I have no conflicts of interest to declare.
WE LIVE IN A CHEMICAL STEW
EDCs are exogenous chemicals or chemical mixtures that interfere in some way with hormone action.
SUSPECTED ENDOCRINE DISRUPTING CHEMICALS

- Metals
- Industrial chemicals
- Personal care products
- Synthetic & naturally occurring hormones
- Pharmaceuticals drugs
- Pesticides
  - Herbicides
  - Fungicides

Some chemicals from the "families" above are potentially endocrine disrupters
THE ENDOCRINE SYSTEM: WHAT DOESN’T IT CONTROL?
LOW DOSES OF HORMONES CAN INDUCE PERMANENT ALTERATIONS IN DEVELOPMENT

“From the day of conception until an individual is born or hatched, the development of each stage of life is fully under the control of hormones.

Changes that happen during development are far less reversible [than those occurring in an adult]; you can't go back and rewire the brain”.

-Theo Colborn, zoologist, writer
<table>
<thead>
<tr>
<th>Early Prenatal</th>
<th>Mid-Prenatal</th>
<th>Late Prenatal</th>
<th>Postnatal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central nervous system (3wks - 20 years)</td>
<td>Ear (4-20 wks)</td>
<td>Kidneys (4-40 wks)</td>
</tr>
<tr>
<td></td>
<td>Heart (3-8)</td>
<td>Limbs (4-8 wks)</td>
<td>Immune system (8-40 wks; competence &amp; memory birth-10yrs)</td>
</tr>
<tr>
<td></td>
<td>Skeleton (1-12 wks)</td>
<td>Lungs (3-40 wks; alveoli birth-10yrs)</td>
<td>Reproductive system (7-40wks; maturation in puberty)</td>
</tr>
<tr>
<td>Week 1-16</td>
<td>Week 17-40</td>
<td>Birth</td>
<td>– 25 years</td>
</tr>
</tbody>
</table>
Early Life Exposure to EDCs

The effects of early exposures to EDCs – when organ systems are developing – may be manifested any time in life.
WHEN TALKING ABOUT ENVIRONMENTAL CHEMICALS, WHAT ARE LOW DOSES?

“LOW DOSES” FOR EDCs:

- BELOW THE NOAEL
- IN THE RANGE OF HUMAN EXPOSURES
- REPLICATE HUMAN SERUM LEVELS
THE LOW DOSE HYPOTHESIS

• EDCs have effects, especially on reproduction and development, at low doses

• Effects observed in exposed animals are occurring at doses similar to human exposures (i.e. at doses that are thought to be safe)

• Humans environmentally exposed to EDCs are affected by low doses
“LOW DOSE” STUDIES TYPICALLY...

- SMALL SCALE
- NON-TRADITIONAL ENDPOINTS
- EXPERIMENTAL DESIGN TAILORED TO QUESTION.
IN 2002, THE NTP AGREED THAT THERE WERE “LOW DOSE EFFECTS” FOR 4 EDCs

• DES (adult prostate weight)

• Genistein (brain sexual dimorphisms, male mammary gland development, immune responses)

• Methoxychlor (immune responses)

• Nonylphenol (brain sexual dimorphisms, immune response, estrus cyclicity)
Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses

Laura N. Vandenberg, Theo Colborn, Tyrone B. Hayes, Jerrold J. Heindel, David R. Jacobs, Jr., Duk-Hye Lee, Toshi Shioda, Ana M. Soto, Frederick S. vom Saal, Wade V. Welshons, R. Thomas Zoeller, and John Peterson Myers

