Endocrine Disruptors: Definitionen

- WHO/IPCS definition(s) 2002
  "An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."

  "A potential endocrine disrupter is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations."

- Adversity ? WHO 2009, BfR Workshop
  "A change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences."
2 ENDOCRINE DISRUPTORS: Def. & Risk Assessment

- WHO/IPCS Definition(s) ... endorsed by EFSA 2013 ...
- Important considerations in risk assessment:
  - windows of susceptibility
  - dose-response relationship
  - exposures (external, internal)
  - mechanisms / mode of action
- Toxicokinetics
- Toxicodynamics

Question:
Endocrine Active Substances (EAS*)
Should EAS be treated like other chemicals of concern for human health (and the environment), i.e. be subject to risk assessment and not only to hazard assessment?

3-1 ENDOCRINE DISRUPTORS: KONTROVERSEN

- Thresholds? (Wirksschwellen? Mechanism; MoA)
- Nicht-monotone Dosis-Effekt Beziehungen
- LOW Doses – in Relation zu Exposition (und PFAD!)
- Regulation – auf Basis von Hazard oder RA?

3-2 RISK Assessment

Steps in toxicological risk assessment (RA)

- Hazard Identification
- Hazard Characterization
- Exposure Assessment
- Risk Characterization

To inform Risk Management
DIETHYLSTILBESTROL (DES)

Epidemiology revealed adverse effects

- "DES Daughters"
- "DES Sons"
- "DES Mothers"
  -> transplacental carcinogen and teratogen ...
  -> (non-cancer) lesions in sons
  -> increased risk of breast cancer in mothers

The Dilemma:
- difficult to define a 'safe dose' for carcinogens
- dose response data ... threshold ?

Animal Studies

4-1 Diethylstilbestrol in utero Exposed Cohorts and Estimated Maternal Exposures

<table>
<thead>
<tr>
<th>Group / Type of study</th>
<th>Estimated mean total DES dose (g)</th>
<th># DES-exposed</th>
<th># unexposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayo Clinic Sons (Cohort)</td>
<td>1.4</td>
<td>828</td>
<td>676</td>
</tr>
<tr>
<td>Connecticut Mothers (Cohort)</td>
<td>2.1</td>
<td>1531</td>
<td>1404</td>
</tr>
<tr>
<td>DESAD Study (Cohort)</td>
<td>4.2 ≈</td>
<td>4014</td>
<td>24</td>
</tr>
<tr>
<td>Boston Collab. Study b (Cohort)</td>
<td>6.4</td>
<td>217 1033</td>
<td></td>
</tr>
<tr>
<td>DES Efficacy Trial a (rand. clin. trial)</td>
<td>11.6</td>
<td>840</td>
<td></td>
</tr>
<tr>
<td>Britisch Rand. Trial (rand. clin. trial)</td>
<td>11.5</td>
<td>650 806</td>
<td></td>
</tr>
<tr>
<td>Br. Med. Res. Council (rand. clin. trial)</td>
<td>17.9</td>
<td>70 66</td>
<td></td>
</tr>
</tbody>
</table>

- University of Chicago
- 80% of cohort from Boston Lying-In Hospital; DES mean total estim. for total cohort
- Median dose based on 26% of cohort with known DES doses


4-2 Diethylstilbestrol Exposure of Women

- Prenatal exposure to DES:
  - increased risk of CCAC, clear cell adenocarcinoma of vagina and cervix
  - increased risk of breast cancer after the age of 40

- Maternal total doses : between 1.4 - 18 g

- DES use in pregnancy associated with a modest, but statistically significant increased risk of breast cancer in "DES-mothers".
  Risks do not appear to increase greatly over time.
4-3  Studies in cohorts of DES Sons  

(b)  

Dose and timing of exposure ?

