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Environmental endocrine disruptors

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Abstract: Environmental endocrine disruptors (EEDS) are those endocrine modifying chemicals that are also environmental pollutants. A brief description of known and suspected effects of these chemicals is presented along with a table of chemicals that are of interest for further study by three organisations. The future challenge to environmental chemists will be to develop sensitive, selective, and accurate methods for monitoring EEDs with low levels of false positives and false negatives.

INTRODUCTION

Environmental Endocrine Disruptors (EEDS) are endocrine modifying chemicals that are also environmental pollutants. While most research on this topic continues to focus on the *effects* of EEDS, the focus of this paper is on the *analysis* of EEDS. The analysis of EEDs in environmental matrices (e.g., water, air, soil, wastes, and biota including both plants and animals) is critical to future regulatory monitoring of them as well as to an understanding of their occurrence, transport and migration within the environment, and their ultimate degradation.

In order to be able to analyse for EEDs several prerequisites are necessary:

1. The EEDs subject to monitoring and/or regulation need to be identified*;
2. Methods for EEDs need to be effective at concentration levels desired for monitoring†; and
3. Analytical reference materials must be available to calibrate the instruments used for the analyses.

DEFINITION OF ENDOCRINE DISRUPTING CHEMICALS

The endocrine system refers to the complex system that involves the brain and associated organs and tissues of the body. These include the pituitary, thyroid, and adrenal glands and the male and female reproductive systems, all of which release hormones into the bloodstream. In particular the sex hormones include estrogens in females and androgens in males. Endocrine disrupting chemicals (EDCS) consist of synthetic and naturally occurring chemicals that affect the balance of normal hormonal functions in animals. Depending on their activity they may be characterised as estrogen modulators or androgen modulators. They may mimic the sex hormones estrogen or androgen (thereby producing similar responses to them) or they may block the activities of estrogen or androgen. (i.e., be anti-estrogens or antiandrogens). [1]

There are three categorical sources of EDCS: [2]

1. Pharmaceuticals - One of the first recognised synthetic EDCs was diethylstilbestrol (DES), a pharmaceutical product given to pregnant women from 1948 to 1972 to help prevent miscarriages. It

* Developing screening methods is an important effort that hopes to bypass initial needs to identify specific individual EEDS. However, as discussed below, screening methods for EEDs will have to accommodate a wider variety of diverse chemicals than have ever been subjected to screening methods before. This is an extremely complex challenge.

† Concentration levels, at which EEDs are presumed to be effective and therefore potentially monitored, are at those of hormones. These are typically a thousand or more times below levels at which most current analytical methods are able to measure analytes in the environment with reasonable rates (e.g., <10% at a 95% confidence level) of false positive and false negative identifications. Thus, EED levels of concern may be at parts per trillion (10^{-12}) or below.

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caused clear-cell carcinoma in the vagina, reproductive abnormalities in female offspring, and a much higher than normal rate of genital defects in male babies.

2. Naturally Occurring EDCs - This source of EDCS, collectively called "phyto-estrogens," includes foods such as soybeans, apples, cherries, wheat, and peas.

3. Environmental EDCs - The third group of EDCs are some environmental pollutants. These *environmental endocrine disruptors* (EEDS) are the subjects of this symposium.

ANALYTICAL CHALLENGES

The analytical challenges can be summarised as:

1. Determining which analytes are EEDs so that their effects can be studied and those of importance can be monitored and/or regulated in the environment and in food.

2. Developing screening analytical methods that will accommodate a wide variety of analytical functional groups at extremely low detection levels.

3. Developing qualitative and quantitative analytical methods to confirm, as necessary, the identity and quantity of EEDs in the environment and in food.

4. Assuring, through appropriate use of analytical reference materials and QA/QC procedures, an acceptable level of false positive and false negative determinations at regulatory levels.

Analytical Challenge #1 is the subject of this paper.

Challenge #1 - Determining which pollutants are environmental endocrine disruptors

A major problem is determining which chemicals in the environment should be labelled as environmental endocrine disruptors. This is a critical question because these materials will be the subjects of future regulations. Obtaining information on them will also require very significant expenditures of time and money. In addition, traditional environmental analytical methods require specific known analytes for identification and quantification. If you don't know what chemical(s) to analyse for then you can't analyse for them -- a trivial statement but a difficult problem with EEDS. Thus, identifying which chemicals.are EEDs is the first of four analytical challenges. Currently, the process for deciding if a chemical is an environmental endocrine disruptor is to determine the effects of that chemical on the endocrine systems of humans and other animals.

