a result all selected block groups had to be evaluated for residential

density prior to sampling. Numerous census block groups that were reported empty in 1990 had residential development in 1995.

Logistics of Large-scale Environmental and Biological Sample Collection: Pesticides are semi-volatile organic compounds that are particularly sensitive to volatilization by heat or degradation by ultraviolet light. Availability of cold storage either in a refrigerator, freezer or ice chest is an essential component of sample collection, transport and storage. This includes shipping samples for analysis on "ice" by an overnight carrier. The need for cold storage and transport influences the size of the field vehicle, number of samples collected and the distance and timing of field trips.

Complexity of Methods: When working in a field environment use the simplest method possible to accomplish the job. Select sturdy, lightweight, durable equipment that provides consistent, reliable, precise and accurate measurements. Complex systems are more likely to break, require extensive cleaning between operations or be inappropriately operated. Some problems we have experienced: large vacuum systems that are not easily transported and require cleaning of multiple parts, programmable pumps with digital displays that do not function at high temperature and mechanical pump systems that fail to hold flow rate.

Students as Professionals: The purpose of research in a university setting is to provide learning experiences for students. Advantages of student labor include a low cost, partially trained work force that is reasonably intelligent. Disadvantages include limited flexibility of scheduling field work, maturity level and work force turnover. We are constantly training new students to provide the simplest skills.

Quality Assurance Issues: With a highly mobile work force it is essential to provide consistent training, cross checks to unravel problems, and adequate supervision including an independent quality assurance officer. An overall quality assurance plan should be implemented, all procedures and changes should be documented and all documents should be available to project staff at all times. The more complex the project, the more important the plans and procedures become.

Research Projects and Clinical Practice: Many procedures and treatments are available to the physician treating a patient for a given disease, syndrome or symptom. Physicians routinely prescribe medications and laboratory evaluations viewed as nearly risk-free. However, procedures used on the general population for research purposes, are judged more critically. Low risk procedures used routinely in the clinic may not be allowed in the field where there is no health risk to the patient. Expectations of field implementation must be realistic relative to what the researcher can get approved by an institutional review board.

Selected Preliminary Results from the NHEXAS Arizona Project

Several surveys are described above, but only the NHEXAS Survey is advanced to the point where any results are currently available. These are all **PRELIMINARY** results. Not all results are currently available.

Table 1 is derived from data in the NHEXAS Descriptive Questionnaire. The descriptive questionnaire briefly assesses the demographic characteristics of a each enrolled household. Each household has one subject designated as the primary respondent. This person is assigned an

individual respondent number (IRN) of 01. The IRN 01 is the index case for the study. These percentages apply only to the recruited population.

Tables 2 and 3 examine pesticide use reported inside and outside the homes by responses to questions on the NHEXAS Baseline Questionnaire. Table 2 examines pesticide use throughout the state for all IRN 01s, pesticide use inside homes of those under the age of 18. Unfortunately, only 4 children with IRNs of 01 were under the age of 6 so the percentages are not meaningful. In general, there appears to be lower indoor use of pesticide among the border counties. The reduction may reflect the higher elevation of Santa Cruz and Cochise Counties. The resulting decrease in temperatures may result in fewer indoor pests, fewer lawn treatments or lower incomes.

Table 4 reports specific pesticides used in homes in the non-border counties of the State and in the Border counties. Professional application of pesticides twice as great in the Border Counties over the rest of Arizona and knowledge of specific pesticides applied is low; 3 times as many unknown pesticides are applied in the Border region. Some of this lack of information flows from professional application, but professional application alone cannot explain the difference. I will speculate that proximity to pesticides available in Mexico may account for part of this unknown component. Residents are aware of their exposure to pyrethroids, chlorpyrifos and diazinon.

Additional information about specific use of pesticide can be found in the NHEXAS Time-Activity Questionnaire, and the Follow-up Questionnaire. IRN 01s applied pesticide 2% of the days where information was sought (2560 person days) and 1% mixed the pesticide. During the week we sampled 11.7% reported using or being near applied pesticide (315 person weeks); of these .6% report using pesticides for more than .5 hours and appear to be professional applicators. 1.5% report using protective equipment and 9.2 % washed hands following application.

Environmental Data

With the possible exception of house dust, food and beverage, few samples contained measurable amounts of pesticide. Tables 5 and 6 are provided ONLY as illustrations of potential exposures. They illustrate the population exposure at the 50^{th} and the 90^{th} percentile of distribution for each medium. Several assumptions are inherent in these numbers. First, the example assumes that an individual has the 50^{th} percentile (or 90^{th}) of exposure for all media; a very unlikely scenario. I have assumed a sedentary rate of inhalation at 10 l/min over the course of the day with 85% of time spent indoors and 15% of time spent outdoors. I assume 1 kg of food was consumed and 4 liters of beverage, 3 liters of which were water. Ingestion appears as the most important pathway of exposure in terms of volume of pesticide for chlorpyrifos (Table 5), but not so for diazinon (Table 6).

Summary

The NHEXAS approach will provide a wealth of information to determine the extent of public exposure to target analytes and to evaluate the mechanisms of that exposure. Projects like this will point the way toward exposure mitigation methods once pathways are identified. These projects are also incredibly expensive to perform. The question becomes what proxy data can be used to determine the high end of exposure and what focused study types can be applied?

There are a great many pesticides in use in our environment and we lack the methods to evaluate many of them, Questionnaires indicate that about half the homes in Arizona use

pesticides and 11.7% of the primary respondents report applying or working near applied pesticide each week. Preliminary data suggest that homes with children appear to use less pesticide, but this trend needs additional evaluation.

One approach is to employ screening techniques like urinary biomarkers as an indication of exposure and then evaluate the high end of the exposure for multiple media. In NHEXAS, we found few elevated levels within each medium but the biomarker for chlorpyrifos was found by CDC in every urine sample submitted.

A second, or perhaps joint approach, is to evaluate a proxy media like house dust. Following the meeting I performed a simple chi square evaluation of the relationship between house dust and the urinary metabolite of chlorpyrifos. There appeared to be no significant relationship between the concentration of chlorpyrifos in the house dust and the concentration of the biomarker in urine.

We are currently examining the value of the questionnaires in predicting urinary biomarkers. We are in the process of applying the screening approach within the Children's Pesticide Project.

Table 1. Baseline Questionnaire Arizona Subject Characteristics of State-wide IRN 01s by Age Class. N represents the number of people in each category.

	All IRN = 01 N = 1015	IRN <18 years N = 151	IRN < 6 years N = 21
Gender			
Male	40.5%	53.3%	42.9%
Female	59.5%	47.7%	57.1%
Age			
< 6 years	3.3%	13.9%	100%
>6 & <18 years	13.4%	86.1%	n/a
>18 & < 45 years	39.7%	n/a	n/a
> 45 & < 65 years	27.7%	n/a	n/a
> 65 years	15.9%	n/a	n/a
Race			
White	93.6%	88.1%	81.0%
African American	2.5%	3.3%	9.5%
Native American	2.5%	5.3%	4.8%
Asian/Pacific Islander	0.5%		
Other	1.5%	3.3%	4.8%
Ethnicity			
Hispanic	35.4%	38.4%	28.6%
Non-Hispanic	63.7%	60.9%	71.4%
School Completed			
None	3.6%	19.9%	95.2%
Primary/Middle School	20.2%	63.6%	4.8%
Some High School	8.9%	13.9%	-
High School Grad	20.6%	2.0%	-
Some College	28.5%	0.7%	-
College Grad	10.0%	-	-
Post-Graduate	7.6%	-	-
Smoker			
Yes	18.2%	0.7%	0
No	81.2%	99.3%	100%
Smoke Indoors			
Yes	12.2%	0.7%	n/a
No	5.9%	n/a	n/a

Table 2. Percent Homes Using Pesticides as reported in the Baseline Questionnaire. Results are reported in percentages for the age class of the IRN 01. N represents the number of homes sampled in each area.

	Baseline QX Stage 2 & 3 All IRN 01 N = 359	Baseline QX Stage 2 & 3 IRN 01 <18 yrs N = 53	Baseline QX Stage 2 & 3 IRN 01 < 6 yrs N = 4
Pesticides at Work/School	6.4%	7.5%	0
Work Pesticide= Raid	0.6%	0	0
Work Pesticide= Repellent	1.7%	0	0
Work Pesticide= Chlorpyrifos	0	0	0
Work Pesticide= Malathion	0	0	0
Work Pesticide= Diazinon	0.6%	0	0
Work Pesticide= Carbaryl	0.3%	0	0
Work Pesticide=Other termat/pesticide	0.6%	0	0
Work Pesticide= Atrazine	0	0	0
Work Pesticide= Other Herbicide	0.6%	0	0
Work Pesticide= Fungicide	0.3%	0	0
Work Pesticide= Unknown Pesticide	4.5%	0	0
Work Pesticide= Other—specified	1.2%	0	0
Use Pesticides Indoors @ Home	52.4%	41.5	50.0%
Living Room	37.0%	22.6%	25.0%
Family Room	24.2%	17.0%	0.0%
Dining Room	32.6%	24.5%	25.0%
Kitchen	45.7%	34.0%	25.0%
Bathroom	42.3%	28.3%	25.0%
Bedroom	36.8%	26.4%	25.0%
Treatment Areas			
Floors	22.0%	13.2%	50.0%
Baseboards	39.3%	26.4%	25.0%
Lower Half of Wall	7.0%	5.7%	0
Upper Half of Wall	2.5%	1.9%	0
Ceilings	2.2%	0	0
Cupboards with dishes	4.7%	5.7%	0
Cupboards with Food	4.2%	5.7%	0
Storage Cabinets	9.2%	7.5%	0
Closets	12.0%	3.8%	0
Other	10.0%	9.4%	0

Table 2 (cont.). Percent Homes Using Pesticides as reported in the Baseline Questionnaire. Results are reported in percentages for the age class of the IRN 01. N represents the number of homes sampled in each area.

	Baseline QX Stage 2 & 3 All IRN 01 N = 359	Baseline QX Stage 2 & 3 IRN 01 <18 yrs N = 53	Baseline QX Stage 2 & 3 IRN 01 < 6 yrs N = 4
Personal Indoor Application during the last 6 months	22.0% (1-24 treatments)	3.8% (1 treatment)	0
Professional Indoor Application during the last 6 months	25.9% (1-12 treatments)	15.1% (1-6 treatments)	25.0% (1 treatment)
Other Resident applied Indoors during the last 6 months.	7.2% (1-12 treatments)	20.8% (1-12 treatments)	25.0% (2 treatments)
Pesticide State			
Needs Dilution	15.6%	13.2%	0
Diluted/Mixed by Respondent	2.2%	0	n/a
Diluted/Mixed by Professional	12.3%	11.3%	n/a
Diluted/Mixed by Another	1.7%	1.9%	n/a
Applied directly	25.3%	22.6%	25.0%
Don't Know	11.4%	5.8%	25.0%
Use Pesticides Outdoors @ Home	59.6%	58.5%	50.0%
Personal Outdoor Application during the last 6 months	16.7% (1-24 treatments)	18.9% (1-6 treatments)	0
Professional Outdoor Applic. During the last 6 months	28.1% (1-24 treatments)	15.1% (1-6 treatments)	0
Other Resident applied Indoors during the last 6 months.	16.7% (1-12 treatments)	35.8% (1-12 treatments)	50% (1-3 treatments)

Table 2 (cont.). Percent Homes Using Pesticides as reported in the Baseline Questionnaire. Results are reported in percentages for the age class of the IRN 01. N represents the number of homes sampled in each area.

	Baseline QX Stage 2 & 3 All IRN 01 N = 359	Baseline QX Stage 2 & 3 IRN 01 <18 yrs N = 53	Baseline QX Stage 2 & 3 IRN 01 < 6 yrs N = 4
Pesticide State			
Needs Dilution	23.4%	20.8%	0
Diluted/Mixed by Respondent	8.4%	3.8%	n/a
Diluted/Mixed by Professional	12.8%	11.3%	n/a
Diluted/Mixed by Another	2.2%	5.7%	n/a
Applied directly	23.7%	32.1%	50.0%
Don't Know	12.5%	5.7%	
Lawn Treatment	27.3%	35.8%	0
Lawn treatment with insect control	5.0%	0	n/a
Mothball Use	5.6%	1.9%	0
Pets	68.0%	81.1%	50.0%
Pets treated for Fleas/Ticks	21.2%	24.5%	25.0%
General Health			
Good	75.5%	90.6%	75.0%
Fair	20.1%	9.4%	25.0%
Poor	3.6%		
Reside on a Farm/Ranch	3.3%	5.7%	0
Family Income Level			
< $9,999	7.5%	5.7%	25.0%
$10,000 to $19,999	8.9%	9.4%	
$20,000 to $29,999	13.1%	13.2%	25.0%
$30,000 to $39,999	19.5%	18.9%	25.0%
$40,000 to $49,999	13.9%	7.5%	
$50,000 to $74,999	20.3%	32.1%	25.0%
$75,000 to $99,999	5.0%	1.9%	
>$100,000	3.1%	5.7%	
Don't Know	1.9%	1.9%	
Refused to divulge	4.7%	3.8%	

Table 3. Percent Homes Using Pesticides as reported in the Baseline Questionnaire for the Non-Border counties of Arizona and for the Border Counties. Results are reported in percentages for the region lived in by the IRN 01. N represents the number of homes sampled in each area.

	Baseline QX Stage 2 & 3 All IRN 01 in AZ Non Border Co. N = 329	Baseline QX Stage 2 & 3 IRN 01 Border Co. N = 30
Pesticides at Work/School	6.4%	6.7%
Work Pesticide=Raid	0.6%	0
Work Pesticide=Repellent	1.8%	0
Work Pesticide= Chlorpyrifos	0	0
Work Pesticide= Malathion	0	0
Work Pesticide= Diazinon	0.6%	0
Work Pesticide= Carbaryl	0.3%	0
Work Pesticide=Other termat/pesticide	0.6%	0
Work Pesticide= Atrazine	0	0
Work Pesticide= Other Herbicide	0.6%	0
Work Pesticide= Fungicide	0.3%	0
Work Pesticide= Unknown Pesticide	4.3%	6.7%
Work Pesticide= Other—specified	1.2%	0
Use Pesticides Indoors @ Home	52.3%	41.5
Living Room	36.0%	40.0%
Family Room	24.2%	26.7%
Dining Room	32.5%	33.3%
Kitchen	45.6%	46.7%
Bathroom	42.2%	43.3%
Bedroom	36.2%	43.3%
Treatment Areas		
Floors	22.8%	13.3%
Baseboards	39.5%	36.7%
Lower Half of Wall	7.0%	6.7%
Upper Half of Wall	2.7%	0
Ceilings	2.4%	0
Cupboards with dishes	4.9%	3.3%
Cupboards with Food	4.6%	0
Storage Cabinets	9.1%	10.0%
Closets	11.6%	16.7%
Other	8.2%	30.0%

Table 3 (con't). Percent Homes Using Pesticides as reported in the Baseline Questionnaire for the Non-Border counties of Arizona and for the Border Counties. Results are reported in percentages for the region lived in by the IRN 01. N represents the number of homes sampled in each area.

	Baseline QX Stage 2 & 3 All IRN 01 in AZ Non Border Co. N = 329	Baseline QX Stage 2 & 3 IRN 01 Border Co. N = 30
Personal Indoor Application during the last 6 months	22.2% (1-24 treatments)	20.0% (1-24 treatments)
Professional Indoor Application during the last 6 months	25.8% (1-12 treatments)	26.7% (1-6 treatments)
Other Resident applied Indoors during the last 6 months.	13.7% (1-12 treatments)	10.0% (1-12 treatments)
Pesticide State		
Needs Dilution	15.8%	13.3%
Diluted/Mixed by Respondent	2.4%	0
Diluted/Mixed by Professional	12.2%	13.3%
Diluted/Mixed by Another	1.8%	0
Applied directly	25.2%	26.7%
Don't Know	11.2%	13.3%
Use Pesticides Outdoors @ Home	59.0%	58.5%
Personal Outdoor Application during the last 6 months	25.5% (1-24 treatments)	33.3% (1-24 treatments)
Professional Outdoor Applic. During the last 6 months	28.2% (1-24 treatments)	26.6% (1-6 treatments)
Other Resident applied Indoors during the last 6 months	16.4% (1-12 treatments)	20.0% (1-3 treatments)

Table 3 (con't). Percent Homes Using Pesticides as reported in the Baseline Questionnaire for the Non-Border counties of Arizona and for the Border Counties. Results are reported in percentages for the region lived in by the IRN 01. N represents the number of homes sampled in each area.

