NATIONAL CHILDREN'S STUDY
PERI-CONCEPTION ENDOCRINE ACTIVE AGENTS CORE HYPOTHESIS

I. Proposed Hypothesis

Pre- and peri-conceptional exposure to endocrine active compounds (EACs), specifically phthalates and certain pesticides, is associated with increased risk for selected congenital anomalies, including hypospadias, cryptorchidism, transposition of the great arteries (TGA), and limb reduction defects.

II. Workgroup

Fertility and Early Pregnancy Working Group (Possible collaboration with Chemical Agents)

III. Contacts

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IV. Public Health Significance

Prevalence of these congenital anomalies: *Hypospadias* is a congenital anomaly in which the urinary meatus is abnormally positioned, varying in severity from 1) anterior placement (glandular, occurring on the inferior surface of the glans penis, in the balanopenile furrow, or on the distal third of the penile shaft; 2) middle hypospadias, with the opening on the middle third of the shaft; or 3) posterior hypospadias, extending from the proximal third of the shaft to the perineum (1). Increased incidences have been documented in several countries, including the United States, with these increases occurring during different time intervals by region. At the end of the 1980s, the incidence of hypospadias appeared to level off at between 15-20 per 10,000 births in Norway, Denmark, and Sweden, with Finland reporting only one-third this rate. Hungary reported an increase from 5.5 to 23.9 per 10,000 births over the period 1960-1970s, while England and Wales saw a doubling in their rates from 7.3 to 16 per 10,000 from 1960-1980s (2).

According to the Birth Defects Monitoring Program, the incidence of hypospadias in the U.S. has been steadily rising, from 20.2 to 39.7 per 10,000 births during 1970-1993. Moreover, this increase cannot be explained on the basis of improved ascertainment of the milder forms, as the ratio of severe to mild forms reported by the Metropolitan Atlanta Congenital Defects Program increased from three to five fold over this same time period (2). Currently, hypospadias occurs in approximately 1 out of every 125 live male births in the U.S. (1)

Cryptorchidism is the failure of the testicle to migrate to the scrotum (3). In the United States, the prevalence of cryptorchidism is approximately 1.1% of livebirths, with some controversy over possibly increasing incidence of this defect (4).

Cardiovascular malformations (CVMs) encompass a large and diverse group of congenital malformations of varying degrees of severity. The Baltimore-Washington Infant Study (1981-1989) reported prevalences of 3.26/10,000 births for tetralogy of Fallot, 2.64/10,000 for transposition of the great arteries (TGA), and 1.78/10,000 births for hypoplastic left heart syndrome, all of which are severe defects (5). An analysis conducted by the Metropolitan Atlanta Congenital Defects Program (MACDP) to describe trends in major CVMs over time indicated prevalences of 4.7, 2.4, and 2.1/10,000 births, respectively during the period 1995-1997. These

investigators concluded that the prevalence of CVMs is increasing and called for research to determine potential environmental exposures that could be related to these increases (6).

Finally, *limb reduction defects* were noted in 0.69/1000 livebirths, stillborn infants, and pregnancies ending in elective terminations in the Active Malformations Surveillance Study in Boston, covering the interval 1972-1974 and 1979-1994 (7). While many of these defects were ascribed to vascular disruption and genetic/chromosomal abnormalities, 4% were due to teratogenic exposures, and 32% were idiopathic. Limb reductions defects have been associated with pesticide exposure in recent investigations (8-10), although not in every investigation of this issue (11).

Prevalence of Human Exposure to EACs: Over the past several years, concerns regarding adverse reproductive effects due to exposure to environmental chemicals that modulate endocrine functions (so-called "endocrine disruptors" or endocrine active compounds) have received intense global attention. These EACs are synthetic chemicals or natural compounds in plants that influence the endocrine system's function (12). These compounds may disrupt hormonal function in different ways. Some chemicals act similarly to sex steroid hormones and bind to hormone receptors. Xenoestrogens are compounds that act like estrogens in the body; other chemicals behave like androgens. Alternatively, there are endocrine active compounds that function as hormone antagonists, called anti-estrogens and anti-androgens (13). The relation between exposure to pesticides that are EACs remains highly controversial (14,15).

