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TOXICOLOGY
NEWSLETTERS

"The journey to a different future must begin by defining the problem differently than we have done until now. The task is not to find substitutes for chemicals that disrupt hormones, attack the ozone layer, or cause still undiscovered problems, though it may be necessary to use replacements as a temporary measure. The task that confronts us over the next half century is one of redesign."

- ***Theodora Emily Colborn****
(28 March 1927 - 14 December 2014)

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Endocrine Disruptors and Safety Assessment

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1. Preamble

Chemicals (industrial cleaning agents, by-products of industrial process etc.), synthetic resin and their products are widely used for various purposes. Numerous materials such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), phenolic resin, melamine resin, polyester resin, and polycarbonate (PC) are used as major ingredients for the manufacture of plastic bottles, food containers, food packaging, cosmetics containers, appliances, packaging foam, plastic film, and microwavable packaging. *Most of these contain additional chemicals to bestow properties such as flame resistance, color, flexibility, and softness. In this regard, these chemicals have been a source of concern due to the possible presence of endocrine-disrupting chemicals (EDCs).* Additionally, the media coverage concerning the presence of phthalates, some of which are reported to be EDCs in infant milk formula in Great Britain is further evidence of the public's attention and concern.

Endocrine disruption (ED) has achieved its visibility from both a sociological and scientific basis. The fact that the endocrine system is tightly regulated during certain life stages, particularly during the differentiation process of the central nervous system, reproductive tract, and other organ systems, means that *small perturbations in hormonal status for short periods can have profound and lasting effects on the exposed individual.* From the scientific perspective, there is an accelerating accumulation of information related to the significance of the interaction of environmental chemicals with the endocrine system. The publication of the consensus statement from the first Wingspread Conference

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(1992) and the meta-analysis describing a decline in human sperm counts followed by the development of the hypothesis that in utero exposure to estrogens may be involved were landmark contributions in raising the debate. *In some cases, there are clear indications that human and wildlife populations exposed to high levels of certain persistent organic pollutants, including DDT, PCBs, and dioxins, have experienced adverse health effects*. A variety of efforts are underway in several countries testify as to the global concern.

2. Definition of an Endocrine Disruptor

Endocrine disruption is an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of endogenous hormones responsible for the maintenance of homeostasis and the regulation of developmental processes. Effects involving the steroid receptor superfamily (especially the sex steroids, thyroid, and adrenal hormones) have been the principal focus of attention, but effects on peptide hormones (e.g., GnRH, LH, FSH, and prolactin) are also of concern. Hence, endocrine disruptors are chemicals that interfere with the hormone systems and produce adverse developmental, reproductive, neurological, and immunological effects in both humans and wildlife.

3. Origin of Endocrine Disrupting Chemicals (EDCs)

The dawn of EDCs is associated with Diethylstilbestrol (DES), a "synthetic estrogen" with similar potency to 17β-estradiol, was first prescribed in 1938 to prevent miscarriage and premature births. This drug was particularly significant as the daughters of women who took DES during pregnancy were found to have an increased incidence of clear-cell carcinoma, a rare vaginal cancer. As a result, in 1971, the Food and Drug Administration (FDA) advised physicians to stop prescribing DES to pregnant women. Further research has revealed an increased risk for breast cancer in DES mothers and also possible links with reproductive tract abnormalities in DES sons and daughters.

A wide range of substances, both natural and man-made, do cause endocrine disruption, which includes pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, DDT and other pesticides, and plasticizers such as bisphenol A (BPA) and Di(2-ethylhexyl) phthalate (DEHP). *BPA - imitates the natural estrogen- is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins, and DEHP- androgen antagonist- is a high production volume chemical used in the manufacture of a wide variety of consumer food packaging, some children's products, and some polyvinyl chloride (PVC) medical devices.*

Additionally, EDCs may be found in many everyday products– including plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides.

Phytoestrogens are naturally occurring substances in plants that have hormone-like activity. Examples of phytoestrogens are genistein and daidzein, which can be found in soy-derived products. Genistein having a similar structure of 17β-estradiol (estrogen) which competes with receptor binding and produces a similar effect. *Food is a major route of exposure to EDC.* Several environmental substances including heavy metals which seem to act as EDC.

4. How Do Endocrine Disruptors Work?

Endocrine disruptors (ED) can:

- *Mimic or partly mimic naturally occurring hormones in the body* like estrogens (the female sex hormone), androgens (the male sex hormone), and thyroid hormones, potentially producing overstimulation.
- *Bind to a receptor within a cell and block the endogenous hormone from binding due to which normal signal fails to occur*, and the body fails to respond properly. Examples of chemicals that block or antagonize hormones are anti-estrogens and anti-androgens.
- *Interfere or block the way natural hormones or their receptors are made or controlled,* for example, by altering their metabolism in the liver.

