Dioxin and Its Effects on Reproductive Systems

Claude Hughes, Ph.D., M.D.*

Reproductive and developmental toxicology refers to the effects of exposure to particular compounds on many aspects of reproductive function and development. Toxic outcomes include disruption in male or female animals of normal processes that are known to be essential for reproduction and detrimental effects on the developing fetus that may appear at birth or much later in life. These detrimental effects include malformations and abnormal function of organ systems, such as alterations in learning and other behaviors, including sex-appropriate behaviors.

In the Environmental Protection Agency's (EPA) reassessment of dioxin conducted as open public meetings in September 1992, the injurious effects of dioxin on reproduction and development were analyzed. Various human and animal studies of the reproductive or developmental effects of dioxin were evaluated. In addition, the levels of dioxin that had effects on reproduction and development were compared to the amounts of dioxin and related chemicals that we currently bear.

The people of Maine face a real health concern

* CH is Associate Professor of Obstetrics and Gynecology, Duke University School of Medicine. Address correspondence and reprint requests to Claude Hughes, Ph.D., M.D., Duke University Medical Center, P.O. Box 3418, Durham, NC 27710

regarding current exposure, current body burdens, and current intake of dioxin and related compounds. The intake of contaminated fish would incrementally add to that exposure and those hazards.

There currently exist several reliable studies [1-8] that demonstrate reproductive and developmental effects from dioxin in animals and people at relatively low levels of exposure. The levels of dioxin that can be realistically expected to occur in people in Maine now and the levels in these studies are similar. Thus it is reasonable to be concerned that the effects reported in these studies may occur in similarly exposed populations in Maine.

In 1990, when EPA decided to approve state dioxin water quality standards up to 1.2 parts per quadrillion (ppq), the Agency still was not focusing on reproductive, developmental, and other noncancer effects of dioxin. Researchers at EPA, National Institute of Environmental Health Science (NIEHS), and universities, however, had concerns and had been actively investigating these kinds of effects from the 1970s to present. EPA's current attention to these noncancer end points has been exemplified by a number of activities, including dedication of an entire half-day at the EPA's reassessment of dioxin to reproductive and developmental effects and immunotoxicity effects of dioxin as well as updating developmental toxicity guidelines and publishing them in the *Federal Register* in 1991

The effects of dioxin toxicity on reproduction and development may be more important than carcinogenesis because when one talks about effects on the

developing brain one is talking about the functional competency of the next generation Developmental biology issues are framed differently than carcinogenesis issues. During the development of the brain or other organ systems, the window in time during which critical events occur is in fact usually quite limited. Narrow time-limited exposures may have profound effects in terms of disrupting normal organization of tissues or systems within the body. Exposure to dioxin or other agents can have an important effect over a short period of time. Thus, an exposure in early or midpregnancy can indeed be transient for the adult mother but have permanent effects on the offspring.

This approach to the effects of toxic exposure is different from our thinking about cancer as the end point. With regard to cancer, it may take years of ingestion or exposure to elicit a modest increase in cancer risk. The developmental effects resulting from dioxin exposure are stochastic, rather than probabilistic. In this regard, the risk from exposure to dioxin is expressed in the same way as the risk from exposure to radiation. The risk of experiencing developmental effects from a given exposure is more homogeneously spread out across the population exposed Everybody in the exposure group is assumed to run the same risk of experiencing an effect.

Scientists do not know at this time whether in addition to early exposures in fetal or intrauterine development there are other periods in human life that are also exquisitely sensitive to perturbation. The peripubertal interval is a period that may also prove pivotal in terms of susceptibility to toxic exposures. Other important unknowns concern the fate of absorbed toxins once they are mobilized or metabolized in the body. For toxins that accumulate in body fat such as dioxin, continued ingestion over time results in an age-associated depot of the compound in that tissue. During intervals of weight loss when body fat is mobilized, dioxin must mobilize as well. The consequences of shifting this toxicant from fat stores to other metabolic compartments in the body are currently unknown. Furthermore, 30% to 50% of pregnant women commonly have an interval of anorexia early in pregnancy. We do not know how high the peak blood levels of dioxin may be when those women in the first trimester, especially late in the first trimester, mobilize fat stores. There are no data that assess the changes in blood levels, or other target tissue organ levels, when fat mobilization occurs

Dioxin and related chemicals exert effects by binding to very specific nuclear receptors called Ah receptors, which are similar to steroid hormone receptors. These receptors are proteins that are located in the nucleus of cells, and generally there are 1,000 to 2,000 per cell For these types of receptors, if a few hundred are occupied, biological effects are elicited. An increased response occurs with an increasing percentage of receptors occupied. Dioxin works by binding to these Ah receptors. At a concentration of 1 ppq, a teaspoon of water contains over 1.6 million molecules of dioxin. While all 1.6 million molecules of dioxin will not end up in one single cell such as a neuron in the brain, it is clear that a very dilute solution contains a large number of dioxin molecules, which are more than enough to occupy the active receptors in the cells and hence to produce adverse effects. This mode of action is very different from that of a compound like aspirin, which is a fairly general, weak inhibitor of a widely dispersed enzyme that involves prostaglandin synthesis Aspirin does not have very specific effects, whereas dioxin does.