Serious concerns have been raised in recently with regard to human health effects due to exposure to phthalates, a ubiquitous EAC in the environment. Dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid, commonly called phthalates, are most commonly used as industrial plasticizers of polyvinyl chloride (PVC) to impart flexibility and are found in a variety of commercial products such as blood storage and transfusion equipment, dentures, children's toys and food containers. Phthalates are also used for nonpolymeric purposes as solvents, fixatives, detergents, and lubricating oils and consequently are present in a variety of commercial products such as cosmetics, paints, and wood finishing products. A recent examination of urinary phthalate metabolites in the NHANES III revealed that exposures to these compounds are much higher and more common than had been expected (16). Using a novel and highly selective technique, monester metabolites of seven commonly used phthalates were measured in 289 adults from the NHANES III study population. This sample was comprised of adults aged 20-60 years (mean 37.4, SD 10.6 years), 56% female, with about one-third Caucasian, African American, and Mexican American. An important finding of this study was the significantly higher urinary levels of monobutyl phthalate (MBP) among women of reproductive age (n=110; MBP 46.9 ug/g creatinine), compared to other age/gender groups (31.4 ug/g creatinine), P=.003. In addition, rural women of childbearing age had higher levels of benzyl butyl phthalate (MBzP), 31.1 ug/g creatinine, compared with older rural women (21.1 ug/g creatinine.

Morbidity: All of the above congenital anomalies are associated with significant morbidity. The potential for urogenital problems resulting from surgery to reverse hypospadias is a concern, as is the psychological trauma associated with this condition and it's treatment (1). Cryptorchidism is acknowledged to be one of the most important risk factors for the development of testicular cancer (17). The cardiovascular malformations can have life-long deleterious impacts on health and quality of life (see below).

Mortality: A population-based cohort study in Oregon (1958-1989) assessing survival and cause of death among individuals who had undergone corrective surgery for CVMs indicated an increased risk for cardiac mortality over this time period; delayed cardiac mortality was 15% for

TGA, and 5% for tetralogy of Fallot (18). An extension of this study included follow-up through 1996 and reported that the risk of late sudden death among these individuals was 25-100 times greater than age-matched controls (19).

Quality of life: The above description of the morbidity/mortality associated with these congenital malformations provides strong evidence for a decreased quality of life among those who survive with these defects.

Cost: The burden of these anomalies to the health care system varies by type of malformation, but all contribute substantially to the costs of medical care in the U.S. The average cost for corrective surgery for hypospadias is $14,000; given a prevalence of 125 cases/125 livebirths, this totals to approximately $221,760,000 per year (20). The costs for surgery to correct CVM varies widely according to the type of malformation as well comorbidities, ranging from $15,819 for repair of uncomplicated ventricular septal defect to $22,241 for tetralogy of Fallot with no accompanying complications (21). These figures averaged $48,252 and $114,202 when comorbidities were present. Thus treating the most common CVM, uncomplicated ventricular septal defect, contributes about $156,608,100 to the annual cost of health care in the U.S.

Perceived importance: The increasing contribution of congenital malformations to overall infant mortality in the United States renders their investigation to be of paramount importance. Congenital malformations were a leading cause of infant mortality in the U.S. in the 1990's, accounting for 22.1% of infant deaths in 1997 compared with 15.1% in 1970 (22). The etiology of major birth defects remains unknown in many instances, and the call to determine the proportion that are due to exposure to environmental toxic agents has been issued by several leading scientists and scientific organizations (see Section V). In 1999, the Teratology Society Public Affairs Committee issued a position paper calling for research on EACs and malformations of reproductive tract (23).

The potential influence of periconceptional exposures on subsequent reproductive health, thus impacting the subsequent generation(s) as well, emphasizes the urgent need to examine these relations. A paper describing the conclusions reached by a recent Workshop to Identify Critical Windows of Exposure for Children's Health (24) stated the following:

> *"Furthermore, although early embryogenesis is clearly a critical window during which exposure to exogenous chemicals, both naturally occurring and man-made, may have profound and long-lasting adverse effects on reproductive structure and function, preconceptional and postnatal exposures may also adversely affect the reproductive system and progeny outcome."*

V. Justification for a Large, Prospective, Longitudinal Study

The ability of epidemiologic studies to evaluate the relation between congenital malformations and specific environmental exposures has been severely limited by the rarity of these outcomes, and the resultant recall bias inherent in the case-control study methodology, the default design under these circumstances. A large, prospective study design could accommodate both of these limitations by: 1) providing a population of sufficient size to enable a valid study of environmental exposures on selected prevalent congenital malformations that have major impact on the quality of life of those affected, and 2) by permitting the ascertainment of critical exposure data during the most relevant susceptible time periods, thus eliminating the recall bias that has plagued previous research attempts in this area.

