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The FRIENDS Children's Environmental Health Research Center: Assessing the Risks to Children from Environmental PCB and MeHg Exposure*

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ABSTRACT

The FRIENDS Children's Environmental Health Research Center is one of eleven Research Centers funded by the National Institute of Environmental Health Sciences and the US Environmental Protection Agency. Concerned with environmental justice, these Centers focus on child populations most vulnerable to environmental chemicals, and use multidisciplinary approaches to assess and reduce the health risks from environmental exposures. The FRIENDS Center is assessing the neurodevelopment of Southeast Asian refugee children exposed to PCB and methyl mercury contaminated fish from Wisconsin waters. Animal models are used to identify the critical outcomes to assess in exposed children and early cognitive assessments are being explored as possible indicators of later neurodevelopmental problems.

The FRIENDS Children's Environmental Health Research Center: Assessing the Risks to Children from Environmental PCB and MeHg Exposure

Recognizing the vulnerability of children to the effects of environmental contaminants, the National Institute of Environmental Health Sciences (NIEHS) and the U.S. Environmental Protection Agency (EPA) have been funding Children's Environmental Health Research Centers since 1997. These Centers conduct multidisciplinary basic and applied research with the goal of reducing environmental threats to children's health and development. Besides combining the expertise of scientists from a wide range of areas, from basic sciences to behavioral and clinical fields, four other key components make the work of each of the Children's Centers unique and innovative.

First, the Children's Centers' research addresses issues of environmental justice. The Centers investigate the effects of environmental contaminants on the health of children living in disadvantaged and underserved communities, who are disproportionately exposed to contaminants and who often suffer from unusually high rates of illnesses and developmental delays and deficits. Currently, the Children's Centers are researching asthma in inner city children exposed to air pollutants, and various aspects of neurodevelopment in children of migrant farm-workers, Southeast Asian refugees, and Native Americans who are exposed to pesticides, PCBs, and metals. Two Centers are also evaluating the potential role of environmental contaminants in the etiology of autism.

Second, all Children's Centers are required to incorporate strategies of community-based participatory research to ensure that the interests of the community are addressed in the design and planning of the research, and that their needs are a critical part of the short and long-term research objectives. Thus, each Center works closely and continuously with community representatives from various local community organizations, including a Community Advisory Board. This partnership between researchers and local community representatives informs and supports all phases of the Centers' work from the writing of grant proposals, to the implementation and evaluation of research projects, to the development of new research directions.

Third, the Children's Centers are not only charged with the tasks of assessing the health effects of children's exposure to environmental contaminants and understanding the mechanisms of biological action of these contaminants, they are also highly committed to searching for effective strategies to prevent future exposure and lessen the effects of exposure. Aside from systematic tests of the effectiveness of different educational strategies, the intervention piece of the Centers' work also involves community outreach efforts that are designed to inform the community about the research findings in ways that are meaningful and usable in their daily lives. Through systematic research in conjunction with creative public education programs, each Center seeks to improve the environmental health of the community by increasing the community's awareness and understanding of environmental health threats and teaching them preventive techniques to reduce their children's exposure to contaminants.

Finally, many of the Children's Centers are conducting basic, laboratory research aimed at understanding the mechanisms through which environmental chemicals affect child health and development, uncovering genetic vulnerabilities that may enhance children's susceptibility to environmental chemicals and identifying specific health outcomes that are important to assess in exposed human populations. By working together within the context of the Centers, laboratory researchers and epidemiologists are able to use these basic laboratory research findings to inform and guide the direction of epidemiological studies in exposed children.

THE FRIENDS CHILDREN'S ENVIRONMENTAL HEALTH CENTER

The FRIENDS Children's Environmental Health Center was established in 2001. FRIENDS stands for Fox River Environment and Diet Study. The Lower Fox River in northeastern Wisconsin is one of the most highly PCB (polychlorinated biphenyl) contaminated sites in the entire Great Lakes basin. PCBs are persistent environmental contaminants that were commercially produced from 1929 until the late 1970s (Tanabe, 1988). Their primary use was as dielectric fluids for transformers such as those on power lines and capacitors such as those used in fluorescent lighting, but PCBs were also used as hydraulic lubricants, heat-transfer fluids, flame retardants, sealants, plasticizers, pesticide extenders, and in carbonless copy paper (Safe, 1994). Due to their high lipophilicity and low biodegradability, PCBs from the environment bioaccumulate in the adipose tissue of living organisms. Human exposure occurs primarily through consumption of contaminated fish (Erickson, 2001).