Research shows that EDCs may pose the greatest risk during prenatal and early postnatal development when organ and neural systems are forming

5. Endocrine Disruptor Screening Programme (EDSP)

Environmental Protection Agency (EPA) was mandated by the US Congress to implement a screening and testing program to detect endocrine disruptors, through an advisory committee known as the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) established in 1996. EDSTAC recommended that a tiered approach should be adopted to examine endocrine activity with a focus on the potential of compounds to interact with the EAT (estrogen, androgen, thyroid) hormone systems. The Tier I assays were designed to screen for potential interaction with these endocrine systems and designed to be an efficient screening process to screen a large number of chemicals for endocrine activity. EPA launched the EDSP validation program. Based on results of methods development and validation work, some of Tier I *in vitro* and *in vivo* assays were adopted (listed below):

U.S. EPA EDSP Tier I Assays

***In vitro* assays**
Estrogen receptor (ER) binding - rat uterine cytosol
ER—(hERα) transcriptional activation - human cell line (HeLa-9903)
Androgen receptor (AR) binding - rat prostate cytosol
Steroidogenesis - human cell line (H295R)
Aromatase - human recombinant microsomes
***In vivo* assays**
Uterotrophic (rat)
Hershberger agonist and antagonist (rat)
Pubertal female (rat)
Pubertal male (rat)
***In vivo* (non-rodent assay)**
Fish short-term reproduction
Amphibian metamorphosis (frog)

The goal of the Tier I EDSP is to screen and identify chemicals that have the potential to interact with the EAT hormone system for further evaluation in Tier II. Given that there are apical endpoints in Tier I assays that may be altered by

non-specific effects and there is some redundancy to detect endocrine MOAs across different Tier I assays. It is beneficial to use a **Weight-of-Evidence (WoE)** approach to determine whether there is sufficient data to warrant **Tier II testing.**

*Based on a **weight-of-evidence** approach, chemicals deemed positive in **Tier I** will undergo **Tier II** testing to identify, characterize hazards, and quantify EDC adverse effects and establish dose-response relationships for hazard assessment*. Possible tests under consideration for inclusion in Tier II include the extended one-generation (EOGRT rat), Medaka-EOGRT, Larval amphibian growth and development assay, the two-generation avian reproductive toxicity study, fish life cycle toxicity study, and the invertebrate (mysid) life cycle toxicity study.

6. Major Endocrine Disrupting Chemicals (EDCs)

Three chemicals are focused on in this review: BPA, NP (nonylphenol) and DEHP. These chemicals are typical endocrine disruptors and also the numbers of studies are the most abundant. In fact, some government agencies take these three chemicals as representative substances of all the endocrine disruptors. Researches show these chemicals can disrupt reproductive and developmental systems, increase cancer risks and damage the immune systems in experimental laboratory animals.

1.Bisphenol A (BPA)

The major human exposure route to BPA is leached from the lining of food and beverage cans, *where it is used as an ingredient in the plastic used to protect the food from direct contact with the can and including ingestion of contaminated food and water.* BPA can enter the environment either directly from the chemical, plastic coating, and staining manufacturers, from paper or material recycling companies, foundries who use BPA in casting sand or indirectly leaching from plastic, paper, and metal waste in landfills or ocean-borne plastic trash.

BPA is an endocrine disruptor that can mimic estrogen and has been shown to cause negative health effects in animal studies. Early developmental stages appear most sensitive to its effects, and some studies have linked prenatal exposure to later physical and neurological effects. *BPA has been proposed to increase the risk of obesity, brain diseases, disruption of the hormone system/reproduction system, cancer, asthma, and heart disease.*

2.Di-(2-ethylhexyl) phthalate (DEHP)

DEHP is widely used as the plasticizer in the manufacturing of articles made of Polyvinyl Chloride (PVC). Three billion kilograms are produced annually worldwide and its environmental exposure has been issue. *It can be absorbed from food and water, with higher levels found in milk and cheese.*

DEHP is known as a potential endocrine disruptor, which can affect development and produces obesity, and cardiotoxicity. *In general, the exposure of children to phthalates is greater than for adults because of infants' and toddlers' mouthing behavior.*

Phthalate inhibits the action of testosterone and causes endocrine disruption. Cardiac muscle has been studied that associate cardiovascular disease with

phthalate. The PPARα is key regulator of lipid metabolism and a peroxisome proliferator, and one common gene that produces significant changes in response to phthalate in cardiac muscle.