Much of the research on EEDs to date has focused on the effects of EEDs on wildlife. There are many studies involving certain pesticides, polychlorobiphenyls (PCBS) and polychlorodibenzo-p-dioxins (e.g., 2,3,7,8-TCDD) that link them to birth defects and aberrant sexual behavior. Laboratory tests have also produced genital defects, reduced testicular weights and low sperm counts in rats fed with DDE, PCBS, Vinclozolin, and 2,3,7,8-TCDD. [3]

Some researchers also believe that EEDs may be the cause of similar types of recent observations in humans. The Centers for Disease Control and Prevention (CDC) in Atlanta, GA has performed surveys that show that the average US resident has hundreds of chemicals accumulated in their fat tissues including polychlorodibenzo-p-dioxin and polychlorodibenzofuran isomers ("dioxins" and "furans"). [4]

Some chemicals, including many EEDS, can "bioaccumulate" or build up in animals. Once they are incorporated into the tissues and fat of animals and humans, they can remain there for long periods of time until they are ultimately metabolised. Thus, chemicals the mother was exposed to weeks or years earlier can damage an embryo, the most sensitive stage of life. [5]

Another factor that is important with EEDs is that timing of exposure to them can be critical. In fact, timing of exposure may be more important than the dose or concentration of their exposure. A single exposure at a vulnerable moment for a developing embryo has the potential to cause damage and, of course, long term exposure to relatively small amounts of them also could cause damage. [5]

Some combinations of two or three EEDs may be many times more potent than any one of them by themselves. Thus, in addition to the timing of exposure, combinations of EEDs that people are exposed to may cause a synergistic effect that can magnify the damage they can cause. However, it should be noted that the initial research that led to this hypothesis has not been able to be repeated.

Lastly, there is the problem of the naturally occurring EDCS. How does the exposure of EEDs relate to exposure to naturally occurring EDCS? How do humans and animals metabolise or neutralise the effects of each categorical source of EDCS? These effects are briefly described later. The above are unresolved issues that will have to be worked out with time and additional research.

Table I reflects the enormity of the first analytical challenge: deciding which environmental pollutants are EDCS. There are at least 103 suspect EEDs identified by various organisations; no doubt this list will change rapidly in the near future. Some of the chemicals on this list will probably be dropped from future consideration and other new ones are expected to be added. The U.S. EPA is interested in 60 suspect EEDS, 25 of which are targeted by the EPA NERL Endocrine Disruptor Exposure Team for multi-media environmental analysis this year. [6] The CDC in Atlanta, GA is interested in 48 suspect EEDS. [7] Finally, the World Wildlife Fund Canada (WWF) has expanded the approximately 50 suspect EEDs listed in *Our Stolen Future* [8] to 68 suspect EEDS. [4] As seen in Table 1, many of these chemicals are on all three lists, some are on only two of the three lists, and others are on only one of the three lists. In addition, these chemicals may be known by other names or synonyms so it is very important to characterise any list of chemicals by their unique Chemical Abstract Service (CAS) numbers.

USAGE CLASSIFICATIONS OF EEDS

One of the characteristics of EEDs mentioned in the introduction is the wide variability of their chemical class characteristics. This is also reflected in a classification of their uses. Some suspected EEDs are various types of pesticides, others are common metals, and many fall into the classification of useful organic industrial chemicals. The result provides a real challenge for developing screening methods.

There are at least nine different usage classifications of EDCs that are listed below and also in Table 1.

1. Biocides,
2. Insecticides,
3. Herbicides,
4. Nematocides,
5. Fungicides,
6. Industrial Chemicals (e.g., solvents, plasticizers, etc.),
7. Metals
8. PCBs (i.e., Specific PCB isomers), and
9. No Commercial Use (i.e., compounds that are a degradation product or impurity of other chemicals).

EFFECTS OF EEDS

Earlier it was mentioned that the determination of which pollutants are environmental endocrine disruptors depends on their effects on the endocrine systems. However, the exact effect of hormone exposure, both natural and unnatural, is greatly dependent on factors such as species, age, and gender.