	Baseline QX Stage 2 & 3 All IRN 01 in AZ Non Border Co. N = 329	Baseline QX Stage 2 & 3 IRN 01 Border Co. N = 30
Pesticide State		
Needs Dilution	23.7%	20.0%
Diluted/Mixed by Respondent	8.4%	6.7%
Diluted/Mixed by Professional	12.8%	13.3%
Diluted/Mixed by Another	2.2%	0
Applied directly	22.8%	33.3%
Don't Know	12.5%	13.5%
Lawn Treatment	28.9%	10.0%
Lawn treatment with insect control	5.2%	3.3%
Mothball Use	4.6%	16.7%
Pets	69.3%	53.3%
Pets treated for Fleas/Ticks	21.6%	16.7%
General Health		
Good	75.4%	76.4%
Fair	20.1%	20.0%
Poor	3.6%	3.3%
Reside on a Farm/Ranch	3.6%	0
Family Income Level		
< $9,999	7.0%	13.3%
$10,000 to $19,999	8.5%	13.3%
$20,000 to $29,999	12.5%	20.0%
$30,000 to $39,999	19.5%	20.0%
$40,000 to $49,999	14.9%	3.3%
$50,000 to $74,999	19.8%	26.7%
$75,000 to $99,999	5.5%	0
>$100,000	3.3%	0
Don't Know	1.8%	3.3%
Refused to divulge	2.1%	0%

Table 4. Types of pesticide applied inside and outside NHEXAS homes in the Non-Border Counties of Arizona and the Border Counties. Results are reported in percentages for the region lived in by the IRN 01. N represents the number of homes sampled in each area.

Households from Non-Border Counties in Arizona N = 329		Specific Pesticides Reported Used	Households from Border Counties in Arizona N = 329	
Indoor	Outdoor		Indoor	Outdoor
4.5%	4.5%	Professional	10.0%	10.0%
3.9%	2.4%	Pyrethroids	13.1%	3.3%
1.3%	1.2%	Chlorpyrifos	0	3.3%
1.3%	3.3%	Diazinon	3.3%	10.2%
0.1%	1.2%	Other OPs	0	0
0.1%	0.4%	Carbamates	0	0
3.0%	1.2%	Hydromethylon	0	12.2%
1.0%	1.2%	Herbicides	0	0
0.1%	0	Other	3.3%	0
7.3%	1.6%	Unknown	20.0%	26.9%

Table 5. An idealized view of exposure to chlorpyrifos through multiple media and along several pathways

Media	50th Percentile	90th Percentile
Floor Dust	0.1 μg/g	0.2 μg/g
Sill Wipe	0.1 $\mu g/m^2$	0.3 $\mu g/m^2$
Dermal	0.0 μg/sample	0.2 μg/sample
Foundation Soil	0.0 μg/g	0.2 μg/g
Yard Soil	0.0 μg/g	<0.1 μg/g
Food/Beverage	0.0 μg/kg	2.0 μg/kg
Air Indoors	0.0 $\mu g/m^3$	0.1 $\mu g/m^3$
Air Outdoors	0.0 $\mu g/m^3$	<0.1 $\mu g/m^3$

Table 6. An idealized view of exposure to diazinon through multiple media and along several pathways

Media	50th Percentile	90th Percentile
Floor Dust	0.0 μg/g	0.2 μg/g
Sill Wipe	<0.1 $\mu g/m^2$	0.3 $\mu g/m^2$
Dermal	0.0 μg/sample	0.9 μg/sample
Foundation Soil	0.0 μg/g	<0.1 μg/g
Yard Soil	0.0 μg/g	<0.1 μg/g
Food/Beverage	0.0 μg/kg	0.0 μg/kg
Air Indoors	0.0 ng/m^3	4.2 ng/m^3
Air Outdoors	0.0 ng/m^3	27.4 ng/m^3

Issues in Studying Populations along the U.S. - Mexico Border

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Introduction

The purpose of this paper is to discuss some of the unique conditions found along the U.S.-Mexico border and their implications for the design and conduct of epidemiologic studies. While the views and experiences presented are primarily those of the authors, approximately 15 other researchers with extensive experience along the U.S.-Mexico border were contacted during the development of this paper for their input.

General Considerations

The U.S.-Mexico Border: Large, Diverse and Dynamic

Over the last five years, the U.S.-Mexico border has received much attention, primarily due to issues of illegal immigration and the development of the North American Free Trade Agreement (NAFTA). While the use of the term "U.S.-Mexico border" can give an impression of a well-defined homogenous area, the area is, in fact, quite large, diverse, and very dynamic. The La Paz Agreement, signed by the U.S. and Mexican governments in 1983, defines the border region as that encompassed by a 100-km buffer on each side of the political boundary between the United States and Mexico. This region is approximately 125,000 square miles, about the same area as the states of Maine, New Hampshire, Vermont, Massachusetts, Connecticut, Rhode Island, New York, New Jersey, and Delaware combined. This area stretches along 2,000 miles of border and transects several ecological zones.

The main urban areas developed from small towns along traditional north-south trade routes. There are 19 major population centers (>50,000 population) within the 100-km buffer, 12 of which are in San Diego County, California. Of the remaining seven population centers, four are in Texas (El Paso, Brownsville, McAllen, and Laredo), two are in Arizona (Yuma and Tucson), and one is in New Mexico (Las Cruces) (1990 U.S. Census figures). Of the 25 counties that are on the border, only three have populations greater than 500,000. Ten counties have a population greater than 90,000 people, and the 15 remaining all have populations less than 15,000.

There is significant variation in the demographic characteristics of the U.S. population living in the border region. In 9 of the 25 border counties, more than two-thirds (66%) of households are predominantly Spanish speaking, with a majority being Spanish speaking in an additional 13 counties. Only three counties are predominantly (>66%) English speaking.

Low educational attainment is common in border communities. For example, in 22 of the 25 border counties, over 25% of the adult population (> 25 years of age) do not have a high school diploma; in seven counties, more than half of the adult population have not graduated from high school. While the area is predominantly Hispanic, the proportion of Hispanics ranges from less than 10 to over 80 %, with the highest proportions found in the lower Rio Grande Valley.

People Cross the Border, Existing Data Systems Don't

For many decades, the pairs of "sister cities" along the border acted much like single social and economic units. For example, until four years ago residents of Cuidad Juaréz would freely cross the Rio Grande into El Paso for work, returning to their homes and families each night. Citizens from one side would (and still do) shop and eat in the other. Even with the increased efforts to prevent illegal crossings into the U.S., the border can be likened to a "semi-permeable membrane," with the "permeability" varying from day to day and location to location.

This in itself presents unique difficulties for designing and conducting epidemiological studies. What is the "population at risk"? Does it include Mexican nationals who cross into the U.S. temporarily, or even for extended periods of time? If health outcomes occurring to such individuals are counted in a study, how does one account for the denominator from which these health outcomes have been generated? Complicating the situation is the fact that, while the individuals do cross, information systems generally do not. Thus information about these individuals is often difficult, if not impossible, to access.

Immigration Status Is Always an Issue

Many families along the border have members living in both countries. However, because of the economic opportunities on the U.S. side, and the consequences of not having the appropriate immigration status to remain or work in the U.S., many residents are not willing to provide accurate information to researchers, government agencies, and health care providers. This leads to serious selection and observation bias as an individual's decisions regarding whether to seek health care, where to seek health care, whether to participate in clinic- or community-based epidemiological studies, as well as the accuracy of information provided to health care practitioners or researchers, will certainly be influenced by a person's immigration status. In addition, immigration status may well be correlated with other potential risk factors (e.g., income, education level, years lived in the U.S.).

Structural Barriers to Binational Research

There are several structural barriers to conducting binational research. Few sources of funding exist for conducting research in both countries. When such funding is obtained, transferring money, equipment, supplies, biological samples, and even the researchers themselves, can be complicated and time consuming.

There are a number of structural differences which make binational studies challenging. There are significant differences in institutions, especially in health care systems, making it difficult to recruit comparable populations and generate comparable estimates of disease occurrence. There are problems in identifying appropriate review boards for research on human subjects in Mexico. Universities are not comfortable having such studies reviewed solely by institutional review boards (IRBs) in the U.S.. It can take an enormous amount of time to develop an appropriate review board and get a protocol reviewed and approved.

There are many well-trained and dedicated scientists in the border cities of Mexico who are quite eager to conduct research on public health problems. Many of them have very limited access to computers or adequate laboratories. There are often differences in the underlying expectations, and

in the sense of what is timely and adequate progress on a project. This can be a source of frustration and conflict.

Factors Affecting Study Design

Arising from the situations described above are a number of specific factors that can directly affect study design.

Prospective and Cross-sectional Cohorts
Identifying Cohorts.

There are several problems which arise when trying to identify sampling frames for selecting representative cohorts. The first issue is the definition of the target population. If the target population is defined on the basis of location of residence, then there is the potential that a small, yet a significant proportion of the population in some areas is not U.S. citizens or permanent residents. In such situations, many of the civil databases commonly used as sampling frames, such as voter registration and driver's licenses, would not be representative of the target population. Furthermore, many newer immigrants have a fear of dealing with government officials for any reason (e.g., registering to vote) even though they have the appropriate documentation. Thus, if the target population is limited to individuals who are found in such civil databases, then newer immigrants and Mexican nationals living in the study area will not be included in the study.

Using telephone listings may result in similar biases. For example, according to 1990 Census figures, there were four census tracts in El Paso County where the proportion of households lacking phones was greater than 30%, and these were in the lower income inner-city and peri-urban areas of the county. Community-based surveys have found the proportion of households lacking phones to range from 20% to 41% in rural West Texas.

Recruiting cohorts through clinics is often problematic. On several occasions we have encountered clinic administrators who wanted clear evidence that the research was going to directly benefit the clinic or the clinic population before agreeing to participate. Some administrators feel that researchers have used clinic resources for recruiting and data collection, but have failed to involve the clinic in the analysis or follow-up. In some instances, we have been told that the clinic never even received a copy of the study results. In addition, clinics rarely have any form of IRB in place, and yet feel the desire to have some form of oversight in addition to university IRBs.

It is our experience that once the clinic administration agrees to participate, there is a very high level of cooperation. Many institutions, however, (including hospitals) do not have sophisticated information systems and it can take significant time and effort to work with information system managers to locate and extract the desired data.

A more fundamental issue is the population served by any given clinic. Many border residents have limited access to health care. A recent Behavioral Risk Factor Survey conducted by the Paso del Norte Health Foundation in El Paso, Texas, found that a third of the adults contacted by phone did not have any kind of health care coverage (Paso del Norte Health Foundation, unpublished data, 1997). Sixteen percent reported that they had not seen a doctor when they needed to, and just under half of these cited cost or lack of insurance as the main reason. Three percent said that they didn't see a doctor because of "fear."

Many residents use a variety of providers, so that the records from a single provider may not contain all information about a given patient's complaints, diseases, or treatments. For example, there has been a rapid increase in the number of providers serving areas south of El Paso where many colonias are located. This has led to some degree of competition and the use of multiple providers.

The situation is further complicated by the use of health care providers in Mexico. Estimates of the number of people using health care providers in Mexico vary significantly. A recent household survey in Presidio found that 11% of the respondents used medical care in neighboring Ojinaga, 65% sought dental services, and 61% purchased medicines (Serrano, personal communication, 1997). A survey in Brownsville, Texas, found that 41% of the respondents went to Mexico for medicines and health care (Zveleta, 1985). In another survey in San Diego County just over 40% said they had used medical services in Mexico (Nichols, 1991). Cost and the quality of treatment are the primary reasons cited in most studies for the use of health care services in Mexico (see Warner and Reed, 1993 for further information on this issue).

Of equal importance is the use of U.S. health care by Mexican nationals. A 1987 study of 2,954 randomly-selected Tijuana residents found that 2.5% had used the U.S. health care system in the past 6 months, and that half of these were U.S. citizens or legal residents (Geundelman and Jasis, 1990). Such cross-border utilization of health care makes it difficult to use hospital or clinic records to gather consistent information for a sample of individuals representative of a population living in a specific geographical region, such as on the U.S. side of the border.

Cross border utilization also impacts the identification of birth cohorts. In Nogales, almost 11% of U.S. residents receive some sort of prenatal care and 1% deliver in Mexico, while 4% of women from Nogales, Sonora, receive prenatal care in the U.S., and 8% deliver in the U.S. (Homedes et al., 1992). Preliminary findings from a recent survey of women giving birth at a public hospital in El Paso found that 5% said they lived in neighboring Cuidad Juaréz (Byrd, personal communication, 1997).

In some areas, a high proportion of pregnant women do not receive prenatal care until late in their pregnancy. The El Paso study found that only 57% of the women had their first prenatal visit in the first trimester, while 11% had their first prenatal visit in the third trimester or never received prenatal care. NCHS estimated that in 1992 between 5.3% and 9.9% of pregnant women in the four border states have late or no prenatal care (HRSA, 1997). As a result, generating birth cohorts through prenatal care may miss up to 11% of the women, and these women are quite likely to be systematically different than the rest of the population. WIC clinics have the potential of being a good means for recruiting birth cohorts as eligibility for WIC is based solely on proof of place of residence, not immigration status.

Public schools are another potential source of information. As with WIC clinics, proof of place of residence is required to enroll children in public school in Texas; however, school officials usually do not request information regarding immigration status. Such populations are relatively stable. Further, in many smaller areas the school is an important focus of the community and school officials are respected community leaders. If school officials recognize the importance of a study and are involved in the planning stages, their active support and participation can be of immense value (Redlinger, O'Rourke, and VanDerslice, 1997).

Farmworker organizations may provide a basis for identifying cohorts. Local organizations are keenly interested in pesticides as an issue and many groups are very well organized. Church-

based organizations are also important in many communities and might be used to generate a cohort of individuals. It is our experience that church leaders are highly respected and trusted.

Selecting cohorts based on a specific geographical region can be problematic. There are few accurate maps available for the border regions, particularly in peri-urban areas where colonias and sub-divisions have been developed on former agricultural lands. Aerial photos are generally available and can easily be used to generate a sampling frame of houses in a community. However, it can be quite difficult to contact residents through household visits. A recent household survey in Presidio, Texas, found almost no one at home during the first weekend of data collection (Serrano, personal communication, 1997). In a survey of households in four colonias near El Paso, interviewers approached every home during daytime hours, but were only able to make contact with an adult resident in 53% of the attempts (VanDerslice, Byrd, and Mroz, 1995). While scheduling household visits for the evenings and weekends will be necessary, it does not guarantee high rates of contact. In a study of randomly-selected households in Sunland Park, New Mexico, 20 of the 296 selected households could not be contacted after five or more attempts, including visits on the weekend, in the evening, and in the early morning (VanDerslice and Shapiro, 1996).

Follow-up: Long term or short-term follow-up can be quite difficult due to the dynamic nature of the population, particularly in out-lying areas. In Sunland Park, New Mexico, 29% of the residents had lived there for less than 5 years (VanDerslice and Shapiro, 1996), while in Presidio, Texas, 35% of the residents had lived in the town and 48% had lived in the same house for 5 years or less (Serrano, personal communication, 1997). A survey of women delivering in the public hospital in El Paso found that 20% had changed their residence during pregnancy (Byrd, personal communication, 1997).

Addresses and phone numbers provided by residents are frequently incorrect. For example, in a study of Hepatitis A seroprevalence among school children in San Elizario, Texas, experienced promatoras from the community were unable to locate 20% of the children's families using the phone number and address provided to the school (Redlinger, O'Rourke, and VanDerslice, 1997). In many cases families will use the address of a relative, particularly for billing information. Obviously, loss-to-follow-up in these situations will not be random, but will occur more frequently among families with unskilled workers, as well as among newer immigrants and immigrants lacking documentation.

Follow-up may be more efficient if the school system or health care system is used as the means of continued contact. However, there seems to be significant loss to follow-up after birth, even for 2-month post-partum check-ups (Shapiro, personal communication, 1997). WIC clinics may be a useful mechanism for maintaining contact, and it appears that mothers who enroll in WIC prenatally are more apt to continue to use WIC after childbirth (O'Rourke, personal communication, 1997).

Case-control Studies

Case Ascertainment: In general, there is limited availability of computerized health outcomes in the four border states. The statewide registries which do exist are relatively new. A notable exception is New Mexico which has had a tumor registry for over 20 years. Unfortunately, many other centralized health data sets are poorly organized or suffer from inadequate reporting. Datasets from hospitals on the border vary widely in quality. Most computerized records exist for billing purposes, and as such, the degree of medical information is limited. Few of these databases have ever been used for research, and as a result, it can take significant time and effort to get approval to access such data, and work with the information specialists to generate the correct dataset in the

desired format.

Selection of Controls: While many of the problems described above regarding the identification of cohorts also apply to the selection of population-based controls, the more fundamental issue is how to actually sample from the same population that generated the cases. Based on tumor registry or hospital records it may be impossible to tell which cases were from Mexico. Further, Mexican nationals coming to the U.S. for health care would not be proportionately represented among those presenting to the hospital for the types of conditions used to generate controls (e.g., minor trauma) as compared to those presenting for more serious conditions defining the cases (e.g., cancer). Thus hospital controls as a group may not represent the population from which the cases were generated.

Unique Study Factors

There are a variety of unique study factors in border areas which may complicate the design and analysis of epidemiologic studies of environmental risk factors. Studies which rely on place of residence as a proxy for exposure may be confounded by the high degree of mobility along the border. A household survey in Presidio, Texas, found that 43% of the respondents had lived in Ojinaga, Mexico, before moving to Presidio (Serrano, personal communication, 1997). A survey of women delivering at the public hospital in El Paso, Texas, found that 76% had previously lived in Mexico (44% from neighboring Ciudad Juaréz, 15% from other parts of Chihuahua, and 17% from other parts of Mexico). It is possible that living in or working in Mexico, particularly in agricultural areas, may have led to exposure to pesticides not licensed for use in the U.S.

