International Symposium on Environmental Endocrine Disrupters 2000

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低用量問題

Low Dose Issue in the ED-Reaction

On Low Dose Issues in the ED Reaction

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Three general areas of research support concern for the effect of endocrine disrupting chemicals on health: field studies in various contaminated ecosystems, status and trend studies of endocrine-related diseases, and laboratory studies of specific endocrine disrupting agents in test species. Each of these brings particular strengths and weaknesses, and each brings noticeable uncertainties in terms of quantitative risk assessment. In the former two areas, the primary limitations are usually associated with limited exposure assessment, so that when a disease is seen to be occurring in a particular place or time in a population, it has been difficult to establish a cause-and-effect relationship with a particular agent in the environment during the critical period of susceptibility. In the later case, that of controlled laboratory studies, this limitation is readily overcome by the inherent nature of the study design: specific agents are administered at specific times, and particular outcomes are observed after the exposure. Instead, one of the biggest uncertainties in laboratory studies pertain to understanding the magnitude of the risks to populations at dose levels below those normally utilized in toxicity tests. Questions such as do standardized tests incorporate the most sensitive endpoints, do mixed agents exhibit synergic and/or additive response, and are dose-response relationships linear or non-linear as the dose approaches the levels encountered in the environment. This session will review the outcome of a recent peer-review workshop on the matter of low dose effects, as well as explore in detail a few examples focused on effects of several specific EDCs on development and function of the reproductive tract. A final presentation will point to what impact these findings and discussions will have on the overall issue of endocrine disruption.

Summary of the NTP/NIEHS Endocrine Disruptors Low-Dose Peer Review

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To determine whether or not its guidelines for reproductive and developmental toxicity testing are adequate for endocrine active chemicals, the U.S. Environmental Protection Agency (EPA) asked the National Toxicology Program (NTP)/National Institute of Environmental Health Sciences (NIEHS) to organize and conduct an open scientific peer review to evaluate reported low-dose effects and dose-response relationships for endocrine disrupting chemicals. For this meeting, "low-dose effects" referred to biological changes that occur in the range of human exposures or at doses that are lower than those typically used in EPA's standard testing paradigm for evaluating reproductive and developmental toxicity. The Panel examined data from more than 50 selected studies that support either the presence or absence of low-dose effects of endocrine disruptors in laboratory animals. To provide an objective scientific perspective on this important environmental health issue, a unique approach was selected for this review. At the request of the organizng committee, principal investigators of research groups active in this field willingly provided their raw data on selected parameter values for independent statistical re-analysis by the Panel. In addition, investigators from these groups were available to give formal presentations of their findings and to have informal discussions with the Panel. Preliminary conclusions from this review include the following:

- Low-dose effects have been clearly demonstrated for some estrogenic compounds. In other studies, low-dose effects were not apparent. In most cases, positive and negative findings were considered to be credible and sound within the context of the experimental designs. Study design differences were identified among laboratories that might account for discrepancies in experimental outcomes. With regard to the observed low-dose responses, there is uncertainty about the overall and long-term health consequences of those effects (i.e., whether or not these effects represent risk factors for later developing diseases).
- Low-dose effects of androgenic compounds have not been studied. For antiandrogenic compounds, the dose-response curve for some endpoints appears linear to the lowest dose tested; however, available studies were not designed to evaluate low-dose effects as defined for this review.
- To clarify uncertainties and better characterize low-dose effects, the Panel recommended using pharmacological and genetic approaches to determine mechanisms of action and to characterize dose-response relationships, characterizing response longevity from gestation through adulthood, evaluating long-term health outcomes, and determining the impact of variations in endogenous hormone levels.
- Special consideration should be given to study design and biologic factors that might affect experimental outcomes. Animal models that are highly responsive to endocrine active compounds, particularly to positive controls, should be selected for study.
- The shape of the dose-response curve for endocrine disrupting agents might be non-monotonic (*e.g.*, inverted-U), linear, or threshold-appearing. Numerous factors influence the dose-response curve including the endpoint being evaluated, the chemical being studied, the dosing regime, and biological characteristics of the target tissue.

