Title
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Journal
Environmental Health Perspectives, 111(13)

ISSN
1542-4359

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Publication Date
2003-10-01

DOI
10.1289/ehp.5800

Peer reviewed

An Approach to Assessment of Endocrine Disruption in the National Children's Study

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In this article we consider the importance of assessing endocrine disruption in a large new cohort that has been proposed, the National Children's Study (NCS). We briefly review evidence that endocrine disruption is a potentially important hypothesis for human studies and weigh the need to assess endocrine disruption in the NCS. We note the salient features of earlier, similar cohort studies that serve as reference points for the design of the NCS. Finally, we discuss features of the NCS that would allow or enhance assessment of endocrine disruption, even if endocrine disruption were not a primary hypothesis motivating the study. At this time, the evidence supporting endocrine disruption in humans with background-level exposures is not strong. Thus, a compelling rationale for the NCS will probably need to be based on core hypotheses that focus on other issues. Nonetheless, if properly designed, the NCS could serve as an excellent resource for investigating future hypotheses regarding endocrine disruption. *Key words:* **chemical actions, child, cohort studies, endocrine disruption, environment, epidemiology, health.** *Environ Health Perspect* **111:1691–1697 (2003). doi:10.1289/ehp.5800 available via** *http://dx.doi.org/* **[Online 18 March 2003]**

In 1999, the National Research Council's Committee on Hormonally Active Agents in the Environment concluded that "in human populations suspected of being affected by hormonally active agents, prospective and cross-sectional studies using cohorts tracked from conception through adulthood are particularly needed" (National Research Council 1999). Subsequently, as part of the conference "Endocrine Disruptors and Children's Health: A Workshop to Examine the Effects of Endocrine Disruptors on Child Development for a National Longitudinal Study," held 16–17 March 2000 (New York, NY), the authors jointly presented material for a session titled "Epidemiology and Assessment of Outcomes." The objective of the session was to develop recommendations on how to design and execute a national longitudinal study of childhood development that incorporates the latest information on the potential impact of endocrine disruptors on human development. The longitudinal study referred to in the workshop has since been named the National Children's Study (NCS).

We assume that the NCS will be a multicenter study of at least 100,000 children followed from before birth. Because the primary hypotheses motivating the study (core hypotheses) are still under discussion, the precise design of the study has not yet been specified. Nonetheless, a comprehensive study of the determinants of health and development is envisioned. As the NCS is still being designed, the role that investigation of endocrine disruptors might play in the rationale and design is very much a salient issue. A more complete description of the proposed study appears elsewhere (Branum et al. 2003).

In this paper we consider the importance of assessing endocrine disruption in the NCS. First, we briefly review the evidence that alterations of the early, normal endocrine environment has adverse effects and the evidence that endocrine disruption is a potentially important hypothesis for human studies. We then weigh the need for assessment of endocrine disruption in the NCS. Next, we note the salient features of earlier, similar cohort studies that serve as reference points for the design of the NCS. Finally, we discuss features of the NCS that would allow assessment of endocrine disruption, even if endocrine disruption were not a primary hypothesis motivating the study.

Long-term Effects of the Endocrine Environment during Pregnancy and Early Life

In this section, we consider primarily exogenous effects of the endocrine environment, apart from those associated with normal differentiation and function. In humans, major alterations in the endocrine environment during pregnancy are known to have adverse consequences in offspring. The classic example is maternal diethylstilbestrol use causing adenocarcinoma of the vagina in daughters (National Research Council 1999). Other examples are type 1 diabetes in pregnancy causing birth defects in offspring (Becerra et al. 1990) and hypothyroidism during pregnancy causing mental retardation in offspring (Haddow et al. 1999). Lesser degrees of maternal hypothyroidism may also adversely affect the cognitive function of offspring (Haddow et al. 1999).

