Session 6 Causal Criteria for Assessing Endocrine Disrupters

Opening Session

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The purpose of this session is to provide a forum to discuss the utility of the causality criteria presented in Chapter 7 of the WHO/IPCS Global Assessment of the State-of-the-Science of Endocrine Disruptors (2002), relative to determining whether a particular biological outcome is being influenced by environmental factors, and the extent to which an endocrine-disruptive mode of action is likely to be responsible for the outcome.

Participants should be familiar with the overall logic and case studies (http://ehp.niehs.nih.gov/who/) presented in Chapter 7, and be prepared to provide the audience with an in depth examination of the application of the criteria to specific case studies.

There will be two case studies for human health outcomes by Lizbeth Lopez-Carillo and Gerhard Winneke, and two case studies for wildlife outcomes by Masatoshi Morita and Glen van der Kraak.

Discussion panels will provide additional comments on the applicability and utility of the criteria for the human health and wildlife outcomes, respectively.

Causal Criteria for Assessing Endocrine Disruptors: Overview

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Given the complex, interactive nature of the endocrine system, it is extremely unlikely that any single set of studies or research strategy can provide definitive answers. Studies on EDCs need to evaluate all the relevant scientific information. Therefore, the WHO/IPCS Global Assessment of the State-of-the-Science of Endocrine Disruptors introduced a weight-of-evidence framework utilizing objective criteria to 1) evaluate causality between exposure to specific EDCs and particular health outcomes and 2) to determine whether these associations involved endocrine-mediated mechanisms of action. The five causal criteria used to evaluate the database included: temporality, strength of the association, consistency of the observation, biological plausibility, and evidence of recovery. The strength and weaknesses of this framework and causal criteria will be summarized.

Dichlorodiphenyldichloroethane Burden and Breast Cancer Risk: a Meta-Analysis of the Epidemiologic Evidence

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The exposure to DDT and breast cancer (BC) risk received increasing attention since the beginning of the 90's. Contradicting results are published regarding the relationship between body burden levels of p,p'-DDE -the main DDT metabolite- and BC, we argue that such differences stem from methodological differences crosswise those studies.

A meta-analysis of 22 articles was performed using the DerSimonian and Laird method for random effects models. The Q-statistic was used to identify heterogeneity in the outcome variable across studies. The gradient of p,p'-DDE exposure in epidemiological studies was homogenized to serum lipid bases (ng/g). The potential for publication bias was examined by means of the Begg's test. Methodological features of the studies are discussed as an attempt to reconcile the findings.

The summary odds ratio for selected studies was 0.97 (95% CI, 0.87 - 1.09) and the gradient of exposure ranged from 84.37 to 12,948 ng/g. No overall heterogeneity in the OR was observed ($\chi 2=27.93$; df=23; p=0.218). Neither the study design, nor the lack for breastfeeding control or the type of biological specimen used to measure p,p'-DDE levels, were causes of heterogeneity throughout the studies. Evidence for publication bias was not found (p=0.253).

Overall these results should be regarded as a strong evidence to discard the putative relationship between p,p'-DDE and BC risk. Nevertheless, the exposure to DDT during critical periods of human development, from conception to adolescence, and individual variations in metabolizing enzymes of DDT or its derivatives are still questions to be researched in regards to BC development in adulthood.

Pah-Related Neurodevelopmental Deficit and Endocrine Disruption: What is the Evidence in Terms of Causality?

Gerhard Winneke

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Developmental neurotoxicology deals with adverse chemical-induced alterations in the nervous system during development. Although developmental changes in the nervous system do take place all over the life span and would, thus, relate to both the developing as well as the ageing brain, it is the immature brain which has received particular attention as a possible target for chemical insult. This is so because insult to the developing brain is likely to result in longlasting or even permanent neurobehavioral alterations later in life.

Endocrine disruption during early ontogenesis is a case in point. It is well established that hormones, as e.g. thyroid hormones or sex steroids, play an organizational role in brain development, and that interference with their orchestrating the sequence of neurobiological events of brain maturation may give rise to neurodevelopmental disruption. Thyroid hormones can be used to illustrate the point. They have been shown to interfere with proliferation, differentiation and migration of neurons, and severe hypothyroid conditions during brain development, if untreated, are known to result in cretinism (Porterfield, 1994). At the same time, however, some PAHs - and polychlorinated biphenyls (PCBs) in particular - have been shown to give rise to hypothyroid effects by interfering with the release, metabolism or transport carrier function of thyroid hormones (Brouwer et al., 2001). It has, therefore been proposed that PCB-induced hypothyroid conditions might underly the PCB-related neurodevelopmental adversity observed in several studies worldwide (Tilson, 1998).