Three-Generation Reproductive Toxicity Study of Bisphenol A (BPA) Administered in the Diet to CD® (Sprague-Dawley) Rats

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BPA is a high production volume chemical used principally as a monomer in the manufacture of polycarbonate plastics and epoxy resins. It can produce some "estrogen-like" effects at high gavage and parenteral doses; initial studies (not replicated) have reported that it is also active at low doses. Therefore, BPA (>99.5% pure) was evaluated in a multigeneration reproductive toxicity study (under U.S. EPA OPPTS 1998 testing guidelines with additions and in compliance with EPA GLPs) at dietary concentrations of 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, available *ad libitum* to 30 animals/sex/dose for three generations, one litter/generation, through F3 adult offspring. Adult systemic toxicity was present at 750 ppm (approximately 50 mg BPA/kg/day) and 7500 ppm (500 mg/kg/day) for all adult generations, expressed as reduced body weights (BW) and BW gains during prebreed (both sexes) and gestation and lactation, reduced absolute and increased relative weanling and adult organ weights, and increased incidences of renal tubular degeneration and chronic hepatic inflammation only at 7500 ppm in F0, F1, and F2 (but not F3) females. Reproductive organ histopathology and function were not affected in either sex in any generation. Paired ovarian weights and total and live pups/litter on pnd 0 were decreased at 7500 ppm (in the absence of any increase in prenatal postimplantation loss) in the presence of substantial maternal toxicity in all generations. There were no treatment-related effects in any generation on parental or offspring mortality, clinical signs of toxicity, or on mating, fertility, or gestational indices, ovarian primordial follicle counts, estrous cyclicity, precoital interval, gestational length, offspring sex ratios or survival, on nipple/areolae retention in preweanling F1, F2, or F3 males, on adult F0, F1, F2, and F3 male epididymal sperm number, motility, or morphology, on adult F0, F1, F2, and F3 male testicular homogenization-resistant spermatid head counts, daily sperm production (DSP), or efficiency of DSP. At 7500 ppm, there were also delays in acquisition of puberty (vaginal patency and preputial separation) in F1, F2, and F3 offspring of both sexes, associated with reduced BW, which began during lactation (pnd 7) and continued through adulthood. Anogenital distance on pnd 0 was unaffected for F2 and F3 males and F3 females, and increased (by 0.03-0.05 mm) in F2 females at 0.015, 0.3, 4.5, and 750 ppm, in the absence of any effects on pnd 0 pup body weights, with no dose-response pattern, and no developmental or reproductive sequela. There were no consistent or persistent treatment-related effects of dietary BPA exposure on any adult or offspring parameters at 0.015, 0.3, 4.5, or 75 ppm (equivalent to approximately 0.001, 0.02, 0.3, or 5 mg BPA/kg/day) in rats in this study.

In conclusion:

- The adult systemic toxicity NOAEL for both sexes was 75 ppm (approximately 5 mg BPA/kg/day)
- The reproductive and postnatal toxicity NOAELs were 750 ppm (approximately 50 mg BPA/kg/day)
- There were no treatment-related low dose effects.

- There was no evidence of a non-monotonic dose response
- The absence of treatment-related low dose effects in this study are consistent with the findings from a two-generation reproduction study in rats recently completed by the Chemical Compound Safety Research Institute, Hokkaido, Japan, in which CD® rats, 25/sex/group, were gavaged daily with 0, 0.2, 2, 20, 0r 200 μg BPA/kg/day; there were no treatment-related effects of BPA on any parameters examined at any dose.

This work was sponsored by the Society of the Plastics Industry, Inc., Washington, DC.

In vivo Effects of Nonylphenols on Reproductive Development in Pre-Adolescent Rats: Dose Response Considerations.