That very small differences in hormone exposures during a critical period can affect an animal's development is demonstrated by the intrauterine position phenomenon. Female mice adjacent to two male mice *in utero*, compared with those adjacent to two females, differ in terms of anogenital distance, activity level, body weight, estrous cycle length, age at sexual maturity, and other factors (vom Saal 1989). Similarly, among human females some evidence suggests that modestly higher *in utero* androgen exposure has detectable effects. A female dizygotic twin with a twin brother, compared with a female dizygotic twin with a twin sister, has fewer spontaneous otoacoustic emissions (clicking sounds generated by the ear) (McFadden 1993) and less craniofacial symmetry (Boklage 1985), and exhibits more risk-taking behavior (Resnick et al. 1993). The association with risk-taking behavior was found in a twin study designed to compare males with females; thus, biased observations depending on the sex of the co-twin seem unlikely, although other reasons besides an *in utero* androgen effect accounting for the association cannot be excluded. In another type of study, stored maternal pregnancy serum was analyzed for sex hormone–binding globulin and total testosterone, and the female offspring, as adults, completed questionnaires about masculine and feminine behavior before their blood was analyzed (Udry et al. 1995). Lower sex hormone–binding globulin levels (implying higher free testosterone) were associated with less femininity. Nonetheless, whether

This article is part of the mini-monograph "Endocrine Disruptors and Children's Health."

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The opinions expressed herein are those of the individual authors and do not necessarily reflect the views of their respective organization, sponsoring institution, or the National Children's Study Interagency Coordinating Committee.

The authors declare they have no conflict of interest.

Received 30 May 2002; accepted 16 December 2002.

in humans very small differences in hormone exposures *in utero* have any measurable, long-term effects remains to be established. We know that in childhood, small increases in plasma concentrations of endogenous estradiol at very low concentrations (nanograms per liter) increase prepubertal growth rates (Brucker-Davis et al. 2001).

Endocrine Disruption as a Hypothesis in Human Studies

Endocrine disruption, expressed as a general hypothesis, suggests that environmental chemicals have effects on health that are mediated by the endocrine system (Harrison 2001). The significance of endocrine mediation lies in the biologic plausibility that low-level exposures can have effects. Because some environmental chemicals can act like hormones (for example, by binding with a receptor), effects of low-dose exposures are biologically plausible. The consequences of low-dose exposures to certain chemicals has recently come under greater scrutiny (National Toxicology Program 2001). Whether natural, hormonally active constituents of food, including alcohol, or nonchemical agents such as light should be considered potential endocrine disruptors is unclear. For the purposes of this discussion, we consider these outside the scope of endocrine disruption.

Recognition that environmental contaminants can have adverse effects on human health dates back at least to the time of Paracelsus (Haeublein 1982). London's coal smoke–induced lethal smog in the winter of 1952 showed dramatically the importance of environmental contamination (Bell and Davis 2001). Subsequently, we realized that less obvious pollutants or pollutants at lower concentrations can have insidious untoward consequences. An example in wildlife is the recognition in 1962 of the effects of dichlorodiphenyltrichloroethane (DDT) on reproduction (Carson 1962); an example in humans is the discovery of the effect of methyl mercury on neurodevelopment, primarily in the late 1950s (Nishigaki and Harada 1975), and the effect of lead on neurodevelopment in the 1970s (Landrigan et al. 1975; Needleman et al. 1979). Advances in analytical chemistry have made it increasingly clear that the general population is exposed to hundreds of man-made chemicals at low doses [Centers for Disease Control and Prevention (CDC) 2001].

Some have voiced concern recently that low-level exposure to certain chemicals can lead to effects on the endocrine system (Colborn et al. 1996). This concern stems in part from temporal trends involving end points in humans that imply endocrine-mediated mechanisms: decreasing age at menarche (Kaplowitz et al. 2001), decreasing semen quality (Auger et al. 1995), decreasing male-to-female sex ratio at birth (Davis et al. 1998), and increasing rates of hypospadias (Paulozzi 1999) and testicular cancer (McKiernan et al. 1999). Also contributing to this heightened concern is the increasing body of knowledge about the toxicologic properties of the compounds to which people are exposed and about developmental biology.

As noted above (CDC 2001), measurable levels of a host of environmental chemicals are present in the general U.S. population, including pregnant women. Several of these compounds are known to interact with the endocrine system in animal and *in vitro* experiments (Andersson et al. 2000; Hester and Harrison 1999), with periods of special vulnerability occurring during development (Bigsby et al. 1999). Some of these chemicals have estrogenic, antiestrogenic, antiandrogenic, thyroid-depleting, and hyperglycemic effects (Andersson et al. 2000). Furthermore, animal research suggests that some of the neurobehavioral and developmental effects of endocrine disruptors may be amplified over successive generations (Crews et al. 2000). That is, endocrine disruptors transmitted from the mother not only influence the morphologic and physiologic development of the offspring, but also the reproductive behavior of the offspring as adults (Meaney 2001). These altered behaviors accentuate the effects of contaminants on the sexual development of their young.