Apart from studies in children from poisoning episodes in Japan (Yusho) and Taiwan (Yu-Cheng), characterized by high accidental exposure to PCBs and other PAHs and subsequent polysymptomatic sequelae, including neurological and neurobehavioral deficit, such prospective cohort studies in newborns and young children exposed to moderate environmental PCB-levels have been or are being conducted throughout the world, namely in the US (Michigan, North Carolina, New York) and in Europe (Denmark, Germany, The Netherlands; see review by Schantz et al., 2003). Despite some discrepancies in terms of effect spectrum, effective PCB-matrix and effect persistence this set of studies rather consistently shows negative associations between pre-/neonatal PCB-levels and developmental adversity, mainly in terms of mental and motor development until at least 3½ years of age. However, there is little or no information about the mechanisms possibly underlying this link. The hypothyroid hypothesis, although biologically plausible and supported by animal experiments, has received little support in epidemiological studies, so far, because the few reported associations between elevated PCB-levels in children at birth and hypothyroid function are only partly consistent.

As for PAH/PCB-interactions with the sex steroid system during development and associated neurobehavioral outcome clearcut findings are available from animal experiments for both sexual and non-sexual endpoints (WHO, 2002). It has been shown, for example, that the activity of the enzyme aromatase (CYP 19), which converts androgens to estrogens, is reduced in the brain of neonatal rats following maternal exposure to a PCB-mixture reconstituted according to the congener-pattern in human milk, and that this is associated with behavioral feminization in the male offspring (Hany et al., 1999). It is, however, uncertain if aromatase has a role in sexual brain differentiation in humans, as well, and behavioral feminization following pre-/neonatal PAH-exposure has rarely been studied in human infants, so far. A recent but still isolated example is feminized play behavior and altered sex role-characteristics in boys with high pre-/neonatal PCB-exposure (Vreugdenhil et al., 2002).

It may, thus be concluded that the explanation of PAH-induced developmental neurotoxicity in terms of endocrine disruption, although biologically plausible, is not sufficiently consistent and coherent to pass the Bradford-Hill criteria of causal inference; more supporting evidence is needed.

Gerhard Winneke
Heinrich-Heine-Universität Düsseldorf, Germany

Questionnaire for Session 6 Presenters/Commentators

Title of Presentation:

"Pah-related neurodevelopmental deficit and endocrine disruption: What is the evidence in terms of causality"? (Target organ is the developing brain and associated mental/motor development)

Abstract has already been submitted

Observation Period
Recruitment period: 1993-1995
Observation Period: 1994-1999

Human cases

Newborns and young children up to age 6 years. Recruitment of 170 healthy mother/infant pairs from the obstetrical wards of three Düsseldorf hospitals. This is a normal population with background PCB-exposure. From the dose-response curves it may be estimated, that between 1993 and 1995 the upper 10-20% of all newborn children in the catchment area of the hospitals present with PCB-levels likely to be associated with delay of mental/motor development.

Geographical Point

City of D?sseldorf, a densely populated urban area with 600 000 inhabitants.

Possible EDC compound and evidence for involvement of the endocrine system

PCBs (congeners 138, 153, 180 as indicators) and related PAH-compounds. Interference of PAHs with the thyroid and the sex steroid system has been demonstrated experimentally. Whether or not such interference underlies human developmental delay or deficit is still uncertain, although there is a certain degree of biological plausibility.

Increase or decrease of the strength of evidence since the early studies

Apart from the studies based on the Yusho (Japan) and the Yu-Cheng (Taiwan) poisoning episodes with presumably very high levels of PAH-exposure, the first studies examining infants and children at more background levels of exposure were those from Michigan (Jacobson et al.) and from North Carolina (Rogan et al.). These early studies used the less developed analytical and epidemiological tools of their time. In this respect considerable improvement has taken place. The more recent studies conducted and published since then have, in my judgement, strengthened the evidence of PAHs (particularly PCBs) to exhibit developmental neurotoxicity in humans at elevated background levels of exposure, although the underlying mechanisms still need to be elaborated.

Relevance to humans

Does not apply here

Gaps in knowledge to be addressed in future research

The role of thyroid hormones and sex steroids as known regulators of brain development should be addressed in relation to human neuro-developmental studies in relation to the outcome of early PAH-exposure of mother and child. This could best be done by making use of archived tissue-banks of children now in puberty or adolescence.