3.**Nonylphenol (NP)**

NPs are a family of closely related organic compounds called alkylphenols. *They are used in manufacturing antioxidants, lubricating oil additives, laundry and dish detergents, emulsifiers, and solubilizers.* Commercially important non-ionic surfactants alkylphenol ethoxylates and nonyl-phenol ethoxylates, which are used in detergents, paints, pesticides, personal care products, and plastics are produced from NP. NP has attracted attention due to its prevalence in the environment and its potential roles as an endocrine disruptor and xenoestrogen-. *NPs act as xenoestrogen by binding to estrogen receptors and competitively inhibiting natural estrogens*. *NPs have been shown to mimic the natural hormone 17β-estradiol and to compete with the endogenous hormone for binding with estrogen receptors ERα and ER.β, and thus produces endocrine disruption.*

7. Risk Assessment Paradigm For EDCs

In general, the risk assessment procedure can be carried out in two ways:

1. *(1) Risk assessment through the exposure assessment of specific environmental media such as food and water (or)*
2. *(2) Risk assessment through biomonitoring of EDCs in human samples (urine, blood, or tissues), which reflect total exposure to EDCs regardless of source or route of exposure.*

For example, risk assessment for EDC in plastic food containers is based on the exposure scenario when the Estimated Daily Intake (EDI) of each food item and the concentration of EDCs in the food are available. In the case of risk assessment using biomonitoring data, the assessment of human exposure to EDCs is possible when toxicokinetics information about chronic exposure to a specific chemical is available for extrapolation or interpretation. To assess chemical risks for humans, several approaches can be applied depending on the carcinogenic or non-carcinogenic nature of the chemicals. For an assessment of the risk associated with EDC (BPA, phthalates, and so on) from the use of plastic food containers, estimation of human exposure to EDCs can be compared with acceptable exposure limits established by the European Food Safety Authority (EFSA) or the United States Environmental Protection Agency (U.S. EPA). *Current Tolerable Daily Intake (TDI) and Reference Dose (RfD) values for BPA are each 50 (μg/kg/day) and are derived from body weight changes in two- and three-generation studies in mice and rats.* The EFSA has allocated TDI values for Dibutyl phthalate (DBP - 10 μg/kg/day), Di(2-ethylhexyl) phthalate (DEHP - 50 μg/kg/day), Di-isodecyl phthalate (DIDP -150 μg/kg/day), and Di-isononyl phthalate (DINP - 150 μg/kg/day) based on liver effects and developmental and reproductive toxicity. The U.S. EPA has also established RfD for BPA (50 μg/kg/day), benzyl butyl phthalate (BBP- 200 μg/kg/day), DBP (100 μg/kg/day), DEHP (20 μg/kg/day), and DEHA (600 μg/kg/day).

1. Margin of safety (MOS) approach

To determine the MOS, data on the no observed adverse effect level (NOAEL in µg/kg body weight/day) and the estimated daily intake (EDI in µg/kg body weight/day) are provided by the ratio of the specified dose (NOAEL; obtained from animal data) to the level of human exposure (MOS = NOAEL ÷ EDI). NOAELs in mg/kg derived from animal studies can be converted to Human Equivalent Doses (HEDs) in mg/kg based on body surface area and the application of HED for the estimation of MOS will be more appropriate to minimize species variation between humans and animals.

The risk posed to human health by exposure to phthalates, BPA, and styrene via air, water, and food can be assessed in this way. *A MOS ≥100 is generally considered to indicate no risk, while a MOS <100 indicates that there may be a risk that needs to be regulated.* As food is a major exposure source of phthalates (for example, DEHP, DBP) in consumer products. Consumers may have very few opportunities to effectively reduce their exposure. However, food and beverage manufacturing companies can contribute to the reduction in consumer exposure by avoiding the use of phthalates in food packages (including adhesives, imprints) and in food processing equipment. Although there have been some indications of adverse effects of plastic ingredients in humans exposed in the workplace or natural environment (for example air), the exposure levels of plastic ingredients using plastic food containers are so low that they do not appear to pose a health threat to humans.