Center for Regenerative and Developmental Biology and Department of Biology (L.N.V), Tufts University, Medford, Massachusetts 02155; The Endocrine Disruption Exchange (T.E.D), Pocatello, Idaho 83208; Laboratory for Integrative Studies in Amphibian Biology (T.I.S.A.B), Molecular Toxicology, Group in Endocrinology, Energy and Resources Group, Museum of Vertebrate Zoology, and Department of Integrative Biology, University of California, Berkeley, California 94720; Division of Extramural Research and Training (L.I.R.T), National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, North Carolina 27709; Division of Epidemiology and Community Health (D.E.C.H), School of Public Health, University of Minnesota, Minneapolis, Minnesota 55455; Department of Preventive Medicine (D.P.M.), School of Medicine, Kyungpook National University, Daegu 702-701, Korea; Molecular Profiling Laboratory (T.S.), Massachusetts General Hospital Center for Cancer Research, Charlestown, Massachusetts 02129; Department of Anatomy and Cell Biology (A.C.B.), Tufts University School of Medicine, Boston, Massachusetts 02111; Division of Biological Sciences (P.S.E.S) and Department of Biomedical Sciences (W.K.W.), University of Missouri Columbia, Columbia, Missouri 65211; Biology Department (T.Z.), University of Massachusetts Amherst, Amherst, Massachusetts 01003; and Environmental Health Sciences (U.P.M.), Charlottesville, Virginia 22902

For decades, studies of endocrine-disrupting chemicals (EDCs) have challenged traditional concepts in toxicology, in particular the dogma of “the dose makes the poison,” because EDCs can have effects at low doses that are not predicted by effects at higher doses. Here, we review two major concepts in EDC studies: low dose and nonmonotonicity. Low-dose effects were defined by the National Toxicology Program as those that occur in the range of human exposures or effects observed at doses below those used for traditional toxicological studies. We review the mechanistic data for low-dose effects and use a weight-of-evidence approach to analyze five examples from the EDC literature. Additionally, we explore nonmonotonic dose-response curves, defined as a nonlinear relationship between dose and effect where the slope of the curve changes sign somewhere within the range of doses examined. We provide a detailed discussion of the mechanisms responsible for generating these phenomena, plus hundreds of examples from the cell culture, animal, and epidemiologic literature. We illustrate that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Whether low doses of EDCs influence certain human disorders is no longer conjecture, because epidemiologic studies show that environmental exposures to EDCs are associated with human diseases and disabilities. We conclude that when nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Thus, fundamental changes in chemical testing and safety determination are needed to protect human health. (Endocrine Reviews 33: 378–455, 2012)

I. Introduction
A. Background: low-dose exposure
B. Background: NMDRCs
C. Low-dose studies: a decade after the NTP panel's assessment
D. Why examine low-dose studies now?
E. Mechanisms for low-dose effects
F. Intrauterine position and human twins: examples of natural low-dose effects
II. Demonstrating Low-Dose Effects Using a WoE Approach

A. Use of a WoE approach in low-dose EDC studies
B. Refuting low-dose studies: criteria required for acceptance of studies that find no effect
C. BPA and the prostate: contested effects at low doses?
D. BPA and the mammary gland: undisputed evidence for low-dose effects

Abbreviations: A4, Androstenedione; AHR, Aryl hydrocarbon receptor; BPA, bisphenol A; CDC, Centers for Disease Control and Prevention; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DES, diethylstilbestrol; EDC, endocrine-disrupting chemical; EPA, Environmental Protection Agency; ER, estrogen receptor; FDA, Food and Drug Administration; G.P., good laboratory practices; GAOL, lowest observed adverse effect level; MFR, membrane-associated ER: RNASES, National Health and Nutrition Examination Survey, NS, sodium valproate syrup; NAOM, nonmonotonic dose-response curve; NDA, no observed adverse effect level; NOEL, no observed effect level; NTP, National Toxocology Program; PNC, prostate intraepithelial neoplasia; PDB, persistent organic pollutants; p,p', par in billion; SERM, selective estrogen receptor modulator; TCDD, 2,3,7,8-tetrachlorodibenzop-dioxin; WoE, weight of evidence.