Border populations also have access to many products and pharmaceuticals not readily available in other parts of the U.S. A recent study found several highly potent arsenic based rodenticides available from hardware stores in Ciudad Juaréz (Lugo, personal communication, 1997). Border residents frequently cross the border to purchase pharmaceuticals without a prescription. Lead based paints, and a variety of foods may be obtained in border towns and easily brought into the U.S. Other important factors include the use of pesticide containers or industrial 55-gallon drums to store drinking water, and unique dietary patterns.

Dealing with People

Recruiting

Research Not Seen as Important: We have encountered many residents with negative attitudes regarding research, particularly during community-based household surveys. Comments we have heard include: "we have been studied to death and nothing has changed," "we are used and nothing is left behind," and "all studies, no action." This appears to be particularly true in colonias, where in a convenience sample of 269 households, 16% remember being interviewed in their home (VanDerslice et al., 1995).

In contrast to water and sewerage which are almost universally viewed as important issues to residents of colonias, pesticides are not seen as much of a threat. In a community survey conducted just south of El Paso, "pesticides in food" ranked 15th out of 20 named environmental and social problems potentially posing a "risk to myself and my family" with only 38% of the respondents rating this risk as "high" (Byrd, VanDerslice, and Petersen, 1997). In a subsequent phone survey covering all of El Paso County, only 13% felt that pesticide use was an "extremely serious" or "very serious"

problem in their community (VanDerslice et al., unpublished data, 1997). A similar study in Arizona and Sonora found that 33% of Arizona residents and 39% of Sonoran residents felt that they were "at risk a lot" from pesticides and herbicides in foods (Udall Foundation, 1996). As one resident of Soccorro, Texas, responded when asked if she was concerned about planes applying pesticides to fields near her home: "How can I smell the pesticides over the smell of sewage from the (failed) septic tank next door?"

In contrast, farmworker groups both in the U.S. and Mexico that we have contacted are very concerned with this issue, and a local group organized and hosted a three-day conference last year to promote interaction between their organization and researchers interested in initiating studies.

Contacting Potential Participants: Being "introduced" to the community before starting recruiting is extremely important for maximizing the level of cooperation. Ideally, such an introduction should be made through local, well-respected community groups (e.g., churches, schools, civic groups, local clinics). The introduction adds a degree of legitimacy and sends the message that the work is worthwhile.

Even an introduction through the mail, phone, or via the mass media can improve legitimacy and remove some of element of suspicion and surprise when a interviewer knocks on the door. Researchers with the University of Arizona found that an introductory letter stating approximately when they would be visiting the household was quite effective (M. O'Rourke, personal communication, 1997).

A number of surveys have collected data on the types of mass media used by border residents and the results vary substantially. While television appears to be the most commonly used media, results on the proportion who relied on television for environmental or health information ranged from 10% in Sunland Park, New Mexico (VanDerslice and Shapiro, 1996), 64% for El Paso as a whole (Byrd, VanDerslice, and Petersen, 1997), and 35% in Arizona and 81% in Sonora (Udall Foundation, 1996). Radio appears to be the second most important media, with 27% of El Paso residents, 7% of Arizona residents and 57% of Sonoran residents using radio to get information.

Interviewers and Interviewers: The perceived identify of the interviewers is crucial for gaining some level of immediate trust. In some communities there is widespread distrust of strangers, and in particular, a fear that any unknown person is an INS agent trying to locate illegal aliens. In a recent pilot project, two professors were observing drivers leaving a school parking lot in an effort to estimate the level of seat belt use. Many drivers would not stop and the school received several complaints about the "government agents." Local universities are well known and generally well-respected. Using university identification badges and university hats and shirts provides a means of instantaneous recognition.

Female interviewers seem to be trusted more than male interviewers, and pairs of interviewers (one male and one female) can provide security as well as a level of trust. Many communities have informal health educators (*promatoras*), and these workers have been effective interviewers. During training the difference between being an educator and data collector needs to be discussed explicitly, and consistency with data
collection protocols between interviewers needs to be checked in the field. Some *promatoras* have now been working intermittently as interviewers for various surveys over three years. Being from the community or from nearby communities, they are familiar with study areas, and are very good at approaching and convincing residents to participate in a study. While using Hispanic interviewers

helps recruiting in Hispanic communities, there have been instances where such interviewers were treated with hostility in predominantly white communities (M. O'Rourke, personal communication, 1997).

Incentives for Participation: Cash or product incentives are often used as a means of increasing participation. In our experience, such incentives seem to have only a marginal effect on participation rates, but may be justified more on grounds of being a symbol of compensation for a person's time. Researchers with the Arizona National Human Exposure Study (NEXUS) report that potential study subjects seemed to either immediately see the value of the study and chose to participate, or did not see the study as having value and refused to participate. Offering an incentive did not appear to change a person's mind (M. O'Rourke, personal communication, 1997).

Community incentives (e.g., health fairs, free walk-in consultations, talks at community groups) seem to be as, if not more, effective at increasing participation. Such measures give the message that the researcher wants to see action that addresses the community's health problems and is willing to make an investment to help bring about such changes. While there are always concerns that such community activities might affect the study results, such potential effects can be minimized by the sequencing of activities and the types of community incentives used.

Participation Rates from Selected Studies: Participation rates have been estimated for a number of studies conducted along the U.S.-Mexico border. Raw participation rates measure the overall effectiveness of locating and contacting the specific persons or households chosen to be in a study as well as the participants decision whether to participate. Raw participation rates varied from just under 50% to just over 90% (Table 1). Adjusted rates, which measure only the person's decision to participate, ranged between 70 and 90 percent.

Data Collection

Instrument Development: The most obvious issue regarding instrument development for the border region is language. Depending on location, between 20 and 90% of border residents speak Spanish as their preferred language. However, there is substantial local variation in word usage, particularly for words describing foods and products.

Of particular difficulty is gathering comparable data from different ethnic groups using different languages. Standard instrument development techniques include translation and back-translation. Few investigators, however, have assessed whether different cultures or regional groups assign different meanings to the same word. For example, a local study found that 50% of monolingual Spanish speakers either reversed or equated the meaning of the words "probable" and "possible" (L. Cohn, unpublished data, 1997). Further, a similar study found that 42% of English-speaking adolescents had the same type of misunderstanding (Cohn et al., 1995).

In bilingual populations, language use varies over time, and there are clear differences in the use of Spanish between young adults and older adults. In addition, there are differences between recent and less-recent immigrants, as well as for immigrants from different parts of Mexico. Such variability in language use makes it difficult to develop valid survey instruments.

Table 1: Participation Rates in Selected Surveys along the U.S.-Mexico Border

Location	Study Design Participation Rates (%)	Citation
San Elizario, TX	School-based, Entire School 76.6 (raw[a])	Redlinger et al., 1997
Santa Cruz, AZ	Community, Cluster, Convenience 83.7 (adjusted[b])	Clark et al., 1994
Webb County, TX	Colonias, Random Sample 69.7 (raw) 97.2 (adjusted)	Rogers et al., 1994
El Paso County, TX	Colonias, Random Sample 70.0 (raw) 93.3 (adjusted)	Rogers et al., 1994
El Paso County, TX	Colonias, All Households 49.2 (raw) 92.4 (adjusted)	VanDerslice et al., 1995
AZ-Sonora border	Mail Survey to All Dentists 74.0 (raw)	Homedes et al., 1994
AZ-Sonora border	Mail Survey to All Dentists 70.0 (raw)	Homedes et al., 1994
Brownsville, TX Zavaleta, 1986	Community 87.3 (raw)	
TX border	Cluster Sample 83.8 (adjusted)	Dutton, unpublished
Sunland Park, NM	Community, Random Sample 66.9 (raw) 72.5 (adjusted)	VanDerslice et al., 1996
El Paso, TX	WIC Clinic, Eligible Clients ≅90.0 (adjusted)	O'Rourke, unpublished
El Paso, TX	Community, Convenience 90.0 (adjusted)	Byrd et al., 1997

[a]raw rate = # completed interviews / total number of units selected.
[b]adj. rate = # completed interviews / number of units contacted.

Using language-based to instruments to measure psychological development in children and changes in development over time presents unique challenges and difficulties in bilingual populations such as in the border region. Children often use both languages to communicate, and the relative use of one language over the other changes as they get older and are encouraged to develop the use of English in school. As such, subtle differences in language-based ratings over time may reflect changes in language use rather than changes in development. This is an area needing much new research.

Common data collection techniques may also be used differently by different social or ethnic groups. Some researchers have observed that Hispanics are less apt to select extreme values (e.g., strongly agree, strongly disagree) on Likert scales (L. Cohn, personal communication, 1997). Personnel in local emergency rooms report that when they ask patients to rate their pain on a scale of 1 to 10, many Hispanic patients are unable to translate the intensity of pain into a numeric score (Z. Green, personal communication, 1997). These examples point to the need for more in-depth

instrument development and validation when working with populations along the U.S.-Mexico border.

Finally, instruments to be used along the border, or in any specific region, need to be more than just accurate translations of the original; they need to reflect the reality of the region, from the types and names of foods being consumed, to beliefs and about specific health events. Key informants and focus groups are crucial for finding out about these aspects of "local reality."

Interviewing

Many of the factors and problems discussed above regarding recruiting in the border region apply equally well to interviewing. One unique issue is the use of computer-assisted interviewing. It is our experience that the presence of a portable computer distracts the respondent, while others feel that the use of computers has had minimal effect on the interview process.

Collecting Biological Samples

While collecting biological samples, particularly using invasive techniques, is always more problematic than asking questions, with close cooperation from community groups or clinic staff it can be quite successful. In the Hepatitis A seroprevalence study of children 3 to 7 years of age, we were able to obtain blood samples from 561 of the 682 children registered in the school, an 85% participation rate. In a recent study of folate levels in women attending a WIC clinic, approximately 90% provided a blood sample (O'Rourke, unpublished data, 1997). In the Texas Department of Health border survey, preliminary figures indicate that while only 16% of the families contacted refused to be in the study, 40% refused to let a blood sample be taken from their child, in spite of a $40 cash incentive. Native Americans appear to be extremely reluctant to provide blood samples (M. O'Rourke, personal communication, 1997).

Conclusions

A truly successful large research project will require concerted effort in preparation and in follow-through. It should be designed as part of a larger commitment to working with a community to help them improve health conditions over a sustained period of time. Interventions developed as a result of research must be culturally appropriate and acceptable to the community. They should be involved not only in the research, but in the development of interventions. This will require listening to the community and responding to their "felt needs," as well as developing working relationships with agencies serving the community. All groups in the community, including industries, health care agencies, and community leaders should be involved in the process of assessment and planning. This inclusion of all stakeholders will increase the likelihood that programs will be adopted and institutionalized. Without this intersectoral commitment, programs cannot be sustained.

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Some Observations on Studies of Pesticides and Children on the U.S.-Mexico Border

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Cross Cultural Considerations

Although there is an abundant literature on the challenges of cross cultural studies, these challenges are relatively new for environmental epidemiology. Three examples from the environmental health literature are presented.

The British Medical Research Council Respiratory Questionnaire is available in many different languages, and it is widely used for evaluation of environmental pulmonary effects. However, the standard practice of translation and back translation is not sufficient to guarantee comparable responses across cultures. Data from Central American banana plantation workers demonstrated high prevalence rates of dyspnea among unexposed, healthy workers (McConnell, in press). South African miners interviewed in their mother tongue have much higher prevalence of dyspnea than when interviewed in another language with which they are also fluent (Becklake, 1987). These culturally based differences in reporting may be relevant to the interpretation of respiratory questionnaire data obtained from parents of differing ethnic backgrounds (or from different ethnic sub-populations) about their pesticide exposed children.

The application of the WHO Neurobehavioral Core Test Battery, which was designed to be applicable cross culturally for evaluation of neurotoxic exposures, has shown unexpected dramatic differences in neuroperformance in different parts of the world (Anger, 1993). These differences were not explained by exposure to neurotoxins and were particularly marked for a group of Central American farm workers, a population from which there have been large migrations to the United States in recent years. These findings would be relevant to the evaluation of neurobehavioral effects of pesticides, at least among older children from some border sub-populations.

Finally and perhaps most important for the proposed border studies, the common practice of grouping all Hispanic populations in epidemiologic studies may mask important differences between sub-populations. The high incidence of a birth defect with clear environmental determinants, neural tube defects, among offspring of Mexican women provides an example of the complexity of these differences between different Hispanic populations (Shaw, 1997). Although children of U.S.-born women of Mexican descent have a risk of neural tube defect similar to non-Hispanic white women, children (born in the U.S.) of Mexican born mothers have a risk 2.4 times that of non-Hispanic white women. A review of rates of neural tube defect within Mexico has shown that there are marked differences between different parts of the country, differences which may be related to the wide variety of ethnic sub-populations within Mexico or to environmental factors.

These examples demonstrate some of the difficulties in identifying appropriate populations for cross sectional study of potentially pesticide related respiratory, neurobehavioral, and developmental effects among border children. One strategy for controlling for potential confounders to such associations, many of which are likely to be unidentified and unmeasured,

would be to restrict the study population to a group as homogeneous as possible with respect to cultural and ethnic factors, for example children of U.S. born parents of Northern Mexican descent. Alternatively, prospective studies of children, using each child as his or her comparison for evaluation of the outcome of interest, would help to control for confounders associated with different ethnic subgroups.

Identifying Heavily Exposed Populations

Previously poisoned patients, if accessible, may provide attractive high-dose population groups for study of some outcomes of pesticide exposure, especially neurobehavioral effects. Adults previously poisoned with cholinesterase inhibitors have demonstrated clearly and consistently the neurobehavioral sequela of heavy exposure (Savage, 1988; Rosenstock, 1991; Steenland, 1994). Because the results of studies of heavily occupationally exposed, but not poisoned, groups have been less conclusive, it would make sense first to evaluate children previously poisoned with pesticides for neurobehavioral (and perhaps developmental or immunologic) sequelae. If such a study were to identify associations between previous pesticide poisoning and measurements of specific neurobehavioral or other outcomes, these would be the specific outcome measurements which might be further developed as instruments for evaluating the effect of pesticides among children heavily exposed environmentally, but not poisoned, with cholinesterase inhibitors.

The difficulty with this proposal is in identifying and finding previously poisoned children for study. The California pesticide poisoning reporting system, an international model for surveillance of occupational poisoning for many years, receives reports from physicians, who are required by law to report any illness they suspect of being caused by pesticides. Nevertheless, this surveillance system leaves most poisonings unreported, and the system is biased toward capturing occupational exposures, although surveillance reports from poison control centers, where poisoned children are often treated, are now being included routinely. Active surveillance of pesticide poisoning, in Fresno County in California (Maizlish, 1994) and internationally (McConnell, 1994) suggests that it would be worth exploring the feasibility of identifying a large enough cohort of children previously poisoned with cholinesterase inhibitors from several border counties to evaluate chronic neurobehavioral sequelae.

California also has a pesticide use data base which contains geographically referenceable records of all agricultural applications in the state by date. The design for a potentially relevant pilot study linking this little known resource to a data base of school absences will be presented.

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Resources for Pediatric Research in the Border Region

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For the past 30 years, I have been a private pediatrician in California's Imperial Valley. The Imperial Valley is located on the Mexican border about 100 miles east of San Diego. The land is desert, but because it is irrigated by the Colorado River, it has a year-round growing season. The population of the Imperial Valley is approximately 140,000 on the United States side of the border and close to one million on the Mexican side. Both areas are extremely active agriculturally. My experience in the Imperial Valley leads me to share with the U.S. Environmental Protection Agency (EPA) in order to assist their efforts to identify and measure health end points associated with pesticide usage and its effect on young children.

Since the health organization resources for measuring these end points will vary from area to area, I will not attempt to catalogue these resources. I will instead offer guidelines to EPA on how to best access resources and also foster community cooperation, understanding, and acceptance of environmentally beneficial projects whether the project is short lived or extends over a period of years. As a private pediatrician, I have experience in all the areas that I will address.

Cooperation is essential between EPA and the community. In conducting studies, both EPA and the community have needs that must be met to successfully complete a project. EPA needs:

1) access to community resources,
2) access to information, and
3) access to the study population.

The community needs:

1) trust in the outside entity and
2) trust in the benefit to be derived from the project.

It is imperative that both sets of objectives be reached for their mutual benefit. The key to meeting these objectives is in building relationships between the community and the entity (in this case EPA).

Outsiders in any area can be met with reserve. Along the U.S./Mexican border, outsiders, especially government representatives, are viewed as a possible threat by some segments of the population. Therefore, knowledge of and involvement in any studies must involve the entire community, including health care providers, in order to assure the best outcomes.

Accessing the community can be difficult. A crucial step is developing a relationship with key physicians in the community. The physician can join as a member of the team and assist in making rounds to other key entities with information and recruiting participants as necessary. The County Medical Society can provide information and contact with local physicians. Working with the society and referred physicians can build relationships that will extend into and involve the community. The most intimate knowledge of the health community with access to unpublished data can be obtained from the health department, clinics, private practitioners, and local hospitals. In areas where centralized structures do not exist, the creation of an advisory committee comprised of health care components within the community might prove productive.

