The Animal Evidence: critical effects, and dose-response

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a persistent and bioaccumulative toxicant produced as a byproduct in the manufacture of chlorinated chemicals such as pesticides[1]. TCDD is also liberated through the incineration of plastics such as hospital and municipal waste[2-4]. The prototypical polyhalogenated aromatic hydrocarbon and aryl hydrocarbon receptor ligand, TCDD is generally accepted to be a highly toxic chemical with adverse effects on the reproductive, immune, and endocrine systems[5-7]. Animal studies have documented adverse effects of TCDD treatment over the life course including fetal development, the peripubertal transition, and adulthood[8-17]. Reproductive effects of developmental exposure demonstrated in animal studies[8;16-18] illustrate the sensitivity of the developing reproductive system to the life-long consequences of the effects resulting from exposure to this toxicant. However, the relevance of these findings to human health is unclear.

Epididymal sperm counts have been used as the key outcome measure in animal studies and underlie the current tolerable daily intake (TDI) for TCDD of 2pg/Kg BW/day established by the WHO[19]. This TDI was arrived at through an evaluation process that relied on the criteria established in the human relevance framework, a weight-of-the-evidence process employed by numerous scientific groups including the IPCS, ILSI and the USEPA. While such data are invaluable in establishing hazard it is proposed that their relevance to risk assessment is more difficult to apply. Furthermore, it is suggested that differences in the pharmacodynamics and pharmacokinetics of the test agent in animal models vs. humans and the animal models used must be considered in assessing the reproductive and developmental toxicity of all toxicants including TCDD. Moreover, comparative physiology and differences in key mechanistic pathways that operate in experimental animals relative to humans raises concerns regarding the relevance of animal data and the weight to apply to such evidence in establishing TDI values. For example, differences in the mechanisms regulating reproductive senescence and gonadotropin secretion in rodent models relative to humans[20] can contribute to confusion and misguided conclusions about risk to human health.

While the weight-of-evidence approach as advocated in the Hill criteria[21] is a good start, there are limitations to this approach. A process for establishing the strength of individual studies contributing to the overall weight-of-evidence has not been defined and thus creates opportunity for confusion and disagreement concerning the outcome of the evaluation. As an example, it is unclear how to deal with inconsistencies in the literature as found for TCDD treatment-induced changes in epidiymal sperm counts where initial studies[12;22-24] have demonstrated decreased sperm counts whilst other investigators have reported no adverse effects of TCDD treatment on epididymal sperm counts[25-27]. Furthermore, the biological relevance of experimental outcomes used and the potential impact of the changes on the distribution of the outcome are two important areas that require attention. For example, although statistical differences can be documented, the

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biological relevance of the change is often ignored or simply assumed leading to differences in opinion regarding the strength of the findings. The relevance of treatment-induced changes within the normal range for the outcome of interest such as body weight or hormone measure has not been discussed and thus no consensus exists within the scientific community concerning what is and what is not an adverse effect. Moreover, it may not be appropriate to assume that all individuals have equal sensitivity to the test agent and that exposure will result in a simple shift in the normal distribution and thus an increase in the number of affected individuals[28]. It is proposed that key criteria important to the evaluation of the animal literature should include but not be limited to: the relevance of the animal model; appropriateness and justification of the dose selection; the experimental design employed; outcome measures used and their relevance to human health; comparative endocrinology including the similarities vs. differences between the experimental animal model and humans; sample size employed to insure that the evaluation is robust and the absence of an effect is not due to inadequacy of the sample size employed; and appropriateness of the statistical methods of analysis.

In summary, animal studies are indispensible in establishing hazards associated with exposure to chemical hazard toxicants and elucidating potential mechanism of action important to human health. However, the relevance of animal data for establishing risks to human health are difficult to assess due to limitations in study design, lack of consistency in the literature, failure to address differences in physiology owing to comparative endocrinology, and limitations of the animal models employed. The weight-of-evidence approach is without question the route to follow, however there is need for guidance in evaluating the literature as it applies to different criteria. Clearly further guidance in the application of weight-of-evidence evaluations is necessary to reduce uncertainty and lead to transparent and generally acceptable evidence based decisions.

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