Fish in the Fox River are contaminated with unacceptably high levels of PCBs and fishing advisories for the river have been in effect continuously since 1976. The PCB concentrations in some popular fish, including walleye and white bass, are so high that they are not considered safe to eat (Wisconsin Department of Natural Resources [WDNR], 2005).

Although not as severe a problem as PCBs, MeHg (methylmercury) has also been detected in fish species from the Fox River and other local bodies of water. Mercury in the environment originates from both natural and anthropogenic sources. In nature, mercury can be released from mineral deposits, volcanoes, forest fires, oceanic emission, and crust degassing.

Human activities such as mining, mineral processing, chloroalkali production, and combustion of fossil fuels also release mercury into the environment (Renzoni, Zino, & Franchi, 1998). Inorganic mercury compounds can then enter waterways where they undergo a process of methylation via microorganisms to produce methylmercury (MeHg). MeHg has a high affinity for protein sulfhydryl groups causing it to bioaccumulate in aquatic organisms (D'Itri, 1991). Like PCBs, the primary sourceof MeHg for humans is contaminated fish.

Individuals who regularly eat PCB- and MeHg-contaminated fish can accumulate these chemicals in their bodies, and pregnant women can then pass them to the developing fetus. Both contaminants have a number of health effects, but one of the more serious concerns with PCBs and MeHg is the risk of impaired neurocognitive development following in utero exposure.

Impact of PCBs on Neurodevelopment

A number of epidemiological studies have assessed the impact of PCB exposure on children's cognitive function. Jacobson and colleagues reported PCB-related cognitive deficits in Michigan children whose mothers consumed PCB-contaminated Lake Michigan fish, including impairments in visual recognition memory during infancy (Jacobson, Fein, Jacobson, Schwartz, & Dowler, 1985), poorer performance on verbal and memory tests at 4 years of age (Jacobson, Jacobson, & Humphrey, 1990), and lower IQ scores at 11 years of age (Jacobson & Jacobson, 1996).

Similar effects were observed in children from the Oswego, NY area whose mothers consumed contaminated fish from Lake Ontario. PCB exposure was associated with reductions in visual recognition memory at 6 and 12 months of age (Darvill, Lonky, Reihman, Stewart, & Pagano, 2000), and poorer cognitive function at 3.5 years of age (Stewart, Reihman, Lonky, Darvill, & Pagano, 2003). Similarly, a study with Dutch children (Patandin, Lanting, Mulder, Boersma, Sauer, & Weisglas-Kuperus, 1999) and one with German children (Walkowiak, Wiener, Fastabend, Heinzow, Kramer, Schmidt, Steingruber, Wundram, & Winneke, 2001; Winneke, Bucholski, Heinzow, Kramer, Schmidt, Walkowiak, Wiener, & Steingruber, 1998) have found cognitive impairments associated with PCB exposure at 4.5 years of age and at 2.5 and 3.5 years of age, respectively. Although PCB exposure was no longer associated with cognitive impairments at 4.5 years of age in the Oswego cohort (Stewart, Reihman, Lonky, Darvill, & Pagano, 2003) and at 6.5 years of age in the Dutch cohort (Vreugdenhil, Lanting, Mulder, Boersma, & Weisglas-Kuperus, 2002), impairments in attention and inhibitory control were observed in the Oswego cohort at 4.5 years of age (Stewart, Fitzgerald, Reihman,, Gump, Lonky, Darvill, Pagano, & Hauser, 2003) and impairments in attention and executive function were observed at 9 years of age in a subset of children in the Dutch cohort (Vreugdenhil, Mulder, Emmen, & Weisglas-Kuperus, 2004).