Commentary on Dr. Winneke

Noriyuki Koibuchi

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As a neuroendocrinologist studying on thyroid hormone (TH) and brain development, I would like to make a specific comment on Dr. Winneke's presentation. Lipophilic hormones such as thyroid/steroid hormones play an important role in brain development. In particular, TH plays a crucial role. Perinatal deficiency of TH causes abnormal brain development known as cretinism in human. As exposure to PAH/PCBs during such period shows neuronal abnormalities partly similar to those seen in cretinism patient, a close interaction between TH system and PCB has been considered. In fact, in experimental animals, exposure to PCB reduces plasma T4 levels, which has been considered to induce abnormal brain development. However, as stated by Dr. Winneke's presentation, such interaction is not always evident in human studies. Even in animal model studies, the interpretation that should be more careful may be required. Although PCB exposure during perinatal period causes significant reduction of TH levels in neonates, their general growth is not greatly affected, indicating that they are generally euthyroid. Another study has shown that the tissue concentration of T3, a bioactive compound of TH, is within normal range in such animals. In addition, as several hydroxylated form of PCBs have high affinity to TH-carrier protein in plasma, PCBs have been considered to inhibit TH delivery to peripheral organ by competitively binding to such proteins. However, these carrier proteins do not play major role in TH action, and therefore, animal models and human cases, in which such protein genes are deleted, are generally euthyroid. Taken together, we consider that PCB may not act to thyroid gland to reduce TH secretion that causes abnormal brain development. We do not conclude, however, that PCB is ineffective on TH system. Recently, we have obtained an evidence that PCBs may act directly to TH receptor (TR) to inhibit ligand-induced transcriptional activation. The effect seems to be greater in neuronal cells. Our recent findings will be introduced in my commentary.

Wildlife Exposure to Endocrine Disrupting Chemicals

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National Institute for Environmental Studies

Endocrine disrupting chemicals (EDCs) are chemicals that produce adverse effects on human health and wildlife through interfering with internal information systems by mimicking hormones, or inhibiting/stimulating hormone metabolism, processes which result in functional and often irreversible physical changes. Although EDCs have long been a fundamental subject of chemical toxicology, it is only in recent years that society has become increasingly aware of an array of possible adverse effects, such as in the areas of reproduction, intellectual development, and immune systems.

Impact on wildlife has been reported in a variety of forms and animal species. Examples include the feminization of fish, the masculinization of marine snails, abnormal behavior in birds, deformities in frogs, reduced penis size in alligators, sex organ abnormalities in mammals, and so on. Wildlife surveys designed to investigate such phenomena, including exposure levels, have been conducted in recent years in Japan.

This paper will report on current observations of wildlife in relation to EDC exposure. It is not an easy task to establish a cause-effect relationship since this would require an extensive body of information including on the activity and concentration of suspected EDCs, on additive, synergistic and antagonistic effects, on exposure routes and duration in different animal species, species characteristics and levels of sensitivity, and more. However, a cause-effect relationship will be discussed for selected cases including imposex in marine snails and exposure to organotin compounds. Biological and chemical monitoring of wildlife, together with an understanding of the biology of different species of animals, is a prerequisite to identifying the substances responsible and assessing their impact.

Ecotoxicological Risk Assessment of Atrazine in Amphibians

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Atrazine is a widely used broadleaf herbicide that is generally applied to crops in the spring when amphibians are congregating and preparing for the breeding season. While atrazine is not acutely toxic at environmentally relevant concentrations, there is uncertainty as to whether chronic exposure to atrazine affects reproduction and development in amphibians. The purpose of this presentation is to provide an overview of available lab and field data applicable to the question of whether atrazine causes adverse effects in frogs through endocrine-mediated mechanisms. These data will be evaluated using the criteria for the cause and effect relationships described in the WHO/IPCS Global State of the Science of Endocrine Disruptors in order to address whether the outcome is being influenced by environmental factors and the extent to which an endocrine-disruptive mode of action is likely to be responsible for the outcome. The hypotheses tested were that atrazine causes adverse effects in amphibians through: 1) estrogen-mediated mechanisms, 2) androgen-mediated mechanisms, 3) thyroid-mediated mechanisms, 4) adverse effects on gonadal development or 5) adverse effects at the population level. Overall the biological plausibility of proposed mechanisms of endocrine disruption was not supported by laboratory or field observations. The data do not support evidence of effects through thyroid hormone mediated mechanisms and little evidence for mechanisms mediated through estrogens or androgens. No temporal correlation exists between occurrence of gonadal effects and the introduction and use of atrazine. Similarly the strength of association is not strong when considering the concentration-responses for endpoints such as gonadal development, incidence of intersexuality, or laryngeal growth. The incidence rate of gonadal abnormalities and other effects on populations was not related to atrazine exposures. Controlled studies in different laboratories produce inconsistent results and observations were also not consistent between lab and field studies. In summary, available data to date do not provide evidence to show that atrazine produces a consistent, reproducible effect on amphibian development. This example illustrates the utility of the cause and effect criteria described in the WHO/IPCS document in not only evaluating the relative strength of data but in setting direction for further studies to reduce the uncertainty in ecological risk assessments.