Generally, the offspring of exposed adults are the most vulnerable to these effects. Foetuses and newborns are especially susceptible to environmental contaminants. In addition to regulating sexual differentiation during foetal development, sex hormones play a role in the organisation of specific areas of the brain. Less is known about this action, but studies have shown a correlation between levels of estrogen and brain morphology, as well as with sexual behaviour in male rats and mice. The brain and central

nervous system continue development throughout the foetal stage and early natal period making them particularly susceptible to chemical exposure. [5]

EEDs affect humans as well as wildlife. There are many documented effects on humans and even more suspected effects. These include decrease in male fertility, defects in male sexual development, increases in prostate cancer, female reproductive problems, increases in breast cancer, endometriosis, immune system damage, increased incidence of goiters, and behavioural and developmental problems in children. Some examples of these are described below. [1]

Male fertility

The most likely effect of endocrine disruption in men may be a reduction in sperm production and also in the sperm's ability to fertilise an egg. In normal human males, the number of sperm produced per ejaculate is normally close to the level required for fertility. Thus, even a small reduction in daily sperm production can lead to infertility. [5] Sperm production by the average man in western countries, including the U.S., today is reported by some to be half of what it was in 1940. One report indicates that average sperm count has declined 42% and average volume of semen diminished by 20%. [9]. [5] Another report showed an increase in infertility in the last twenty years concluding that one in twenty men are either subfertile or infertile. [10], [5] However, these may be an over simplification and later reports have questioned these kind of conclusions. [1]

Male sexual development defects and cancer

There appears to be an increase in sexual development defects in recent years and some of these may be linked to exposure to EEDS. For example, more baby boys have to undergo operations to correct undescended testicles ("cryptorchidism") now than 30 years ago; the rate appears to have increased 2- to 3-fold during the past 30 years. A birth defect called "hypospadias," in which the male urinary canal is open on the underside of the penis, also is increasing. "Inter-sex" features in baby boys, where the penis is covered with a layer of fat and genitals have a cleft resembling female features, also appear to be increasing. In some cases where pregnant mothers were exposed to very high levels of toxic chemicals, the mothers' boys have shorter than normal penises, similar to Lake Apopka's alligators in Florida. Boys born to women who were exposed to PCB-poisoned rice bran cooking oil in 1978-79 in central Taiwan, the so-called "Yucheng" boys, were found to have significantly shorter penis lengths at ages 11 to 14. [11], [5]

Studies in some industrialised western nations show that cancer of the testicles, relatively more common in young men than older men, has increased at least 3-fold in the past 30 years. Another possible effect of exposure to estrogen-like contaminants is prostate enlargement in older men. This condition affects 80% of men 70 years and older. The exact cause of prostate enlargement, however, is often unknown. Prostate cancer in men also has increased by 80% in the last 20 years. [12], [5]

Female reproductive effects

Women normally are exposed to estrogen, but the effects of EEDs on females are more difficult to track due to the oestrous cycle and the resulting huge differences in circulating hormone concentrations at different stages of the cycle. The presence of estrogen mimicking compounds in adult women can impair reproductive capacity by interfering with natural hormone cycles, potentially rendering women unable to conceive or to maintain pregnancy. [5]

Female breast cancer

Breast cancer may also have links to the estrogenic contaminants. [5] Women in the US and Canada who live to age 85 have a one in nine risk of contracting breast cancer in their lifetime, double the risk in 1940 [13]. Furthermore, breast cancer mortality since the 1940s has increased by 1% per year. [14] Two leading theories of the primary risk factors for breast cancer are exposure to estrogen and high fat diets. [5] It also may be possible that some chemicals are promoters or inducers of cancer rather than being direct carcinogens. This theory is supported by the findings that some EEDs have estrogenic properties, and that estrogen is known to promote abnormal cell growth. If estrogen exposure after maturation plays a role in the full expression of early

developmental changes then this could provide an explanation for both the increased risk of breast cancer to women exposed to estrogens in utero and the rare cancers initiated at maturation in the women whose mothers took DES. [5]

Endometriosis

Recent animal studies strongly suggest that human exposure to dioxin may be linked to endometriosis, a painful disease currently affecting 10% of reproductive-age women. Endometriosis causes bits of uterine lining to migrate generally to other pelvic organs and can cause infertility, internal bleeding and other serious problems. The disease appears to becoming more common and afflicting women at younger ages. [5]