Whether any endocrine disruptor, with the possible exception of dioxins, affects human health is controversial—because effects have not been studied in humans, because epidemiologic results are equivocal, or because exposure–outcome relations are not necessarily endocrine mediated. To illustrate these latter points, in the remainder of this section we briefly discuss selected evidence regarding some of the compounds frequently implicated as endocrine disruptors and consider whether this evidence suggests that further study be a focus of the NCS.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent of the dioxins and a recognized human carcinogen [International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Carcinogenic Risks to Humans 1997], has been associated with at least one endocrine-related malignancy—breast cancer. In experimental data, TCDD had antiestrogenic effects (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 1997); thus, an endocrine-based mechanism by which TCDD might cause human breast cancer is not obvious. The association with breast cancer has been seen in occupationally exposed populations (Flesch Janys et al. 1999; Kogevinas et al. 1997; Manz et al. 1991) and in residential populations in Chapaesk, Russia, and Seveso, Italy, who received high-level TCDD exposure (Revich et al. 2001; Warner et al. 2002). High-level TCDD exposure has also been associated with a dose-related change in the sex ratio (Mocarelli et al. 2000; Ryan et al. 2002), which appeared to be mediated through paternal exposure. TCDD exposure has been associated with endometriosis in the monkey (Rier et al. 1993), and there is some, albeit inconsistent, evidence for the same in humans (Eskenazi et al. 2002a; Mayani et al. 1997; Pauwals et al. 2001). A relation between TCDD exposure and type 2 diabetes has also been reported in some studies, among both those with high- and background-level exposure (Longnecker and Daniels 2001). By background-level exposure for TCDD, we mean exposure experienced by the general public, resulting primarily from normal diet, not from unusual circumstances of occupation or accident. A mechanism by which TCDD might cause diabetes is that binding of TCDD with its receptor could antagonize the action of another nuclear receptor, peroxisome-proliferating activator gamma, which in turn has antidiabetic actions (Remillard and Bunce 2002). Although dioxins may well have endocrine-mediated effects on health, evidence for such effects at background levels of exposure is weak. Furthermore, if trends in the United States are like those in other developed countries, level of exposure to dioxins in the general population is decreasing (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 1997), rendering studies of their effects in the NCS even less compelling as core hypotheses.

Levels of polychlorinated biphenyls (PCBs) have been examined in relation to thyroid hormones in at least seven studies (Hagmar et al. 2001; Longnecker et al. 2003a; Persky et al. 2001). No consistent associations with a specific thyroid hormone have been observed, although positive results of some sort have been found in several studies (Hagmar et al. 2001; Koopman-Esseboom et al. 1994; Osius et al. 1999; Persky et al. 2001). In animal experiments, PCBs decrease serum levels of thyroxine (Brouwer et al. 1999). Many investigators have reported associations of PCBs with neurodevelopmental delays (Gladen et al. 1988; Jacobson and Jacobson 1996; Longnecker et al. 2003a, 2003b; Patandin et al. 1999), although relations were not present in all such studies (Daniels et al. 2003; Grandjean et al. 2001). In other epidemiologic studies where exposure to PCBs was associated with adverse outcomes, whether the relations were endocrine mediated was unclear. For example, PCBs have also been related to reduced growth (Patandin et al. 1998) and reduced weight (Blanck et al. 2002; Jacobson et al. 1990), though, again, results vary. In animals, PCBs have some effects that are not endocrine mediated (Seegal 1996). Levels of PCBs are falling in the United States (Longnecker et al. 2003b).

