



Improving the Nation's Reproductive Health: Updating the Guidelines for Reproductive Toxicity Risk Assessment

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Background: Exposure to a steadily-increasing number of anthropogenic chemicals in the environment can have a profound negative impact on reproductive function and health, including the production of high quality gametes and a functioning endocrine system which is critical to an individual's general health [1]. In fact, trends in reproductive health demonstrate that **reproductive function has declined since the mid-20th century** - a time that corresponds to a significant increase in chemical production [2]. One class of chemicals that has been especially problematic for reproductive health has been endocrine disrupting chemicals (EDCs). EDCs include a wide array of chemicals and mixtures such as PFCs, PBDEs, PCBs, organochlorine pesticides, environmental phenols, phthalates, and perchlorate [3]. These chemicals have been shown to interfere with the production, transport, activity, and metabolism of natural hormones in the body and can have deleterious effects on developmental, reproductive, and neuroendocrine functions [4, 5]. Perhaps the most concerning is that humans are surrounded by and exposed to EDCs via air, water, food (including breast milk), and consumer household and personal care products [3, 6].

Significance: There is a critical need to update and revise the Guidelines for Reproductive Toxicity Risk Assessment (Federal Register 61 (212) 56274-56322) to **reflect today's state of knowledge of reproductive science, medicine, and health**. These guidelines were developed based on expert recommendations from the National Academy of Science to help scientists at the United States Environmental Protection Agency (US-EPA) assess the risks to human health following exposure to chemicals and other environmental contaminants. These guidelines, written 16 years ago, are outdated. Although these guidelines were designed to a) "provide guidance for interpreting, analyzing, and using the data from studies that follow [the guidelines]...", b) "provide information for interpretation of other studies and endpoints...", and c) promote consistency in the Agency's assessment of toxic effects...", it is unclear what manner they are actually being used currently (Application of the Guidelines: pages ix-xi). Moreover, it is particularly troubling that several assumptions made in this document that are meant to guide reproductive toxicity assessment may be misleading in light of data that is now available. We thus advocate that the scientific basis for the document be revisited, that the role of this guideline be clearly specified, and that the specific points outlined below be prioritized for immediate consideration.

Proposal: We request that the US-EPA either assemble a panel of experts in the field or request the National Academy of Science to **re-evaluate and update the Guidelines to accurately reflect current scientific thinking and practice**. This document should then be **referenced and linked in all future funding opportunities** to ensure that scientists are pursuing research in manners that are consistent with US-EPA definitions and goals. This update will ultimately better **protect the nation's reproductive health** because reproductive toxicity testing will be done using broader endpoints and with more appropriate animal or *in vitro*



model systems. This will ensure that research ***funding is targeted*** to the most important knowledge gaps and that policies that impact the general public are based on ***accurate information***.

Specific focus areas:

The new version of the Guidelines, in addition to being updated in general, should:

1) Expand the definition of male and female reproductive health beyond the ability to merely conceive and produce offspring to include related general health outcomes.

Problematic statement in current Guidelines:

"...secondary adverse health effects that may result from toxicity to the reproductive organs (e.g., osteoporosis or altered immune function), although important, are not included (page 14)."

Reproduction is the biological process by which new "offspring" are produced from their "parents". Reproduction is a fundamental feature of all known life and each individual organism exists as the result of reproduction. Therefore, reproduction itself is of utmost importance. However, reproductive health encompasses more than just the ability to produce offspring. The term *reproductive health* addresses the functioning of the male and female reproductive systems during all stages of life, not merely during childbearing years [7]. An impaired reproductive system can affect the endocrine system, and lead to diseases, disorders and conditions that impact general health. For example, defects in reproductive health can have ramifications on bone, cardiovascular, neurological, and sexual health, resulting in osteopenia and osteoporosis, heart attack, stroke, cognitive changes, and/or urological disorders such as incontinence [8-10]. Therefore, to discourage studying the secondary adverse health effects that may result from toxicity to reproductive organs limits the ability to gain important knowledge of how chemicals and other environmental toxins impact men and women throughout their life span.

2) Address that the reproductive toxicity in one species may not be representative of others.

Problematic statement in current Guidelines:

"Because similar mechanisms can be identified in the male and female of many mammalian species, effects of xenobiotics on male and female reproductive processes are assumed generally to be similar across species unless demonstrated otherwise (page 2)."

The current guidelines language understandably extrapolates positive toxicity results across species in order to be protective of understudied species and populations; however, this language risks extrapolating negative results in the same way. To be truly protective, negative toxicity testing in a single species cannot be applied to other, unstudied species. Failure to examine the effects of a toxin across multiple mammalian model organisms may prevent the identification of species-specific differences with consequences on the human population and ecosystem. It is long and well-established that toxic effects can vary dramatically from one species to the next. A classic example of this is observed in the case of dioxin toxicity, specifically 2,3,7,8-tetrachlorodibenzodioxin (TCDD), where certain toxic effects have been measured in some species (dogs, monkeys, guinea pigs, rats and mice), but not others (hamster) and shown to be related to a receptor model [11, 12]. In addition, certain toxic effects also depend on the hormone status of organisms indicating that TCDD may act in combination with other hormones or chemicals to produce adverse effects. Thus, there are both species and



individual differences in TCDD's receptor based model of action and these differences provide insight into the mechanisms of gene regulation.

3) Implement sex-based toxicity testing given the key biological differences that exist between males and females.

Problematic statements in current Guidelines:

a) *"In the absence of specific information to the contrary, it is assumed that a chemical that affects reproductive function in one sex may also adversely affect reproductive function in the other sex (page 2)."*

b) *"Many of the mechanisms controlling important aspects of reproductive system function are similar in females and males, and therefore could be susceptible to the same agents (pages 3-4)."*

In recent years, there has been increasing recognition that sex and gender are key variables that influence the response to exogenous drugs and/or chemicals. In fact, an entire field has been developed to explore and understand these sex-based differences, and the emerging data is compelling [13]. For example, in clinical studies, "being female" is a risk factor for having an adverse drug reaction [14]. Women are more likely than men to experience life-threatening cardiac arrhythmias, drug-induced liver toxicity, gastrointestinal adverse events due to NSAIDs, and allergic skin rashes after being prescribed drugs; and a report from the Government Accounting Office showed that the majority of drugs recalled from the market had more health risks for women than men [15, 16]. Thus, a treatment, clinical protocol, or diagnostic technique being tested may likely show differences in the safety and efficacy between males and females (effective for one and not the other, effective in one and potentially harmful in the other, effective in different ways for each sex, or effective for each sex under different circumstances). Failure to examine findings in both males and females could result in errant conclusions about the efficacy or even about adverse effects, potentially putting the targeted treatment population at risk.

Congress has recognized the importance of sex-specific investigation, and federal laws now mandate the inclusion of both males and females in National Institutes of Health (NIH)-funded clinical research. These laws transcend just enrollment into trials as they also require that the research be designed in such a way that its relevance to women and minority groups can be assessed and used to inform personalized clinical decision-making [17]. Recent data suggests that sex differences exist in the study of environmental chemicals as well. For example, signaling pathways critical for gonadal function are sexually dimorphic in male and female alligators and respond differently to exposure to environmental contaminants [18]. In addition, sexually dimorphic responses to EDCs have been reported for cardiovascular and neuroendocrine brain functions [19-21]. Given sex-specific biological differences and based on the model established in NIH-funded clinical trials, we request that the EPA require that toxicity testing be performed in both sexes rather than to rely on extrapolation.



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