The background level in humans for dioxin is approximately 1.3 ng/kg, and the sum total of activity of all dioxin-like chemicals in humans (known as toxic equivalents) is approximately 7 ng/kg [9-11]. Keeping these figures in mind, it is instructive to look at the studies that have been done in the last 15 years that have examined some developmental end points and some reproductive effects

In a National Institute of Occupational Safety and Health (NIOSH) study [7], men who were occupationally exposed to dioxin showed suppression of testosterone levels The body burdens in the men showing suppression were in the range of 5 to >19 ng/kg. Even allowing for our inability to determine whether current body burdens or previous exposures were the mechanistic cause for these reduced testosterone levels, such effects are of concern and are clinically significant.

Reasonable criticism of the NIOSH study includes noting that it is likely that these men were exposed to other chemicals. Although the researchers looked for other possible chemical mediators of the observed low testosterone levels, they did not find any, and the other known chemicals these workers were exposed to are not known to affect testosterone levels.

In a study by Mably and colleagues [3-5], pregnant rats were given a single dose of dioxin on day 15 of pregnancy. The lowest dose studied was 64 ng/kg, compared to background levels in the U.S. population of around 7 ng/kg. That experimental dose did not affect birth weights or adult weights of these off-

spring. It did, however, alter male fetal development, such that these male rats were observed to have compromised sperm production and diminished size and weight of other hormone-dependent tissues, such as prostate and epididymis. In addition, these males exposed to dioxin *in utero* showed demasculinization, with altered sexual behavior including markedly increased mount and intromission latency and increased lordosis response after estrogen priming. These behavioral changes are a brain effect. When studies of similar design using other agents have shown perturbations in normal sexual behavior, subsequent studies in every instance have confirmed that these observed effects are associated with structural or biochemical changes in how the brain functions [13,14]. Anatomic and chemical studies of the brains of dioxin-exposed rats are underway.

In a study of monkeys by Bowman and colleagues [12], the offspring who were exposed *in utero* to 22 ng/kg of dioxin showed specific defects in learning ability, with disordered object learning but unimpaired spatial learning. In this primate model, this behavioral index of learning in the offspring was compromised by a dose that is only about three times higher than what we humans currently bear as a result of environmental exposure.[1]

If levels shown to exert adverse effects in animal studies are somewhat higher than the current levels of dioxin in human populations, then why is there concern about human health? In part, the answer is that none of these studies identified levels at which reproductive or developmental effects did not occur. Therefore, we do not know whether the levels that exert effects are truly different than the dioxin levels we currently bear. At this time there is very little information to allow us to establish whether humans are more or less sensitive to dioxin than other animals, or whether wide differences in sensitivity to the effects of dioxin occur within the human population.

This issue is not clarified in the only epidemiological study available that examines birth defects in a human population exposed to dioxin as a result of the 1976 industrial explosion in Seveso, Italy [15]. The comparison of human and animal data summarized here would suggest that humans are much more sensitive to these effects than are laboratory animals, since comparable effects occurred in men at body burdens of dioxin that were several times lower than in

experimental animals. Given these observations where the body burden in human study subjects or the animals are only three to nine times greater than present human body burdens, it is prudent to conclude that there is little margin of safety between levels that all of us currently bear and the level of dioxin and related compounds shown to produce adverse effects on male reproduction and central nervous system development, as manifested in sexual behavior and learning. This conclusion was presented at the EPA's reassessment of dioxin in September 1992 and none of the assembled panel members expressed disagreement at that time with these correlations and conclusions. The acknowledgment of that conclusion by EPA is described in the October 9, 1992, memo that Erich W. Bretthauer, Assistant Administrator for Research and Development, sent to then EPA Administrator William Reilly:

> My interpretation of some salient features of the discussion by the panel members is:
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> Risk characterization should encompass the broad range of health effects attributable to dioxin exposure and not focus just on cancer.
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> Certain noncancer effects, including changes in endocrine function associated with reproductive function in animals and humans, behavioral effects in offspring of exposed animals, and changes in immune function in animals have been demonstrated. Some data suggest that these effects may be occurring in people at body burden levels that can result from exposures at, or near, current background.
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> While recent epidemiology studies indicate that dioxin and related compounds may be carcinogenic in humans, a focused review of these studies by a panel of epidemiologists is required. The Agency should then reconsider its current classification of dioxin which is based primarily on the results of laboratory animal studies.
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> Based on the key role of the Ah receptor in mediating toxic responses to dioxin and related compounds (other dioxins, furans and biphenyls) the full range of compounds which bind to this receptor should be considered in the risk characterization. Additional work will be required to better understand the impact of dioxin-like PCBs.
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> Risks from the ubiquitous background levels of dioxin in the general population need to be carefully considered.

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[1] The reliability of this study was questioned at one point; however at the EPA's reassessment of dioxin in September 1992, Linda Birnbaum and others at EPA, who have audited it, report the study to be reliable.

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