The DDT metabolite dichlorodiphenyldichloroethene (DDE) has been associated with another outcome that, at face, appears to be caused by an endocrine mechanism. In two studies, maternal DDE levels were related to a shorter duration of lactation (Gladen and Rogan 1995; Rogan et al. 1987). Whether the association is causal remains unresolved. Associations of DDE with assorted other outcomes are less clearly endocrine mediated. For example, associations of DDE with impaired immunity (Dewailly et al. 2000), decreased height (Karmaus et al. 2002), increased body mass index (Gladen et al. 2000), and preterm birth (Longnecker et al. 2001b) have been reported, although these relations are also not established. As with PCBs, levels of DDE are falling in the United States (Smith 1999).

Three environmental chemicals in particular have generated much recent concern: phthalates, polybrominated diphenyl ethers (PBDEs), and bisphenol A. Di(2-ethylhexyl)phthalate (DEHP) and dibutylphthalate (DBP) are high-volume production chemicals used primarily as plasticizers, and 75% or more of the U.S. population have detectable levels in their urine (Blount et al. 2000). Both compounds have antiandrogenic effects and cause hypospadias in laboratory animals (Gray et al. 2000). Of the phthalate esters known to be antiandrogenic (Mylchreest et al. 2002), such as DEHP and DBP, exposure to DBP appears to be the highest in the United States (Blount et al. 2000). However, even the highest estimated exposure in U.S. women of childbearing age (Kohn et al. 2000) "is more than two orders of magnitude lower than the lowest no observable adverse effect level of DBP from animal studies" (Mylchreest et al. 2002). In the absence of animal data showing abnormalities in male offspring, such as hypospadias, at doses typically experienced in humans, and with no human data to support that low-dose effects exist, the hypothesis that phthalates have adverse effects on male offspring in humans seems too weak to serve as a core rationale for the NCS.

PBDEs are manufactured primarily as flame retardants and are used in plastics and foam rubber (Darnerud et al. 2001). These are persistent organic pollutants, and levels in the environment and in human tissues are rising exponentially. The PBDE chemical structure resembles that of thyroid hormone, and PBDEs interfere with thyroid metabolism (Hallgren et al. 2001; Zhou et al. 2001). Although levels of PBDEs are increasing rapidly in this country (Hale et al. 2001), levels are still 1–2 orders of magnitude lower than those of PCBs. PBDEs, especially those present in people at highest concentration, appear to be less potent thyroid disruptors than are PCBs, though toxicity via other mechanisms cannot be excluded.

Bisphenol A is another high production volume chemical, used in a variety of applications including manufacturing of flame retardants, resins, and plastics. Bisphenol A is a weak estrogen (Pottenger et al. 2000). Human exposure arises, e.g., when foods are contaminated by heated plastics. New data show that blood levels of bisphenol A in pregnant women (Schonfelder et al. 2002) are similar to those found in pregnant rats that give birth to offspring with bisphenol A–induced reproductive toxicity (Howdeshell et al. 1999; Pottenger et al. 2000; Rubin et al. 2001). None of the animal experiments showing low-dose endocrine toxicity from bisphenol A, however, have been replicated (Ashby J. Personal communication); thus, the importance of investigating effects of low doses on humans is debatable.

The Role of Endocrine-Disruption Studies in the NCS

Although the theoretical possibility of endocrine disruption in humans is based on an excellent scientific rationale, the importance of searching for low-dose endocrine-mediated effects of environmental contaminants in humans remains arguable (Neubert 1997; Safe 2000; Safe et al. 1997). Via diet, humans are exposed to a huge variety of xenobiotics, many of which are endocrine active, and our bodies may be well equipped to handle routinely encountered doses of endocrine-active compounds whether from natural or anthropogenic sources (Safe et al. 1997). Although specific human studies of endocrine disruption may be well justified, overall the evidence supporting endocrine disruption in humans is not sufficiently strong that endocrine disruption studies should be a primary motivating factor for the NCS. Nonetheless, although the NCS (if it is ever realized) is likely to be justified on the basis of core hypotheses that are not centered on endocrine disruption, if properly designed the NCS could serve as an excellent resource for investigating hypotheses regarding endocrine disruption in the future.