Although the weight of evidence strongly suggests an enduring effect of early PCB exposure on cognitive development (Schantz, Gardiner, Gasior, McCaffrey, Sweeney, Humphrey, 2004), not all studies have reported PCB related cognitive deficits. In a cohort of North Carolina children, no effects of PCBs on cognitive function were observed between 3 and 5 years of age (Gladen & Rogan, 1991), even though PCB exposure in these children was similar to the levels seen in the Michigan cohort and significant enough to be associated with delayed psychomotor development through 2 years of age (Rogan & Gladen, 1991; Rogan, Gladen, McKinney, Carreras, Hardy, Thullen, Tinglestad, & Tully, 1986a & b; Gladen, Rogan, Hardy, Thullen, Tingelstad, & Tully, 1988).

Impact of MeHg on Neurodevelopment

Longitudinal studies assessing the effects of environmental MeHg exposure on cognitive functioning in children have also been conducted. In the Faroe Islands, prenatal MeHg exposure from maternal consumption of MeHg-contaminated pilot whale meat was associated with deficits in memory, language, and attention at 7 years of age (Grandjean, Weihe, White, & Debes, 1998; Grandjean, Weihe, White, Debes, Araki, Yokoyama, Murata, Sorensen, Dahl, & Jorgensen, 1997). Similarly, prenatal exposure to MeHg in a cohort of New Zealand children was associated with language, perceptual, and IQ deficits at 6 years of age (Kjellstrom, Kennedy, Wallis, Stewart, Friberg, Lind, Wutherspoon, & Mantell, 1989). In contrast, no cognitive deficits were found in a cohort of Seychellois children prenatally exposed to dietary MeHg from contaminated ocean fish when tested at 5.5 and 9 years of age (Davidson, Myers, Cox, Axtell, Shamlaye, Sloane-Reeves, Cernichiari, Needham, Choi, Wang, Berlin, & Clarkson, 1998; Davidson, Palumbo, Myers, Cox, Shamlaye, Sloane-Reeves, Cernichiari, Wilding, & Clarkson, 2000).

Combined Impact of PCBs and MeHg

PCBs and MeHg tend to co-occur in the environment and some recent work suggests that the combined PCB and MeHg exposure has significantly greater effects than exposure to either contaminant alone. For instance, in terms of neurochemistry, an in vitro study has shown that exposure to a PCB and MeHg mixture caused significantly greater reductions of the neurotransmitter dopamine than exposure to either contaminant alone (Bemis & Seegal, 1999). In terms of neuropsychological function, Faroe Islands children exposed to both PCBs and MeHg from maternal consumption of pilot whale meat exhibited deficits in several different aspects of cognitive and sensory function, whereas Seychelle Islands children exposed to MeHg alone did not show similar deficits (Davidson et al., 1998; Grandjean et al., 1997; Grandjean et al., 1998).

Although PCBs and MeHg tend to co-occur in the environment and recent evidence suggests that they act in an additive or interactive manner, there is very little research investigating the health effects of their combined exposure. The FRIENDS Center is one of the first research programs specifically designed to address this issue.

The overall research goal of the FRIENDS Center is to evaluate neuropsychological and auditory outcomes in East Asian American children exposed perinatally to PCBs and MeHg, through maternal consumption of fish from the highly polluted Fox River. The research includes community-based projects and complementary basic biomedical projects. The biomedical projects, especially the laboratory animal studies, are used to guide the selection of outcomes to be assessed in the exposed children. Working on this combined applied/basic research effort is a large team of investigators representing various fields of expertise—epidemiology, toxicology, biostatistics, and developmental psychology—and various institutions—University of Illinois at Urbana-Champaign, the University of Illinois at Chicago, Texas A & M University, the University of Oklahoma, the State University of New York at Buffalo, the New York State Department of Health, and Michigan State University.

Biomedical Projects

Animal Studies

The animal studies conducted within the FRIENDS Center were designed to generate data that would guide the selection of outcome measures for use in the epidemiological research. An important goal was to model PCB and MeHg-exposure in the human population as closely as possible. One important aspect of this was to model the pattern of PCB exposure in these individuals. Commercial PCBs were complex mixtures, each containing 50 or more individual PCB congeners with different chlorine substitution patterns. Laboratory research has shown that these individual PCB congeners differ in toxicity depending on the number and position of the chlorines on the basic biphenyl ring structure (Hansen, 1999). Some of the individual PCB congeners break down quite readily in the environment whereas others are very stable (Hansen, 1999). As a result, the pattern of PCB congeners present in the environment does not resemble that of any of the original commercial mixtures.

