Session 4 Exposure Assessment / Risk Assessment

Recent Evidence for Low Dose Effects of Bisphenol A

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Bisphenol A is a monomer used in the manufacture of plastic and resins, and it has been known for decades that it mimics the activity of the hormone estradiol. Until recently, bisphenol A had been considered to be a very weak environmental estrogen, since in some bioassays (for example, the uterus or breast cancer cells) it is 10,000-100,000 - fold less potent than estradiol. These assays focused on binding of estradiol to estrogen receptors (ER α and ER β) located in the cell nucleus. Effects of compounds that occur as a result of binding to nuclear receptors depend on recruitment of co-factors that are specific to each cell type. These co-factors can dramatically influence the dose of an estrogenic chemical required to stimulate responses in the cell, as well as the type of responses that occur. It is now clear that the dose required for a hormone mimicking chemical to elicit effects in one type of cell cannot be used to predict the dose of the chemical that will elicit responses in other types of cells. For example, a number of studies have demonstrated that administering pregnant mice a daily dose of bisphenol A in the low part per billion (ppb) range produced permanent changes in reproductive organs in male offspring. In contrast, effects of bisphenol A in the uterus of developing female mice and rats only occur at doses dramatically higher than those required to alter reproductive organ function in males. Chemicals that exert different effects by interacting in unique ways with estrogen receptors in different cell types (and thus have a different effect on recruitment of co-factors) are referred to as " Selective Estrogen Receptor Modulators or SERMs". Bisphenol A has been shown to interact with nuclear estrogen receptors in a manner different from estradiol; thus, bisphenol A is a SERM.

There is now also extensive evidence that some effects of estradiol occur through the activation of nongenomic cell signaling systems as a result of binding of estradiol to receptors associated with the cell membrane. A characteristic of transmembrane cell signaling systems is a very rapid response combined with a high level of amplification, such that a very low concentration of a compound can activate large changes in cell function. Another characteristic of transmembrane cell signaling systems is a dramatic decrease in response as the dose of a ligand exceeds a " physiological" range for receptor activation, resulting in an inverted-U (biphasic) dose-response curve. Recent studies have shown that bisphenol A can act via cell membrane receptors to activate rapid cell signaling pathways at very low concentrations (1 nM or 228 parts per trillion, ppt). Bisphenol A has also been reported to produce an inverted-U dose-response curve for stimulation of proliferation of human prostate cancer cells, with maximum stimulation occurring at 0.23 ng/ml (1 nM) and no stimulation occurring at a 100-fold higher dose of 23 ng/ml (100 nM).

Over the last two years, there have been a large number of other studies that have reported effects of bisphenol A in the " low dose" range, below the previously reported no effect level, including effects in mollusks in the ppt range. The focus of current research is on determining which effects of bisphenol A are mediated by the nuclear receptors ER α and ER β , and which effects are mediated by activation of transmembrane cell signaling systems.

Determining the mechanisms responsible for effects of chemicals that are only detected in a narrow low-dose range should be a priority for regulatory agencies. This recommendation is based on the fact that published scientific findings do not support the current risk assessment model regarding the use of only very high doses in toxicological studies. The current model predicts that by testing only very high doses of chemicals and using linear extrapolation (specifically, the use of safety factors), valid estimates of human risk can be determined for effects at much lower doses that are in the range of human exposure. For chemicals such as bisphenol A that can produce an inverted-U dose response curve, this current testing method can result in the false conclusion that low doses of the chemical, within the range of human exposure, are safe. Only by directly testing a predicted " safe" dose can the possibility of adverse health effects be determined.

Newly Arising Endocrine Disruptors: UV Filters in Cosmetics

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The lipophilic character of UV filters used in sunscreens and cosmetics allows bioaccumulation of these persistent compounds in the environment. Contamination of fish and human milk (1, 2) demonstrates their presence in human tissues and wild life.

Identification of endocrine activity We examined 7 frequently used UV filters for their endocrine activities. Six of the chemicals were found to be estrogenic on MCF-7 breast cancer cells: homosalate (HMS), octyl-dimethyl-p-aminobenzoic acid (OD-PABA), benzophenone-3 (Bp-3), octylmethoxy cinnamate (OMC), 4-methylbenzylidene camphor (4-MBC) and 3-benzylidene campher (3-BC) (3). *in vivo* estrogenicity was demonstrated for orally administered 4-MBC, 3-BC, OMC and Bp-3 by the uterotrophic assay on immature LE (Long Evans) rats. Dermal application of 4-MBC and OMC in olive oil to immature hairless rats (hr/hr) also increased uterine weight at concentrations used in sun screen products. In addition, antiandrogenic potency of Bp-3 and HMS was demonstrated in MDA-MB-453-KB2 cells, a human breast cancer cell line expressing both androgen receptor (AR) and glucocorticoid receptor (GR), transfected with the MMTV luciferase gene construct.

Risk Assessment: Developmental Toxicity of 4-MBC The potential sensitivity of developmental processes to endocrine active chemicals prompted us to study the developmental toxicity of 4-MBC in LE rats at 4 different doses: 1.0g, 0.66g, 0.33g and 0.1g per kg chow yielding daily doses of 70mg/kg, 47mg/kg, 24mg/kg and 7mg/kg body weight. Following prolonged exposure (12 months), adult animals of the parent generation did not exhibit signs of adverse effects. In contrast, the F1 offspring showed a marked, dose-dependent reduction of survival rate during the first 14 postnatal days (significant at 24 mg/kg/day and above) (4). Offspring body andthymus weights were dose-dependently reduced at postnatal day (PN) 1. Male puberty was delayed in a dose dependent manner. Testis weight was found to be reduced in PN 14 pups, but increased in adult offspring. The proportion of sperm with normal morphology was decreased at increasing doses of 4-MBC. Additional organ weight changes were seen in epididymis, seminal vesicles and ventral prostate.