WORKGROUP REPORTS

Introduction to Workgroup Reports

The workshop was structured to bring together experts from a variety of fields and attempt to draw on their collective expertise to identify priority pediatric research questions related to pesticide exposure. Workgroups were organized around five health endpoint domains: neurology, developmental effects, pulmonary, immunology and cancer. After day one, the pulmonary and immunology groups combined to discuss areas of mutual interest.

Workshop participants were assigned to the multidisciplinary workgroups on the basis of their areas of expertise. Each group had a least one epidemiologist, exposure assessment expert, physician and representative from the border area (e.g., health department). EPA facilitators conducted the workgroup sessions and were responsible for producing the workgroup reports.

Day One - Health Effects

The purpose of the day one breakout group was to generate a comprehensive list of child health endpoints under the health domain of interest that could be studied in young children and infants. The groups were also charged with assembling sufficient information to rank the priority of each endpoint for Phase II (pilot) study. The discussions were fueled by the health effects speakers presentations given earlier in the day.

The groups were encouraged to list as many health endpoints as possible which could be measured in infants and young children. Although certainly important, facilitators tried to avoid an overemphasis on "pesticide-related" endpoints at this point. The literature is sparse and the group leaders encouraged people to think about measurement in a relatively broad way. Some groups organized part of the discussion around a validated test which was applicable to multiple endpoints. A facilitators guide listed some relevant points for discussion including the gender and age groups affected; the population prevalence (or availability of population norms); the persistence of the endpoint (acute, chronic, etc.); whether measurement tools were available and if special training was need to measure the endpoint; and the probability that the endpoint could be a result of a pesticide exposure.

Each group rated the priority of the health endpoints they generated on a five-point scale with one as the highest priority and five the lowest. All factors were considered important in the ranking (biologic plausibility, feasibility, etc.). For example, an endpoint could be rated as a high priority because it is easy to measure in children even if there is no strong evidence to link it directly to pesticide exposure (e.g., growth).

A plenary group discussion was held to discuss the highest rated endpoints from each health domain. The group considered whether the constellation of endpoints discussed for Phase II study would adequately assess the likely health effects for children with respect to variations in age; gender; representativeness of the potential sample (citizenship, school participation, access to health care providers); and comprehensiveness across organ systems or endpoint domains.

Day Two - Development of Strawman Study Proposals

The purpose of the day two breakout group was to generate a collection of potential studies for Phase II implementation. Study hypotheses were generated based on the day one

discussion of health endpoints, and the speakers presentations on pesticide exposures and border populations.

A collection of potential Phase II (pilot) studies were generated under each health endpoint domain, rather than designing a "cadillac" study. Generally, the discussion was organized around the study design rather than the endpoint (in some cases multiple endpoints could be addressed in the same study design), but it was also appropriate to generate a series of similar designs which require different measurement strategies (e.g., a series of cross-sectional studies). Groups were not asked to develop a study design for every endpoint generated in the previous days' discussion, but to try to be comprehensive and at least get some ideas about the highest priority endpoints. The facilitators were asked to elicit information and encourage discussion about the main hypotheses or aim of the study; the target population; the appropriate study group; exposure and outcome assessment issues; strengths and weakness of the design; and the timeframe needed for the study.

Groups discussed the relative merits and feasibility of the studies generated and rated the priority of the proposed studies on a five-point scale. One is the highest priority and five the lowest. Ranking was based on the information gathered in the earlier discussion. Groups were instructed to be mindful of the time frame for Phase II studies. If a priority study was a longer term (larger scale, etc.) endeavor, could a pilot test be done at this phase?

Reports on the strawman proposals were made to the entire group on the morning of day three in a plenary session.

Neurobehavioral Workgroup Report

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Introduction

Pesticides are widely used in the border region for agricultural purposes and for residential pest control. Unlike other chemicals encountered in the environment, pesticides are designed to kill and the mechanisms of action are well known. Organophosphorus pesticides are the most widely used class of pesticides along the border (*add ref*). OP pesticides produce acute cholinergic effects and, in some cases, delayed neuropathy. While the acute effects of high level pesticide exposure have been documented extensively, the effects of chronic, low-level exposure in human populations--a situation typical of the border and other agricultural areas--are poorly understood.

Day 1: Recommended Neurobehavioral Tests

The initial question to be examined in the workshop was what measures are available in selected disciplines for studying health effects in young children (aged 1-5 years), without considering the specific features of pesticide exposure. This workgroup was charged with discussing neurobehavioral and psychometric measures. Sensory measures were also considered.

Selection of appropriate tests to assess the neurobehavioral effects of chemical exposure in young children is a challenging task for several reasons including: (1) the range of cognitive and sensorimotor behaviors in young children is limited; (2) the sensorimotor and cognitive abilities of young children change very rapidly over time, necessitating different measures at different ages (see Table 1); and (3) the predictive validity of available sensorimotor tests before age three is very poor (Bellinger, this volume). The logistic constraints of field testing also impose limitations on which tests can be done. The neurobehavioral group reviewed tests of sensory, motor and cognitive functions appropriate for use in testing infants and children of different ages. Test characteristics that were considered included general psychometric qualities (test-retest reliability, concurrent validity, standardization of instrument, and sensitivity to neurobehavioral impairment); applicability for use in field testing (cost, simplicity and time of test administration; training needed to administer and score the test); and age range of children for which the test is appropriate. The group also considered if the test has been adapted for use with Hispanic populations. Results of this discussion are summarized in Table 1. The only tests recommended for use in children under age 3 are the Bayley Scales of Infant Development (Bayley, 1993). The Bayley includes two primary scales, the Mental Development Index (MDI) of cognitive skills and the Psychomotor Development Index (PDI) of motor skills. The sensitivity of these scales to environmental insult has been demonstrated repeatedly in pediatric lead studies (Dietrich & Bellinger, 1994). The strengths and weaknesses of the Bayley and other tests available for assessing neurobehavioral function in young children are discussed in detail elsewhere in this volume (see chapters by Bellinger and Llorente).

TABLE 1a. SUMMARY OF RECOMMENDED NEUROBEHAVIORAL TESTS (Part A)

END-POINT	BAYLEY-II (2nd ed.)	WPPSI-R	WRAML (Memory & Learning)	WRAVMA (Visuo Motor Abilities)	PEABODY
Reliability	High	High	High	High	?
Validity	Concurrent, not Predictive	Concurrent and Predictive	✔	✔	?
Standardized	✔	✔	✔	✔	✔
Simplicity	✔	✔	✔	✔	✔
Admin Time	30-40 min	1 hr	40-45 min	25-30 min	45 min
Cost	$500-$600	$500/kit $1.50/test	$240/kit	$250	Supplies
Training	Moderate	Moderate	simple	simple	simple
Age Range	1-42 mos	3-7 yrs	5-17 yrs	5-17 yrs	3-7 yrs
Sensitivity	Pb/PCB	✔	?	?	?
Spanish Translation	Yes	?	?	Neutral	
Confounders	Iron deficiency; not affected by SES <2 yrs of age	SES			
Priority	1	1	1	1	1

Abbreviation Key for Tables 1a and 1b

Bayley-II	Bayley Scales of Infant Development, 2nd ed. (Bayley, 1993)
NES	Neurobehavioral Evaluation System (Letz, 1994)
Peabody	Peabody Developmental Motor Scales (Folio & Sewell, 1993)
Vineland	Vineland Adaptive Behavior Scales (Sparrow et al, 1984)
WISC-III	Wechsler Intelligence Scales for Children, 3rd ed. (Wechsler, 1991)
WPPSI-R	Wechsler Preschool and Primary Scales of Intelligence-Revised (Wechsler, 1989)
WRAML	Wide Range Assessment of Memory and Learning (Sheslow & Adams, 1990)
WRAVMA	Wide Range Assessment of Visual Motor Abilities (Adams & Sheslow, 1995)

TABLE 1b. SUMMARY OF RECOMMENDED NEUROBEHAVIORAL TESTS (Part B)

END-POINT	VINELAND	VISUAL ACUITY	VISUAL CONTRAST SENSITIVITY	TACTILE SENSITIVITY	WISC-III	NES
Reliability	✔	✔	✔	✔	✔	✔
Validity	✔	✔	✔	✔	✔	✔
Standardized	✔	✔	✔	not for kids	✔	~
Simplicity	✔	✔	✔	~	✔	✔
Admin Time	30 min	3 min	3 min	15 min	1 hr*	
Cost	$50	-----	$500	$2,000	$500-$600	$500+
Training	Simple	Simple	Simple	Simple	Moderate	Simple
Age Range	3 yrs - teens	4+	7+	7+	6-16	5/7+
Sensitivity	?	~	✔	✔	✔	✔
Spanish Translation	culture-neutral	✔	✔	✔		✔
Confounding Factors					SES	
Priority	3	1	1	1	1	1

* Shorter screening test available

For children older than three, a wide range of tests are available. Tests recommended by this group include the Wechsler Preschool and Primary Scales of Intelligence-Revised (Wechsler, 1989) for children aged 3-7, the Wechsler Intelligence Scales for Children, 3rd ed. (Wechsler, 1991) for children aged 6-16, the Wide Range Assessment of Memory and Learning (heslow & Adams, 1990), the Wide Range Assessment of Visual Motor Abilities (Adams & Sheslow, 1995), the Peabody Developmental Motor Scales (Folio & Sewell, 1993), and the Vineland Adaptive Behavior Scales (Sparrow et al, 1984). These tests were selected because they cover most of the major functional domains (except sensory) that one would want to assess when the targets of a toxicant are unknown and the objective is to be comprehensive. Moreover the psychometric characteristics of these tests are very robust. Both Wide Range tests have the advantage of providing for the assessment of multiple factors within a broad domain (memory/learning or visual-motor function). A child's performance on different wide range factors can be compared directly because all parts of these tests were normed on the same group. The Vineland Scales, administered to parents in interview form, provide additional information on four aspects of child behavior--communication, daily living skills, socialization and motor skills.

Traditional psychometric tests, including some of those mentioned above, have been applied extensively in studies of children exposed to lead, methylmercury and to a lesser extent, polychlorinated biphenyls (see Bellinger, this volume). These tests, in some cases, are time consuming, require highly trained personnel to administer and interpret, and often yield only global measures of cognitive function. During the past decade many traditional tests have been adapted for computer administration and are available in rapid, reliable and easy-to-administer form. The Neurobehavioral Evaluation System (NES) is the most widely used computerized system developed specifically for human neurotoxicity testing (Letz, 1994). NES was designed for testing workers with a minimum education level of 5th grade, although many of the tests can be performed by school children (Otto et al, 1996) and even preschool children (Winneke et al, 1994). A pictorial version of the continuous performance test was recently developed specifically for use with preschool children (Dahl et al, 1996). NES tests provide a very useful and efficient alternative to traditional paper-and-pencil tests for children aged six and older.

Tests of sensory function, particularly vision, have also proven to be sensitive indicators for the early detection of neurotoxicity (e.g., Mergler, 1995; Hudnell et al, 1996) and are integral components of several human neurotoxicity testing batteries (Anger et al, 1994; ATSDR, 1995). Three sensory tests are recommended for use with children--visual acuity, visual contrast sensitivity, and tactile sensitivity. Snellen charts for testing visual acuity are available in a variety of forms including pictures suitable for use with young children. Visual acuity is a measure of the ability to focus images on the retina. Contrast sensitivity is a measure of spatial vision--the ability to discriminate line gratings with varying spatial frequencies. Preferential looking methods are available to measure visual acuity and contrast sensitivity in infants and young children (Adams et al, 1992; Adams & Courage, 1993). The Functional Acuity Test, F.A.C.T. 101 (Stereo Optical Co, Chicago, IL), is recommended for measuring contrast sensitivity in children aged seven and above. Tactile sensitivity is a measure of somatosensory function assessed by administering vibratory stimuli to the fingers or toes and determining the intensity threshold for detecting the vibratory stimulus. Several tactile testing devises are available commercially (Amler and Gibertini, 1996). Tactile sensitivity can be measured in children six and older.

Day 2: Proposed Neurobehavioral Study Designs

The Neurobehavioral Workgroup discussed three study designs--(1) a retrospective cohort design, (2) a cross-sectional study, and (3) a longitudinal cohort study. The basic hypothesis addressed by these studies is that exposure to pesticides produces neurotoxic effects in children. A brief description and rationale for these study designs follow.

(1) **Retrospective Acute, High-exposure Cohort Study.** While one of the long term objectives of the pesticide studies in children is to determine if exposure to low levels of pesticides has measurable adverse health effects, one obvious starting place to examine the issue of pesticide health effects in children is in those who are exposed to high levels and who may have suffered acute symptoms from the exposure. Thus, we propose a retrospective cohort study of a fairly small group of children with clearly defined, high-level exposure for one initial study to determine unequivocally whether or not pesticide exposure at acutely toxic levels produces neurotoxic effects in young children. The hypothesis would be that exposure to high levels of pesticides, enough to produce acute toxic effects in children, also will produce measurable adverse neurologic effects. In this study common confounders to be controlled for include socioeconomic status, ethnicity, birth weight, gestational age, parental IQ, diet, and exposure to other potential neurotoxicants such as the heavy metals arsenic, mercury, and lead.

The study would be designed as a retrospective cohort study. Thirty children poisoned during the period 1990-1995 while aged 0-4 years would be selected from the registries of Poison Control Centers. One or two age (+/- 6 months) and gender matched unexposed controls would be selected per case. Exposure would be determined from hospital records. Age appropriate tests would be administered: these would include psychometric tests such as the Bayley-II, WPPSI-R, WISC III, and sensory tests to assess vision, vibration sense, and balance. Results of psychometric tests would be compared to national norms as well as to age and gender matched controls. A positive result of this study would be defined as the finding that children who were poisoned by pesticides during early childhood have measurable adverse neurologic effects on psychometric and sensory neurologic testing. A positive result would lead to cross-sectional or longitudinal studies of children with chronic, low-level pesticide exposure. If the retrospective cohort study yielded negative results, additional neurobehavioral testing of pesticide-exposed children may still be needed, since chronic low level pesticide exposure may lead to different neurological effects than a one time high level exposure. However, further discussion of the value of such studies would be warranted.

(2) **Cross-sectional Chronic, Low-exposure Study.** If results of the retrospective cohort study suggest that acute, high level exposure to pesticides produces neurotoxicity in children, then a further study of exposure in children would be advisable. In this study the hypothesis would be that differences can be detected in the neurobehavioral measures among children in three exposure level groups. Three groups-- high, middle, and low deciles (10%) of exposure-- would be selected based on responses to an exposure and pesticide usage questionnaire which would be administered to mothers. Children in this study would be aged 1.5-2.5 years, and only mothers with children in this age range at the beginning of the study would be eligible for participation. Approximately 100 child participants would be recruited. Possible sources for participants are Women Infants and Children's (WIC) Clinics or state health department well-baby clinics. An alternative approach would be to identify groups of children more likely to be exposed to high levels of pesticides. Possibilities might include children of pesticide

exterminators, children living in apartment buildings sprayed on a regular schedule with chlorpyrifos, or children of farm workers--particularly those whose parents take them to the fields with them. In selecting an exposed group, careful consideration must also be given to an appropriate and readily available non-exposed control group. For instance, finding controls for children of exterminators--e.g., children of appliance repairmen--or children living in chlorpyrifos-treated apartment buildings--e.g., children living in apartments not treated for cockroaches--might be easier than finding comparable non-exposed matches for farm children.

The Bayley-II Test (2nd-ed.) is recommended for neurobehavioral assessment of children in the age range of 1.5 to 2.5 years, and would be administered to child participants. Exposure measures would include house dust as well as urine samples for biological measures of agents such as specific organophosphates (OP), OP metabolites, a-esterases, and an OP screen; carbamates could also be measured. Since the consistency of OP urinary measures over time is not well documented, as a preliminary pilot study a subset of 30 children would be selected to participate in repeated urine sampling at time intervals such as weekly or every two weeks. This would give data regarding the consistency of the urinary pesticide measures over time in the three different exposure groups. A positive result of this study would be defined as the finding that children who were chronically exposed to pesticides at levels less than those considered acutely toxic, during early childhood, have measurable adverse neurologic effects on psychometric neurologic testing. This study would also provide information to determine how well questionnaire derived exposure information correlates with biological measures of exposure. Common confounders that would be controlled for in the neurobehavioral analyses include socioeconomic status, ethnicity, birth weight, gestational age, parental IQ, diet, and heavy metals such as arsenic, mercury and lead.

(3) **Longitudinal Cohort Study.** If neurobehavioral function is shown to be impaired by low-level chronic OP exposure in the cross-sectional study, a logical next question is whether neurobehavioral function changes over time as a result of chronic exposure. We propose to select 100 children, aged 1.5 to 2.5 years, living in a high-risk area, such as an agricultural area, from WIC or well-children clinics, or Head Start or day-care centers. The Bayley Test would be administered to these children at 3-month intervals for one to two years. Urine samples would also be obtained at each testing for measurement of OP levels, metabolites and a-esterases. This would provide a perspective over time of changes in both neurological function and urine pesticide levels. If it were necessary to economize on analysis costs, urine samples could be frozen and then possibly aggregated over time. Frozen urine samples could also be analyzed retrospectively, such as when an abnormal Bayley score was obtained. However, the most desirable study design would be to analyze each urine specimen obtained over the entire time period. A positive result of this study would be defined as the finding that children who were chronically exposed to pesticides at levels less than those considered acutely toxic, during early childhood, have measurable adverse neurologic effects on psychometric neurologic testing, and that these effects can be measured longitudinally over time. Common confounders that would be controlled for in the neurobehavioral analyses include socioeconomic status, ethnicity, birth weight, gestational age, parental IQ, diet, and heavy metals such as arsenic, mercury and lead.