In order to model PCB exposure in the FRIENDS Center study population, we obtained data from the Wisconsin Department Natural Resources (WDNR) showing the pattern of PCB congeners in walleye caught in the Fox River. We then mixed four commercial PCB mixtures to achieve a PCB congener profile that resembled the PCB congener pattern in the Fox River walleye as closely as possible. The final mixture which represented the closest match to the Fox River walleye consisted of 35% Aroclor 1242, 35% Aroclor 1248, 15% Aroclor 1254 and 15% Aroclor 1260. This experimental mixture of PCBs is being used for all of the Center's studies in laboratory rats. Another goal was to model the ratio of PCBs to MeHg in the fish. Additional data from the WDNR suggested that the ratio of PCBs to MeHg in Fox River fish was approximately 6 or 7 to 1. Thus, a 7:1 ratio of PCBs to MeHg was adopted for the laboratory studies.

A final goal was to model the timing of exposure in the human population. Hmong and Laotian women in our study population consume locally caught fish on a regular basis and have been doing so since relocating to the area a number of years ago. They typically continue to eat the fish while they are pregnant and after their children are born. To model this pattern of exposure, we begin exposing adult female rats to PCBs and/or MeHg orally several weeks before they are bred and we continue the exposure throughout gestation and postnatally until pups are weaned. After weaning pups exposed to PCBs alone, MeHg alone or PCBs and MeHg in combination are evaluated in a variety of sensory, motor and cognitive tests to determine the impact of PCBs, MeHg or PCBs and MeHg in combination on these outcomes.

An important goal has been to focus on outcomes that have not been adequately addressed in previous PCB and MeHg studies. One important aspect of this has been to conduct detailed assessments of auditory function in these animals, and to use the results of these assessments to guide the development of auditory assessment strategies for PCB and MeHg-exposed human infants.

Previous studies in laboratory animals had suggested that both PCBs and MeHg might impair auditory function, although the mechanism of action and site of action may be different for the two chemicals. Crofton and colleagues have reported a low frequency hearing loss in rats exposed to a commercial PCB mixture (Aroclor 1254) during early development (Crofton, Kodavanti, Derr-Yellin, Casey, & Kehn, 2000; Goldey, Kehn, Lau, Rehnberg, & Crofton, 1995). They further demonstrated that the deficit might be mediated by PCB-induced reductions in thyroid hormone, since co-treatment of PCB-exposed pups with thyroxine partially ameliorated the deficit (Goldey and Crofton, 1998). This finding makes theoretical sense since PCBs have been shown to reduce circulating thyroxine concentrations (Brouwer, Morse, Lans, Schuur, Murk, Klasson-Wehler, Bergman, & Visser, 1998) and thyroid hormones are critical for normal development of the cochlea (Uziel, Gabrion, Ohresser, & Legrand, 1981; Uziel, Rabie, & Marot, 1980). Histological examination of the cochlea in PCB-exposed rats revealed damage to the outer hair cells (Crofton, Ding, Padich, Taylor, & Henderson, 2000).

Based on these findings we are using distortion product otoacoustic emissions (DPOAEs), which provide an assessment of the functional integrity of the cochlea to measure hearing. DPOAEs also have the advantage that they can be used in newborn human infants. This will allow us to apply the findings from animal studies directly in our epidemiological research. The initial results from PCB-exposed rats suggest hearing loss may extend across a range of frequencies (Powers, Widholm, Gooler, Lasky and Schantz, 2005) rather than being present only at lower frequencies as suggested by earlier studies using a different method to assess hearing (e.g., Crofton, Ding, et al., 2000). Thus, it will be important to test DPOAes across a range of frequencies in infants born to PCB-exposed women.