Palmer JR et al. (2009)  Urogenital abnormalities in men exposed to diethylstilbestrol in utero. Environ Health 8:37 (8p)

Men who were exposed to DES in utero had an increased prevalence of cryptorchidism, epididymal cysts, and inflammation/infection of the testes. The associations were strongest for exposure before the 11th week of gestation and for a cumulative dose of DES of 5 grams or more. Because nearly all women who received a cumulative dose of 5 grams or greater had begun taking DES before the 11th week of gestation, it was not possible to determine definitively which of these factors was more important.

--> DES exposed sons have a higher occurrence of cryptorchidism and epididymal cysts than unexposed sons, and the increased risk is related to timing and dose.

4-4  DES – Proof of principle for ED

Adverse effects studied in:

"DES Daughters"  "DES Sons"  "DES Mothers"

... carcinogen and teratogen

> increased risks related to timing of exposure and dose

The Dilemma:

- difficult to define a "safe dose from epidemiol. data
- mechanistic data ... (threshold ?)

Animal Studies

♦ Estrogen  e.g. Uterotrophic Assays
♦ Antiandrogen  e.g. Hershberger Assay

4-5  A maternal dose threshold for adverse reproductive tract development ?

♦ Studies on in utero DES exposure on structure and function of the male genital tract in mice treated s.c. with 0.1, 1.0, 2.5, 5, 10 or 100 µg / kg bw on GD 9-16:

Only the highest dose was associated with noticeable changes in the reproductive tract.

♦ Fertility ?

In: Reproductive and Developmental Toxicology (Ed. K.S. Korach) Marcel Dekker, Inc. New York, pp. 531-551
4-6 Reduced fertility in female mice exposed transplacentally to Diethylstilbestrol

Dose-dependent reduction in fertility ....
Sterile at high doses
McLachlan et al. 1982
Fertility & Sterility
38: 364-371

4-7 Developmental Exposure to EDCs: DES lessons

"Effects observed in rodents and humans following in utero exposure to sufficient doses of DES are consistent with basic principles of dose response as well as the possibility of maternal levels below which potential non-cancer effects may not occur."

Uncertainty for cancer effects ...
- Low incidence in DES daughters ...

4-8 Exposure: in utero or postnatal

Cancer Risk from ED: The case of DES

- Female offspring of mice treated s.c. with graded doses of DES (0.01 up to 100 microg/kg b.w.) during GD 9–16 → at 12-18 months
  - The number of genital tract tumors was low in all the mice treated with DES at a dose less than DES-100. The combined prevalence of tumors of the vagina, cervix, and uterus ranged from 9.1% (2 of 22) in DES-5 mice to 4.6% (1 of 21) in DES-0.01 mice. The corresponding value for control (female offspring was 0% (0 of 85). In females treated with DES-100 in utero, 35% had genital tract tumors. Moreover, in the 3 highest dose groups, tumors were observed in animals sacrificed at 12 or 13 months of age.
  [Quote from McLachlan JR et al., 1996; see also Newbold RR 1995]

- Mice treated s.c. on PND 1 - 5 with equi-estrogenic doses of DES or Genistein → developed similar incidences of uterine adenocarcinoma at 18 months [Newbold R et al. 2001]
5-1 **Route-dependent estrogenic potency of DES**


3 day uterotrophic assay in rats with s.c. or d.w. application

![Graph showing kinetics have an impact...]

5-2 **Biologische Wirksamkeit : Pfadeffekt**

![Graph showing impact of metabolism and route of administration]

**QUIZ**

**6-1**  
**"unusual" dose-response-curves:**  
Are they new in toxicology?  
What can they tell us?  

Man-made and natural EDCs:  
How to assess xeno-estrogens?  
Which ones are important?  

Are High Soy-eaters different?

