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From Bisphenol A experience:

requirement for an appropriate risk assessment of endocrine disruptors

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Controversy on the risk assessment of bisphenol A

- in 2006, EFSA set a TDI of 0.05 mg BPA/kg bw
  - NOAEL of 5 mg/kg bw/day
  - two-generation reproductive toxicity study in mice based on liver effects (hypertrophy, Tyl *et al.*, 2006).
  - liver toxicity at least as sensitive as reproductive/developmental effects.
  - uncertainty factor of 100

- but many studies suggest toxicity at doses < 5 mg/kg bw/day, although none of them is totally convincing

- Paradoxical position in the opinions:
  - no risk at current level of exposure
  - risk, even at very low level, especially for babies linked to the weak estrogenic activity of BPA

Key points of Afssa's opinion (29 January 2010)

Afssa self-requested in October 2009 to analyse the results of

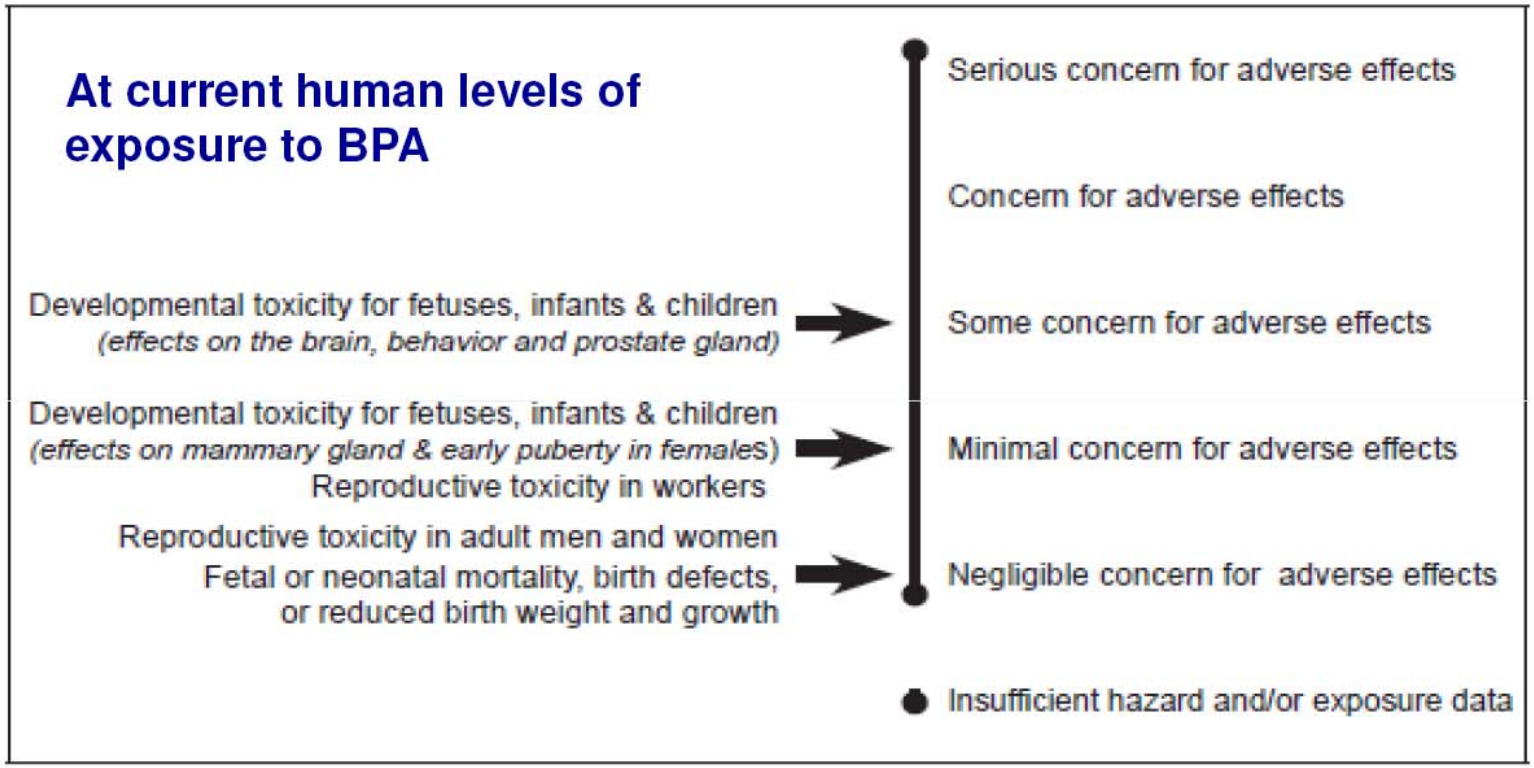
- the full report of a study commissioned by the American Chemistry Council in the context of the REACH procedure (Stump *et al.*, 2009):
  - morphological and functional effects of BPA on the nervous systems of rat from birth to adulthood, following perinatal exposure (*in utero* and lactation).
  - in compliance with OECD 426 and EPA guidelines