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Nonylphenols (NPs) found in the environment has estrogen-like properties. We studied the *in vivo* effects of NPs on pre-adolescent rats. Single i.p. injection of NPs to immature female rats stimulated uterine growth and induced uterine peroxidase activity that mimic the effects of estradiol (E) but at a lower potency (1/1000 to 1/2000). The threshold dose is ～1 mg/animal. Using RT-PCR, increase in uterine ER (estrogen receptor)-mRNA was detected with NPs at 0.5 mg/animal. A dose-dependent increase in ER-mRNA up to 2 mg/animal was seen. At 4 mg/animal, ER-mRNA was down regulated. Gel-shift assays showed increases in complex formation of ER-ERE (estrogen response element) with uterine nuclear proteins from NPs or E treated rats suggesting NPs act similar to E. Male pups given NPs (i.p.) from ages 1-15 days decreased the size of their testis and male accessory organs when measured at 31 days of age. The threshold dose is between 0.8 & 8 mg/Kg. Long term follow-up showed decrease in fertility in males that had been exposed to NPs in their neonatal life.

Bisphenol A Alters Development in Mice at Human Exposure Levels

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Contrary to popular belief, recent research demonstrates that plastic products made from the monomer bisphenol A are unstable and release increasing amounts of free bisphenol A as the products are washed and reused. Human exposure to bisphenol A can thus occurs due to its use as the monomer in polycarbonate plastic, dental sealants, the interior resin lining of food and beverage cans, and in many other commercial applications. Interestingly, bisphenol A was synthesized for use as an estrogenic drug in the 1930s by Dodds, who subsequently synthesized another estrogenic drug, diethylstilbestrol (DES). Decades later, polymer chemists used bisphenol A to make plastics and resins.

The major public health concern is exposure to bisphenol A during development of the brain, reproductive organs, and other estrogen-responsive tissues. During fetal development genes are "imprinted", by estrogen, such that the activity of genes in tissues is determined by the amount of estrogen during "critical periods" in development.

The focus of my talk will be on experiments conducted with mice showing the consequences for fetuses of exposure via the mother to very low doses of bisphenol A that are within the range of exposure by humans. The implications of adverse effects due to fetal exposure to bisphenol A in mice for risk assessment will then be discussed.

We have found that bisphenol A is a potent estrogen in fetuses, although it is typically erroneously described as being a "weak" estrogen. There are a number of reasons for the high potency of bisphenol A in fetuses: 1. Bisphenol A accumulates in pregnant mice, but this does not occur in non-pregnant adult females. This bioaccumulation in pregnant females leads to much higher exposure of fetuses to bisphenol A than is predicted by studies of bisphenol A metabolism in non-pregnant adult females. 2. Estrogen binding proteins in blood that protect fetuses from natural estrogens do not block bisphenol A from entering fetal tissues. This leads to much higher levels of bisphenol A being available to bind to estrogen receptors and stimulate estrogenic responses in tissues than is predicted from *in vitro* screening tests that do not take this into account. 3. Fetuses are more vulnerable to bisphenol A and other estrogenic chemicals than are adults. Even very high doses of potent estrogenic drugs, such as DES, do not damage adult organs. In contrast, fetal exposure to extremely low doses of estrogenic chemicals, including bisphenol A, permanently alters the structure and functioning of the reproductive organs.

Research conducted on mice in my laboratory, independently confirmed by other scientists, has involved the use of doses of bisphenol A within the range of exposure of human fetuses. The dose of bisphenol A that we used is 25,000-times lower than the previously reported lowest observed adverse effect level (LOAEL) for bisphenol A. When the lowest dose tested causes adverse effects, this dose is termed the lowest observed adverse effect level. Exposure during fetal life to a dose of bisphenol A 25,000-times below the prior LOAEL increases the rate of postnatal growth and accelerates the rate of sexual maturation in female mice.