Another extremely valuable advantage to a prospective cohort design enrolling couples prior to pregnancy is that by definition, the fate of all conceptions would be known to the researchers. This is the only methodology that can permit the identification of exposure levels that may prohibit conception from occurring at all, or that may result in subclinical pregnancy losses. If a dose-response relationship does indeed exist along the spectrum of reproductive effects, this is the *only* design that would permit the calculation of a dose-response curve in humans (25,26).

VI. Scientific Merit

As described above, the controversies surrounding the existence of a relationship between EACs and infertility, subfecundity, and congenital malformations are largely a result of the inadequacy of previous study methodologies to appropriately examine these critical questions. Previous studies in this area are consistent in reaching one important conclusion that is that without knowledge of the exposures present during the critical windows of susceptibility, these questions will never be answered with any measure of confidence. Moreover, these exposures are ubiquitous, thus if the association is real, it impacts hugely on public health in the United States. The financial and psychological burdens associated with these outcomes are already acknowledged, and it is possible that they could be prevented through environmental regulation of these contaminants. The ultimate goal is to preserve the health and well being of the population, while continuing to manufacture the many products that improve our quality of life. The development of risk assessment models requires data describing dose-responses to these toxicants, and it is clear that a prospective approach is the only design that will permit the acquisition of this critically needed information.

VII. Potential for Innovative Research

The scientific advances that the prospective pregnancy study methodology yield are tremendous. The development of new and sensitive biomarkers of exposure, susceptibility, and effect for these highly susceptible time periods, which were previously unavailable to researchers for examination, would expand our knowledge of the relation between these chemicals and adverse reproductive outcomes exponentially. The doses required to produce effects, the mechanisms through which the effects occur, the identification of susceptible subpopulations through evaluation of genetic markers, and the ability to examine interactions (gene-gene and gene-environment) would all be greatly enhanced through this approach.

VIII. Feasibility

Critical period: The effects of EAC exposures differ depending upon the timing of the exposure and the target tissues during pregnancy (1). The development of male external genitalia is dependent upon the influence of testosterone early in gestation (27). Therefore, early *in utero* exposure to EACs could conceivably result in altered male urogenital development. Several EACs, including Vinclozolin (a fungicide used on fruits and vegetable crops), certain phthalates, DDE and PCBs have produced hypospadias in laboratory animals (28-30). In human males, the differentiation of the primordial germ cells begins in the sixth week of gestation, with ovarian development occurring in the seventh week for females (3). Likewise, structural heart defects can occur as early as 3 weeks post conception (31); it would be nearly impossible to recruit couples this early in gestation into a population-based study. This makes the enrollment of couples prior to conception imperative if accurate exposure assessment is to be achieved. For example, phthalates, while lipophilic, are rapidly metabolized and do not appear to bioaccumulate (32). Therefore, in order to measure phthalate exposures precisely, it is necessary to obtain biological specimens (urine) close to the time of the

environmental exposure. Fortunately, earlier studies done to assess the feasibility of examining early pregnancy on a population basis, as well as willingness to comply with the stringent protocols necessary demonstrate that this approach is not only feasible but also well accepted with high compliance rates for biological sample collection and survey completion (33,34).

Sampling needs: Thus, in order to allow the measurement of exposures during this critical window of development, at least one-third of the total NCS population should be recruited from the following subgroups of women: women at risk for pregnancy, women planning pregnancy, and women seeking infertility treatment (broadly defined as women seeking treatment following the absence of conception despite at least one year of regular unprotected sexual intercourse or those seeking treatment as a result of recurrent pregnancy loss.) Studies have shown that women at risk for pregnancy can be recruited and that women of reproductive age will comply with intense scientific protocols.

In a study examining 100,000 births, given that approximately 50% will be males and a rate of hypospadias of .008 in the unexposed population, there would be 78%, 77%, and 95% power to detect relative risks of 1.38, 1.5 and 2, respectively, for associations between elevated phthalate exposures and hypospadias. These calculations also assume proportion exposed in the population at 20%, 10%, and 5%, respectively.

Contact: Women will need to be contacted at varying intervals, depending on the nature of the measurements.

Nature of measurement: Various methods of data collection could be utilized, ranging from diaries and interviews to urine and blood sample collection. The specific questions to be answered would influence the frequency of collection.

Burden on the participant and family: The data and specimen collection would be time intensive, but participants would receive a large amount of valuable information. Among women trying to conceive, they would be very interested in knowing how various exposures may compromise their fertility and the health of their offspring.

Ethical considerations: Privacy would be of paramount concern in collecting a large amount of sensitive information. Reporting exposure levels to participants might also represent a dilemma, as researchers may not be able to adequately express what the exposures mean to a particular individual.

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