Neurodevelopmental Effects of Prenatal and Early Childhood Exposure to Chlorpyrifos-Containing Pesticides

A. Study Purpose and Rationale

In the United States, children and adults are exposed to pesticides principally in the home. Ninety percent of all American households use pesticides (1) and it has been estimated that full-time homemakers and young children spend up to 21 hours per day inside the home (2). Such individuals have ample opportunity for contact with indoor contaminants such as pesticides. In New York State, recent reports indicate that the greatest use of pesticides in all counties statewide is in the densely populated urban boroughs of Manhattan and Brooklyn (3). Moreover, heavy applications of pesticides have been required in inner-city neighborhoods due to the age and poor maintenance of urban housing stock. Chlorpyrifos, or CP, (0,0-diethyl 0-[3,5,6-trichloro-2-pyridyl] phosphorothioate) is a semi-volatile broad-spectrum organophosphate insecticide typically used indoors by licensed pest control operators against termite and roach infestation. It one of the two insecticides most heavily used by the New York City Housing Authority over the past twenty years.

CP may persist in indoor environments at higher levels and for longer periods of time than initially thought. In a recent study, Gurunathan et al used standard pesticide application techniques in two apartments and found that plush toys, carpets and furniture are likely to absorb CP, retain it for long periods of time, and release vapors into the air (4). In such circumstances, based on conservative estimates of dermal and non-dietary oral ingestion, children were calculated to receive up to 21 times the US EPA reference dose for CP A chemical reference dose is the level at which no statistically or biologically significant increases in frequency or severity of adverse effects is seen within an exposed population and its appropriate control. Thus, these findings suggest that current pesticide application safety protocols do not adequately protect children against exposure to unacceptably high CP levels in the home.

Children are at higher risk of adverse effects from pesticide exposure for several reasons. In addition to spending large amounts of time in the home, young children play close to the ground with frequent hand-to-mouth activity. Compared to adults, infants and children are less able to detoxify some chemicals, including organophosphates. Moreover, as they undergo rapid growth and development, children may be more susceptible to possible adverse neurodevelopmental effects of pesticides.

Like other organophosphates, CP is a neurotoxin which has been shown in animal models to inhibit cholinesterase, preventing breakdown of acetylcholine. In vitro and animal data indicate that CP may exhibit developmental toxicity to the fetal nervous system in relatively low doses (5). In humans, data is sparse regarding the neurobehavioral effects of pesticides. However, a recent study of young children living in the Yaqui valley of northwestern Mexico showed that those children exposed to high levels of pesticide perfon-ned poorly as compared to their relatively unexposed counterparts on a number of simple tests, such as drawing a picture of a person (6).

Currently six million children live in poverty in inner cities in the United States, at increased risk of exposure to numerous environmental toxins, including indoor pesticides. Our present understanding of the biological effects of pesticides on humans is limited. The results of preliminary studies (6) are concerning and indicate the need for a larger, prospective epidemiologic study.

Alternative pest management techniques are currently being tested in East Harlem at Lehmann Village Houses, an apartment complex of five 20-unit buildings. In this joint project of the State Department of Health, Hunter College Environmental Group, and the New York City Housing Authority, one-half of the buildings are routinely treated with broadcast pesticides, while the other half employ non-aerosolized pesticides and nonpesticide methods of pest control (Hill-Barcelona, personal communication). Using subjects from these two housing groups for a prospective study of the effects of pesticide exposure would help eliminate many confounding variables.

B. Study Design and Statistical Analysis

Two study groups will be formed. Non-randomized group assignment of subjects will be made based on residence in one of two housing groups in Lehmann Village ("A" or "B"). Group A implements a program of routine CP application to each apartment unit, while Group B uses alternative pest control measures. Subjects will be followed through delivery, and with their subsequent infant for four additional years.