Immune system damages

Associations between endocrine-disrupting pollutants and immune system damages in wildlife are well established. [15] Similar associations are being discovered in humans. [5]

Goiters

Another effect of endocrine disruption in both adult males and females may be thyroid gland enlargement, more commonly known as goiter. The thyroid gland controls growth hormones and the hormones that regulate metabolism, and enlargement of the thyroid gland can disrupt metabolism. EEDs that have been implicated in a syndrome known as the "wasting syndrome" include PCBS, dioxin, DDT, toxaphene and lead. [5]

Hyperactivity, learning, and attention problems with children

Recent studies found a dose-response relationship between the quantity of contaminated Great Lakes fish consumed by the mother and such measures in new-born infants as abnormally weak reflexes, reduced responsiveness, motor coordination and muscle tone. [16], [17], [5] Further studies indicated that more highly exposed children had slower reaction times to visual stimuli, made more errors on a memory test and took longer to solve problems. Hyperactivity and learning deficits are among the likely effects in children exposed in utero to endocrine-disrupting chemicals, based on many related studies. If only a small part of the learning and behavioural problems of children can be attributed to endocrine, immune, or nervous system damages caused by maternal or childhood exposure to EEDS, the implications are profound. [5]

SUMMARY

In summary, the analytical challenges are complex and depend first on determining which pollutants are to be labelled as environmental endocrine disruptors and thus studied, analysed, and perhaps regulated. Currently, the only means of determining which pollutants are EEDs is from observing their effects on the endocrine systems of humans and other animals but that also has a separate set of complex factors that affect those decisions (this is where most research is currently focused but it is only the first stage of the process). Once chemicals are selected for analysis as EEDs then the problems of developing appropriate screening and/or individual chemical confirmatory analyses must be solved. Methods must be developed and validated for identification and quantification of EEDs at concentration levels a thousand times lower than most environmental methods can currently function. At those levels there will be more interferences and thus greater possibilities for false positive and false negative conclusions from the data. Thus, programs that incorporate appropriate QA/QC data and reliable analytical reference materials for both qualitative (identification) and quantitative analysis will be critical in order to avoid basing decisions and regulations on data of unknown or unreliable quality. U.S. EPA's Data Quality Objective (DQO) process and associated data quality assessments will be important tools to facilitate the measurement and use of reliable analytical data as environmental chemists cross a new threshold of analytical challenges. It won't be easy.

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Appendix. Table 1 - Comparative list of environmental endocrine disruptors

Compound	Type	EPA	CDC	WWF	CAS Number
Tributyl tin chloride	B,F	X*	X		56-35-9
Triphenyl tin acetate	B	X			900-95-8
Triphenyl tin hydroxide	B	X			76-87-9
2-Acetylaminofluorene	C	X			53-96-3
Acenaphthene	C	X			83-32-9
Anthracene	C	X			120-12-7
Benz(a)anthracene	C	X			56-55-3
Benzo(a)pyrene	C	X*		X	50-32-8
Benzo(b)fluoranthene	C	X			205-99-2
Benzo(k)fluoranthene	C	X			207-08-9
Benzophenone	C			X	119-61-9
Bisphenol-A	C	X	X	X	80-05-7
n-Butyl benzene	C			X	104-51-8
Butyl benzyl phthalate	C	X*	X	X	85-68-7
Butylated hydroxyanisole (BHA)	C	X			25013-16-5
Butylated hydroxytoluene (BHT)	C	X			128-37-0