MeHg exposure has been associated with hearing loss in animal models (Rice & Gilbert, 1992), as well as in children from the Faroe Islands cohort (Murata, Weihe, Araki, Budtz-Jorgensen, & Grandjean, 1999; Murata, Weihe, Budtz-Jorgensen, Jorgensen, & Grandjean, 2004; Murata, Weihe, Renzoni, Debes, Vasconcelos, Zino, Araki, Jorgensen, White, & Grandjean, 1999). In the Faroe Islands study, auditory brainstem evoked responses (ABRs) were used to assess auditory function. The changes that were observed suggested the site of action for MeHg is most likely central rather than at the level of the cochlea. As a result, hearing loss induced by MeHg exposure could be missed if only DPOAEs are used to assess hearing. To address this we are using ABRs to further assess the integrity of the auditory system in our animal models and in PCB- and MeHg-exposed newborn human infants.

<u>Mechanistic Studies</u>

Mechanistic studies are also being conducted in order to better understand how PCBs and MeHg may be acting together to produce neurotoxic effects. These studies are based on earlier work by Center investigators which suggested that MeHg may augment the effects of PCBs on the brain neurotransmitter, dopamine (Bemis & Seegal, 1999), and that the two chemicals have complex interactive effects on intra-cellular calcium signaling (Bemis & Seegal, 2000) which may underlie the neurochemical changes. The same experimental PCB mixture described above is being employed in these mechanistic studies. Various in vitro and in vivo approaches are being used to further understand the mechanisms through which PCBs and MeHg alter functioning of the dopamine system. A particular focus of study is the inhibition of vesicular transport of dopamine and one mechanism of neurotoxicity that is being explored in detail is the production of reactive oxygen species.

Community-Based Projects

Study Population

The focus of the FRIENDS research is on children of Hmong and Lao immigrants residing in Green Bay and Appleton, Wisconsin. These immigrants from Laos began settling in the Fox River valley in the 1970's and there are now over 4,000 Hmong and 2,000 Lao individuals living in the area. Preliminary data suggest that approximately 50% of all Hmong households in the area are eating fish from local waters on a regular basis. The most popular species consumed by this population is white bass, considered not safe to eat by the Wisconsin Department of Natural Resources (WDNR, 2005) because of its high level of PCB contamination. An earlier survey of Hmong families in Green Bay reported that 58% of the households ate fish from local waters on a regular basis (Hutchinson & Kraft, 1994). Fifteen percent reported eating locally caught fish one or more times per week and another 40% reported eating the fish 1-3 times per month.

The Hmong are also notable in that they have more than twice the birth rate of 7% observed in the U.S. general population. The high rate of births in the Hmong and Lao population coupled with their reported high fish consumption from the Fox River and other polluted waters makes this a high-risk population for long-term adverse effects from PCB and MeHg exposure, and an important population in which to investigate the impact of these contaminants on children's health and development.

Birth Cohort Project

The centerpiece of the FRIENDS Center research program is a prospective birth cohort study which tracks pregnancies among Hmong and Lao women to assess the impact of in utero exposure to PCBs and MeHg on infant development through the first year of life. Reproductive age Hmong and Lao couples in Green Bay and Appleton are currently being recruited to participate in this study.

Pregnancy Surveillance Phase

Initially, couples complete a health and diet questionnaire that includes detailed information about their consumption of locally caught fish as well as an extensive demographic and health history. Couples also provide a blood sample for PCB and MeHg analysis. The women are then contacted every two months and asked to take a home pregnancy test. Once they become pregnant, women are contacted monthly to obtain relevant diet and health information, including changes in smoking habits, fish consumption, and caffeine and alcohol intake, as well as any occupational changes. At these monthly contacts women also provide information necessary to create a detailed pregnancy history, including patterns of prenatal care, facilities where care is sought, gestational age at first visit, and number of prenatal visits.

During the third trimester, women are asked to provide another blood sample for analysis of contaminants as well as thyroid hormone levels. Past research indicates that PCBs can decrease the levels of thyroid hormones, and that decreased levels of this hormone can adversely impact the developing fetus (Porterfield, 1994). Thus, analyzing maternal serum for contaminants and hormones will allow us to examine their interactions and the resulting impact on the health of the expectant mother and her fetus. Determining pre-pregnancy levels of contaminants in women's serum is essential because risk to the developing fetus may be dependent on the mother's lifetime exposure to PCBs, not solely on her recent exposure. Finally, comparing contaminant levels in women's serum prior to pregnancy with those in late pregnancy will allow us to examine how gestation might affect the body stores of these chemicals.