- 53 other recent publications: 29 on toxicity and 24 on exposure including studies put forward by a French NGO (invited to present its view)

How did we work?

- Working Group : 12 experts (scientific Panels on Contaminants, FCM, Pesticides + 1 external expert with experience on BPA and endocrine disruptors)
- Critical analysis of the studies:
  - Experimental protocol
  - Results
  - Quality of the study
  - Methodological flaws
- WG conclusions discussed and adopted by 2 scientific Panels (Contaminants, FCM) in January
- Opinion issued on 29 January 2010

Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be effected by exposure to bisphenol A*



NTP (2008). NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A, September 2008, NIH Publication No. 08 – 5994, 321p.

Selected studies:

- *in vivo*
- special attention to low doses
- mainly on behaviour and reproduction/reproductive tract
- no *in vitro* studies

according to the results at doses < 5 mg/kg bw/d
3 kind of studies

- **studies showing the absence of toxicity**
- **studies reporting effects not deemed relevant by the experts**
- **studies considered as "warning signals"**

Studies showing the absence of toxicity

Stump *et al.*, (2009), A dietary **developmental neurotoxicity** study of bisphenol in rats, WIL-186056, September 2009, 4796 p. *American Chemistry Council*
0.15, 1.5, 75, 750, 2250 ppm/d
➔ auditory startle, motor activity, learning and memory using the Biel water maze, brain and nervous system neuropathology, brain morphometry
NOAEL : 75 ppm (eq 5.85 mg/kg bw/d) based on ↘ maternal and offspring bw

Ryan *et al.* (2010), *In utero* and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter **sexually dimorphic behavior, puberty, fertility and anatomy** of **female** LE rats. *Toxicol. Sci.* 114: 133-48.

Howdeshell *et al.* (2008), Gestational and lactational exposure to ethinyl estradiol, but not bisphenol a, decreases androgen-dependent **reproductive organ weights and epididymal sperm abundance** in the **male** Long Evans Hooded rat. *Toxicol. Sci.* 102: 371–382.
2, 20 or 200 µg/kg bw/d, + control: ethinyl estradiol
➔ in ♀ : no effect on anogenital distance, pup bw, age at vaginal opening, F1 fertility and F2 litter sizes, malformations, saccharin preference in ♀ and lordosis behaviour
➔ in ♂ : no effect on on anogenital distance, pup bw, androgen-dependent tissue weights, epididymal sperm counts

Studies reporting effects not deemed relevant by the experts

Bosquiazzo *et al.* (2009), Effects of neonatal exposure to bisphenol a on steroid regulation of vascular endothelial growth factor expression and endothelial cell proliferation in the adult rat uterus. *Biol. Reprod.* 82: 86-95.

Braun *et al.* (2009), Prenatal bisphenol a exposure and early childhood behavior. *Environ. Health Perspect*. 117: 1945-1952.

