2. TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

This section addresses transparency and clarity in the study selection process and identifies key data sets for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dose-response analysis. Section 2.1 summarizes the National Academy of Sciences (NAS) committee's comments specifically regarding this issue. Section 2.2 presents U.S. Environmental Protection Agency's (EPA's) response to those comments and describes EPA's approach to ensuring transparency and clarity in the selection of studies for subsequent dose-response analyses. Section 2.3 describes the TCDD-specific study inclusion criteria and study quality evaluation process EPA used in this document for determining the eligibility of both epidemiologic and experimental animal studies for TCDD dose-response analysis. Section 2.4 summarizes the results of applying the study inclusion criteria to the epidemiologic studies (see Section 2.4.1, Tables 2-1 and 2-2) and the in vivo mammalian bioassays (see Section 2.4.2, Tables 2-3 and 2-4). These results present the key TCDD epidemiologic and animal bioassays that were identified using the study inclusion criteria. Additional details on this process can be found in Appendices C and D. Appendix C summarizes all of the available epidemiologic studies, evaluates the suitability of these studies for TCDD dose-response analyses, and presents the study selection process results. Appendix D summarizes only the animal bioassay data that have met the study inclusion criteria for TCDD dose-response assessment and, in Tables D-1 and D-2, shows the results of the study selection process for all of the animal bioassays identified by EPA. Study/endpoint combination data sets for developing TCDD toxicity values for noncancer effects are further evaluated in Section 4 of this document. Based on the cancer studies identified in this document, study/endpoint combination data sets for developing toxicity values for cancer effects will be explored in a separate document, Volume 2 of this effort.

2.1. SUMMARY OF NAS COMMENTS ON TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

The NAS committee proposed that EPA develop a clear and readily understandable methodology for evaluating and including epidemiologic and animal bioassay data sets in dose-response evaluations. The NAS committee recommended the development and application

of transparent initial criteria to judge whether or not specific epidemiologic or animal bioassay studies be included in TCDD dose-response analysis.

Specific NAS comments on the topic of study evaluation and inclusion criteria include the following:

> EPA should specify inclusion criteria for the studies (animal and human) used for derivation of the benchmark dose (BMD) for different noncancer effects and potentially for the development of RfD values and discuss the strengths and limitations of those key studies (NAS, 2006b, p. 27).
>
> …in its [EPA's] evaluation of the epidemiological literature of carcinogenicity, it did not outline eligibility requirements or otherwise provide the criteria used to assess the methodological quality of other included studies (NAS, 2006b, p. 56).
>
> With regard to EPA's review of the animal bioassay data, the committee recommends that EPA establish clear criteria for the inclusion of different data sets (NAS, 2006b, p. 191).
>
> …the committee expects that EPA could substantially improve its assessment process if it more rigorously evaluated the quality of each study in the database (NAS, 2006b, p. 56).
>
> EPA could also substantially improve the clarity and presentation of the risk assessment process for TCDD…by using a summary table or a simple summary graphical representation of the key data sets and assumptions…(NAS, 2006b, p. 56).

2.2. EPA'S RESPONSE TO NAS COMMENTS ON TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

EPA agrees with the NAS committee regarding the need for a transparent and clear process with criteria identified for selecting studies and key data sets for TCDD dose-response analyses. The delineation of the study selection process and decisions regarding key data sets will facilitate communication regarding critical decisions made in the TCDD dose-response assessment. In keeping with the NAS committee's recommendation to use a transparent process and improve clarity and presentation of the health assessment process for TCDD, Figure 2-1 provides an overview of the approach that EPA has used in this document to develop a final list of key cancer and noncancer studies for quantitative dose-response analysis of TCDD. The steps in Figure 2-1 are further explained below.

Literature search for in vivo mammalian and epidemiologic TCDD studies (2000−2008): EPA conducted a literature search to identify peer-reviewed, dose-response studies for TCDD that have been published since the 2003 Reassessment. This search included in vivo mammalian and epidemiologic studies of TCDD from 2000 to 2008. Additional details describing the conduct of this literature search are presented in Section 1.5.1 of this document.

