Endocrine Disrupter Studies at the National Center for Environmental Health: Laboratory Perspective

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There is much worldwide interest regarding environmental endocrine disrupters (see Table 1)- obviously they are of concern and much more research needs to be done in this area, in order to protect public health and the ecosystem. We at CDC's main mission is to protect public health. In our laboratory, we have research efforts on-going in the areas of exposure assessment to environmental pollutants and their relationship to adverse health effects as well as areas "in between, such as susceptibility markers and preclinical markers of disease. This paper will focus on exposure assessment to selected environmental disrupters, our collaborative studies to relate exposure and adverse health outcomes, and the important role that the laboratory plays in environmental health.

We, in the National Center for Environmental Health believe that human exposure assessment should, if possible, be based on the measurement of the pollutant, its metabolites, or reaction products (such as DNA abducts) in human specimens. Several examples that form the basis of this belief can be cited [1] This presentation deals with two chemical substances that have been deemed as "endocrine disrupters" - 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and lead; and with studies in progress which examine the relationship of serum levels of PCBs and organochlorine pesticides with endocrine effects.

In the mid 1980's, our laboratory developed the ability to measure dioxin in lipophilic human specimens (adipose tissue, milk, and serum) in large scale epidemiologic studies. These studies have included assessing of exposure to the general population; 2,4,5-T sprayers in Australia and New Zealand; veterans of U.S. Army ground troops and U.S. Air Force "Operation Ranch Hand" in Vietnam; occupational workers in the U.S., Germany, and the Netherlands; and residents of Seveso, Italy. One preliminary finding in Seveso Zone A residents is that high levels of serum dioxin in the mother and/or father may lead to an increased number of female births.(2) This is the subject of another presentation at the meeting (Mocarelli). But, to re-emphasize, this is an observation that needs further study, but without the ability to accurately measure dioxin in serum with the needed specificity and sensitivity this observation would not have been possible, for based on the exposure index of dioxin soil levels, all of these Zone A residents would have been classified as similarly exposed.

Another endocrine disrupting substance is lead. The literature on lead may surpass that of

dioxin-certainly it does from a time perspective. Lead exposure in children has long been recognized to be detrimental to human development. In the mid-1970's the amount of lead in gasoline was reduced. There was much debate as to how much this reduction would mean in terms of reducing blood lead levels in Americans. Many models predicted that the mean blood level would decrease only slightly from its 15-16 µg/dL level. The NCEH Laboratory is the central laboratory for the National Health and Nutrition Examination Survey (HNHANES). NHANES is designed to assess the health and nutritional status of Americans; CDC's National Center for Health Statistics (NCHS) is responsible for it. NHANES II took place from 1976-1980; one of the measurements was blood lead in a representative sampling of Americans. In the early 1980s there was much discussion in Washington about increasing the amount of lead in gasoline. At about that time NCHS published its findings that showed the "marked parallel" between the decrease in blood lead levels (9-10 µg/dL) and the decrease use of lead in gasoline.[3] Now in the U.S. as well as many other countries, lead is essentially no longer used in gasoline. Also, the FDA has banned the use of lead solder in "tin cans" containing foods. As a result the first phase of NHANES III (1988-1991) has shown a mean blood lead level of less than 3 µg/dL[4] This has been a public health success story that again shows the utility of measuring the internal dose (blood level) of environmental toxicants, and using it to evaluate temporal trends. However, we are not finished with the lead story because in a segment (low SES, inner city, minority children) of our population, blood lead levels remain unacceptably high. Steps must be made to reduce these exposures.

Many endocrine disrupting effects may reveal themselves several years after exposure to the pollutant. Therefore, it is often necessary to assess past exposures, rather than present exposures, to these compounds. Primarily for this reason most of the human studies have focused on the persistent compounds (those with a long biologic half-life). Three studies [5,6,7] in the early 1990s were studies that garnered much interest in environmental public health in linking an effect, breast cancer, with human levels of persistent pesticides and PCBs. Findings from these studies heightened interest in this topic of endocrine disrupters. We at CDC feel that this is a very important topic in the environmental public health area that needs more research. Our laboratory is collaborating with various investigators (primarily epidemiologists) in studying the relationship between persistent organochlorine pesticide serum levels and adverse health outcomes in case control studies. These studies (Table 2) include studying the relationship between breast cancer in women and serum levels of individual congener and total PCBs and selected organochlorine pesticide. In all cases, blood was drawn from the women prior to diagnosis of breast cancer. Each of these studies has unique features. For example, the Native Alaskan population consists of a population that may have higher levels of these environmental pollutants because of their subsistence on foods known to contain relatively high levels of these persistent environmental pollutants. The Asian-American Study is examining a population that historically has low incidence of breast cancer. The Maryland and Missouri Studies include examining biomarkers of susceptibility in addition to biomarkers of exposure and health effects (breast cancer). The Denmark and Norway breast cancer studies are of similar design except that the latter also includes assessing serum levels of dioxins and furans.