Some Earlier Cohort Studies of Children's Health

The two largest cohort studies of children's health in the United States were the Child Health and Development Studies (CHDS), a single cohort, providing the setting for multiple studies, which enrolled more than 20,000 pregnant women from 1959 to 1966, and the Collaborative Perinatal Project (CPP), which enrolled more than 55,000 pregnant women from 1959 to 1965 (Broman 1984; van den Berg et al. 1988). The CHDS focused on women delivering at primarily one facility of the Kaiser Permanente Medical Center in Oakland, California, whereas the CPP recruited women from 12 medical centers at various locations across the country. Both studies had comprehensive follow-up during childhood (to age 5 years in the CHDS and to age 7 years in the CPP), with additional assessments among subgroups at later ages, including adolescence and adulthood in some instances. Both studies also collected blood specimens at multiple times during pregnancy.

These previous cohort studies have provided extremely valuable prospective information on pregnancy and children's health. They were constructed to allow a broad range of research questions to be investigated, many of which were not originally envisioned. Recently, the stored serum specimens have been analyzed to examine exposure to tobacco (English et al. 1994), caffeine (Klebanoff et al. 1999), PCBs (Longnecker et al. 2001a), organochlorine pesticides (Longnecker et al. 2002), hormone levels (Udry et al. 1995), and antibodies to herpes simplex type 2 (Buka et al. 2001) in relation to a broad range of outcomes. Thus, these studies provide a useful benchmark for designing the proposed NCS; the NCS, however, should be charged with improving upon the design of these earlier studies.

NCS Features Allowing Assessment of Endocrine Disruption

Although a broad-based cohort along the lines of prior investigations is recommended for the NCS, the prior studies can be improved upon in significant ways. Five specific improvements especially pertinent to investigation of endocrine disruptors include better assessment of societal context, the use of an intergenerational design, linkage to other data sources, addition of selected exposure and outcome measures, and use of genomics and proteomics. Rapid advances in genomics and proteomics offer great promise for evaluating low-level effects on the hormonal axes during development, and have been recently discussed in detail (Henry et al. 2002). The specimens to be collected from subjects that will facilitate genomic and proteomic analyses will be addressed in the section on addition of selected exposure and outcome measures. While vital to the investigation of endocrine disruptors, these five improvements would be equally beneficial for the study of many other exposure–outcome relations.

Societal context. One way in which previous longitudinal cohort studies can be improved upon is in the comprehensiveness of the model of child development used in evaluating the health impact of a chemical exposure. Many of the end points being considered, particularly neurobehavioral and cognitive, are final common pathways for the expression of influences at many levels. Sociodemographic and economic factors affect academic performance, IQ, and cognitive development (Duncan et al. 1994; Sameroff et al. 1993; Schonkoff and Phillips 2000). Overall, past studies have done a reasonably good job in

characterizing sociodemographic and economic factors that can be considered proximal to the child (e.g., those pertaining to the immediate home environment). However, improvement is required in characterizing these proximal sociodemographic and economic factors and those of the community in which the child lives. Although these latter factors are more distal to the child, recent work in epidemiology suggests that they are likely to be important aspects of a child's ecology and can improve models of child development (Leventhal and Brooks-Gunn 2000; Sampson et al. 1997). These factors include physical, socioeconomic, and cultural aspects of the neighborhood and the broader cultural community in which a child grows up, such as the school and day care experiences (Schonkoff and Phillips 2000).

The need to capture such features of a child's world can be inferred from the results of previous studies. In studies of continuously distributed end points such as IQ, multiple regression models rarely account for more than 40–50% of the variance, and often much less (10–15%), even in populations that are reasonably heterogeneous in terms of conventional indices of socioeconomic status (Bellinger et al. 1992; Cooney et al. 1989; Wasserman et al. 1994). Some of the unexplained variance undoubtedly reflects genetic factors, but a portion of the variance is likely due to aspects of the broader social and cultural context that have generally been neglected. It will be particularly important to pay attention to these factors if the population sampled for inclusion in a longitudinal study is ethnically and culturally diverse, or if particular groups are targeted for inclusion by virtue of some notable exposure (e.g., children of migrant farmworkers exposed to pesticides).

Several benefits will follow if the NCS is able to characterize these aspects of a child's environment more completely. One is that it may reduce the amount of unexplained variability in an outcome, thereby increasing the statistical power of tests of the association between the outcome and the chemical of interest. This is particularly important when trying to detect subtle effects, which, based on our past experience with other chemical exposures, is likely to be the case. Another is that it will provide the ability to control more effectively for potential confounders. Perhaps most important, it will advance the field by creating new opportunities to identify effect modification (e.g., factors that increase a child's vulnerability to a chemical exposure and factors that are protective). Currently little is known about what makes some children more resilient than others to insults. The type and breadth of community supports available to the child and family may be particularly important in this regard.

