# Workshop on Perinatal Exposure to Dioxin-like Compounds. II. Reproductive Effects

Brenda Eskenazi[1] and Gary Kimmel[2]

[1]School of Public Health, University of California, Berkeley, California; [2]U.S. Environmental Protection Agency, Washington, DC

This summary report focuses on current studies on reproductive effects reported at the workshop on Perinatal Exposure to Dioxin-like Compounds and supporting data noted in the discussion. Recent laboratory studies have suggested that altered development (e.g., low birth weight, spontaneous abortion, congenital malformation) and reproductive health (e.g., fertility, sex organ development, reproductive behavior) may be among the most sensitive end points when examining the effects of dioxinlike compounds. Thus, future research should target the reproductive health of both males and females exposed postnatally and prenatally. Studies in humans are needed and are on-going. In animal models, postnatal exposure to dioxin or dioxinlike compounds has been associated with abnormal spermatogenesis and abnormal testicular morphology and size in males and with reduced fertility and endometriosis in females. *In utero* exposure may also produce profound reproductive consequences in both males and females including delays in sexual maturation, abnormalities in development of sexual organs, and abnormal sexual behavior. The mechanism by which dioxin-like compounds cause reproductive effects is not well delineated. — Environ Health Perspect 103(Suppl 2):143–145 (1995)

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## Introduction

The effects of high level exposure to dioxin (TCDD) and TCDD-like compounds on reproductive and developmental end points have been recognized for many years. Recent laboratory studies have further suggested that altered development and reproductive function may be among the most sensitive end points. Members of the "Workshop on Perinatal Exposure to Dioxin-like Compounds" discussed the scientific implications of published, on-going, and planned studies in humans and in animal models. This summary report focuses on identifying the future research needs from those studies that were reported at the workshop and supporting data noted during the discussion. For a more complete review of the literature on TCDD's potential reproductive and developmental toxicity (*1*,*2*).

This paper was presented at the Workshop on Perinatal Exposure to Dioxin-like Compounds held 13–15 June 1993 in Berkeley, California.

The Reproductive Effects Working Group: Pierre Ayotte, Laval University Hospital Center, Quebec, Canada; Germaine Buck, Department of Social and Preventive Medicine, SUNY, Buffalo, NY; Theo Colborn, World Wildlife Fund, Washington; John Cicmanec, U.S. Environmental Protection Agency, Cincinnati, OH; L. Earl Gray, Jr., U.S. Environmental Protection Agency, Research Triangle Park, NC; Paolo Mocarelli, University Department of Clinical Pathology, Hospital of Desio, Milan, Italy; Anne Sweeney, University of Texas-Houston, School of Public Health, Houston, TX.

Address correspondence to Dr. Brenda Eskenazi, University of California School of Public Health, 312 Earl Warren Hall, Berkeley, CA 94720. Telephone (510) 642-3496, 642-9544. Fax (510) 642-5815.

## Identification of Research Needs

Future research on the effects of TCDD and TCDD-like compounds on reproduction and development should target reproductive function and behavior and associated endocrinologic effects, and should be carried out in both males and females. Characteristics of exposure including the timing, route, and duration should be relevant to the human population. The workshop participants provided a number of examples of the range of research in the area, including both laboratory animal and human studies, and timing of exposure ranging from prenatal to the adult.

### Animal Postnatal Effects

In male animals exposed postnatally, TCDD and TCDD-like compounds alter testis and accessory gland weight, testicular morphology, spermatogenesis and fertility (*1*). Given the effects of these compounds on endocrine function, it is possible that subtle effects on males could also be observed at lower postnatal exposure levels; however, these lower dose effects on male reproduction have not been adequately studied.