378 eendr.endojournals.org Endocrine Reviews, June 2012, 33(2):378–455
REVISITING THE ‘LOW DOSE HYPOTHESIS’

Epidemiology studies continue to suggest associations between EDC exposures and human disease

- Phthalates: neurobehavior, adult fertility, metabolic syndrome, anogenital distance
- Dioxin: metabolic syndrome, male infertility, age of pubertal onset (males)
- DDT: body weight, cancer, neurodevelopment, oxidative stress
- Atrazine: size at birth, pre-term birth, abdominal defects, cancer, sperm quality
- Heptachlor: diabetes, asthma & chronic bronchitis, male reproductive tract defects
- PBDEs: thyroid hormone levels, neurodevelopment, autism
- BPA: metabolic syndrome, infertility, neurodevelopment
- Dieldrin: neurotoxicity, cancer, diabetes, infertility
- Toluene: bronchitis & asthma
- Simazine: cancer
- Chlorpyrifos: neurodevelopment, behavior, asthma
Many chemicals have effects at low doses.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Affected endpoint</th>
</tr>
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<tbody>
<tr>
<td>Maneb</td>
<td>Testosterone release</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>Immune system</td>
</tr>
<tr>
<td>4-Methylbenzylidine camphor</td>
<td>Sexual behavior</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>Uterine tissue organization</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Incidence of cryptorchidism (Humans)</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>Testosterone metabolism</td>
</tr>
<tr>
<td>Octylphenol</td>
<td>Testes endpoints</td>
</tr>
<tr>
<td>Parathion</td>
<td>Cognitive &amp; emotional behaviors</td>
</tr>
<tr>
<td>PBDE-99</td>
<td>Thyroid hormone levels in blood</td>
</tr>
<tr>
<td>PCB180</td>
<td>Diabetes (Humans)</td>
</tr>
<tr>
<td>PCB mixtures</td>
<td>Thyroid hormone levels</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>TSH levels (Humans)</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>Bone mass &amp; strength</td>
</tr>
<tr>
<td>Tributyltin oxide</td>
<td>Obesity</td>
</tr>
<tr>
<td>Triclosan</td>
<td>Altered uterine responses to ethinyl estradiol</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>Male fertility</td>
</tr>
</tbody>
</table>
ATRAZINE & MALE SEXUAL DIFFERENTIATION, A FASCINATING EXAMPLE
MALE SEXUAL DIFFERENTIATION IN AMPHIBIANS

On W chromosome, DM-W gene \rightarrow aromatase expression
ATRAZINE: A GENDER-BENDING CHEMICAL?

Hayes et al. 2010
BISPHENOL A (BPA), AN ESTROGEN WITH MANY USES
BPA ALTERS MAMMARY GLAND DEVELOPMENT & INDUCES PRE-CANCEROUS LESIONS

Vandenberg et al. Repro Tox 2008
BPA INDUCES DCIS, FRANK CARCINOMAS, & ALTERS RESPONSE TO CARCINOGENS

BPA ALTERS BRAIN DEVELOPMENT & BEHAVIOR

Rubin et al. Endocrinology 2006
TWO-HIT MODEL OF DISEASE INDUCTION

1st hit

Mammary cancer (carcinogens)
Prostate cancer (hormones)
Asthma (dust mites)
Leiomyoma (hormones)
Diabetes (high fat diet)
Stress responses (induced metamorphosis)

2nd hit
TWO-HIT HYPOTHESIS: PROSTATE

1st

BIRTH

2nd

DAY 20 → ADULT

AGING

Undifferentiated progenitor

Differentiated secretory cell

Dysplasia (PIN)
PROSTATE GLAND: ESTROGEN DRIVEN CARCINOGENESIS
DEVELOPMENTAL REPROGRAMMING INCREASES LEIOMYOMA RISK

Uterine leiomyomas: 100% of animals
WE ALSO NOTED THAT BOTH HORMONES AND EDCs INDUCE NON-MONOTONIC DOSE RESPONSES
NON-MONOTONIC CURVES ARE COMMON IN MEDICINE, PHARMACOLOGY & ENDOCRINOLOGY

