THE APPLICATION OF STUDY SELECTION CRITERIA TO TCDD EPIDEMIOLOGIC STUDIES AND ANIMAL BIOASSAYS FOR DEVELOPMENT OF A REFERENCE DOSE AND CANCER ORAL SLOPE FACTOR

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Introduction

The NAS review *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment*[1] of the EPA's draft *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds*[2] (herein referred to as the 2003 Dioxin Reassessment) recommended that EPA utilize a clear and transparent process for the selection of key studies and data sets for dose-response assessment. EPA responded to the NAS review in a draft document titled, *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments*.[3] In the response, EPA developed detailed TCDD-specific criteria for the selection of key dose-response studies and applied them to approximately 2,000 potentially relevant studies. These criteria are based on common practices and current guidance for point of departure (POD) identification and reference dose (RfD) and oral slope factor (OSF) derivation; they also consider TCDD-specific issues. The goal was to identify scientifically sound studies that provide the most relevant kinds of information needed for quantitative human health risk analyses of TCDD and screen out those studies that did not.

EPA derives RfDs and OSFs for a chemical from a health-protective perspective, generally using data sets that demonstrate the occurrence of adverse effects, or their precursors, in the low-dose range. Thus, when a group of studies is available on a chemical for which a number of such effects are observed at various doses across those studies, the studies using the lowest exposures that show effects will typically drive the RfD and OSF derivations, all other considerations being equal. Studies conducted at higher exposures relative to other available studies are used as supporting evidence for the final RfD or OSF because they were conducted at doses too high to impact the numeric derivations of toxicity values. These low-dose requirements do not imply that TCDD studies conducted at higher doses are of poor quality, simply that they are not quantitatively useful in the development of toxicity values because other studies with lower exposures will drive the RfD and OSF derivations under current EPA practice.

For the study evaluation and key data set selection, EPA undertook different approaches for the epidemiologic studies and non-human *in vivo* animal bioassays because the significant differences between animal and human health effects data affect their use in RfD and OSF derivation. This extended abstract describes, and briefly discusses, these two sets of criteria.

Criteria for Selecting TCDD Epidemiologic Studies

EPA first evaluated the available epidemiologic studies based on the five following considerations:

1. The methods used to ascertain health outcomes are clearly identified and unbiased, with high sensitivity and specificity.
2. The risk estimates generated from the study are not susceptible to important biases arising from an inability to control for potential confounding exposures or other sources of bias arising from either the study design or statistical analyses that were used.
3. The study demonstrates an association between TCDD and an adverse health effect (assuming minimal misclassification of exposure and absence of important biases) with some suggestion of an exposure-response relationship.

4. The exposure assessment methodology is clearly described and can be expected to provide adequate characterization of exposure, with assignment of individual-level exposures within a study (e.g., based on biomarker data, or based on a job-exposure-matrix approach). Limitations and uncertainties in the exposure assessment are considered.
5. The size and follow-up period (for a cohort study) are large enough and long enough, respectively, to yield sufficiently precise estimates for use in development of quantitative risk estimates and to ensure adequate statistical power to detect associations that might be present.

After careful evaluation of all identified epidemiologic studies published on TCDD and dioxin-like compounds (DLCs) using these five TCDD-specific epidemiologic study considerations, EPA further screened the studies for dose-response assessment by applying the following three study inclusion criteria:

1. The study is published in the peer-reviewed scientific literature and includes an appropriate discussion of strengths and limitations.
2. The exposure is primarily to TCDD, rather than DLCs, and is properly quantified so that dose-response relationships can be assessed. All epidemiologic cohorts will have background exposures to DLCs through the food chain, and these exposures are not included in this criterion.
3. The effective dose and oral exposure must be reasonably estimable. The measures of exposure must be consistent with the current biological understanding of dose. For TCDD dose-response assessment, it is critical that reported dose is consistent with a dose that is likely to be toxicologically relevant. The timing of the measurement of effects (i.e., the response) also must be consistent with current biological understanding of the effect and its progression.

EPA developed PODs from the epidemiologic studies that met these three inclusion criteria.

Criteria for Selecting TCDD Animal Bioassays

EPA also developed study selection criteria for the non-human *in vivo* animal bioassays. If the study was published in the peer-reviewed scientific literature, then EPA first evaluated the bioassay using the following two TCDD-specific study inclusion criteria:

1. The lowest dose level tested is ≤1 μg/kg-day for cancer bioassays and ≤30 ng/kg-day for noncancer bioassays.
2. The study design consists of orally administered TCDD-only doses, and specifies the purity and matrix used to administer the doses.