Similarly, females exposed postnatally to TCDD and TCDD-like compounds have not been well studied. The primary reproductive end points among female animals that have been examined include decreased fertility and an inability to maintain pregnancy. At the workshop, Cicmanec et al. reported reduced fertility of female macaques exposed to 100 μg/kg of Aroclor 1254 for 6 months when mated with unexposed males (one live birth for five of eight females surviving the dosing period. By comparison, males receiving this same dose did not show impaired reproductive performance when bred to untreated females.) Decreased fertility may be related in part to an increase in endometriosis—a finding observed in a few recent studies (*3–5*). These reports in macaques have demonstrated that postpubescent females exposed to polychlorinated biphenyls (PCBs) (Aroclor 1254) (*4*) and to TCDD (*3*) develop endometriosis. In the study of TCDD exposure (*3*), the incidence and severity of endometriosis are dose-related.

### Animal Prenatal Effects

Developmental toxicity end points have been observed at lower exposure levels than are end points of adult male and female reproductive toxicity (*1*). This is consistent with findings that, in general, the unborn animal appears to be more sensitive than the adult to TCDD and TCDD-like PCB and polychlorinated dibenzofurans (PCDF) congeners. For example, fish, birds, and mammals exposed to a range of organochlorines that include TCDDs and PCBs exhibit precocial development and never reach full sexual maturity (*1*,*6*). Given that postpubescent female monkeys exposed to dioxin-like compounds develop endometriosis, it is also plausible that females exposed *in utero* may be at increased risk and should be further investigated.

At the workshop, Cicmanec and colleagues reported that macaque females

exposed to 25 μg/kg of Aroclor 1254 for 6 months prior to conception and then for the 5.5 months during gestation had offspring that showed a 15% reduction in birthweight compared to controls and a 22% reduction in body weight at 2 months. Most exposed at 25 and 100 μg/kg had abnormal finger- and toenails. The offspring exhibited more severe clinical hematologic changes than adults, and renal and pancreatic changes were seen at postmortem examination in infants but not in adults. These effects are similar to those observed in the children exposed *in utero* in the Yusho and Yu-Cheng incidents (*7–9*). In these cases, pregnant women were among those who accidently consumed rice oil contaminated with a complex mixture of TCDD-like congeners. The offspring of these women were shown to have reduced birthweights and significant clustering of effects in organs derived from ectoderm, including skin, nails, and meibomian glands.

Future research should specifically investigate the reproductive health of offspring exposed *in utero*. Mably et al. (*10*) reported that TCDD exposure on the 15th day of gestation at doses ≤1 μg demasculinized Holzman male rats. That is, male offspring had reduced anogenital distance and number of testicular and caudal epididymal sperm, and smaller androgen-dependent sex accessory glands. They also showed abnormalities in mounting behavior. At the workshop, Gray et al. (*11*) reported on their replication of these findings in male LE hooded rats exposed *in utero* and via lactation to a maternal dose of 1 μg TCDD/kg on days 8 and 15 of gestation. In addition to the findings reported by Mably et al. (*10*), the rats of Gray et al. (*11*) showed a 60% reduction in ejaculation sperm counts and delays in puberty. Gray et al. (*11*) also reported adverse reproductive effects in female offspring exposed following *in utero* and lactational exposure to TCDD. These rats had severe clefting of the clitoris and incomplete to absent vaginal openings, although estrous cyclicity was not significantly affected. Gray et al. have suggested that TCDD produced "estrogen-like" malformations in gestationally exposed female rats.

## Studies of Humans

Based on the above animal evidence, adults exposed to TCDD and TCDD-like compounds may show reproductive effects such as decreased sperm count in males and reduced fertility, endometriosis, and poor pregnancy outcomes in females. Children exposed *in utero* may show delayed maturation, e.g., delayed onset of menses or puberty, delayed development of secondary sexual characteristics, or growth retardation. Upon sexual maturation, they may show reduced fertility or other endocrinologic effects, e.g., longer time to conception, abnormal cyclicity, alterations in semen quality including sperm count, or premature menopause. Little is known about these reproductive and developmental end points in humans.