Houshmand et al. 2009
WE FOUND SEVERAL HUNDRED EXAMPLES OF NON-MONOTONIC RESPONSES TO EDCS

<table>
<thead>
<tr>
<th>DES</th>
<th>Lead</th>
<th>Atrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trenbolone</td>
<td>Cadmium</td>
<td>Endosulfan</td>
</tr>
<tr>
<td>R1881</td>
<td>Genistein</td>
<td>dieldrin</td>
</tr>
<tr>
<td>BPA</td>
<td>Coumesterol</td>
<td>DDT</td>
</tr>
<tr>
<td>DEHP</td>
<td>daidezin</td>
<td>DDE</td>
</tr>
<tr>
<td>Octylphenol</td>
<td>Resveratrol</td>
<td>hexachlorobenzene</td>
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<tr>
<td>Nonylphenol</td>
<td>Biochanin A</td>
<td>Prochloraz</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>Licoflavone C</td>
<td>Ketoconazole</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>Quercetin</td>
<td>PBDE-49</td>
</tr>
<tr>
<td>Retene</td>
<td>TCDD</td>
<td>PBDE-99</td>
</tr>
</tbody>
</table>
WHY THIS MATTERS: ATI/RFD DOSES ARE CALCULATED FROM NOAELs WITH AN ASSUMPTION OF LINEARITY.
EXAMPLES IN THE CONTEXT OF THE NOAEL AND RFD

Angle et al. 2013
EXAMPLES IN THE CONTEXT OF THE NOAEL, RFD & HUMAN EXPOSURES

Do et al. 2013
EXAMPLES IN THE CONTEXT OF THE RFD & HUMAN EXPOSURES

Vandenberg et al. 2013
Epidemiology studies reveal NMDRCs – presumably all of which occur below the RFD.

Human exposure range

Lee et al. 2014
RISK ASSESSMENT FOR ENDOCRINE DISRUPTORS: IS IT INSUFFICIENT?

- IDENTIFY LD50, MTD, LOAEL, NOAEL
- CALCULATE RFD (NOAEL/10, 100 OR 1,000)
- COMPARE THE RFD TO HUMAN EXPOSURE LEVELS
AN ILLUSTRATION: PERCHLORATE

NHANES 95th percentile: 0.234 µg/kg/day

<table>
<thead>
<tr>
<th>NOAEL</th>
<th>0.007 mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>RfD</td>
<td>0.0007 mg/kg/day (0.7 µg/kg/day)</td>
</tr>
</tbody>
</table>
AN ILLUSTRATION: PERCHLORATE

MATERNAL PERCHLORATE IN TOP 10%

OFFSPRING IQ IN LOWEST 10% AT AGE 3

NHANES

perchlorate

serum T4

serum TSH
DIISONONYL PHTHALATE (DINP)

Testing range in rats

Human exposure range

AGD

Nipple retention
AGD

NOAEL

1

10

100

1,000

10,000

100,000

300,000

900,000

10

100

1,000

10,000

Testing range in rats

Human exposure range
HOW WE ADDRESS UNCERTAINTIES MATTERS: EXPERIMENTAL DESIGN IN LOW DOSE STUDIES
WHAT IS AN ADVERSE OUTCOME?

• BIOCHEMICAL
• PHYSIOLOGICAL
• GROWTH
• DEVELOPMENT
• BEHAVIOR
• LIFESPAN
• RESPONSE TO “STRESS”
STANDARD ASSAYS
STANDARD ASSAYS

• TOXICITY

• ADULT DISEASE
RELATIVELY SMALL CHANGES CAN MAKE A DIFFERENCE

Changes in the composition of can coating
AN EMERGING PROBLEM: REGRETTABLE REPLACEMENTS
CHANGING HOW WE TEST EDCs

- Without appropriate testing, ‘safety’ should not be assumed
- ‘positive’ effects should trigger chemical abandonment
BUT WE’VE ALL BEEN EXPOSED AND WE’RE ALL FINE

Over recent decades there has been:

- significant increase in reproductive problems in some regions of the world, suggesting a strong role for unidentified environmental factors in disease etiology
- increase in endocrine cancers
- significant decrease in human fertility rates
- increase in use of assisted reproductive services
- Increases in neurobehavioral disorders
- increasing number of chemicals to which all humans in industrialized areas are exposed

Top: Richiardi et al., Cancer Epidem. Biomark. (2004);
Bottom: based on data from http://data.euro.who.int/hfadb/
ACKNOWLEDGEMENTS

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