Federal Register Notice—Web publication of literature search for public comment: In November 2008, EPA published a list of citations from results of this literature search (U.S. EPA, 2008a) and invited the public to review this preliminary list of dose-response citations for use in TCDD dose-response assessment. EPA requested that interested parties identify and submit peer-reviewed studies for TCDD that were absent from this list. Two parties identified additional references that were not included in the 2008 Federal Register notice and submitted additional references for EPA to consider. These references were included in the final TCDD literature database considered by EPA for TCDD dose-response analysis.

Initial study inclusion criteria development for TCDD in vivo mammalian bioassays: EPA developed an initial set of draft criteria for evaluating the extensive TCDD database of in vivo mammalian bioassays. These initial study inclusion criteria had three purposes. First, they provided a method to transparently and rigorously evaluate the scientific quality of each study in EPA's database, a deficiency in the 2003 Reassessment identified by the NAS committee. Second, their application provided an efficient way to initially screen the vast number of TCDD mammalian bioassays for consideration in TCDD dose-response analyses. Third, they served as a starting point for discussions of study inclusion criteria by expert panelists who were convened by EPA for its scientific workshop on TCDD dose-response analysis (the Dioxin Workshop), described next [also see the workshop report in Appendix B, U.S. EPA (2009b)].

Dioxin Workshop and expert refinement of TCDD in vivo mammalian study inclusion criteria: In February 2009, EPA convened "A Scientific Workshop to Inform EPA's Response to NAS Comments on the Health Effects of Dioxin in EPA's 2003 Dioxin Reassessment" [see workshop details in Section 1.5.2 and Appendix B (U.S. EPA, 2009b)]. At the workshop, EPA presented the draft set of study inclusion criteria; the workshop panelists evaluated the study inclusion criteria in relation to the various toxic endpoints that were discussed and made recommendations for their revision.

Final development of study inclusion criteria for TCDD in vivo mammalian studies: Based on discussions and recommendations made at the Dioxin Workshop, the initial draft study inclusion criteria for evaluating the TCDD mammalian bioassay literature were revised and are presented in Section 2.3.2.

Development of study inclusion criteria for epidemiologic studies: Following the Dioxin Workshop, EPA determined that an evaluation process was also needed for selection of epidemiologic studies for TCDD dose-response assessment. These criteria were developed and are detailed in Section 2.3.1.

Final literature collection (October 2009): Additional literature was collected as it was identified by EPA following the Dioxin Workshop through October 2009 to ensure the consideration of all recently published data for this report.

Studies screened using study inclusion criteria: The two sets of TCDD-specific study inclusion criteria for epidemiologic studies and in vivo animal bioassays presented in Sections 2.3.1 and 2.3.2, respectively, were used to evaluate all studies included in the 2003 Reassessment, studies identified in the 2000−2008 literature search, studies identified through public comment and submission, and studies collected in 2009 as identified by EPA during the development of this document. Section 2.4 and Appendices C and D present results of EPA's evaluation of epidemiologic and mammalian bioassay literature for both cancer and noncancer endpoints.

Final list of key cancer and noncancer studies for quantitative dose-response analysis of TCDD: Application of the study inclusion criteria concludes in Section 2.4 with development of a list of key noncancer and cancer studies to be considered for quantitative dose-response analyses of TCDD. In Section 4, points of departure (PODs) are developed and evaluated for all biologically relevant noncancer study/endpoint combinations from these final key study lists, and key data sets and PODs for the development of TCDD noncancer toxicity values are identified. Similar analyses will be undertaken in Volume 2 of this effort for TCDD cancer dose-response assessment.