Analyses of **mRNA expression of estrogen target genes** by Real Time PCR with Cyclophilin as reference gene, reveal significant changes of steady state levels in 4-MBC exposed offspring for IGF-1 mRNA in uterus (from 7mg/kg/day) and ventral prostate, progesterone receptor (PR) mRNA in uterus, and androgen receptor (AR) in uterus and ventral prostate. Marked sex- and region- dependet changes are seen in PR mRNA and ER alpha mRNA levels in ventromedial hypothalamus region and medial preoptic region (from 7mg/kg/day 4-MBC), with a loss in sex different pattern in the ventromedial hypothalamic region. The seven tested UV filters represent only a small fraction of the 30 UV screens admitted for use, indicating an evident need for a more detailed toxicological evaluation of high volume cosmetics.

Reference

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Endocrine Disruption - the Trouble with Mixtures

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It has been argued that risks arising from exposure to individual estrogenic chemicals, so-called xenoestrogens, are negligible because their potency is rather low. Another argument used to dismiss concerns of health effects is that endogenous steroidal estrogens are too potent for xenoestrogens to contribute significantly to estrogenic effects. We have tested these ideas experimentally by assessing the ability of combinations of xenoestrogens to modulate the actions of 17 β-estradiol. Great care was taken to ensure that each xenoestrogen was present at a level well below its no-observed effect concentration (NOEC). Next, concentration-response relationships for each xenoestrogen and 17 β-estradiol were recorded. These data were used to predict entire concentration-response curves of mixtures of xenoestrogens with 17 β-estradiol, assuming additive combination effects. It was found that the experimentally observed responses were in excellent agreement with the model predictions. This suggest that xenoestrogens and 17 β-estradiol act together in an additive manner. However, the joint additive effect of the xenoestrogens led to a dramatic enhancement of the hormone ' s action, even when each single agent was present below its NOEC. Our results show that not even sub-NOEC levels of xenoestrogens can be considered to be without effect on potent steroidal estrogens, when they act in concert with a large number of similarly acting chemicals. It remains to be seen to what degree these effects can be neutralised by environmental chemicals with anti-estrogenic activity.

Environmental Endocrine Disrupters Assessment: Strategy of the European Commission

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In December 1999, a first Community Strategy for Endocrine Disrupters was published in which the European Commission stated its intention to direct efforts on this issue. This strategy identified four key elements: the need for further research; international cooperation; hazard and risk communication to the public and appropriate policy actions. On this basis, an appropriate set of actions has been recommended.

One of the first key short-term actions identified in the Commission publication was the establishment of a priority list of substances that needed further scientific, in depth, evaluation of their role in endocrine disruption ("priority" in the context of this work refers to making the best use of available resources in the process of further evaluation of all candidate substances). In order to establish this First for this priority-setting exercise, a review of evidence of endocrine disrupting effects and human/wildlife exposure was performed which was then submitted for consultation with stakeholders and the Commission Scientific Committees

The first phase of the strategy resulted in a study report entitled ' Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption'. The report identified a candidate list of 553 substances, from which evidence of endocrine disruption or potential endocrine disruption was found for 118 substances. An analysis of the legal status of these 118 substances revealed that 109 were already subject to bans or restrictions or were being addressed under existing Community legislation, although it should be noted that such reasons were not necessarily related to endocrine disruption mechanisms.

In a further phase, two studies were launched simultaneously. The first concerned an in-depth evaluation of the 9 candidate substances with evidence of endocrine disruption or potential endocrine disruption which were neither restricted nor being addressed under existing Community legislation. This study includes the identification of specific cases of consumer or ecosystem exposure that might warrant special consideration in the short-term. In addition, an analysis of 3 synthetic/natural hormones present in the environment is prepared.

The objectives of the second study were to focus on the gathering of data/information on persistence, production volumes and legal status on another 435 candidate substances for which a crucial lack of data was identified in the first study. It should however be noted that the 109 substances already regulated or being addressed under existing Community legislation are not excluded from the initial candidate list and might become future candidates for definitive testing when agreement will be reached regarding the testing methodologies for endocrine disruption.

Results gained by these two approaches will give robust and strategic information that will be used by the Commission, -in consultation with the Member States and other stakeholders - to provide input to policy discussions at the European level.

The approach taken in Existing Community legislation on environmental and human health aspects of chemicals is based on a tier approach. Stage 1: the hazard identification concerns the **identification** of the potential and/or intrinsic properties of a substance to cause adverse effects on human health and the environment. This process is mainly covered by the Directive 67/548/EEC relating to classification, packaging and labeling of dangerous substances. The second stage consists of a **risk assessment**, which comprises of not only the hazard assessment but also the exposure assessment to the chemical substance. Different legislative instruments have addressed this step, i.e. the Directive 91/414 or the Regulation (EEC) No. 793/93. The third and final stage is the **risk management**, in which risk reduction strategies are developed and comprises of three broad categories of instruments: product oriented (Directive 76/769/EEC), process oriented (Directive 96/61/EC) and media oriented instruments (Directive 92/72/EEC).