Based on power analysis of prior data by Guillette et al. (6), a minimum of 35 subjects per group is needed to yield a statistically significant difference in outcome between the two groups (alpha--0.05, power--80). Our study proposal will assume a dropout rate of 30%, based on prior and ongoing longitudinal studies of young children with a follow-up time of 5 years or greater (7,8; T. Berkowitz, personal communication). As indicated by prior studies (9, 10), the teratogenic potential of CP-containing compounds is still a matter of considerable debate. This study will use the CDC and EPA consensus of no demonstrable effect (11), and assume the same rate of adverse pregnancy'outcomes as the general population (<I%). Finally, we estimate that 10% of subjects will change residence over 4.5 years. Given the above considerations, a total of 60 subjects per group will be recruited.

Statistical analysis of outcomes will use t-test and chi-square methods to test for baseline differences in control variables. These variables will include socioeconomic status, materrial age at child's birth, race, marital status, sex and birthweight of child, duration of breastfeeding, number of children in household, maternal education, HOME inventory, familial stress, measured levels of other environmental toxins (lead, mercury, PCBs), matemal smoking and alcohol use during pregnancy, as well as individual household use of pesticides. Analysis of covariance will be done controlling for those variables which differ significantly between the two groups.

C. Study Procedures

Please see flowsheet for procedure sequences and appropriate references.

At the time of entry into the study, a questionnaire and Raven's test will be administered to each maternal study subject. When each study child is two years of age, a home visit will be made to assess the quality of the childrearing environment. This will be done in structured interview format with administration of the preschool version of the Home Observation for Measurement of the Environment (HOME) to each family. At four years of age, the McCarthy Scales of Children's Abilities will be administered to each study child. This test consists of a General Cognitive Index (interpretable as a measure of IQ) as well as specific subscales (Perceptual Performance, Verbal, Quantitative, Memory, and Motor) and has been used in studies of lead exposure (16). At this time, each mother will be interviewed regarding quality of life at home and any chemicals to which the child may have been exposed. All tests should take one hour or less, and will be administered at the study center with exception of the HOME assessment. Subjects must be willing to allow a study investigator to enter their home on this occasion.

Urinary and blood samples will be collected at the times indicated. As a marker of CP absorption, levels of the urinary metabolite 3,5,6-trichloro-2-pyridinol (TCP) will be measured. The variability of urinary TCP levels in a given individual with reference to time and environmental CP exposure is currently unknown. In an attempt to adjust for this variability, three urine samples will be taken over a one-month interval, and the average of these values will be used for each TCP level data point. Subjects will be given light-proof urine collection containers for samples (second micturition of the day) which will subsequently be collected by study investigators for analysis. Breastmilk samples will be collected on the second post-parturn day. A blood sample and hair sample will be taken from each child subject at 4 years of age. With exception of the blood sampling, all tests are non-invasive. Efforts will be made (e.g. topical anesthetic) to minimize discomfort to children during the blood draw. Urine sampling does not require catheterization, although collection may be inconvenient in subjects using diapers.

D. Study Drugs

None.

E. Medical Devices

None.

F. Study Questionnaires

The maternal questionnaire will consist of questions regarding demographic background (SES, age, race, marital status, parity, educational level), intended duration of breastfeeding, as well as drug, alcohol and tobacco history. The follow-up questionnaire (Year 4) will assess familial stress level (six-point scale in any of four domains: financial, health, marital, other) as well as one-year recall of household pesticide use and amount of time that the study child spent away from home.

G. Study Subjects

A total of 120 women residing in Lehmann Village Houses in East Harlem will be recruited during their second trimester of pregnancy. Half of these women will be residing in a housing group which implements a program of routine pesticide application to each apartment unit. The other fifty women will be the control group, residing in a housing group in which alternative pest control measures are being used. Subjects must be healthy women between 18 and 35 years of age, without a history of illicit drug or excessive alcohol use during the pregnancy. The children resulting from these 120 pregnancies will entered into the study at birth, excluding those of gestational age <28 or >44, from multiple births, or with documented CNS defects or chromosomal abnormalities. All subjects must continue to live in their respective residences (at least 9 months/year) for the duration of the study. Subjects who change their residence (outside of the original housing group) during the study will be excluded.