Ideally, prospective studies of exposed populations should be conducted to determine the adverse reproductive effects of dioxin-like compounds in human populations. Key to these studies are adequate and reliable exposure measurements. Prospective studies would reduce many of the exposure recall problems associated with retrospective studies. To obtain sufficient numbers of pregnancies, these studies should begin with an exposed and an unexposed population that are planning pregnancy; however, information about reproductive health of a population also can be gleaned from those not actively trying to reproduce. Because of the growing evidence that paternal exposure can affect fetal health, exposure information on both mother and father should be obtained. To fully understand the adverse reproductive effects, it is necessary to conduct studies in different populations, since effects may differ depending on the mixture of congeners to which the population is exposed and the source of their exposure. Two studies are currently underway of anglers in New York state and of the Inuit population of Quebec. The extent, the source, and mixture of PCB congeners differ for these populations.

The New York state (NYS) angler study described at the workshop by Buck et al. (this supplement) focuses on a population that consumes sport fish from Lake Ontario, the most polluted Great Lake. Buck et al., using a mailed questionnaire, obtained reproductive history information and lifetime fish consumption from 11,717 licensed anglers and 6579 wives or partners of male anglers and 18 to 40 years of age. By linking cohort members to the New York state live birth and fetal death registries, they will be able to assess fertility patterns and fetal death rates. Using birth certificates and hospital records, they will assess the relationship between fish consumption and birth weight and other developmental effects. On a subset of 3000 women, Buck et al. are conducting detailed telephone interviews to obtain information on reproductive end points such as time to pregnancy. Once a clearly exposed population is delineated, this population could be followed for fertility, fetal loss, and other reproductive outcomes. Offspring could be examined for neurodevelopment and reproductive health.

Another example of a heavily exposed population is the Inuits who live in Arctic Quebec and traditionally eat large amounts of fat from sea mammals (Ayotte and Dewailly, described them at the workshop). Organochlorine compounds that reach the Arctic by long-range atmospheric transport accumulate in the fatty tissues of Arctic marine animals. The total concentration of 10 PCB congeners in breast milk of the Inuits in northern Quebec is about seven times higher than in women from southern Quebec (*12*). Using Aroclor 1260 as the standard, PCB concentration is considerably higher than the 1 mg/kg "NOAEL" for visual recognition memory impairment reported by Tilson et al. (*13*). Ayotte and Dewailly and coworkers will be prospectively following a cohort of Inuit women and a control group of women from southern Quebec. Placentas and cord blood samples will be analyzed for cytochrome P450, Ah receptor, organochlorines, and stress assays and the infants will be clinically evaluated for growth retardation.

## Mechanism of Action

The mechanism by which TCDD-like compounds cause adverse reproductive and developmental effects is not well delineated. Clearly, studies in animal models are needed to help elucidate the toxicology and mechanism of effect. Discussion of this issue during the workshop outlined the current focus on the Ah receptor as the mechanism of action for TCDD and its congeners. Although there appears to be an association between binding to the Ah receptor and structural malformations in various mouse strains, the mechanism of effect may differ depending on the end points as well as the congeners to which a population is exposed (*1*). For other end points of reproductive and developmental toxicity, there has been no direct or indirect association of receptor binding with specific effects. An Ah-receptor mechanism may be demonstrated for many more of these effects but not necessarily for all effects. Toxic potency relative to TCDD has not been determined for the dioxin-like polychlorinated dibenzodioxin (PCDD), PCDF, or PCB congeners for effects other than structural malformations. All reproductive and developmental events are a culmination of a large number

of processes at various levels of biologic complexity. Therefore, future studies should attempt to elucidate the underlying mechanisms that combine to produce a toxic effect at the level of the whole organism. Mechanistic models must take into account the ever-changing physiologic status and developmental stage of the individual.

## Conclusions

In sum, the findings reported to date indicate that continued study of TCDD and TCDD-like compounds and their effects on human reproduction is needed. Results of animal studies lend support for adverse reproductive effects of these compounds in those exposed postnatally or *in utero*. As the results from studies on humans continue to emerge, added insight may be gained regarding potential human reproductive health effects of specific congeners and their mechanism of effect. This avenue of research will benefit from the continued collaboration of scientists from multiple disciplines including toxicology, embryology, endocrinology, genetics, and epidemiology.

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