2.3. STUDY SELECTION PROCESS FOR TCDD DOSE-RESPONSE ANALYSIS

In this section, EPA describes the study selection process that includes both TCDD-specific study selection criteria and methodological considerations that have been developed to evaluate epidemiologic studies and animal bioassays for quantitative TCDD dose-response assessment. These criteria and considerations reflect EPA's goal of developing an RfD and a cancer OSF for TCDD through a transparent study selection process; they are intended to be used by EPA for TCDD dose-response assessment only. The TCDD in vivo mammalian literature base differs from most other chemicals in magnitude and comprehensiveness. It comprises ~1,500 studies that evaluate multiple cancer and noncancer endpoints, many species including humans, and covers an expansive dose range, including doses at and below 1 nanogram per kilogram body weight per day (ng/kg-day). Thus, the study inclusion criteria and considerations developed in this document are specific to evaluating the TCDD literature and cannot necessarily be generically applied to other chemicals. Further, TCDD has a long half-life in humans (~7 years) and bioaccumulates in fat tissue, resulting in the specification of study inclusion criteria for estimating exposures during the critical windows for adverse health effects. In this effort, EPA sought to identify a group of studies for TCDD dose-response evaluation that would span the types of adverse health effects associated with TCDD exposures and encompass the range of doses in the lower end of the dose-response region most relevant to human health protection. Detailed study inclusion criteria have been developed that consider

TCDD-specific issues and reflect EPA methods for POD identification, noncancer RfD derivation, and cancer OSF derivation. (The effort in this document contrasts with EPA's 2003 Reassessment where the focus was on individual endpoints and the goal was to compare dose response across studies.)

The study inclusion criteria and considerations were applied to each of the studies listed in the "Preliminary Literature Search Results and Request for Additional Studies on 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Dose-Response Studies" (U.S. EPA, 2008a); studies identified and submitted by the public and by participants in the Dioxin Workshop (U.S. EPA, 2009c); studies included in the 2003 Reassessment; and other relevant published studies collected by EPA scientists through October 2009. In this effort, the goal was to identify the most relevant studies for TCDD quantitative human health risk analyses. Those that did not qualify were not used quantitatively, but some of these were still considered relevant to the qualitative evaluations of the noncancer and cancer assessments. Similarly, some types of studies were not screened, i.e., studies on dioxin-like compounds (DLCs), mixtures toxicity, mode of action, in vitro toxicity, nonmammalian toxicology, and risk assessment; however, they were considered to be important supplemental information to be used as needed, for example, in discussions of biological significance.

For the study selection process, EPA has focused on TCDD studies and has not included studies on DLCs or DLC mixtures because inclusion of the DLC literature would likely increase the uncertainty in TCDD dose response unnecessarily, given that the TCDD database is quite robust. In addition, EPA believes that using studies evaluating information primarily or exclusively on TCDD dose response provides the most appropriate data for the risk assessment of dioxins and DLCs using the TEF approach. Because TCDD is used as the index chemical in the TEF approach, the most relevant and accurate information that specifically addresses quantitative dose response of individual TCDD exposures is needed. The WHO expert panel assigned TEF values from a conservative perspective that was intended to be health protective (Van den Berg et al., 2006). In the development of the TEFs, the WHO expert panel considered data from Haws et al. (2006a, b), who present summary statistics of relative potency values assembled from selected in vivo and in vitro studies. For each individual DLC, the WHO expert panel typically assigned TEF values using an in vivo study whose relative potency value was above the 50th percentile of the ranges presented by Haws et al. (2006a, b). Thus, when these

TEFs are used in a dose-response study, they produce total TEQ estimates that may be biased high for certain combinations of DLCs. If a RfD for TCDD were derived based on TEQ dose-response data, that RfD would likely also be biased high and, in that case, would underestimate health risk from environmental exposures. Thus, using the TEQ data to estimate TCDD toxicity values would not accurately reflect TCDD dose response.

Text Box 2-1. EPA Risk Assessment Guidelines and Guidance Documents for Toxicity Assessment
Risk Assessment Guidelines of 1986, including chemical mixtures, mutagenicity, cancer, exposure assessment, developmental effects (U.S. EPA, 1986a, b)
Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991)
Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996)
Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998)
Benchmark Dose Technical Guidance Document [external review draft] (U.S. EPA, 2000)
Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a)
Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005b)

Finally, there is uncertainty in how the underlying data were used to derive the TEF values that complicates the extrapolation of TEQ dose-response data to inform TCDD dose response. The kinds of information available for calculating relative potencies within a study are highly variable across DLCs, including many types of and numbers of in vivo (including different test species) and in vitro studies. In addition, a number of different methods are employed to calculate the range of relative potencies presented by Haws et al. (2006a, b), ranging from comparing dose-response curves, to developing ratios of effective doses that cause an effect in 50% of the test units (ED_{50s}), to estimating values from graphs of dose-response data. The uncertainty in the TEFs can be a substantial issue for dose-response modeling when effect levels in a study occur at doses close to background TEQ levels and TCDD is not a dominant component of the mixture. In this case, the contribution of TCDD dose to the observed toxic effect may not be feasible to estimate as it is confounded by other TEQ concentrations and impacted by other TEF uncertainties.