Infant Health Phase

In the infant health phase of the study, the infants born to the participating women will be followed longitudinally from birth to assess the impact of in utero exposure to PCBs and MeHg on their neurocognitive and auditory development. To determine each infant's in utero exposure to these contaminants, a sample of umbilical cord blood will be collected at birth.

Auditory assessment. Between 18 to 24 hours after birth, infants' auditory function will be assessed. Cochlear function will be assessed across a range of frequencies with distortion product otoacoustic emissions (DPOAEs. Because a large majority of hearing impairments are consequences of cochlear pathology (Schuknecht, 1994) and because previous studies relating auditory impairment to prenatal PCB exposure suggest a cochlear site of action (Crofton, Ding, et al., 2000; Herr, Goldey, & Crofton, 1996; Powers et al., 2005), assessing cochlear function is particularly relevant to this study.

MeHg exposure can also cause hearing loss (Rice & Gilbert, 1992), but as discussed earlier the site of damage appears to be central (Murata, Weihe, Araki, et al., 1999; Murata, Weihe, Renzoni, et al., 1999) rather than at the level of the cochlea. This type of hearing loss would not be detected by measurement of otoacoustic emissions. Thus, auditory brainstem evoked responses (ABRs) will also be measured in newborn infants.

After birth, infants' hearing will continue to be monitored during follow-up assessments at 6, 9, and 12 months of age, when parents will be queried about their infant's hearing, developing speech and vocal capabilities, and middle ear problems. In addition, at 12 months of age, infants' auditory function will be re-assessed DPOAEs and ABRs, as well as, a behavioral assessment of auditory thresholds

Cognitive Assessments. At birth, newborns' speed of processing will be assessed in a visual fixation paradigm in which infants will be shown a black and white checkerboard stimulus for 60 s. Previous research with premature infants found infant visual fixation time in this paradigm to be negatively correlated with performance on intelligence tests at 5, 8, 12, and 18 years of age and with some tests of executive function at 12 and 18 years of age (Sigman, Cohen, & Beckwith, 1997; Sigman, Cohen, Beckwith, Asarnow, & Parmalee, 1991). Given that both general intelligence and executive function are negatively affected by PCB exposure, this visual fixation paradigm has good potential as an early indicator of PCB induced cognitive deficits.

After birth, infants' cognitive development will also be assessed at 6, 9, and 12 months of age using several different measures. Long-term plans are to continue to follow these infants through childhood and adolescence and examine the association between cognitive performance at birth and throughout the first year of life with performance in standardized intelligence tests and executive function tests in childhood and adolescence.

Two of the measurement tools that will be used to assess infants' cognitive function, the Bayley Scales of Infant Development (Bayley, 1969) and the Fagan Test of Visual Recognition Memory (Fagan, Singer, Montie, & Shepard, 1986), have been used in a number of other studies of PCB exposure and child development (e.g., Gladen et al., 1988; Jacobson, Fein, Jacobson, Schwartz, & Dowler, 1984; Jacobson et al., 1985; Koopman-Esseboom, 1995; Lonky, Reihman, Darvill, Mather, & Daly, 1996; Rogan et al., 1986a), and hence will allow for comparisons of the infants in our study cohort with other PCB exposed cohorts in the Great Lakes Basin and elsewhere. Additionally, the Fagan Test is sensitive to a number of at risk conditions and is also predictive of later verbal IQ (Fagan et al., 1986; Rose & Wallace, 1985), which was found to be adversely affected by PCB exposure.

The Bayley Scales involve a number of tasks, some of which require infants to search for and retrieve toys. As in other PCB studies, this tool will be administered at 6 and 12 months of age. The Fagan Test consists of measuring infants' visual fixation to novel vs. previously viewed pictures. Initially, the infant is presented with a picture to look at. After a brief period, the original picture is paired with a new picture and infants' length of fixation to each of the two pictures is measured. Infants' visual recognition memory function is assessed in terms of the degree to which infants show, through their visual fixations, a preference for the novel over the previously viewed picture. Several PCB studies have found a significant relationship between elevated PCB exposure and reduced preference for novelty in this test (e.g., Jacobson et al., 1985; Darvill et al., 2000). As in previous PCB studies, the Fagan Test will be administered at 6 months of age.