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Developmental Workgroup Report

Workgroup Members: Pauline Mendola (facilitator), Asa Bradman, R.J. Dutton, Steve Hern, Antolin Llorente, Andres Lugo, Anne Sweeney.

Day 1 - Health Endpoints

Introduction and Synopsis of Discussion

The purpose of the first breakout group discussion was to generate a list of health endpoints in pediatric populations which could be classified as "developmental". Our task was to try and enumerate potential health effects without giving much attention to exposure or study design issues at this stage. This proved difficult and there was considerable productive discussion of the need to begin with a prenatal, longitudinally-followed cohort in order to adequately assess exposure and pediatric developmental health while controlling for a variety of confounding factors. Even with a prenatal cohort, health effects occurring earlier in the reproductive spectrum (e.g., fertility, fecundability) could not be ruled out.

There was substantial concern that it would be difficult to attribute any effect of pesticide exposure to child developmental measurements without understanding more about the potential mechanisms of action and controlling for factors that are known to influence child development (e.g., gestational age, maternal education, social class, etc.). The timing of exposure, particularly during gestation, may influence developmental outcomes as well as the dose. In this case, the issues are particularly complex because of the limited information on pesticide-related health risks for children. Exposure could be directly and independently related to a developmental health risk, but it seemed likely that the relation between exposure and child development would be more complex. Exposure could be related to social class, education and income and could also be related to other risk factors such as congenital anomalies, birthweight, nutrition and general health status. The probability of biologic relation between pesticide exposure and developmental effects was discussed in general as were issues regarding latency (time between exposure and health effect). Prenatal exposure was thought to be important for many developmental endpoints, either through a direct mechanism or as a potential initiator or confounder in the presence of a later postnatal exposure.

In the absence of a clear understanding of the likely pathway and mechanism for pesticide exposure to influence child development, the group proceeded to discuss potential health endpoints for study that could help define the relation or provide background information on the health status of border children to aid in the interpretation of future study findings.

The workgroup identified nine health endpoint groups, which are outlined in table 1 below. A more detailed discussion of each endpoint follows the table. Endpoints are presented in order of the priority ranking given by the group. Rankings were based on a five-point scale with "l" as the highest and "5" as the lowest priority. Priority scores were based on relevance of the endpoint for a study of pesticide-related effects and need for baseline health status information on the population.

Birth Defects, Stillbirths, Spontaneous Abortion (priority ranking: 1)

Gender/Age. This endpoint addresses males and females from recognition of pregnancy through the newborn period (primarily). Some birth defects may not be diagnosed until the second year of life.

Population prevalence. The population prevalence of major birth defects among live born children is about 3-4/100 live births. Neural tube defects (NTDs) are a particular concern in the border area. The general prevalence of NTD in the United States is about 1-1.5/1000 live births. In the border area, the prevalence may be higher (1.4-2.8/1000 live births). One to two percent of births are stillbirths and 12-15 percent of recognized pregnancies end in spontaneous abortion. Anomalous embryos and fetuses are at a higher risk of loss, particularly early in gestation. This, as well as the prevalence of prenatal diagnostic procedures and elective terminations will effect the birth prevalence of major birth defects, reinforcing the importance of prenatal assessments to reduce survivor bias.

Measurement. Potential sources of data discussed were: birth defects registries, birth certificates and fetal loss registries; hospital discharge data; and clinic and OB/GYN medical records.

Modification/pilot testing needed. There was interest in learning about the availability of genetic information, both to potentially classify aborted fetuses based on karyotype and to potentially develop genetic markers of susceptibility, exposure and/or effect. The need for standardization of methods across the border area was expressed for all studies using abstraction of registry or medical record data.

Mental, Motor, Adaptation (priority ranking: 1)

Gender/Age. Appropriate for both males and females. Different instruments are available to assess these endpoints from newborns through age 18. The group focused discussion on children from 4 months to 42 months.

Population norms. Norms are available for all tests. Children would be assessed for age-appropriate development.

Measurement. The Bayley scales (0 to 42 months) have been adapted for Spanish language populations and should be used here. Other inventory measures such as the Minnesota Infant Development Inventory (4 to 15 months), Child Development Inventory (≥15 months), Child Behavior Checklist (two forms: ages 2-3 years and ages 4-18 years) should also be included in the assessment. The idea is to get a relatively comprehensive picture of child development using both infant/child examinations and parental reports. Indices will include fine and gross motor skills, an index of mental function, and adaptation. Other possible tests for young children include the Peabody Motor Scale (1 year to 6 years old), and the Vineland Adaptive Behavior Scales (0 to 18 months). The Woodcock-Johnson test of cognitive abilities can be conducted on children three years of age or older.

Examiner needs. Testing must be done by trained personnel. Minimally, a masters level psychologist with special training or certification in the test protocols is needed.

Modification/pilot testing needs. The Bayley scale and Woodcock-Johnson test have been adapted for Spanish speaking populations. Other measures will need adaptation and pilot testing.

Gender/Age: Males and females of all ages could be addressed under this endpoint. Main focus is on young children (under 42 months).

Population prevalence: This was hard to determine. There is a consensus that acute pesticide poisonings in children are grossly underreported. It was estimated that greater than 20 cases per year would be reported to poison control centers; national average was estimated at 1/2500 children. Emergency room visits were discussed as another potential source of cases and also as a datasource to estimate prevalence.

Measurement. The idea behind this endpoint is to establish some notion of the natural history of acute pesticide exposure. Children with documented acute high exposures can be carefully examined for subsequent health effects. The developmental status of children would be characterized using age appropriate measures (see Mental, Motor, Adaptation above). Children would be followed over time to observe persistence of developmental effects and potential sequelae of acute poisoning.

Examiner needs. Testing must be done by trained personnel. Minimally, a masters level psychologist with special training or certification in the test protocols is needed.

Modification/pilot testing needs. Case finding through poison centers and hospitals must be piloted. The Bayley scale and Woodcock-Johnson test have been adapted for Spanish speaking populations. Other measures will need adaptation and pilot testing.

Growth (priority ranking: 1.5)

Gender/Age: This endpoint can be measured in males and females of all ages. The most interest is in birth measurements and longitudinal measures from birth through age five.

Population norms. Gender-specific norms are available. There was some discussion of the appropriateness of the available growth curves for border populations. Children can be compared to curves but there is a benefit to following children over time so that they can serve as their own controls as to rate of growth, etc.

Measurement. Birth measurements, particularly head circumference, were of interest. All birth measurements (weight, length, etc.) should be interpreted relative to gender and gestational age. These measures might be found in medical records and birth certificates but there was concern about error in measurement (particularly for gestational age, head circumference, and length). Height (length) and weight could be measured prospectively over time or could be ascertained from pediatric medical records for children with regular care at the same clinic/site over time.

TABLE 1: ENDPOINTS RECOMMENDED FOR DEVELOPMENTAL ASSESSMENT

Health Endpoint	Priority	G ender	Age	Prevalence/Norms Available	Measurement Tools Available	Pilot needed
Birth defect, stillbirths, spontaneous abortion	1	M & F	Gestation to birth	Y	Y	N
Mental, motor, adaptation	1	M & F	Birth to 42 mo.	Y	Y	Y except for Bayley
Acute poisoning sequelae	1.5	M & F	Focus < 42 mo.	Y	Y	Y except for Bayley
Growth	1.5	M & F	Birth to 5 yrs.	Y	Y	N
Language	1.5	M & F	Birth to 30 mo.; 36 mo. to preschool	Y	Y	Y except for CELF
Birthweight, gestational age	2	M & F	Birth	Y	Y	N
Social development	4	M & F	Birth to 3 yrs.	Y	Y	Y except for Bayley
Infant mortality	5	M & F	Birth to 1 yr.	Y	Y	N
Puberty	5	M & F	8 to 18 yrs.	Y	Y	Y
Hearing	Not rated	M & F	Birth to 18 yrs.	Y	Y	Y

Acute Poisoning Developmental Sequelae (priority ranking: 1.5)

Examiner needs. Standardization of measurement techniques is needed. Substantial error in measurement could obscure the potential relation between exposure and outcome. Most of these measurements are typically done by nursing staff, hospital house staff, or pediatricians. If measurements were made prospectively, someone with training to follow a measurement protocol (no special educational requirements) would be needed.

Language (priority ranking: 1.5)

Gender/Age: Males and females could be evaluated. Our focus was on children from birth through 30 months and 36 months through preschool.

Population norms. Norms are available for this test. Children can be compared to age-appropriate norms.

Measurement. MacArthur Scales can be used to assess language development in children from 0 to 30 months. The Comprehensive Evaluation of Language Fundamentals can be used with children 36 months or older.

Examiner needs. Testing must be done by trained personnel. Minimally, a masters level psychologist with special training or certification in the test protocols is needed.

Modification/pilot testing needs. The Comprehensive Evaluation of Language Fundamentals has been adapted for Spanish speaking populations. The MacArthur Scale needs adaptation and pilot testing.

Birthweight, Gestational Age (priority ranking: 2)

Gender/Age. Appropriate for all newborns, male and female.

Population norms. Available for general population in gender-specific form. Less well established for preterm infants and applicability of general norms to border population is unknown.

Measurement. Birthweight could be measured using birth certificate data or medical records. There was concern about the validity of gestational age reported on the birth certificate. In the absence of research protocols for recording gestational age, the clinical or pediatric estimate of gestational age is assumed to be more accurate in the medical record.

Examiner needs. Standardization of measurement techniques is needed. Ideally, a research protocol would be in place to aid in the assessment of gestational age.

Social Development (priority ranking: 4)

Gender/Age. Males and females could be evaluated. Our focus was on infants and toddlers (0-3 years old).

Population norms. Norms are available and children can be compared to age-appropriate norms.

Measurement. Bayley Scales of Infant Development, Child Development Inventory and the Vineland Adaptive Behavior Scales.

Examiner needs. Testing must be done by trained personnel. Minimally, a masters level psychologist with special training (certification?) in the test protocols is needed.

Modification/pilot testing needs. Instruments need adaptation and pilot testing for Spanish speaking populations.

Infant Mortality, Neonatal and Postneonatal (priority ranking: 5)

Gender/Age. Appropriate endpoint for male and female liveborn infants. Neonatal mortality occurs within the first 28 days of life and postneonatal mortality occurs between day 29 and one year.

Population prevalence. There is substantial variation in infant mortality by race and ethnicity. Infant mortality was approximately 8.4/1000 live births in the United States in 1994. The rate for Mexican Americans is similar to Caucasians (6.5 and 6.6/1000, respectively). There is also a suggestion in the literature that Mexican-born mothers have better birth outcomes than US-born mothers (may or may not impact on this question?).

Measurement. It was thought that all border states had linked birth-death files. Concern was expressed about error in recording the underlying cause of death for infants. The reliability and similarity of matching procedures across states should be assessed to ensure that the data are of comparable quality.

Puberty, Age at Menarche, Secondary Sex Characteristics (priority ranking: 5)

Gender/Age. Males and females, aged 8 to 18 could be assessed. There was some interest in looking at younger children for signs of precocious puberty, but it was felt this endpoint would have to be studied on a more focused basis rather than on a population level since the incidence would likely be low.

Population norms. Age-specific norms are available for pubertal development for both males and females (Tanner scales).

Measurement. Mailed questionnaires have been developed for pubertal self-assessments based on the Tanner scales. Physical examinations could also be conducted by physicians.

Modification/pilot testing needs. The acceptability and adaptability of existing measures to the border population would need pilot testing and potential adaptation.

Hearing (no ranking)

This endpoint was raised during the plenary discussion after the first workgroup session. The workgroup saw some relevance for including hearing as a sensory endpoint, but in the absence of data to suggest that hearing might serve as a target endpoint for pesticide exposure, there was more interest in general developmental motor and cognitive outcomes.

Day 2 - Study Designs

Introduction and Synopsis of Discussion

The purpose of the workgroup session on day two was to generate a collection of ideas for Phase II studies that addressed the developmental health domain. The summary sheets from the health endpoint session on day one were available for reference. The group began with a discussion of "priority" populations including: poisoned children (who could also serve as sentinels for areas of high pesticide exposure and who are more likely to experience and thus identify health endpoints with the strongest relation to exposure); farm families (farm workers, people who live in agricultural areas); children in homes/schools/daycare with regular structural (or regular indoor) use of pesticides; siblings of poisoned children; children in colonias; clinic populations with nonspecific symptomatology consistent with pesticide exposure; and clinic populations with identified developmental problems. Building on this discussion and the previous dialogue on developmental health endpoints, a series of potential study designs were assembled.

For the purpose of this report, the eight study designs are described in the order that they were presented at the strawman report on day three. There are three analytic studies, two descriptive studies and three "capacity building" studies. The actual day two discussion was much more dynamic, with ideas going back and forth between priority populations, endpoint and exposure measurement, and other design issues. As the products of a dynamic process, all of the study designs described here have some relation to the overall program of research proposed.

Analytic Studies - Prospective Prenatal Cohort

Study design: Prospective prenatal cohort.

Level of inquiry: Formal test of hypothesis.

Main hypothesis: Pesticide exposure is related to delayed and/or altered development and long term developmental problems.

Secondary hypothesis: Prenatal exposure to pesticides is more relevant than postnatal.

Target (referent) population: Exposed children.

Study group: Enroll women prenatally from a "priority population" such as an occupational group (farm workers) or from a clinic in an agricultural area; compare these women and their children to women and children from a group/clinic assumed to have low exposure.

Exposure measurement: Prenatally: questionnaire exposure/residence history and current practices; maternal blood and urine for exposure assessment and cholinesterase levels; house dust. Perinatally: maternal blood and urine, cord blood for exposure and cholinesterase levels; house dust. Collect breast milk samples from lactating women. Every six months for 2-3 years, characterize the environment, house dust, pesticide questionnaires, biologic samples from child.

Table 2: Outline of Proposed Studies

Proposed Study	Level	Design	Target Population	Time Frame
Prospective Prenatal Cohort	Analytic	Prospective cohort	Exposed children	4-5 yrs. to conduct; 2 yr. pilot
Poisoning Case Followup	Analytic	Case Series	Children with acute exposure	2-3 yrs.
Symptomatic Children	Analytic	Nested Case-Control	Children with chronic exposure	2-2.5 yrs.
Biologic Sample Correlations	Descriptive	Cross-sectional	General population of mothers and infants	1 yr.
GIS Infant Health Status	Descriptive	Cross-sectional	Border area infants	3-6 mo.
Compile Pesticide Dose Information	Capacity Building	N/A	Young children and juvenile animal research	< 3 mo.
Adaptation of Neurodevelopmental Tests	Capacity Building	N/A	Spanish speaking children and families	1 yr.
Border Physician Training	Capacity Building	N/A	Health care providers	1 yr.

Outcome measurement: Start with the prenatal maternal blood and urine measures to further identify (+) and (-) mother-infant pairs. Follow all (+) mothers and selected (-) mothers (this assumes that + mothers will be less common). Assess infant development using a standard battery of tests (see discussion under health endpoints). Neonatal tests should be conducted to assess alertness, habituation, reflexes, etc.. Developmental testing should occur at the following intervals, months 4, 9, 12, 15, 18, 24, 30, 36, 42. Tests at 12 and 15 months are anticipated to be particularly informative. House dust and other exposure measures should be tied to developmental testing whenever possible. It would also be possible, within this design, to assess a number of other reproductive outcomes including spontaneous abortion, preterm delivery, and birth weight.

Overall strengths: Longitudinal follow-up beginning as close to conception as possible with repeated exposure and developmental measures could address the main research question definitively.

Overall weaknesses: More information is needed to be able to design this study efficiently, such as: how to identify truly exposed versus unexposed (or high versus low) women and children for study; how to maximize the relevance of repeated exposure assessments in a variety of media; which developmental tests will be more sensitive to the effects of pesticides, etc.

Time frame: Approximately 4-5 years to conduct. Needs extensive piloting prior to beginning the study (2 years?).

Analytic Studies - Poisoning Case Series

Study design: Case series.

Level of inquiry: Formal test of hypothesis.

Main hypothesis: There are long-term neurobehavioral and neurodevelopmental sequelae of acute pesticide exposure that persist over time.

Secondary aim: Characterize the natural history of acute poisoning and determine neurobehavioral and neurodevelopmental presentation of poisoned children. Children could also be followed for alterations in sexual maturation which may be associated with exposure.

Target (referent) population: Age-specific general population on whom the norms for developmental tests are based.

Study group: Acutely poisoned children identified through poison control centers and/or hospital emergency room visits/admissions. Enroll children reported to be poisoned who are aged 0 to 42 months. Interest was expressed in including Mexican children who are reported to US health care facilities.

Exposure assessment: Based on poisoning reports (medical records, etc.). There is a potential to add followup pesticide exposure characterization to ensure that observed effects are sequelae of the poisoning incident and not due to ongoing exposure.