2. Hazard index (HI) calculation

To carry out a risk assessment for plastic ingredients (for example, EDCs), another popular approach for non-cancer agents is the calculation of HI, employed in this study. Although the hazard index (HI) approach leads to a similar result as the margin of safety (MOS) approach. *HI can be used when acceptable exposure limits (for example, tolerable daily intake [TDI; µg/kg body weight/day], acceptable daily intake [ADI], or RfD) are available. Most HI values {HI = (total EDI) ÷ (ADI, TDI, or RfD)} estimated from human exposure to plastic ingredients such as BBP, DEHA, DEP, DEHP, DBP, and BPA are far less than 1, suggesting that these chemicals are consumed at safe amounts*. One example is a DEHP retort-pouched baby food case that exhibited an HI of 1.068 in a study in which the TDI of several types of phthalates were estimated from packaged lunch, olive oil, butter, and retort-pouched baby foods. Two other examples of DEHP showed HIs of 3.656 and 1.678, respectively for infants (0–12 month old) and toddlers (1–3 year old) exposed to DEHP higher than the safe limit of 37 µg/kg b.w./day. In the case of DBP, HI values were estimated to be 1.48 and1.64 via exposure to DBP contaminated packaged lunch at the heating condition and infant formula, respectively. In each case of Di-isodecyl phthalate (DIDP) or Di-isononyl phthalate (DINP), an estimated HI value was higher than 1 (1.4 or 1.44), suggesting exposure to DIDP or DINP higher than the safe limit of 150 µg/kg body weight/day.

As with the risk assessment process for all chemicals and stressors, human and ecological risk assessments differ significantly in the latter case, the focus is on population and community-level responses. Risks need only be extrapolated to a single species. In contrast, with the notable exception of endangered species, ecological risk assessments are generally more concerned with the overall status of populations and communities than individuals, and effects must be extrapolated from few test species to the many species present in an

environment. This engenders a different focus in terms of assessment and measurement endpoints. For example, the occurrence of low levels of cancer or terata in wildlife generally is not of great concern if the overall population is healthy. *Hence, for ecological risk assessments, measurement endpoints typically focus upon biological responses related to reproductive success, such as survival, normal development, and fecundity.* Although some component of concern about the effects of EDCs on humans is related to reproductive fitness (e.g., declines in sperm quality), an important aspect of the issue, at least from a public health perspective, is the occurrence of diseases such as cancer or learning disabilities in individuals. This does not imply that there should be no interest in effects at the level of the individual in wildlife populations. For example, increases in cancer/terata rates in specific wildlife populations could serve as an important indicator of (a) effects that could occur in humans, given appropriate exposure scenarios and homology, and/or (b) potentially more pervasive effects in the population under consideration.

8. Conclusion

Endocrine disrupting chemicals [EDCs] can migrate as residual monomers (for example, styrene for polystyrene or BPA for polycarbonate) presented in polymers, as additives (for example, phthalates for PVC) used in polymer manufacturing, and/or as contaminants from the polymers depending on physicochemical conditions such as temperature, UV light, pH, microwave, and mechanical stress. Endocrine disruptors are known to cause harmful effects to human through various exposure routes. These chemicals mainly appear to interfere with the endocrine or hormone systems. As importantly, numerous studies have demonstrated that the accumulation of endocrine disruptors can induce fatal disorders including obesity, cardiotoxicity and cancer.

The focus on endocrine disruption represents a new paradigm in toxicology, wherein the MOA, rather than toxicological outcome, is the focus of chemical screening. *As endocrine issues have increased in scope, increased research has led to a better understanding of receptor biology*. To take full advantage of this new knowledge and efficiently meet the needs to screen compounds for endocrine activity, the EDSP should be iterative and allow for a reevaluation of earlier assumptions based on the state of the science. Clearly, the past 15 years have demonstrated that legislative mandates for specific types of toxicity testing that precedes scientific understanding are difficult to implement and limit the latitude to adapt to evolving science. *Even so, the significant resources dedicated to developing and validating the panel of EDSP assays have led to an improved understanding of the challenges involved with implementing and interpreting EDSP assays, and these lessons will be beneficial to eventual implementation of the EPA's ToxCast assays and human primary cell assays as proposed in the National Research Council report Toxicity Testing in the 21st Century.*

Questions

1. *Define Endocrine Disruptor?*
2. *What are Phytoestrogens?*
3. *How do Endocrine Disruptors work?*
4. *What is EDSTAC?*
5. *List out U.S. EPA EDSP Tier I Assays?*
6. *Name three major Endocrine Disrupting Chemicals?*

7. *What are the Current Tolerable Daily Intake (TDI) and Reference Dose (RfD) values for BPA?*
8. *How to calculate Margin of safety (MOS)?*
9. *Write Hazard index (HI) calculation?*

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