Infant cognitive development will also be assessed in four types of object-retrieval tasks designed to tap several important cognitive functions including planning, decision making, working memory, and inhibition of perseverative responses (i.e., the tendency to repeat a previously successful response when it is no longer appropriate). These executive functions are considered to be indicative of the development and integrity of the frontal lobes (e.g., Diamond, 1985, 1991). They are critical for complex thinking and problem solving, and animal studies have found several of these functions to be adversely affected by PCB exposure. Further, when compared to the skills assessed through the Bayley Scales or the Fagan Test, the cognitive skills tapped in these object-retrieval tasks are more akin to the skills tapped in most tests of child and adult intelligence. Thus, performance on these object-retrieval tasks may potentially serve as a better early index for later PCB-related cognitive deficits or delays.

All four object-retrieval tasks will require infants to decide which of two similar alternatives will allow them to retrieve a desired object. In the first two tasks, to be administered at 6 months of age, infants will have to reason about spatial relationships and the physical properties of objects they are presented with to decide which of the two alternatives will allow them to retrieve a toy. These tasks are single-step retrieval tasks because infants will be able to retrieve the toy simply by reaching directly for it. In one task, the object will remain in full view; whereas, in the other task, the object will be hidden, so that infants will have to remember certain key features of this object in order to decide how to correctly retrieve it once it is no longer in view.

In the other two tasks, to be administered at 9 and 12 months of age, infants will not be able to reach for the target object directly; they will have to carry out a two-step action sequence and use another object as a tool or a means to retrieve the target object. Thus, in these two-step retrieval tasks, infants will have to plan means-ends action sequences, as well as reason about spatial relationships, in order to decide which of the two alternatives presented will yield the target object. In addition, the two-step retrieval tasks will involve trials in which infants will be required to inhibit a previously used or pre-potent response in favor of a novel response in order to correctly retrieve the target object. The ability to inhibit prepotent responses has been found to be lowered in animals and humans as a result of PCB exposure (Rice, 1998; Stewart, Fitzgerald, et al., 2003). In one of the two-step retrieval tasks the target object will remain in full view, whereas in the other task the target object will become hidden. Thus, this second task will test both infants' inhibitory control and their working memory capacity, which has also been found to be adversely affected by PCB exposure (Levin, Schantz, & Bowman, 1988; Rice & Hayward, 1997; Widholm, Villareal, Seegal, & Schantz, 2004).

Adolescent Project

While the biggest risk from PCBs and MeHg is believed to be associated with in utero exposure, older children may also be at risk. Therefore, the FRIENDS Center is also investigating the neuropsychological functioning of adolescents, 14 to 16 years of age in Hmong and Lao families residing in Green Bay and Appleton who are exposed to PCB and MeHg contaminated fish. Our research project is one of the first to investigate whether PCB or MeHg exposure during adolescence affects the development of brain functions that are maturing during that period.

Adolescents, 14 to 16 years of age, who are the children of couples participating in our study will be assessed on a battery of cognitive tests including a subset of tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computerized neuropsychological assessment (Fray & Robbins, 1996). All tasks involve non-verbal, abstract, visual stimuli that are presented on a computer screen. The tasks assess visual recognition memory, spatial short-term memory, working memory, planning, and inhibitory control.