Outcome assessment: Conduct a baseline assessment within two weeks of reported

poisoning episode. The sequence of developmental followup testing will be based on age at enrollment and use a standard battery of tests (see discussion under health endpoints). Depending on age at enrollment, testing should occur at the following intervals, months 4, 9, 12, 15, 18, 24, 30 36, 42. Tests at 12 and 15 months are anticipated to be particularly informative.

Overall strengths: Description of the natural history and potential long-term neurobehavioral and neurodevelopmental sequelae of acute poisoning is needed. Longitudinal followup can assess effects over time. This study may help further refine the developmental endpoints which are most sensitive to pesticide exposure and help to define the testing time frames and protocols which best discriminate the effects of pesticides on child development. Studying poisoned children as sentinels may provide information on geographic areas and/or behaviors of high risk children. Modeled after occupational studies like those of Zweiner.

Overall weaknesses: Need to pilot case ascertainment process. Comparison to general population norms may be inappropriate. Dependent on our ability to develop a "fast response" team or individual psychologist who can travel to testing sites. The assumption is that information from acute poisoning provides information on potential effects of chronic low dose exposure - may or may not be true.

Time frame: Collect cases within the first year and follow all children at least one year or until age 42 months. Total time will be less than two years.

Analytic Studies - Prospective Nested Case-Control Study of Symptomatic Children

Study design: Nested case-control within a cohort of symptomatic children seen at a health care facility (or multiple sites?). Symptoms of pesticide poisoning are strikingly similar to the flu.

Level of inquiry: Formal test of hypothesis.

Main hypothesis: There are no developmental differences between symptomatic children with (+) pesticide urine screens and symptomatic children with (-) pesticide urine screens.

Secondary aim: Characterize the prevalence of nonspecific illnesses treated in a clinical setting which are likely due to pesticide exposure.

Target population: Children with chronic low dose exposure to pesticides. We assume children with chronic exposure will be more likely to present with clinical symptoms than children who are reported to poison control centers. No data are currently available to confirm this assumption.

Study group: Symptomatic children who present in a clinical setting for treatment. "Case definition" will be developed based on the flu-like symptoms associated with pesticide exposure. All children aged 2 - 3 1/2 years who fit the definition will attempt to provide a urine sample for pesticide screening and possibly a finger-stick blood sample for cholinesterase testing. Children with (+) screens will be compared to selected children with (-) screens.

Exposure measurement: Urine at the time of presentation will be screened for pesticides. Turnaround time for the initial screen will be a few weeks. Finger stick blood samples may be

available for measurement of cholinesterase and related enzymes. Subsequent to enrollment into case-control study (+/- screen), house dust and urine samples will be collected at a baseline approximately four-six weeks after clinic visit. Quarterly house dust and urine samples will be collected after the baseline visit. Possible "complete characterization" of selected (+) children to determine likely pathways of exposure including: air, duplicate diet and other environmental measures.

Outcome measurement: Establish a case definition based on symptomatology. Assess case (+screen) and control (- screen) children within six weeks of index clinic visit. For children under 42 months, use the standard tests described above. For children over 42 months, more complex testing could be done. Developmental testing should be timed with environmental and biological sample collection. Medical records can be reviewed to examine previous growth information and look for differences between cases and controls. Maternal education, socioeconomic status and access/utilization of health care are important confounders.

Overall strengths: Identifies children for study on the basis of "high risk" presentation. Allows for longitudinal follow-up of children with symptoms related to pesticide exposure who were not identified as "poisoned". As a companion to the poisoning case-series, this study can identify factors which may predict whether or not the pesticide exposure experienced by a child will come to the attention of health care providers, parents, caregivers.

Overall weaknesses: Dependent on clinic staff to identify cases, provide informed consent and collect urine and blood samples. Should we provide an incentive? Need a quick turnaround time from laboratory for urine analysis. Need a "fast response" team/individual similar to poisoning project. Older "young" children studied, don't have information on prior exposures.

Time frame: Training of physicians, medical staff should be done first (see below) - three to six months. After that, collect cases for six-nine months and follow cases and controls for at least one year. Total time - approximately 2 -2 1/2 years.

Descriptive Studies - Correlation Between Maternal and Infant Biologic Samples

Study Design: Cross-sectional.

Level of Inquiry: Feasibility pilot.

Primary aim: Describe the correlation and variability between pesticide levels (as well as cholinesterase and related enzymes) in maternal and infant biologic tissue samples in the general population.

Target (referent) population: General population of mothers and infants.

Study group: NTD control group in the Texas Department of Health NTD study. Approximately 140 general population control mother-infant pairs have provided multiple biologic specimens. Another potential study group is the population-based controls from a University at Texas - Houston, School of Public Health which has prenatal as well as postnatal samples from approximately 120 mother-infant pairs.

Exposure measurement: A variety of tissues are potentially available including maternal, fetal and infant. Some samples are banked and some have already been analyzed (possibly including pesticide data). Potential to collect house dust samples at the enrollment of new control subjects.

Outcome measurement: No health effects measured.

Overall strengths: Fills an important data gap. Will help us determine the appropriate biologic specimens to collect to adequately assess exposure. Data can be used to refine a biologic sampling strategy.

Time frame: Depending on what has already been done, could have results in about one year.

Descriptive Studies - GIS Studies of Infant Health Status

Study design: Cross-sectional.

Level of inquiry: Primarily descriptive but potential to test formal hypothesis.

Main hypothesis: Infant mortality and birthweight are not different in areas with high agricultural pesticide use compared to geographic areas with lower agricultural pesticide use.

Target (referent) population: Infants born in the border area.

Study population: Infants with live birth certificates filed in the border area (for the past year? Multiple years?) for studies of birthweight. Infants who died in the first year of life and have a death certificate linked to their birth certificate (most recent year available, usually a lag of 2-3 years; multiple years?)

Exposure measurement: Geographic mapping of pesticide usage including pounds per acre of active ingredients. Attempts will be made to map the best available pesticide usage data in the smallest area.

Outcome measurement: Vital registry data will be used to locate the place of residence at birth. Birthweight and mortality will be assessed in high pesticide use areas compared to low pesticide use areas.

Overall strengths: May help to identify high risk areas and provides baseline data on the health status of the border population. It is a concern that national statistics may not apply as a good reference population for the border. This exercise will provide data to address that concern. All border states have developed compatible GIS systems and this project makes good use of that capability.

Overall weaknesses: Pesticide use data is non-specific exposure data. No information on individual maternal/paternal/infant exposure is available.

Time frame: Depending on the availability of data, approximately three-six months.

Capacity Building - Pesticide Dose

Our assumption is that a summary of pesticide dose information in young children (and juvenile animals) is needed. The summary should include LOAL, NOAL, RfD, and LD_{50} for a variety of pesticides. This information would be very helpful in determining which pesticides to measure in children (assuming exposure to multiple compounds) and provide some information about susceptibility. The focus should be on pesticides known to be used or present in the border area.

Time frame: Less than 3 months.

Capacity Building - Adaptation of Neurodevelopmental Tests

Our assumption is that many of the currently available neurobehavioral and neurodevelopmental tests are inappropriate for use in the border population. Back translation is not sufficient and tests should be adapted for use with Spanish speaking populations. These efforts will increase the validity of studies of developmental endpoints in this population.

Time frame: approximately one year?

Capacity Building - Training of Border Physicians

Our assumption is that children suffering from the effects of pesticide exposure are not identified by health care workers or their parents/caregivers as having been exposed to pesticides. Training should be provided to physicians and health care providers in the border area. This program has relevance nation-wide as well. It is possible to stagger implementation, to use pre and post-testing to evaluate the effectiveness of the training and provide CME credits for training. This exercise is an important precursor to our proposed nested case-control study based on a cohort of sick children.

Exposure Assessment Issues Raised

Although the focus of the meeting was on the potential health effects which could be studied in young children in relation to pesticide exposure, the measurement of exposure was a recurrent theme. The following issues were raised by members of the developmental workgroup.

The difficulties of exposure assessment were a big focus of concern in various discussions. Investigation of methods and standardization of exposure measurement was seen as a high priority. Since the organophosphate pesticides were of particular interest, yet have a very short biologic half-life, it is difficult to classify exposure with one measurement. Serial measurements and modeling efforts to describe the relation between exposure and environmental levels of pesticides were stressed. Environmental samples are likely to be more persistent, particularly indoors, and may give an indication of usual or long-term exposure.

Housedust was perceived as a particularly useful media for exposure assessment. Many more analytes can be measured in environmental media than can be measured in biologic samples. Until the methods for biologic fluids are developed, thoughtful assessment of the environmental samples can provide otherwise unavailable data on commonly used pesticides.

The focus on acetylcholinesterase inhibitors, particularly common organophosphate compounds was also the topic of discussion. While restricting our focus to one class of compound or one mechanism of action may help to refine the study question, it is critically important that we remember there are others which are operating also. Other mechanisms of action and other compounds may have independent effects on the health outcomes we study and which may interact with the exposures we choose to measure. Aggregate exposures and multiple pathways are important issues.

Most of the biologic assays which were discussed are conducted on urine samples. The difficulties of collecting urine from small diapered children and infants seem daunting but the pediatricians think it is feasible to try. First morning voids are most problematic and may mean less for young children who urinate during the night.

There is a need for more qualified laboratories that can analyze research samples (biologic and environmental). Contract labs may not have the equipment and quality control procedures to achieve a limit of detection appropriate for a non-occupational study. The few research level laboratories available may not be able to keep up with the demands. Perhaps there should be a regional laboratory program to fund (or otherwise support) facilities whose purpose is to support epidemiologic exposure studies.

Immunology and Pulmonary Report

Immunology Workgroup Members: Betsy Hilborn (facilitator), Susanne Becker (rappateur), Lina Balluz, Donald Echobichon, David Camann, Anthony Horner.

Pulmonary Workgroup Members: Rebecca Calderon (facilitator), Jerry Blondell (rappateur), Bob Bornschein, James Ellis, Maria Martinez, Mary Kay O'Rourke, Enrique Paz, Jim VanDerslice.

On day one of the workshop, the immunology and pulmonary workgroups initially met as two separate groups. Toward the end of the session that day, the two groups met together to discuss relevant health endpoints identified by both workgroups. On the second day, the two groups met together to discuss both the immunology and pulmonary study designs. For the purposes of this report, each group has a separate report for day one (health endpoints) even though there may be some redundancy. For day two there is a single report from the group that covers both immunology and pulmonary study designs.

Day 1: Health Endpoint, Pulmonary

The pulmonary workgroup discussed both the utility of validated disease endpoints and self-reported symptomatology in assessing overall pulmonary health. Also, the group discussed physiologic measurements for use in either a clinical or an epidemiologic setting in order to accurately classify individuals' pulmonary health. The workgroup discussed four pulmonary disease classifications along with an extensive list of symptoms and physiologic measurements. Signs and symptoms of high interest were either suggestive or pathognomonic for each of the diseases. The issue of whether symptoms or disease could be ascertained by a questionnaire or had to be diagnosed in a clinical setting was considered in evaluating each disease. The four diseases and their measurement by symptomatology are summarized in Table 1. Physiologic measurements that could be used are summarized in Table 2. For each of the endpoints additional covariates were identified, whether the illness was acute, chronic or persistent and special considerations related to diagnosis and presentation of the disease by age group. Finally the group considered whether it was biologically plausible that exposure to pesticides could be associated with the health endpoint of concern.

Table 1. Pulmonary Disease and Symptoms

Signs and Symptoms	Upper Respiratory Infections	Asthma	Acute Bronchitis	Interstitial Lung Disease
cough	X	X- dry	X- productive	X
wheezing		X	X	
bronchorrhea	X		X	
shortness of breath	X	X	X	X- exertion
cyanosis		X- rarely		
congestion	X		X	
malaise	X		X	
fever	X		X	

Table 2. Pulmonary Disease and Physiologic Measures or Diagnostic Tests

Physiology Measurement	Upper Respiratory Infections	Acute Bronchitis	Asthma	Interstitial Lung Disease
PEFV			X	X
PEFR			X	
Spirometry			X	X
D2CO				X
Pulse oximetry				X
Swabs- microbial	X	X		

Upper Respiratory Infections (URIs). Many of the symptoms could be ascertained through the use of a questionnaire. The incidence of these infections is highest in infants and declines to about the same incidence as adults by six years of age. The majority of these infections are acute and transient. Other important risk factors to consider in epidemiologic studies include: prematurity, overcrowding, day care, seasonality, and nutritional status. The majority of the workgroup felt there was a low probability that pesticide exposure would be related to an increase incidence of URIs.

Acute Bronchitis. Many of the same symptoms associated with URIs are also characteristic of acute bronchitis. Similar to URIs many of these symptoms could be ascertained through questionnaire. Unlike URIs the incidence of this disease increases as children become older. Important risk factors to consider include: prematurity, overcrowding, day care, seasonality, and nutritional status. The issue of biological plausibility split the workgroup between suspected plausibility and a frank unknown.

Asthma. Many of the symptoms of asthma could be ascertained by a questionnaire but asthma should be diagnosed by a physician. The condition of asthma is generally intermittent after an acute onset. Asthma is a rare diagnosis in children under the age of three. After three years of age, the incidence increases, peaking at about 10-12 years of age and then it begins to decline slowly in young adults, ages 18-21. Important risk factors to consider in studies of asthma include: smoking (active and passive), viral respiratory infections, genetic disposition, air pollution, breast feeding, allergens, low socioeconomic status, and parental occupations. It was the overwhelming consensus of the workgroup that there was a biologically plausible relationship between pesticide exposures and asthma. The group had a small discussion about the difference between exacerbation and induction of asthma. The workgroup felt that pesticides could be involved in causing an asthma condition as well as exacerbating an existing condition.

Interstitial Lung Disease. Similar to asthma, signs and symptoms could be obtained by questionnaire, but cases must be confirmed through a physician diagnosis. Physiologic measurements (PEFV and spirometry) could be used in identifying possible cases or measuring severity of the condition. This disease is rare in infants and children, since it is often the result of a long term chronic exposure. Important risk factors to consider include: dust, air pollutants, parental occupation

and other underlying disease. The majority felt there was a low probability that pesticide exposure was related to interstitial lung disease.

Day 1: Health Endpoints, Immunology

In order of priority, the four diseases and methods for clinical and laboratory diagnosis are summarized in Table 3.

Table 3. Priority Immunologic Health Endpoints and Physiologic Measures or Diagnostic Tests

Physiology Measurement	Asthma	Allergy	Immunodeficiency	Contact Dermatitis
Pulmonary function tests	X			
Total and/or specific IgE	X	X		
Skin testing	X	X		
Provocation testing	X			
Total Ig levels			X	
Antibody titer response to antigenic stimulation			X	
Delayed type hypersensitivity testing			X	
T-cell subset measures			X	
B-cell subset measures			X	
Lymphocyte proliferation assay			X	
Patch testing				X
Physical exam	X	X	X	X
Medical records	X	X	X	X
Questionnaire	X	X	X	X

1. **Asthma (reactive airway disease)** This health endpoint was of major interest to researchers and clinicians who work along the border. The incidence of asthma is on the rise with an estimated 30% of children experiencing at least one episode of wheezing during their first 3 years of life. All ages and both sexes are potentially affected. Some children 'outgrow' their asthma symptoms. There is some evidence that low socioeconomic status is associated with higher rates of childhood asthma. Approximately 5% of the adult population was estimated to be diagnosed with asthma.

Specific measures of disease were discussed. Pulmonary function testing was considered the best clinical measure. This test has some limitations of use in the youngest age groups. Some other clinical measures discussed were: measurement of total IgE, skin testing for allergies,

allergen-specific IgE, and provocation testing. These tests would all require skilled clinical and/or laboratory personnel to perform. The usefulness of data derived from questionnaires and medical records was also discussed. Questionnaire administration would require some training.

2. **Allergic diseases (allergic rhinitis, eczema, allergic broncho-pulmonary aspergillosis)** Allergies manifest themselves in multiple organ systems; the workgroup focused on the respiratory and dermatologic systems as among the most commonly affected. Eczema is most common in infants, but all ages are potentially affected. The sexes were considered to be equally affected. There is some variability in the duration of allergic disease. Discussion focused on allergic children and the fact that they may cease to have symptoms of allergy during mid-childhood, but that these symptoms may reappear during later years.

Allergen skin testing is a valuable tool in the diagnosis of allergy. There was some controversy in the group about the use sensitivity/usefulness of skin testing in children under 5 years of age. Other measures that would require clinically trained personnel include: measurement of total IgE, physical exam and interpretation of findings. Questionnaires and medical records may also be useful.

3. **Immunodeficiency** There was much interest in the possibility of pesticide exposure and the development of primary or secondary immunodeficiency in an exposed child. Males are more likely than females to experience primary immunodeficiency due to X-linked disease. It was unknown if there was any modifying effect on the association between pesticide exposure and immunodeficiency due to socioeconomic status or ethnicity.

Tests to assess immunologic competency include: total Ig levels, with a result less than 2 standard deviations below the mean was considered abnormal. Antibody titer response to antigenic stimulation, T-cell and B-cell subset measures, a complete blood count with differential, delayed type hypersensitivity testing, and lymphocyte proliferation studies were all considered to be potentially useful measures of immune structure and function. These tests would require a phlebotomist and trained laboratory personnel to perform and interpret the assays. An accurate infectious disease history obtained during physical exam or questionnaire interview may provide valuable information about functional immune status.