In addition to breast cancer in women, other cases control studies are examining the relationship between endometrial cancer (PCBs and organochlorine pesticides) and endometriosis (TCDD). These studies are also on-going. Not to leave men out, we are also involved in examining the relationship between serum levels of organochlorine pesticides and PCBs with prostrate cancer. We are also examining the link between serum levels of these compounds and other effects, such as non-Hodgkins lymphoma.

In addition to the body burden level of the toxicant, the timing of the exposure may also be

relevant. One well known example is diethylstilbestrol (DES), which was given as a synthetic estrogen to pregnant women to prevent miscarriages. Unfortunately, babies (both male and female) exposed to DES during gestation have a higher risk of reproductive defects and some types of cancer. This example also shows that "timing of the exposure" (in this case, the developing fetus) is an important factor along with the amount and frequency of the dose. Therefore, we are also collaborating in case-control studies involving body burden levels of PCBs and pesticides with genital abnormalities, including hypospadias and cryptorchidism.

Finally, where do we go from here? As for human health, we need to conduct the next epidemiologic studies, using the best science available. At the same time, we must learn from animal studies and in vivo and in vitro tests; we must learn which end-points need to be examined and more about the "timing" of the exposure. As mentioned, more research must be done, but can we afford research for research sake? The research must have as its basis sound science that has as its ultimate goal the protection of humans and the ecosystem. From a more narrow laboratory standpoint more methods are needed to assess human exposure to these chemicals (see Table 1). We need to know what are the "normal levels" of these endocrine disruptors in the general population (use of the NHANES population would be of benefit). We need to know more about the pharmacokinetics of these compounds in humans (in order to properly interpret the exposure assessment data). We need methods which meet the needed sensitivity, specificity, accuracy and precision backed by quality assessment/quality control. In summary, good science should be the rudder that keeps the endocrine disruptor studies on the proper course.

References

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Table 1 Environmental Endocrine Disrupters[8]

Insecticides	Herbicides	Fungicides	Industrial Chem
* DDT/Metabolites	* 2,4-D	*Hexachlorobenzene	*+ Cadmium
* Dieldrin	+ 2,4,5-T	Benomyl	* Lead
* Mirex	*+ Alachlor	Mancozeb	*+ Mercury
* Chlordane	*+ Altrazine	Maneb	* PCBs/PBBs
* Oxychlordane	Amitrole	Metiram	* Dioxins
* Heptachlor/HE	Metribuzin	Tributyl tin	*+ Pentachlorophenol
* Trans-nonachlor	Nitrofen	Zineb	Alkylated phenols
* Toxaphene	Trifluralin	Ziram	Phthalates
β-HCCH			Styrenes
*+ Lindane	Nematocides		Bisphenol-A
Dicofol	Aldicarb		
Methoxychlor	Dibromochloropropane		
Endosulfan			
Pyrethroids (Syn)			
+ Carbaryl			
Methomyl			
+ Parathion			

* Measured in serum in our laboratory
+ Measured in urine in our laboratory
Methods are needed for others and for naturally -occurring endocrine disrupters.

Table 2 Endocrine Disrupters Case-Control Studies at NCEH

Population	Collaborators	#Sera	Data Samples Collected	Effect Studies
Native Alaskan*	IHS	120	Since 1967	Breast Cancer
Asian-American*	NCI	500	1983-1987	Breast Cancer
Maryland*	Johns Hopkins, NCI	800	1974-1989	Breast Cancer
Denmark*	NIEHS, Odense U.	1300	1976-78;1981-83	Breast Cancer
Missouri*	NCI	350	1977-1987	Breast Cancer
Norway*	NIOSH, Norwegians	300	Since 1973	Breast Cancer
Norway*	NCI, Norwegians	1400	Since 1973	Multiple Cancers
Columbia, MO*	NCI	606	1989-1992	Endometrial Cancer
Seveso+	NIEHS,EPA,UC	400	1976	Endometriosis
Seveso+	Italians	500-1000	1976-1985	Sex Ratio
Spanish Children*	U. Granada	250	1990's	Cryptorchidism
Danish Children*	Odense, U.	300	1996	Thyroid Disorders
Children of Ag. Workers*	Odense, U.	300	1996	Genital Abnormalities
Danish Children*	Odense, U.	360	1996	Cryptorchidism
Maryland Men*	NCI	240	1996	Prostrate Cancer
Taiwan, MI +	EOHSI, Taiwan, MCPH, EPA	135	1996	Abnormalities

*PCBs and selected pesticides are measured
+Dioxins are measured