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**6-2**  
**Non-monotonic (Inverted U-shaped) Dose-response**

*Genistein can exert more than one effect:*

- ER-dependent and independent

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**6-3**  
**U-shaped Dose-response: Cadmium**

Uterine complement C3 expression  
C3, an estrogen responsive gene

Toxicol Lett 191:123-131

Is this effect "adverse"?
**6-4 Nicht monotone (U-shaped) Dosis-Wirkungs-Kurve**

Präneoplastische Foci in Rattenleber: DEN (Initiator) - / + TCDD (Promotor) Hormesis

**6-5 Human Exposure to Estrogenic Chemicals**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Exposure</th>
<th>[μg/day]</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol A</td>
<td>in food cans</td>
<td>6.3</td>
<td>Howe &amp; Borodinsky 1998; Howe et al. 1998; GNUt et al. 2002</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>in total diet</td>
<td>10</td>
<td>Gunderson 1995 (Inuit data from Kuhnlein et al. 1995)</td>
</tr>
<tr>
<td>DDT (isomers)</td>
<td>in total diet</td>
<td>0.01</td>
<td>Gunderson 1995 (Inuit data from Kuhnlein et al. 1995)</td>
</tr>
<tr>
<td>PCP</td>
<td>in total diet</td>
<td>0.002</td>
<td>Gunderson 1995 (Inuit data from Kuhnlein et al. 1995)</td>
</tr>
</tbody>
</table>

**6-6 Analysis of Estrogenicity by -Omics.....**

- Moggs JG et al. (2004) The need to decide if all estrogen receptors are intrinsically similar. Environ Health Perspect 112: 1137-1142

**Conclusions**

- Qualitatively similar gene expression profiles, level of regulation differs... (ref. potency)
- Compounds operate via a molecular mechanism analogous to that of estradiol and display tissue-specific responses
- Use "phenotypic anchoring"
Hazard or Risk based approach?

Some conclusions

- In rodent models (potential) endocrine disruptors, of natural or synthetic origin, can be identified and characterized according to criteria such as hormonal activity and adversity of effects.
- A possible impact of EAS on human health will depend upon (internal) exposure at critical windows of susceptibility.
- Endocrine disrupters should be treated like most other chemicals of concern, i.e. be subject to risk assessment and not only to hazard assessment.
- Risk assessment - taking into account hazard and exposure predictions - makes best use of available information to inform on risk and level of concern for the purpose of risk management decisions.

Scientific Opinion on the hazard assessment of endocrine disruptors:
On request from the European Commission, Question No EFSA-Q-2012-00760
Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment.
EFSA (2014) on BPA


Die Sachverständigen der EFSA empfehlen, die tolerierbare tägliche Aufnahmemenge (TDI) für BPA vom aktuellen Wert von 50 µg/kg KG pro Tag (bzw. 0,05 mg/kg KG pro Tag) auf 5 µg/kg KG pro Tag (0,005 mg/kg KG pro Tag) zu senken und für einen vorläufigen Zeitraum festzulegen. Die EFSA ist der Ansicht, dass das Gesundheitsrisiko für sämtliche Bevölkerungsgruppen – einschließlich Föten, Säuglinge, Kleinkinder und Erwachsene – gering ist, da die höchsten Schätzwerte für eine kombinierte orale und nicht-orale BPA-Exposition je nach Altersgruppe drei- bis fünfmal niedriger sind als der vorgeschlagene TTDI-Wert. Für alle Bevölkerungsgruppen ist allein die orale Exposition fünfmal geringer als der vorgeschlagene TTDI-Wert.

Human Biomonitoring

Time trend of urinary BPA in young adults living in Germany in comparison to BPA production data

Endocrine active chemicals: Mode of Action A/B

17ß-Estradiol

Testosteron

Hormonal Mimic

Hormonal Block

Classical genotropic actions of sex steroid receptors and/or nongenotropic signaling

HBMOS: Definition and Purpose

→ HBMOS has been defined as quotient of estimated human daily intakes weighted by the respective relative (rodent) in vivo hormonal potencies.

HBMOS values provide an estimate of estrogen exposure by man-made chemicals in relation to an existing dietary exposure of phytoestrogens and help to identify compounds which should be given priority for further evaluation.