4. **Contact dermatitis** Contact dermatitis was considered of interest because it is common and easy to measure in all ages, including infants. There is no known difference in prevalence by age, sex, ethnicity or socioeconomic status. It is known to be associated with exposure to phenoxy herbicides, fungicides such as maneb, mancozeb, and some 'inert' petrochemical ingredients of pesticide formulations.

Contact dermatitis manifests as a rash that may be difficult to distinguish from eczema. A trained clinician would be required to make the diagnosis. Patch testing is the method of choice to confirm the diagnosis of contact dermatitis.

5. **Autoimmune diseases-** There was concern voiced by a clinician that works along the border, that there is an increased number of lupus erythematosus cases being diagnosed in children living along the border. The overall prevalence in the U.S. pediatric population is 'very low'. Females are affected more frequently than males. African-Americans are affected more frequently than Caucasians. The disease is usually diagnosed during adolescence, so this would not be a useful

health endpoint in very young children. The disease is chronic, but symptoms may remiss, then recur.

Diagnosis is made by documenting the presence of a number of signs and symptoms. Clinical samples such as blood and urine may be examined for: anti-nuclear antibody, an elevated erythrocyte sedimentation rate, an abnormal complete blood count with differential, and a complete urinalysis with examination of sediment. A clinician is needed to perform a physical exam, a phlebotomist to draw blood samples, and a laboratorian to analyze the clinical assays.

6. **Inflammatory bowel disease (inflammatory colitis)** Inflammatory bowel disease (IBD) was discussed as a health endpoint believed to be related to a disorder of the immunological system. There was discussion about the possibility of a genetic component in the development of IBD because of familial and racial predilection in some disorders (e.g.: Crohns disease within some Jewish families). It was unknown if there was a difference in prevalence based on gender or socioeconomic status. IBD is a chronic disease of long duration; no one knew of any association between pesticide exposure and the development of IBD.

Diagnosis of IBD is made by a trained clinician with history, physical exam, and colonoscopy. A nonspecific test for blood in the stool (guaiac assay) may be may also be performed by anyone with minimal training.

7. **Infectious diseases** During group discussions, a hypothesis was made that an individual with immunocompromise would be more susceptible to and experience more frequent infectious diseases. It was unknown if there would be any difference in rates in males and females, but it was felt that children under the age of 2 would be more susceptible to infection. There was also some discussion that children from families with low socioeconomic status may experience more infections due to the potential lack of safe food, water, proper sanitation, and adequate nutrition.

Infectious diseases may be diagnosed by a variety of methods depending on the infectious agent. In most cases a trained clinician would be required to perform a physical exam, and there would be a need for clinical sample collection and analysis. Laboratory capabilities in microbiology, microscopy, immuno-cellular techniques, and possibly molecular methods may be needed. Questionnaires may also be used.

8. **Adverse reproductive outcomes-** The group also briefly discussed that immunopathology in an adult female may contribute to adverse reproductive outcomes, ranging from fetal death to prematurity. There are known associations of maternal race and some maternal behaviors with adverse reproductive outcomes. We did not know of any association with exposure to organophosphates.

This health endpoint could be extracted from medical records or by questionnaire so minimal training would be required to assess reproductive history.

Day Two - Development of Strawman Study Proposals

The purpose of the day two breakout group was to generate a collection of potential studies for Phase II implementation. Study hypotheses were generated based on the day one discussion of health endpoints, and the speakers presentations on pesticide exposures and border populations.

A collection of potential Phase II (pilot) studies were generated under each health endpoint domain, rather than designing a "Cadillac" study. Generally, the discussion was organized around the study design rather than the endpoint (in some cases multiple endpoints could be addressed in the same study design), but it was also appropriate to generate a series of similar designs which require different measurement strategies (e.g., a series of cross-sectional studies). Groups were not asked to develop a study design for every endpoint generated in the previous days' discussion, but to try to be comprehensive and at least get some ideas about the highest priority endpoints. The facilitators were asked to elicit information and encourage discussion about the main hypotheses or aim of the study; the target population; the appropriate study group; exposure and outcome assessment issues; strengths and weakness of the design; and the time frame needed for the study.

Groups discussed the relative merits and feasibility of the studies generated and prioritized the proposed studies. Ranking was based on the information gathered in the earlier discussion. Groups were instructed to be mindful of the time frame for Phase II studies. If a priority study was a longer term (larger scale, etc.) endeavor, could a pilot test be done at this phase? Reports on the strawman proposals were made to the entire group on the morning of day three in a plenary session.

Day 2: Proposed Immunology and Pulmonology Studies

On the second day, the Immunology and Pulmonary groups were merged to discuss study protocol development since the two immune-related diseases of most concern, asthma and allergy were identified as the #1 and #2 health endpoint of concern by both groups. There was some discussion about the diagnosis of asthma in young children, so the diagnosis "reactive airway disease" was adopted to describe asthma-like disease in infants and young children.

The first issue to be discussed was of an ethical nature; individuals may be motivated to participate in community studies by the promise of information about the health and exposure status of the community as a whole. Communication of study results may evolve into a sensitive issue. If results are released in an inappropriate manner, it may lead to concern and distress beyond what is warranted. However, part way through the study, the data may indeed indicate an association between exposure and health problems. Should the community be informed of preliminary results, or should all the data be collected and analyzed before community involvement?

Epidemiological studies require a long period of data collection, then analysis and interpretation. The long lagtime between participation in a survey and feedback from investigators may frustrate community members anticipating an immediate interpretation of study results. Some participants felt that subjects should be kept informed on an ongoing basis to avoid the appearance of neglect and disinterest. Another approach would be to communicate a proposed time-line during the recruitment phase of the study, so that participants will have a realistic expectation of when to expect an analysis of the study data. Any preventive methods to reduce exposure discovered during the course of investigation should be communicated.

A second issue of concern was the use of adequate interview tools. Spanish should be the native language of the interviewers. All health questionaires used should be available in Spanish and proven to be appropriate for the health concerns of interest.

Some study designs were proposed to evaluate the association between pesticide exposure and immunological and pulmonary health effects. It was felt that a cross sectional survey was needed in target communities along the border. This would serve as a pilot study of data and sample collection, and form a basis for future studies with more specific objectives. The approach was the refinement of existing questionnaires for evaluation of pesticide exposure such as the National Exposure Health Assessment Survey, and the use of questions from pre-existing questionnaires for airway disease and other health effects such as those developed by the American Thoracic Society and the National Health Interview Survey. All of these are available in Spanish and are proven appropriate.

Study Questions: Is there excess pulmonary or infectious disease in this community? What is the distribution of pesticide exposure? Is exposure associated with excess pulmonary or infectious disease in this community?

Study Design: Cross Sectional Study, questionnaire derived exposure combined with self-reported health endpoints. Exposure assessment supplemented by GIS and some environmental sampling.

Study population: The goal is to recruit children, less than 11 years of age. Survey questionnaires will be administered to parents of the study population. Recruitment of study population will occur through the Women Infant Children Program (WIC), clinics, day care centers, and schools. It was suggested that highly exposed children could be recruited through the Poison Control center. Benefits to study population; financial incentives could be offered in exchange for study participation.

Questionnaires: The pesticide exposure survey should yield at least the following information: occupation of parents, address, type of residence, presence of livestock or pets, proximity of the home to an agricultural area, if yes, type of agriculture, use of pesticides in home, on property, on pets or children, frequency of use, identity of applicator, home pesticide inventory, known pesticide exposures, sources of food and drinking water.

Parents will be interviewed concerning children's history of and presence of asthma, allergies, eczema, lupus erythematosus, respiratory tract infections, other infections, and gastrointestinal problems.

Survey information may be combined with agricultural pesticide use data obtained from the pesticide control boards and health departments. Currently the most comprehensive pesticide usage data is available in California and Arizona.

Strengths of approach: The strengths of this approach are: 1) standard questionnaires are available, 2) some questionnaires have Spanish versions that may be more useful in predominantly Hispanic communities, 3) surveys can be an economically efficient way to collect information, 4) large samples can be collected in multiple communities and 5) data can be used to generate hypotheses.

The study designs are summarized in Table 4: Proposed Immunologic/Pulmonary Epidemiologic Studies.

Table 4. Proposed Immunologic/Pulmonary Epidemiologic Studies

Proposed Study	Level/ Design	Target Population	Issues	Time Frame
Pilot study of immunologic status and development of infants exposed to pesticide	Cross sections	Border children <1 year old	Feasibility of studying infants and collecting clinical samples	2 years
Longitudinal study of a birth cohort	Longitudinal study	Border families	Loss to follow-up, resource intensiv	5 years, minimum
Survey of border familie	Cross sectional study	Border families	May study large number of subjects cost effectively, but health endpoints not validated. Potential for bias in exposure and outcome measurement. May be useful to develop hypotheses about health endpoints of interest. Use of environmental samples to assess exposure will increase cost of study.	2 years
Case-control study	Case-control study(cases=exposed)	Border children <11 years of age	Difficult to assess chronic exposure levels resulting in mis-classification.	2 years
Case-control study of children with hyper reactive airways.	Case-control study using methacholine challenge	Border children < 6 years old.	Risks to children with preexisting airway disease associated with exposure to methacholine. Exposure assessment costly.	2 years

Hypothesis 1: The prevalence of asthma and other diseases will be higher in individuals with increased pesticide exposure.

Weaknesses of approach: The weaknesses of this approach are: 1) it is a survey, and only one point in time is assessed, 2) cross sectional studies have limited ability to detect rare health events or exposures and 3) conducive to mis-classification, and information bias.

Hypothesis 2: Pesticide exposure increases the incidence of and/ or exacerbates pre-existing asthma.

Case-control study: The next study design to be discussed was a case-control study. A case control study based on health outcome (asthmatics as cases), was proposed. Households would be surveyed for pesticide usage and exposure, and the homes monitored for pesticide residues. Doubt was raised that this design was appropriate since the development of asthma is known to have numerous associations with the presence of other allergens such as house dust mites, cats, cockroaches, and *Alternaria spp.* The group discussed designating cases and controls based on exposure status.

Study design: Case-control study based on exposure. Those persons with highest pesticide exposure would be designated as cases.

Study population: Children < 11 years of age.

The approach would be to select cases and controls based on exposure, and then evaluate these groups for prevalence of asthma. High and low pesticide exposure households with children < 11 years of age would be identified, for detailed pesticide sampling and monitoring. Asthma prevalence in the households would then be assessed by questionnaires, and medical records.

Strength of study: May evaluate specific health outcome hypothesized to be associated with a rare exposure.

Weaknesses of study: The weaknesses are: (1) difficult and expensive to assess exposure levels to assign case or control designation; 2) mis-classification of a problem, as pesticide exposure is ubiquitous and current measures of exposure may have no correlation with past exposure levels.

The group discussed another study related to the association between asthma and pesticide exposure.

Hypothesis 3: Pesticide exposure contributes to airway hyper reactivity.

Study design: Case-control study based on response to a methacholine challenge test.

Study population: Children > 6 years of age.

A methacholine challenge test to objectively assess airway reactivity would be administered to a group of healthy children. Children would be assessed for pesticide exposure. Exposure history (possibly urinary metabolites, household samples) of children with airway hyper-reactivity would be compared to those of children with no evidence of hyper-reactivity.

Strengths of study: 1) Specific, objective outcome measure, 2) strong biologic plausibility.

Weaknesses of study: 1) Difficult and expensive to administer, patient cooperation and trained pulmonologist required; 2) retrospective exposure assessment by questionnaire imprecise, urinary and household measures expensive and current measures of exposure may have no correlation with past exposure levels.

A pilot of a longitudinal study was proposed to study immune system development in infants.

Hypothesis 4: Pesticide exposure affects the development of the immune system in infants and young children resulting in altered antibody response to vaccine administration and increased incidence or severity of infectious disease.

Study design: Cross sectional study as a pilot for a longitudinal study of a birth cohort.

Study population: Children < 1 years of age.

Initially, a pilot study would be performed on approximately 30 subjects in each of four groups; maternal/neonatal, four month, six month, and twelve month old groups. Subjects would be recruited from a community health clinic. In the neonatal group, women near delivery would be interviewed to assess pesticide exposure, samples of blood and urine would be collected. At delivery, cord blood and placental samples would be collected to assess immune system function and pesticide levels. Infants at the age of four, six, and 12 months would be assessed, mothers would be interviewed and urine samples would be obtained at each assessment. Blood, urine, hand wipes and household environmental samples would be collected at six and twelve months. Questionnaires about health status and pesticide exposure can be given to the mother when the child is seen in the clinic. Blood samples would be analyzed for indicators of immune function such as antibody response to vaccination, T-cell immunity, leukocyte marker analysis, and T-cell proliferative response to superantigen. The full-scale study would evaluate a cohort of children in a longitudinal study design, would follow children longer than one year, and would incorporate developmental assessments.

Strengths of study: 1) May evaluate specific immune system parameters in children at an age when immune function is rapidly evolving; 2) may evaluate response to antigenic challenge in the form of naturally occurring infection and planned vaccinations; 3) short-term exposure history for an infant, with few sources of food and water and 4) crawling/mouthing infants have greater potential for exposure.

Weaknesses of study: 1) Invasive assessment (blood draw) of health outcome, may be poorly tolerated by the infant or parent; 2) longitudinal study requires geographically stable population and 3) ethics of exposure results/interpretation/reporting need to be explored.

CANCER WORKGROUP

Workgroup Members: Martha Moore (facilitator), Jim Quackenboss (rappateur), Jonathan Buckley, Luis Escobedo, Debra Gilliss, Luis Ortega, David Camann, and Judy Henry.

Introduction

In the United States, cancer is the second leading cause of death for children between the ages of one and 14 years. Although overall cancer rates have generally been declining, the rate of childhood cancer (specifically acute lymphoid leukemia, tumors of the central nervous system and bone) has increased in North America. The cause of this increase is unclear, but the possibility that it might relate to environmental exposure is real and should be investigated.

There is some evidence that parental occupation is associated with an increased risk for childhood cancers. In addition to paints, solvents, radiation and hydrocarbons, parental exposure to agricultural chemicals has been linked to cancer in children. A number of epidemiological studies involving adults have shown an association between pesticide exposure and cancers (see Buckley, this volume). It seems reasonable to postulate that pesticide exposure would be of particular concern to children who are growing rapidly. Cancer is a disease requiring cellular expansion as a part of its etiology. Thus, the normal growth processes of fetal and child development provide the ideal setting for pesticides, that can modify genetic material and processes, to cause the initiation and/or promotion of tumor development.

Possible Strategies to Determine if Border Children Have a Higher Risk for Cancer

The Cancer Workgroup was tasked with discussing possible strategies to investigate whether children, who live along the U.S.-Mexican border, might be at increased risk for cancer. In approaching this issue, the group formulated the following questions: Does pesticide "exposure" cause (or contribute) to an increased risk of cancer in children who live along the border? Is there a difference in the pattern of childhood cancer and (or) pesticide usage between border regions and non-border regions?

The workgroup outlined and discussed several possible types of studies including: (1) using existing data bases; (2) performing an ecological study that would geographically compare pesticide usage and cancer incidence; (3) performing a case-control study that would identify cases and then determine if the cancers were associated with pesticide exposure; (4) conducting a prospective cohort study that might link exposure to a biomarker and then to the cancer; and (5) conducting a study that could link cancer-relevant biomarkers with pesticide exposure. A series of questions were raised that could be discussed for each approach. These included: (1) the size and characteristics of the population, (2) the existing data and current ongoing studies, (3) existing infrastructure--groups and organizations already in place and working in relevant areas, (4) appropriate strategies for exposure assessment, (5) confounders, (6) study strengths, (7) study weaknesses, (8) time frame and cost, (9) probability of useful outcome and (10) what questions might be answered.

Use of Existing Databases

There are currently a number of cancer registries that can provide cases and incidence rates for various cancers. Some state registries may already have information by county. There are also two national cooperative clinical trial groups that are dealing with children's cancer---the Children's Cancer Group (CCG) and the Pediatric Oncology Group (POG). Both groups provide good resource information and possibilities for collaborative study. Using this approach it would be relatively easy to obtain the relative proportion of cancer types and the number of cancer cases that occur within any given geographic area. It is, however, difficult to get reliable rates due to the small population sizes.

On the exposure side, there are or soon will be GIS maps and data that link agricultural usage, crops and pests. There is also a very active and potentially helpful agricultural extension service. These agents are very familiar with activities within their geographic regions. Pesticide suppliers can also provide information. It is, however, hard to get actual usage information from most border states. California is the one exception; it has a very good reporting system. The real issue, however, is not pesticide use, but exposure levels attained by individuals living along the border. This information is really not available from any existing database.

Ecological Study

It would be possible to look at border and non-border regions and determine if there is a difference in cancer patterns and/or pesticide usage. This might provide a relatively crude ecological assessment. If both pesticide usage and cancer incidence is greater in the border areas, that would provide some additional hypotheses for further testing. The biggest danger from this type of approach would be the possibility of a cancer cluster. Upon further analysis of this approach it was determined that this approach would give little, if any, definitive information. If the study showed a positive association, it might support the need for additional study. If the study showed no association, it would not rule out the need for a further study. There was little enthusiasm for pursuing this approach.