To achieve a solid integration of societal-level factors in the design, it will be necessary to bring in collaborators from fields not usually included in environmental epidemiology and toxicology studies, such as sociologists and cultural anthropologists. These collaborators will be essential in determining how to measure these macrofactors. Their participation in the design of the NCS will strengthen the validity and deepen the interpretation of the findings.

Intergenerational design. The results of experiments in animals, described earlier, suggest that it may be important to study the effects of endocrine disruptors over more than one generation. Thus, if measures of endocrine disruptors and sex hormones are taken during pregnancy of the women in the NCS, long-term follow-up of their children would be illuminating in determining risks for future generations. In addition, if maternal pregnancy serum is sufficient for measuring exposure, then children from previous studies such as the CHDS and the CPP who are now adults can be recruited and followed (Buka et al. 2001; Cohn et al. 2001; Hardy et al. 1997; Udry et al. 1995). Studying intergenerational effects would be a significant advance on prior studies, reflecting the knowledge we have gained from animal studies in recent decades. Although studying intergenerational effects would be ideal, such long-term planning may not be feasible at this point.

Linkage to other data sources. When considering data needs and management in the development of long-term follow-up studies, it is important to incorporate methods that will make the study as flexible as possible for future unanticipated uses. Many studies do not reach their potential because of ineffective linkage to other data sources and inaccessibility of the data. Addressing these issues will be increasingly important in coming years because of increasing technological ability to collect different types of data and to access and share data.

This design feature can be accomplished by *a*) devising the study database so it can be linked easily to other data sets in the future to explore new hypotheses, *b*) developing tools and documentation to make the data easy to use and understand, and *c*) making the data easily accessible to other researchers.

An especially important example of an emerging technology relevant to epidemiology is geographical information systems (Dent et al. 2000). Many environmental contaminants of interest are spatially distributed, and the ability to link and analyze data sets spatially is growing.

Many examples in the environmental epidemiology literature demonstrate opportunistic uses of survey data collected for other purposes to explore relationships between health and environmental exposures. For example, in the field of air pollution epidemiology, a very important study evaluating the long-term effects of particulate matter air pollution on total and cardiovascular mortality used data collected by the American Cancer Society (Pope et al. 1995). The American Cancer Society cohort is an ongoing prospective mortality study of approximately 1 million adults since 1982, intended primarily for studies of cancer.

Addition of selected exposure and outcome measures. To measure exposure to potential endocrine disruptors and their effects in the NCS, some specific assessments are needed that might not otherwise be part of a modern, general-purpose cohort study of children's health. Much of what would be needed, however, would likely be collected regardless of hypotheses about endocrine disruption.

Exposure. The CPP and CHDS restricted specimen collection to blood from pregnancy, stored at –20°C. Laboratory technology has evolved, allowing for the detection of many environmental agents in other media. To test other (non–endocrine disruption) hypotheses, the NCS is likely to collect from the mother multiple samples of urine and blood during pregnancy (Branum et al. 2003). Collection of urine and blood specimens during pregnancy would be especially interesting because it would allow assessment of exposure effects during organogenesis and neurodevelopment. Urine samples would allow assessment of exposure to some quickly excreted xenobiotics, such as phthalate esters, bisphenol A, and organophosphate metabolites. The utility of these samples for assessment of exposure to the specified agents, however, would depend on the frequency of collection, half-life, and exposure pattern. Advances in toxicokinetics could add to understanding of the dose to the fetus during critical windows of susceptibility. Pilot work may be needed to determine the utility of collected specimens and inform decisions regarding the optimal frequency of collection. Collection of a breast milk sample is likely to be part of the NCS protocol, for reasons other than endocrine disruption, and would allow assessment of exposure to persistent organic pollutants present at extremely low levels (e.g., TCDD, PBDEs). Similar considerations hold for the specimens from children, for whom other hypotheses will require cord blood, urine, bloodspots at birth, and blood collection during infancy and childhood. Collection of meconium or amniotic fluid, when available, may be useful for measuring exposures, although additional developmental work is needed (Foster et al. 2000; Whyatt and Barr 2001). Environmental samples obtained in the NCS—for example, to allow assessment of asthma-inducing agents—may also provide material useful in assessing exposure to potential endocrine disruptors.