Fernández *et al.* (2009), Neonatal exposure to bisphenol a alters reproductive parameters and gonadotropin releasing hormone signaling in female rats. *Environ. Health Perspect.* 117: 757-762.

Li *et al.* (2010), Occupational exposure to bisphenol-A (BPA) and the risk of Self-Reported Male Sexual Dysfunction. *Human Reprod.* 25:519-27.

Monje *et al.* (2009), Neonatal exposure to bisphenol A alters estrogen-dependent mechanisms governing sexual behavior in the adult female rat. *Reprod. Toxicol.* 28: 435-442.

Sargis *et al.* (2009), Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. *Obesity* in press, doi:10.1038/oby.2009.419.

Somm *et al.* (2009), Perinatal exposure to bisphenol a alters early adipogenesis in the rat. *Environ. Health Perspect*. 117:1549-1555.

"subtle effects" on behaviour

- In monkeys (Nakagami *et al.*, 2009)

  3/14 behaviours: *clinging, outward looking, social exploration*

- In mice (Palanza *et al.*, 2008)

  ↘ of time dams spent nursing and in the nest, ↗ of time dams resting alone
  but no effect on weaning weight = adequate level of maternal care

  ↘ or elimination of the sex difference in behaviour of offspring (curiosity, anxiety).

➔ **Significance of these effects in terms of human health ??**

Nakagami *et al.* (2009), Alterations in male infant behaviors towards its mother by prenatal exposure to bisphenol A in cynomolgus monkeys (*Macaca fascicularis*) during early suckling period. *Psychoneuroendocrinology* 34: 1189-1197.
Palanza *et al.* (2008), Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ. Res. 108: 150–157.

"subtle effects" on reproductive tract

- In rats (Salian *et al.*, 2009a,b)
  - changes in expression of steroid receptor coregulators and Sertoli cell junctional proteins in the testis
  - effects on 3 generations (only F1 exposed *in utero* and suckling)

➔ **Significance of these effects in terms of human health ??**

Salian *et al.* (2009a), Impairment in protein expression profile of testicular steroid receptor coregulators in male offspring perinatally exposed to bisphenol A. *Life Sci.* 85: 11-18.
Salian *et al.* (2009b), Perinatal exposure of rats to Bisphenol A affects the fertility of male offspring. Life Sci. 85: 742-752.

"subtle effects" on intestine

- In rats (Braniste *et al.*, 2010)
  - in OVX rat:
    - ↘ basal colonic paracellular permeability ,
    - ↗ in epithelial tight junction sealing,
    - ↘ severity of colitis,
    - ↗ pain sensitivity to colorectal stimuli
  - following *in utero* exposure:
    - ↘ CPP in adulthood,
    - ↗ of proinflammatory response of colonic mucosa

→ **Significance of these effects in terms of human health ??**

Braniste *et al.* (2010), Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats. Proc. Natl. Acad. Sci. USA 107: 448-453.

adverse effects on reprotoxicity/reproductive tract

- In rats
  - oral (Salian *et al.*, 2009b):
    ↗ post implantation loss, ↘ litter size, sperm count & motility in F1 ♂ offspring
  - subcutaneous (Salian *et al.*, 2009c):
    ↗ post-implantation loss, ↘ in litter size, changes in sperm count along with hormonal imbalances
- In rat (Murray *et al.*, 2007):
  development of ductal hyperplasias and carcinomas of the mammary gland
- In mice (Newbold *et al.*, 2009):
  ↗ ovarian cysts, ↗ progressive proliferative lesions of the oviduct
  ↗ tumor incidence of reproductive tissues

Salian *et al.* (2009c), Neonatal exposure of male rats to Bisphenol A impairs fertility and expression of Sertoli cell junctional proteins in the testis. Toxicology 265: 56-67.
Newbold *et al.* (2009), Prenatal exposure to bisphenol A at environmentally relevant doses adversely affects the murine female reproductive tract later in life. Environ. Health Perspect. 117: 879-885.
Murray *et al.* (2007), Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod. Toxicol. 23: 383-390.