**Comparison of estrogenic potency of selected synthetic chemicals and naturally occurring estrogens**

**Evaluation of Xenoestrogens**

- **Exposure to xeno-/phytoestrogens**
- **Potency of xeno-/phytoestrogens**

**Comparison of estrogen burden ("estrogen equivalents") for humans**

- **The "HB-MOS concept"**: I. Bolt et al. 2001; II. Bolt & Degen 2002

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**6 Exposure ? Human biomonitoring**

**BPA in urines of German children: Data from GerES IV in µg / L**

Becker K et al. (2009) Int J Hyg Environ Health 212: 685-692

<table>
<thead>
<tr>
<th>Cohort Age</th>
<th>N</th>
<th>N-LOQ</th>
<th>P50</th>
<th>P90</th>
<th>P95</th>
<th>GM</th>
<th>CI GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-8 y</td>
<td>145</td>
<td>143</td>
<td>2.81</td>
<td>9.05</td>
<td>15.4</td>
<td>2.72</td>
<td>2.31-3.21</td>
</tr>
<tr>
<td>9-11 y</td>
<td>149</td>
<td>147</td>
<td>2.13</td>
<td>8.37</td>
<td>13.8</td>
<td>2.22</td>
<td>1.89-2.61</td>
</tr>
<tr>
<td>12-14 y</td>
<td>168</td>
<td>165</td>
<td>2.60</td>
<td>8.87</td>
<td>11.0</td>
<td>2.42</td>
<td>2.07-2.82</td>
</tr>
</tbody>
</table>

**Isolavone Biomarkers in German children (24 urines)**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Median (µg / L)</th>
<th>geom. MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>DONALD</td>
<td>Equol 12</td>
<td>16</td>
</tr>
<tr>
<td>Cohort</td>
<td>Dal 197</td>
<td>188</td>
</tr>
<tr>
<td>510 urines</td>
<td>Gen 113</td>
<td>118</td>
</tr>
</tbody>
</table>

**Similar to values for US children in NHANES Study**


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**Fate of Compound (EAS) in vivo**

**Metabolisms and Route of Administration**

- Oral
- Intestine
- Liver
- Portal
- Kidney
- Circulation
- Target Tissue
- Feces
- Urine
Cadmium Uterine complement C3

Höfer N et al. 2009

Cadmium Uterine weight

Cd content Uterus

Höfer N et al. 2009

Animal and Human Biomonitoring Data

Comparing internal levels...
Genistein stimulates growth of human breast cancer cells in a novel, postmenopausal animal model, with low estradiol concentrations...


**Fig. 1.** Uterine response in prepubescent mice (17 days old) following estrogen challenge with DES. Note an enhanced response in the DES 0.01 µg/kg group but a dampened response in groups exposed to higher doses of DES. and Fig.7. Response of adult (4m) ovx mice ...

Non-monotonic dose effect relationships

Neonatal mice were injected s.c. with DES ...... later challenge with E2 (...) or DES (...) on three days


**5-1** DES: a potent *Estrogen* and *Anti-Androgen*

Jefferson WN & Newbold RR 2000

Testis of Hamsters: treated with DES (top) untreated (bottom)

1.0 or 50 000 µg/kg/day s.c.
Mechanistic studies with DES and ... and epigenetic effects?


Tang WY, Newbold RR, Mardilovich K et al. (2008) Persistent hypomethylation in the promoter of nucleosomal binding protein1 (Nabp1) correlates with overexpression of Nabp1 in mouse uteri neonatally exposed to diethylstilbestrol or genistein. Endocrinology 149: 5922-593

Developmental Exposure to EDCs: DES lessons

Market sales of 25 mg DES vs. cases of CCAC by year of diagnosis ...

Fig 1 from Swan SH (2001) APMIS

Human Exposure to other EAS?

DES and Humans ...

Risk Assessment: Animal and Biomonitoring Data

Comparing internal levels...