Whenever possible, any study of children should include the father and an assessment of

his exposures, including biologic specimens and their metabolites. The contribution of paternal exposures, which may affect mutation rates in the sperm line or contribute directly to exposure of the offspring (Eskenazi et al. 2002b; Hales et al. 1986; Malaspina et al. 2001; Robbins et al. 1997; Wyrobeck 1983), has often been overlooked in past studies.

Outcomes. The outcomes of greatest interest will be those that can be assessed with at least reasonable statistical power in the NCS. Even with a cohort of 100,000 subjects, studies of rare diseases such as childhood cancer and testicular carcinoma would be underpowered. However, useful information could be gained even from assessments of less-common outcomes.

Potentially useful measures of hormone-sensitive end points are standardized examinations for supernumerary nipples, size of breast buds, hypospadias, cryptorchidism, prostate size, anogenital distance and related measures, tooth mineralization, sexual development, gynecomastia, detailed neurologic examinations, and neurodevelopmental assessment of motor development, language, and cognition (Alaluusua et al. 1999; Bigsby et al. 1999; Blanck et al. 2000; Grandjean et al. 2001; Gray et al. 2001; Hannon et al. 1987; Krasnegor et al. 1994; Longnecker et al. 2002; Rogan et al. 1986). Endocrinologic evaluation based on blood specimens would include thyroid function tests, gonadotropins, sex hormones and related measures, insulin resistance and glucose levels, and immunologic assessment of allergy and immune response and markers (lymphocyte subtypes) (Brouwer et al. 1999; Cranmer et al. 2000; Egeland et al. 1994; Longnecker and Daniels 2001; Weisglas-Kuperus et al. 2000). Measures of gendered behavior, such as toy preference, may be sensitive to some endocrine disruptors and appropriate for selected hypotheses (Doering et al. 1989; Udry et al. 1995; Vreugdenhil et al. 2002). Examination of semen might be appropriate as the cohort ages.

Some of the end points we propose for endocrine disruption are subclinical, but may be worthy of study nonetheless. For example, neonatal hypothyroidism is associated with lower IQ. Suppose that PCBs slightly disrupt thyroid homeostasis, decreasing the population mean IQ by a few points. A small decline in IQ may not have any noticeable impact for an individual, but a small decline in mean IQ for an entire population could mean a large increase in the number of people who are learning impaired (Needleman and Bellinger 1991). In addition, studying subclinical end points could provide clues regarding mechanisms and suggest clinical outcomes worthy of additional scrutiny.

Furthermore, for those rare, dichotomous outcomes such as congenital anomalies, evaluation of all NCS children would be needed to obtain reasonable statistical power for study. However, most other outcomes evaluated in the context of endocrine disruption are continuous in nature, and thus measurement in subsets of subjects might be sufficient to allow hypothesis testing.

Conclusion

Endocrine-mediated health effects in humans from exposure to low levels of environmental chemicals are biologically plausible. Given the prevalence of low-level exposure and changing patterns in endocrine-sensitive end points in the population, the extent of endocrine disruption in humans needs to be determined. However, evidence that supports any given specific endocrine disruption hypotheses in humans is weak (Safe 2000). The importance of human endocrine-disruption investigations may thus be subject to debate and influenced largely by one's judgment of whether effects seen in animals, usually at relatively high doses, are worth looking for in humans, who normally experience much lower exposures (Neubert 1997).

At this point we believe that if the NCS can be justified, it will be by virtue of core hypotheses not necessarily related to endocrine disruption. At the same time, as our understanding of endocrine disruption grows, we also believe it is highly likely that excellent hypotheses about human endocrine disruption will arise, and that the NCS should be designed to accommodate as many such possibilities as is feasible. Furthermore, evaluation of societal context and allowance for linkage to other data sources will facilitate examination of not only endocrine disruption but also many other hypotheses. Finally, building on the NCS to follow the next generation may allow greater sensitivity for detecting endocrine disruption than heretofore possible in human studies.

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