but all these studies have major flaws

- route of administration: not oral
  - subcutaneous (Nakagami *et al.*, 2009, Murray *et al.*, 2007; Newbold *et al.*, 2009; Salian *et al.*, 2009c)
- only 1 dose tested
  - 10 µg/kg bw/d (Palanza *et al.*, 2008, Nakagami *et al.*, 2009)
  - 5 mg kg/bw/d (Braniste *et al.*, 2010 for perinatal exposure)
- ovariectomized rat (Braniste *et al.*, 2010)
- no positive control (all studies, except Salian *et al.*, 2009b,c; Braniste *et al.*, 2010)
- no data on actual exposure of the offspring (for all oral studies)
- diet: presence of phytoestrogens (soy-based, Palanza *et al.*, 2008)
  or in-housed prepared (Salian *et al.*, 2009a,b,c)
  or no data on the level of EDC in feed (Nakagami *et al.*, 2009)
- no data on the level of EDC bedding (Braniste *et al.*, 2010)
  corn cob bedding (Palanza *et al.*, 2008), puddy husk (Salian *et al.*, 2009a,b,c)
  hardwood chip (Newbold et al., 2009)
- polycarbonate water bottles (Newbold *et al.*, 2009)

What is our conclusion?

- The methodology of the studies do not enable a formal interpretation to be made that could call into question the previous assessments of BPA.
- **"Subtle" effects**, on behaviour, reproductive tract and intestine following perinatal exposure have been observed.
- The significance of these **"warning signals"** for human health has not been established.
- Consequently, it was not possible to establish a NOAEL/LOAEL on which to set a TDI.

Perspectives and issues for the present meeting

Regarding toxicity studies

Toxicity studies performed in compliance with international standards have not demonstrated any adverse effect below 5 mg/kg bw/d.

➔ But the issue is: are these standards suitable for characterising the toxicity of endocrine disrupting coumpounds (EDCs)?

Regarding the risk assessment approach

EDCs can have different effects depending on the development stage (critical **exposure windows** during which adverse effects can appear, especially the perinatal period)

➔ the TDI does not seem to be the most suitable approach for risk assessment.

The TDI/ADI is the maximum quantity of a compound that can be consumed daily over an entire lifetime without the risk of harmful effects on human health.

Indeed, risk assessment of EDCs is especially complicated due to:

- the nature of effects caused by compounds interacting with the endocrine system

  → the significance of the **"subtle" effects** in terms of human health has to be established: sex difference in behaviour of offspring (curiosity, anxiety, social exploration, clinging, outward looking), time dams spent nursing, changes in expression of coregulators or proteins in testis, intestinal permeability/pain sensitivity…
- suggested non-monotonic dose-response relationship
- potential lack of a threshold for effect
- effects at very low doses
- exposure window: adverse effects following exposure on immature systems
- potential delayed effects

→ **AFSSA recommends** to rapidly develop a methodology to assess health risk associated with very low doses of EDCs such as BPA.

AFSSA recommends:

- studies designed to establish toxicological reference values should include:
  - oral exposure
  - several doses including low doses (dose-response relationship)
  - negative and positive controls
  - internal exposure measurements (*eg* plasma and/or urine concentrations)
  - levels of hormones and their metabolite(s) in blood and/or urine
  - effects on physiological functions identified as critical, depending on the development stage at the time of exposure (consistency, reproductibility)
  - consideration of methodological bias, such as diet (limited level of phytoestrogens), nature of plastics drinking bottles, drinking water, bedding

Margin of Exposure (MOE): a tool for prioritizing EDCs

- margins between LOAEL in animal/human studies and estimated human exposure, on the basis of dietary intake or biomonitoring data
  - avoids the need to determine a safety factor *a priori*.
  - for different population groups (pregnant women, infant, young children, adults)
  - possibility to take into account the particular sensitivity at certain stages of life (different LOAELs)

Thank you for your attention