Pregnant women and minors will be the subjects of this study. These vulnerable populations will be studied in accordance with the hypothesis that it is these two groups who are especially at risk of adverse effects from exposure to pesticides.

H. Recruitment of Subjects

Subjects will be recruited using flyers in the residential common areas, and if necessary, by mail.

I. Confidentiality of Study Data

Results of all questionnaires and tests will be confidential and stored in a secure location, accessible only to study investigators.

J. Potential Conflict of Interest

None.

K. Location of the Study

Subjects will undergo evaluation in their residential homes in East Harlem and at the Irving Center for Clinical Research. Peripartum blood and breastmilk samples will be collected in-hospital, and will require collaboration with each institution.

L. Potential Risks

None.

M. Potential Benefits

None.

N. Alternative Therapies

Not applicable.

O. Compensation to Subjects

Upon completion of the second and third trimester tests, study participants (pregnant women) will be given a gift certificate for a manicure. No compensation will be provided for testing during delivery and post-partum. day 2. After completion of child testing at age 2 and again at age 4, maternal subjects will be given $15 gift certificates to a local toy store. Following completion of the study, maternal subjects will be given a bonus $50 gift certificate to a local department store.

P. Costs to Subjects

Transportation fees to and from the Irving Center will be covered by the study investigators.

Q. Minors as Research Subjects

Prior approval from the Department of Pediatrics Committee on Human Investigation would be required prior to IRB review.

R. Radiation or Radioactive Substances

None.

S. References

1. Wallinga D. Putting Children First: Making Pesticide Levels In Food Safer for Infants and Children. Washington:Natural Resources Defense Council, 1998.
2. National Research Council. Pesticides in the Diets of Infants and Children. Washington, DC:National Academy Press, 19931
3. Thier A, Enck J, Klossner C. Plagued by Pesticides: An Analysis of New York State's 1997 Pesticide Use and Sales Data. Albany, NY:Environmental Advocates, 1998.
4. Gurunathan S et al. Accumulation of chlorpyrifos on residential surfaces and toys accessible to children. Environ Health Perspect 106:1-6 (1998)
5. Whitney KD et al. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. Toxicol AppI Pharmacol 134:53-62 (1995)
6. Guillette et al. An anthropological approach to the evaluation of preschool children exposed to pesticides in Mexico. Environ Health Perspect 106(6): 347-35') (1998)
7. Jacobson JL and Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. N Eng, J Med 335(ll):783-9 (1996)
8. Needleman HL et al. Bone lead levels and delinquent behavior. JAMA 275(5):363-9 (1996)

9. Shepard TH. "Proof' of human teratogenicity. Teratology 50:97-98 (1994)
10. Sherman JD. Dursban revisited: birth defects, US Environmental Protection Agency, and Centers for Disease Control. Arch Environ Health 52(5):332-3 (1997)
11. Jackson RJJ et al. Letters to the editor. Arch Environ Health 54(2)141-2 (1999)
12. Raven JC et al. Manual for Raven's Progressive Matrices and Vocabulary Scales (Section 3)-Standard Progressive Matrices. London:Lewis, 1983
13. Aprea C et al. Reference values of urinary 3,5,6-trichloro-2-pyridinol in the Italian population
14. Validation of analytical method and preliminary results (multicentric study). J of AOAC Int 82(2): 305-12 (1999)
15. Caldwell BM, Bradley RH. Home Observation for Measurement of the Environment. Little Rock, AR: Univ of Arkansas at Little Rock, 1984
16. McCarthy N. The McCarthy Scales of Children's Abilities. New York: Psychological Corp., 1972
17. Factor-Litvak P et al. The Yugoslavia prospective study of environmental lead exposure. Environ
18. Health Perspect 107(l): 9-15 (1999)

Test Schedule

Trimester 2	Trimester 3	Delivery	PP day 2	2 years PP	4 years PP
questionnaire Raven's test (12) urine x 3 (13)	urine x 3	blood x 1 (7)	breastmilk x 1 (7)	urine x 3 HOME (14)	questionnaire urine x 3 blood x 1
					hair x 1 --(7-) McCarthy test (15)