Case-Control Study

Given that cancer is a very rare disease, the group discussed whether it would be possible to do a case control study. The group estimated that there would be about 150 new cancer cases per year in children aged 0-15 living in states along the border. Under the best case scenario, it might be possible to find and enroll about 120 of these cases. Looking just at leukemia incidence, it was estimated that there would be only about 35 cases per year. Cases could be obtained from clinics and hospitals. However, with this small number of cases, the group felt that it was unlikely that the study would have enough power to detect an association with environmental factors.

The limited power of the study was further exacerbated when the exposure assessment component was discussed. Three basic approaches to exposure assessment were discussed: (1) use of questionnaires such as are used by the CCG, (2) environmental samples from the home, particularly house dust, and (3) overall usage of agricultural pesticides. Past and current

studies to assess exposure were briefly discussed. A Brownsville study extensively evaluated 9 households over two seasons. NHEXAS is obtaining data in Arizona from 175 households. In addition, 100 households are being evaluated in Minnesota. None of these studies have really answered the question as to how many and which sampling strategies should be used. Given that the science of exposure assessment has yet to answer some of the basic design questions, it seems prudent to wait on conducting a case control cancer study until such issues can be adequately addressed.

At this juncture further issues that might make the study problematic were identified. It was noted that the majority of cancer cases would likely come from urban rather than rural areas. This would occur because the highest populations live in urban areas, particularly San Diego. This fact further limits the possibility that this study design could evaluate the association between agricultural pesticides and cancer. The number of people available for sample would fall below the number required to make the analysis. Furthermore this design requires that cancer cases be identified and then evaluated as to their possible pesticide exposures. The time between case ascertainment and exposure evaluation means that population mobility and lack of telephones would limit the number of cancer cases that could actually be enrolled and evaluated. It was also noted that there would be confounders to this study including socioeconomic status, infections and other potentially carcinogenic environmental exposures.

As to what questions a case control study might address, it was decided that one might see a weak association between cancer outcome and one or more possible measures of pesticide exposure. A negative study would provide no information due to the low power of the design. Thus, the utility of such a case control study in relation to the resources required would be very low.

The group discussed current projects that might provide insight for the border population. The two national children's cancer networks (CCG and POG) could provide useful information and identify possible cases. For instance in the CCG study, there are approximately 2000 cases of acute lymphoblastic leukemia (ALL) and 600 of acute myeloblastic leukemia (AML). Some of these cases would be in border states. Participants in the CCG study have completed extensive pesticide usage questionnaires. Thus the workgroup recommended waiting for the results of the CCG and POG studies.

Following the El Paso Meeting, Dr. Debra Gillis obtained some of the cancer statistics from the California Cancer Registry. This information is provided to further emphasize the difficulty of obtaining enough border County cases to conduct a case control study for childhood cancer. From 1988 to 1994, there were 47 childhood cancer cases in Imperial County, California. Of these, 15 were leukemias (32%), 10 were central nervous system cancers (21%) and 22 were other cancers (46%). In San Diego County, during this same time period, there were 583 childhood cancers of which 204 were leukemias (35%), 114 were central nervous system cancers (20%) and 265 were other cancers (45%). Thus, the pattern of the main types of childhood cancers is similar between the two counties and is also similar to the rest of the state. It is important to note that this is only 7 cases per year in Imperial County and 83 cases per year in San Diego County. It would therefore take a number of years to accrue adequate numbers of cases for analysis.

Biomarker Approaches

The low incidence of cancer makes it very difficult and expensive to assess whether pesticide exposure contributes to its etiology. Perhaps a better strategy is to utilize cancer-relevant biomarkers. The risk assessment paradigm relating exposure to disease is outlined below:

Usage →	Environ. Conc. →	Exposure "Contact" →	Internal Dose →	Target Dose →	Biol. →	Health Effect & Disease
Agric. Resid.	Dust Surface Food Air Water	Air-Inh. Ingest. Dermal ↕ Activity	Blood Urine	DNA Adducts	V(D)J gene	Diagnosis mutation

The workgroup discussed the possible utility of studying the relationship between pesticide exposure, biomarkers of exposure and biomarkers of biological effect. In the past, researchers have used DNA adducts as a biomarker of exposure and various genetic markers such as gene mutation. Unfortunately DNA adduct analysis requires the development of specific techniques for each pesticide. It is difficult to use this approach in a more generic way. Thus the workgroup recommended that the target dose biomarker not be used and that blood or urine levels be directly associated with the biological effect biomarker (see below).

The genetic markers that have been used, although measuring the types of genetic damage seen in the etiology of tumors, are not directly involved in the development of tumors. More recently, new molecular approaches have been developed to quantitate genetic alterations known to be involved in cancers. Some of these chromosome rearrangements are mediated by V(D)J recombinase. The workgroup recommended that this marker be utilized for the biological effect marker.

Env. Conc. →	Exposure →	Int. Dose →	Target Dose →	Biol. Effect →	Health/ Disease
"Dust"---------->		Blood Urine	----------> V(D)J	---------->	Disease Process

The workgroup considered possible prospective and case control studies that might incorporate the use of biomarkers. For the prospective study, highly exposed and unexposed individuals would be identified and compared as to their biomarker frequency. By focusing first on highly exposed individuals it should be possible to determine the utility of the approach. Such a strategy is already being followed by one panel member, Dr. Moore.

Ideally, one would want to conduct a prospective study that could determine whether the biomarker predicted the development of cancer. Unfortunately, the number of samples that

would need to be taken and achieved, the time required for tumors to develop and be ascertained, and thus the cost of the study, make such an approach prohibitively expensive.

The case control design would identify children with leukemia and ask if they have a higher level of V(D)J recombinase-mediated chromosomal rearrangements. The cases would then be evaluated as to their exposure status. Dr. Buckley, one of the panel members, is currently pursuing such an approach.

Both designs have issues with confounders. In particular, there would be a number of mutagens in addition to possible pesticides that might cause an increase in V(D)J recombinase-mediated rearrangements. It is also possible that V(D)J recombinase activity levels might be under genetic control and that some individuals might be inherently more susceptible to such rearrangements. The normal distribution of V(D)J recombinase activity has not been defined.

The workgroup identified several strengths of this biomarker approach. It seems likely that these types of studies would provide useful information. These genetic markers are non-specific for exposure and therefore might be used to provide insight into cumulative biological effects and thus cumulative risk and disease. Furthermore, the biomarker approach can be applied to a relatively small (compared to cancer assessment) number of cases.

The weakness of this specific biomarker approach is obvious. It is tied to the V(D) J "model" for cancer etiology. In addition, all of the previously described issues concerning exposure assessment apply equally to the biomarker studies. However, a careful coupling of biomarker analysis with exposure analysis might lead to useful conclusions as to the appropriate exposure assessment approach.

Conclusion

Reflecting upon the problems associated with the above approaches, the workgroup focused on the scientific issues underlying them. In all cases, the questions associated with exposure assessment compromised the conclusions that might be drawn from the study. Although databases exist, it is extremely difficult to access even the most fundamental pesticide usage information by geographic region. As already noted, the usage information provides little insight as to the level of exposure that either individuals or groups of individuals might receive. The workgroup, four of whom were representative of the border states, concluded and strongly recommended that the issues associated with proper exposure assessment be solved prior to conducting an analysis of health outcome. This would apply both to cancer and non-cancer. Under the best of circumstances, exposure assessment is difficult. In addition, there are some issues that are "unique" to the border. There are a number of pesticides that are legal for use in Mexico that are not allowed in the U.S. Pesticides from Mexico readily find their way across the border, often without proper labeling as to content. Thus there are products that are used in border communities that are not used in other parts of the U.S. There is essentially no information on these pesticides and their usage levels.

The workgroup concluded that the weakest part of the analysis rests with the exposure assessment. Therefore the group strongly recommended that resources be focused first on improving approaches to exposure assessment. Also, other efforts are already underway investigating childhood cancers, developing databases and evaluating approaches using biological biomarkers. The workgroup recommended waiting for these researchers to complete their work. Once the exposure assessment can be more adequately conducted, and the information from the cancer studies is available, it should be possible to revisit and make recommendations concerning studies to investigate the association of childhood cancers and exposure to pesticides.

Day 3: Group Discussion

The focus was on themes common among workgroups: efficient methods to screen for pesticide exposure, questionnaire development and validation, environmental sampling, and validation of biochemical measures of exposure. There is a need for information about the range of pesticide exposures in this pediatric population. Many outstanding questions remain: how well correlated are current methods of environmental exposure measurement and actual absorbed dose? What is the clinical relevance of metabolite or cholinesterase levels detected in biological samples? What is the population distribution of exposure endpoints for each agent of interest? What are the determining factors that result in a portion of the population being categorized in either 'tail' of the exposure distribution? What are the resources available for performing studies in the border region? What are the difficulties that will be encountered in attempting to study this specific population? What, if any, is the value of studying acutely poisoned individuals, and can this information be extrapolated to persons with chronic, low-dose exposures?

Individual workgroups presented proposed studies for group discussion. The neurobehavioral workgroup raised the question of what the next step would be if a cross-sectional study failed to show any health effects associated with high levels of pesticide exposure. The group suggested initiating the investigation into health effects by studying those children with a history of acute pesticide poisoning. If those children display altered neurolobehavioral function in some area, then at least a health endpoint of interest has been identified, one that can be examined in children with lower dose or chronic exposure. The group raised the issue of there being a scientific need for long term follow-up of those children who have experienced an episode of poisoning; it is unknown if neurobehavioral changes occur and how they may change over time. A cohort of children with repeated neurobehavioral assessment during a longitudinal study was proposed. Participants suggested a cohort could be derived from clinics or a Women Infants and Children program group.

The combined immunology/pulmonology workgroup presented three proposed study designs. A cross-sectional study of border populations would provide essential information about demographic, occupational, geographic charactaristics, and pesticide use. Techniques such as household pesticide inventory, environmental and biologic sample collection for pesticide exposure could be pilot tested. One goal would be to validate questionnaire-derived exposure data through the use of pesticide inventories and environmental and clinical sample collection. Based on the hypothesis that chronic pesticide exposure might increase airway reactivity, a cohort study was proposed. A group of children with quantified pesticide exposure

would be recruited. Children would undergo a methacholine challenge test and pulmonary function testing results from the high and low exposure groups would be compared. Another study was proposed to study immune development in infants. Initially, a pilot study would be performed: women near delivery would be interviewed to assess pesticide exposure. Samples of blood and urine would be collected. At delivery cord blood samples would be collected to assess pesticide levels. Infants at the age of four, six, and 12 months would be assessed, urine samples would be obtained at each assessment. Blood, urine and household environmental samples would be collected at six and twelve months. Blood samples would be analyzed for indicators of immune function such as antibody response to vaccination, leukocyte marker analysis, and T-cell immunity. Health histories would be collected at each assessment to evaluate the frequency of infection. The full-scale study would evaluate a cohort of children in a longitudinal design, would follow children longer than one year, and would incorporate developmental assessments.

The developmental workgroup proposed three study designs. Studies would be composed of a prenatal cohort with a prospective study of infant development, a case series of acutely poisoned children, and a prospective study of toddlers that would incorporate developmental assessment with questionnaire and environmental sampling to assess exposure. Some of the study questions discussed included: what is the relationship between maternal and fetal blood levels of pesticides, associations between geographic information system (GIS) exposure assessment and infant birth weight and mortality. The possible use of serum cholinesterase levels as a measure of exposure was discussed.

The cancer endpoint workgroup raised the question: is there a difference in patterns of pesticide usage between border and non-border regions? One approach would be to examine cancer registries to determine the relative proportion of specific cancer diagnoses in different geographic regions. One drawback of utilizing registries is the problem of under reporting and the difficulty of assessing the true rates of illness. Another approach would be to conduct an ecologic study to determine the association between rates of reported illness and GIS data on agricultural pesticide usage and residential proximity to agricultural areas. One risk associated with this approach would be the possibility of discovering an apparent 'border-related cancer cluster' which may create difficult community communication issues. Another approach would be to perform a case-control study using reported leukemia cases over a 2 year period. Approximately 70 cases would be expected during this time period. Exposure would be assessed by questionnaire, diet and pesticide usage assessment, household sampling, and occupation. Problems with this approach include: recall bias, confounding exposure factors, possible weak association between leukemia and pesticide exposure, high cost, and possible lack of telephones to facilitate contact. Two potential approaches for pilot study include: a retrospective cohort study among those diagnosed with cancer, and a prospective cohort study among those persons categorized as having experienced 'high exposure levels'. A possible source of confounding would be other environmental mutagens.

A comment from a member of the assembled group: the border community is requesting better exposure measurements. Local physicians would like to know the potential health effects associated with pesticide exposure, and to know if current levels of pesticide exposure are causing health problems.

A group discussion was held after the workgroup presentations. The following section is a record of the comments raised in the course of discussion.

1. Participants raised the issue that questionnaire validation was very important for these studies, that it is a difficult process to assess the health effects of mixtures of toxins.

2. Pathway analysis may be useful, but would be difficult to perform with multiple agents. Additionally, pesticides are semi-volatile; they may be found in multiple media.

3. The likelihood of questionnaire data correlating with exposure is low. We must focus on environmental and biochemical measures of exposure to increase precision of exposure assessment.

4. This is state-of-the art work. We may never achieve a linear correlation. More sophisticated modelling techniques may be needed.

5. In our work with measuring exposure to chlorpyriphos, we had 100% of subjects with measurable pesticide metabolites. How do we start to separate out the proportional contribution by media in assessing the source(s) of exposure?

6. We can't afford to analyze a large number of samples for the presence of pesticides when most of them will have no detectable levels.

7. We need to establish 'normal' ranges for health endpoints; how else can we interpret data? We need to consider both exposed and affected persons in the development of ranges. Yes, the 'tails' of ranges are rich in information.

8. It's essential to have a control group.

9. We need a lot of subjects (a high 'N'), not just a control group. We want to measure the whole range of 'normal' endpoints.

10. We know so little about the health effects of environmental contaminants here. The population exposure levels are unknown, the health status of this population is unknown.

11. It may be worthwhile to gather theses, dissertations and other literature about border populations that may contain both exposure and health information. There is not much on Medline.

12. We can target small grant recipients, universities and academic departments in the area. There is ongoing survey work along the border.

13. What is the value of acute poisonings? Answer: the clinical evaluation of poisoning cases. We may need to look outside the border for these. There are databases of poisonings, therefore one method may be to respond to an outbreak of poisonings , and then to study the group longitudinally. For instance, there is currently a follow-up study of methylparathion poisoning of children in the South.

AGENDA

WORKSHOP ON THE ASSESSMENT OF HEALTH EFFECTS OF PESTICIDE EXPOSURE IN YOUNG CHILDREN

DAY 1 Sunday, December 7, 1997

8:00 am	**REGISTRATION**
8:30 am	**Welcome, Introductions, Workshop Objectives** **Dr. Hal Zenick, U.S. EPA Co-chair, Environmental Health Workgroup**
8:45 am	**Keynote Address: Issues in Pediatric Epidemiology - Dr. Robert Bornschein**
9:30 am	**Review of Methods Available for Assessing Toxicity in Young Children - NEUROLOGY - Dr. David Bellinger**
10:15 am	**IMMUNOLOGY - Dr. Anthony Horner**
11:00 am	**COFFEE BREAK**
11:45 am	**DEVELOPMENTAL - Dr. Antolin Llorente**
12:30 pm	**LUNCH**
1:30 am	**PULMONARY - Dr. Maria Martinez**
1:45 pm	**CANCER - Dr. Jonathan Buckley**
2:30 pm	**Charge to Workgroups - Dr. Rebecca Calderon** **Workgroup Breakout** (Neurobehavioral, Immunology, Developmental, Pulmonary, Cancer)
4:30 pm	**Plenary Session: Workgroup Reports**
6:30 pm	**Adjourn**

Day 2 Monday, December 8, 1997

8:30 am	**Keynote: Health Effects of Pesticides - Dr. Donald Ecobichon**
9:15 am	**Increased Sensitivity to Pesticides in Young Children: Possible Mechanisms -Dr. Stephanie Padilla**
10:00 am	**Design of Children's Pesticide Exposure Survey - Dr. Jim Quackenboss**

10:30 am	**BREAK**
10:45 am	**Pesticide Use near the U.S.-Mexico Border - Dr. Gary Robertson**
11:15 am	**Pesticide Use and Assessment along the Arizona Border - Dr. Mary Kay O'Rourke**
12:00 pm	**LUNCH**
1:00 pm	**Issues in Studying Border Populations - Dr. Jim VanDerslice**
1:45 pm	**Cultural Considerations in Conduct of Epidemiolgic Studies - Dr. Robert McConnell**
2:15 pm	**Dr. James Ellis**
3:00 pm	**Charge to Breakout Groups - Dr. Rebecca Calderon** **Breakout Groups for Development of Strawman Study Designs** (Neurobehavioral, Developmental, Immunology-Pulmonary, Cancer)
6:00 pm	**Adjourn**

DAY 3 Tuesday, December 9, 1997

8:30 am	**Reports on Strawman Proposals** **NEUROBEHAVIORAL**
9:00 am	**IMMUNOLOGY-PULMONARY**
9:30 am	**DEVELOPMENTAL**
10:00 am	**CANCER**
10:30 am	**Closing Remarks and Discussion**
11:00 am	**Adjourn**

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