Adolescents will also be tested on a verbal memory test—the California Verbal Learning Test Children's Version (CVLT-C; Delis, Kramer, Kaplan, & Ober, 1994); a continuous performance test—the Integrated Visual and Auditory Continuous Performance Test (IVA;

Sandford & Turner, 1995)— that assesses attention, impulsivity, and hyperactivity; and two scales, vocabulary and matrix reasoning, of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) to measure general intelligence. The CVLT-C is composed of two lists of 15 words each; 5 words from each of 3 semantic categories (e.g., things to wear, things to play with). The test provides measures of repeated trials learning and active mnemonic strategies such as semantic clustering as well as short term and delayed recall. The IVA presents 500 brief trials (the entire test lasts about 13 minutes) of "1"s and "2"s requiring shift of sets between the visual and auditory modalities. The adolescent test battery taps many of the same functions including planning, working memory and inhibitory control that will be assessed in exposed infants, allowing for the assessment of continuity of effects with exposure at different stages of brain development. Exposure to PCBs and MeHg will be determined through analyses of blood samples from the adolescents participating in the study.

Community Intervention Project

In addition to studying the health risks of PCB and MeHg exposure, the project will assess different education strategies to determine which are most effective in lowering exposure to contaminated fish among Hmong and Lao individuals. Hmong and Lao families will be educated about the risks associated with eating contaminated fish, and will be provided with practical information about where it is safe and not safe to fish, which species of fish are safe to eat, and how to prepare fish for cooking so as to reduce contaminant exposure.

The assessment of alternative education strategies will involve distributing an educational video and a fishing advisory in the Hmong and Laotian languages. The advisory was developed by simplifying and translating relevant information from the Wisconsin fishing advisory published by the WDNR. The video teaches basic concepts about chemical contaminants in fish, the health risks associated with exposure to these contaminants and methods for reducing exposure. It instructs viewers about how to use the fishing advisory and how to clean and cook fish so as to limit exposure to PCBs.

One half of the participating couples will receive, in addition to the video and fishing advisory, a more intensive intervention based on what community members have indicated would be most effective as educational approaches. For Laotian couples, additional written information including maps and diagrams that illustrate where it is safe to fish, what size and fish species are safe to eat, and how to prepare fish for cooking will be provided. For Hmong couples, the educational approach will involve meeting with a trained peer educator in small groups of 8 to 10 people to learn the same information in a verbal, interactive format. The peer educator sessions will include live cooking demonstrations and shared meals, as well as help with reading maps and identifying fish species. The effectiveness of the video-alone strategy and of the video-combined-with-supporting-materials strategy will be measured both by knowledge questionnaires that will be administered before and after the intervention.

These education/intervention activities will be an important contribution from an environmental justice perspective since the study population consists of low-income, Southeast Asian refugees who are relatively uneducated, have poor English literacy and are generally unaware of the risks associated with consumption of fish from local waters. Therefore, the Wisconsin fish consumption advisory (WDNR, 2005) and other printed materials that explain these risks are for the most part inaccessible to this population.

CONCLUSION

In summary, the Children's Centers represent a unique and highly innovative approach to assessing the impact of environmental contaminants on child health and development. The

Centers conduct multidisciplinary research which integrates basic laboratory science with epidemiological studies in exposed human populations in order to better understand the mechanisms through which environmental contaminants act to impair children's health. The Centers also address important environmental justice issues by focusing on children living in disadvantaged and underserved communities and they work with these communities in a cooperative manner to insure that the particular health concerns of the community are addressed in the research. Finally, the Centers are also committed to identifying effective intervention and education strategies which will reduce exposure and improve the health of children in these communities.

The FRIENDS Center focuses on Southeast Asian refugees living in the upper Midwest who engage in subsistence fishing in waters contaminated with unacceptably high levels of PCBs and MeHg. These widespread and persistent contaminants tend to accumulate in aquatic ecosystems. Exposure is a particular concern in populations that obtain a large percentage of their protein from fish. Both chemicals are developmental neurotoxicants, yet there is very little research investigating the effects of combined exposure.

At the FRIENDS Children's Environmental Health Research Center in vitro and animal models are being used to characterize the pattern of neurodevelopmental effects from combined exposure to PCBs and MeHg and to understand the mechanisms for these effects. Knowledge gained from these basic laboratory studies is being used to identify specific outcome measures that will be assessed in exposed children. The impact of exposure at several critical stages of brain development including the prenatal period and the adolescent period is being assessed, and an important long-term goal is to identify very early measures that may be indicators of later neurological or neurocognitive problems. Lastly, several different intervention and education strategies are being evaluated to identify effective ways to reduce exposure and improve health outcomes.

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