EPA further evaluated the studies meeting these criteria using the following three study inclusion criteria:

1. The study tests mammalian species, identifying the strain, gender, and age of the tested animals.
2. The study clearly documents testing protocol, including dosing frequency, duration, and timing of dose administration relative to age of the animals.
3. The overall study design is consistent with standard toxicological principles and practices. The control group or groups are appropriate, given the testing protocol, and are well characterized. Clinical and

pathological examinations conducted during the study are endpoint-appropriate, particularly for negative findings.

EPA developed PODs from the animal bioassays that met these five inclusion criteria.

Results and Discussion

EPA developed and applied these two sets of criteria for epidemiologic studies and animal bioassays to meet the NAS' concerns regarding transparency and clarity in the identification of TCDD studies for dose-response assessment. EPA collected and evaluated studies from the 2003 Dioxin Reassessment and also newer studies found via literature searches and through public submissions through October 2009. These criteria provided a transparent and rigorous evaluation of the scientific quality of each study in EPA's database, and, given the vast TCDD mammalian bioassay database, they provided a transparent method for initially screening studies to be considered for TCDD dose-response analyses.

The evaluation of the epidemiologic studies was conducted on a case-by-case basis because each cohort is uniquely defined and has its own set of exposure conditions, distribution of confounders, and different sources of potential bias that need to be considered in dose-response assessment. For TCDD, not all data are from occupational cohorts but include cohorts of residents exposed for relatively short time periods to high concentrations as a consequence of industrial accidents, a scenario that has not commonly been used to establish chronic EPA toxicity values. However, these studies were considered important for inclusion because they provide information on health effects in the human population exposed directly to TCDD. For cancer endpoints, EPA assumed that cumulative TCDD dose estimates are toxicologically relevant measures. Thus, cancer epidemiologic studies needed to provide information about long-term TCDD exposure levels. Further, EPA reasoned that modeling of cancer incidence or mortality provided by the study authors needed to address the timing of the end of the effective exposure prior to death or disease onset. For noncancer endpoints, EPA required that the exposure estimates and analyses allow for examination of issues of latency and other issues regarding the appropriate time window of exposure relevant for specific endpoints. Also, to be consistent with EPA's RfD methodology, only nonfatal endpoints were included.

For the animal studies, TCDD-specific criteria were developed using dose requirements, which were intended to be reasonable cutoffs that restricted the number of studies that would need to be analyzed while ensuring that all study/data set combinations that could provide candidate PODs for the RfD or OSF were identified. Thus, the dose range under consideration allowed for liberal ranges of NOAELs, LOAELs, and BMDLs for assessment of both cancer and noncancer effects. For the animal cancer studies, EPA chose the dose requirements based on an initial evaluation of available average daily doses administered in TCDD animal bioassays in which adverse effects were observed. The linearized multistage model used by EPA to estimate OSFs is most appropriately applied to studies from which PODs can be estimated as closely as possible to the experimental data. Thus, given the dose ranges in the cancer bioassays that are available for modeling, the restriction to $\leq$1 μg/kg-day for cancer was considered to be a reasonable cutoff. For the animal noncancer studies, dose regimens were more complex, and dose ranges varied according to study endpoint. Given the available database at the time the studies were evaluated, it was likely that the same composite uncertainty factor (e.g., of 300; 3 for UF_A [interspecies], 10 for UF_H [intraspecies], and 10 for UF_L [LOAEL to NOAEL]) would be applied to any animal noncancer LOAEL used to derive an RfD for TCDD. This implies that any study that has a LOAEL of 30 ng/kg-day or more would result in a candidate RfD that is more than an order of magnitude higher than those studies exhibiting effects at doses of 1−2 ng/kg-day. BMDLs that might be derived from such data also would not be expected to be lower than these example doses of 1−2 ng/kg-day. Thus, EPA considered a tested dose $\leq$30 ng/kg-day to be a reasonable cutoff where the lowest tested dose would never be used as a POD to derive an RfD given that much lower tested doses (associated with adverse effects) are available from other studies of acceptable quality.

Applying the study inclusion criteria for both epidemiologic studies and mammalian bioassays resulted in lists of key noncancer and cancer studies that were considered for quantitative dose-response analyses of TCDD. Endpoints or precursors identified in these studies that were not considered to be toxicologically relevant were eliminated from consideration. The study/endpoint data set combinations from the remaining studies were then subjected to dose-response assessment, and candidate PODs were developed for use in deriving RfDs or OSFs. PODs included no-observed-adverse-effect levels (NOAELs), lowest-observed-adverse-effect levels (LOAELs), or lower bound benchmark dose levels (BMDLs). The most sensitive PODs were selected as candidates for derivation of the RfD and OSF. [This abstract does not necessarily reflect EPA policy.]

References

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