Chrysene	C	X			218-01-9
2,4-Dichlorophenol	C			X	120-83-2
Dicyclohexyl phthalate	C			X	84-61-7
Diethyl phthalate	C			X	84-66-2
Diethylhexyl adipate	C			X	103-23-1
Diethylhexyl phthalate	C			X	117-81-7
Dihexyl phthalate	C			X	84-75-3
Di-n-butyl phthalate	C	X		X	84-74-2
Di-n-pentyl phthalate	C			X	131-18-0
Dipropyl phthalate	C			X	131-16-8
Indeno(1,2,3-cd)pyrene	C	X			193-39-5
Octachlorostyrene	C			X	29082-74-4
p-Nitrotoluene	C			X	99-99-0
p-Nonylphenol	C	X*	X		25154-52-3
PCBs	C	X*	X	X	1336-36-3
Pentachlorophenol	C	X*	X	X	87-86-5
Phenanthrene	C	X			85-01-8
Pyrene	C	X			129-00-0
Styrene	C	X			100-42-5
Tributyltin oxide	C			X	56-35-9
2,3,7,8-TCDD	C#		X	X	1746-01-6
Dimethyl mercury	C#	X			593-74-8
Benomyl	F		X	X	17804-35-2
Chlorothalonil	F	X			1897-45-6
Hexachlorobenzene	F		X	X	118-74-1
Mancozeb	F		X	X	8018-01-7
Maneb	F		X	X	12427-38-2
Metiram	F		X	X	9006-42-2
Pentachloronitrobenzene	F	X*			82-68-8
Zineb	F		X	X	12122-67-7
Ziram	F		X	X	137-30-4
Alachlor	H	X	X	X	15972-60-8
Amitrole	H		X	X	61-82-5
Atrazine	H	X*	X	X	1912-24-9
2,4-D	H	X	X	X	94-75-7
Metolachlor	H	X			51218-45-2
Metribuzin	H		X	X	21087-64-9
Nitrofen	H		X	X	1836-75-5
Simizine	H	X			122-34-9
2,4,5-T	H		X	X	93-76-5
Trifluralin	H	X	X	X	1582-09-8
Aldrin	I	X			309-00-2
Allethrin	I	X			584-79-2
alpha-BHC	I	X			319-84-6
beta-BHC	I	X	X	X	319-85-7
Carbaryl	I		X	X	63-25-2
Chlordane	I	X*	X	X	57-74-9
Chlorpyrifos	I	X*			2921-88-2
Cyhexatin	I	X			13121-70-5
Cypermethrin	I			X	52315-07-8
Dicofol	I		X	X	115-32-2
Dieldrin	I	X	X	X	60-57-1
Endosulfan	I	X*	X	X	115-29-7
Endrin	I	X*			72-20-8
Esfenvalerate	I			X	66230-04-4
Fenvalerate	I			X	51630-58-1
Heptachlor	I	X	X	X	76-44-8
Heptachlor epoxide	I		X	X	1024-57-3
Kelthane	I			X	115-32-2
Kepone	I			X	143-50-0
Lindane (gamma-BHC)	I	X*	X	X	58-89-9

Malathion	I			X	121-75-5
Methomyl	I		X	X	16752-77-5
Methoxychlor	I		X	X	72-43-5
Mirex	I		X	X	2385-85-5
Oxychlordane	I	X	X	X	27304-13-8
p,p'-DDD	I	X	X	X	72-5-8
p,p'-DDE	I	X*	X	X	72-55-9
p,p'-DDT	I	X*	X	X	50-29-3
Parathion (ethyl)	I		X	X	56-38-2
Permethrin	I	X		X	52645-53-1
Pyrethroids (synthetic)	I		X	X	NA
Toxaphene	I		X	X	8001-35-2
trans-Nonachlor	I	X	X	X	39765-80-5
Vinclozolin	I	X		X	50471-44-8
Arsenic	M	X*			7440-38-2
Cadmium	M	X*	X	X	7440-43-9
Copper	M	X*			7440-50-8
Lead	M	X*	X	X	7439-92-1
Manganese	M	X*			7439-96-5
Mercury	M	X*	X	X	7439-97-6
Tin	M	X*			7440-31-5
1,2- D ibromo-3 -chloropropane	N		X	X	96-12-8
Aldicarb	N		X	X	116-06-3
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB	X*			26601-64-9
3,3',4,4',5-Pentachlorobiphenyl	PCB	X*			25429-29-2
3,3',4,4'-Tetrachlorobiphenyl	PCB	X*			32598-13-3
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Total Number in Column	103	60	48	68	

Abbreviations used in Table 1:
EPA NERL Endocrine Exposure Team List, October 24, 1996 from Tammy Jones
CDC List from Larry Needham @ CDC 8/25/96
WWF = List from World Wildlife Fund Canada from the Internet 8/27/96
X = Present on this list
B = Biocide
I = Insecticide
H = Herbicide
N = Nematocide
F = Fungicide
C = Industrial Organic Chemical
M = Metal
PCB = Specific PCB isomer
* = Analytes that the EPA NERL Endocrine Disruptor Exposure Team plans to examine in a multi-media environment in 1997.
# = No Commercial Use; compound is a degradation product or impurity of other chemicals.