Fetal exposure to low, environmentally relevant doses of bisphenol A also alters the structure and functioning of the reproductive organs in mice and rats. For example, in my lab as well as other labs, research has shown that bisphenol A permanently increases the number and size of prostate glands and also increases prostatic androgen receptors that regulate prostate function. The increase in androgen receptors could lead to the prostate being hyper-responsive to testosterone throughout life, thus increasing the likelihood of prostate disease in old age; our findings

show that these effects persist at least through middle age. There are also dramatic changes in the structure of the urethra, which could have implications for abnormalities in the capacity to urinate later in life. Finally, bisphenol A decreases testicular sperm production.

The traditional method of toxicological testing led to the false assumption that the doses of bisphenol A which caused all of the above effects would not produce any effects. This risk assessment method, which is still in use today, typically involves testing three very high sub-lethal doses of a chemical for effects over a relatively narrow dose range. The lowest dose of bisphenol A that was tested in toxicological studies conducted in the 1980s caused adverse effects, and a LOAEL was reported by the US-National Toxicology Program. However, the assumption was (and still is) that if the lowest dose tested (LOAEL) was divided by a safety factor of 1,000, this calculated dose could be declared "safe". Importantly, this calculated "safe" dose was never directly tested prior to our studies to determine whether it was, in fact, "safe"! Research now shows that when the calculated "safe" dose of bisphenol A is actually tested for effects, this dose is found to cause adverse effects.

Over a dozen research reports from the USA, Japan and Germany now demonstrate that the current method of risk assessment provided a false estimate of the "safe" daily intake dose of bisphenol A for humans. Current research findings would result in the calculation of a "safe" dose far below current human exposure to bisphenol A. It is thus likely that other endocrine disrupting chemicals will also be found to produce adverse effects at doses below the established "safe" daily intake level. I propose that estimated "safe" doses based on testing of high doses of endocrine disrupting chemicals should be directly tested to determine whether, in fact, they really are "safe". In addition, future testing procedures for endocrine disrupting chemicals should include doses within the range of human exposure.

Two-Generation Reproduction Study of Bisphenol A in Rats

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This study was conducted to determine the effects of low dose of bisphenol A (BPA) in a rat two-generation reproduction study, which included reproductive and developmental endpoints, such as sexual development, estrous cyclicity, anogenital distance (AGD), physical and functional development, and sperm counts and motility. Groups of 25 male and 25 female Crj: CD (SD) IGS rats were given BPA at 0.2, 2, 20, and 200 μg/kg by gastric intubation throughout the study beginning at the onset of the 10- and 2-week premating period, in F0 males and F0 females, respectively, and continuing through the mating, gestation, and lactation periods, for two generations. There were adult (F0, F1, F2) and postnatal day (PND) 22 (F1, F2) necropsies; the oldest F2 males and females were killed in postnatal weeks 7 and 14, respectively. No compound-related clinical signs or effects on body weight or food consumption were observed in any generation.

There were no compound-related changes in surface righting reflex, negative geotaxis reflex, mid-air righting reflex, pinna detachment, incisor eruption, eye opening, testes descent, preputial separation, or vaginal opening in F1 or F2 generation, or behavior in the open field or water filled multiple T-maze in F1 generation. No test compound-related changes in estrous cyclicity, copulation or fertility index, number of implantations, gestation length, litter size, pup weight, pup sex ratio, pup viability, or other functional reproductive measures were noted in any generation. A significant decrease in the AGD was found at 0.2, 20, and 200 μg/kg in F1 males and at 20 and 200 μg/kg in F1 females and F2 males and females, and a significant increase in the AGD was observed at 2 and 20 μg/kg in F1 females. However, these changes were consistently small (within 5% of control values) and, no continuous changes in the AGD or AGD/cube root of body weight ratio were found. There were no compound-related changes in epididymal sperm counts or motility in F0 or F1 males. No compound-related necropsy findings or effects on organ weight including the reproductive organs were shown in any generation. Histopathologic examinations revealed no evidence of compound-related changes in the reproductive organs of both sexes. These data indicate that oral dose of BPA between 0.2 and 200 μg/kg over two generations did not cause significantly compound-related changes in reproductive or developmental parameters in rats.