EPA has undertaken different approaches for epidemiologic versus in vivo animal bioassay study evaluation and key data set selection. The significant differences between animal and human health effects data and their use in EPA health assessment support development of separate study inclusion criteria and different approaches to study evaluation. For example, animal bioassays on TCDD are closely controlled experiments where dose and effect are precisely measured and causality is readily apparent; thus, the animal criteria contain precise dose limits and specific limitations on elements of the experimental design. Because

epidemiologic studies on TCDD are carried out within a population setting, these observational studies employ statistical and other analytical techniques to estimate exposures/doses, and to assess dose-response relationships after controlling or accounting for confounding factors and other potential sources of bias. Thus, the epidemiologic criteria contain requirements for being able to reasonably quantify the exposure-response relationship for the biologically-relevant exposure window.[1]

Section 2.4 and Appendices C and D present the results of the study selection process. In Appendix C, all of the available epidemiologic studies on TCDD are summarized and evaluated for suitability for dose-response modeling using the TCDD-specific study inclusion criteria described in Section 2.3.1 below; only studies meeting the study inclusion criteria and study quality considerations are presented as key studies in Section 2.4.1 (see Tables 2-1 and 2-2 for the cancer and noncancer endpoints, respectively). In Appendix D, because summarizing all of the available animal bioassays on TCDD was prohibitive, only studies first meeting the in vivo animal bioassays study inclusion criteria described in Section 2.3.2 below are summarized; Tables D-1 and D-2 present the results of the study selection process evaluations for the studies that met and did not meet the study inclusion criteria, respectively. The selected animal studies are presented as key studies in Section 2.4.2 (see Tables 2-3 and 2-4 for cancer and noncancer endpoints, respectively).

2.3.1. Study Inclusion Criteria for TCDD Epidemiologic Studies

This section describes the process EPA used to select epidemiologic studies for identifying PODs for TCDD quantitative dose-response assessment.[2] This selection process includes specific criteria based on EPA's approaches for deriving OSFs and RfDs (see Text Box 2-1). Additional considerations used in selecting epidemiologic data for quantitative dose-response modeling are also necessary, particularly given EPA's preference to use human studies over animal studies whenever possible (U.S. EPA, 2005a). As described by Hertz-Picciotto (1995), key components needed for the use of an epidemiologic study as a basis for

[1] Critical exposure windows can be identified either through conceptual understanding of the timing of the affected biological process, such as a susceptible life-stage during which the effect is manifested, or empirically, when such critical windows are evident from the results of an epidemiological study. Note that the conceptual understanding can be obtained independently of the epidemiologic study in question.

[2] In general, for these epidemiologic studies, EPA is relating tissue concentrations of TCDD that have been used in conjunction with kinetic modeling to estimate previous TCDD exposures.

quantitative risk assessment include issues regarding exposure assessment (a well-quantified exposure assessment with exposures linked to individuals) and study quality ("strong biases," e.g., with respect to inclusion criteria for membership in the cohort and follow-up procedures "ruled out or unlikely" and "confounding controlled or likely to be limited"). The strength of the association, either within the full study or within a high exposure subgroup, can also be considered in the evaluation of suitability for dose-response modeling (Hertz-Picciotto, 1995). Stayner et al. (1999), however, note that even weak associations could be useful in terms of providing an estimate of a potential upper bound for a quantitative risk estimate.

EPA's study selection process included applying TCDD-specific study inclusion criteria to epidemiologic data which met the five following considerations (also see Figure 2-2 for more details):

1. The methods used to ascertain health outcomes are clearly identified and unbiased (e.g., outcome classification was made "blinded" to exposure levels of the study participants).
2. The risk estimates generated from the study are not susceptible to important biases arising from an inability to control or account for confounding factors or other sources of bias (e.g., selection or information bias) arising from limitations of the study design, data collection, or statistical analysis.
3. The study demonstrated an association between TCDD and an adverse health endpoint (assuming minimal misclassification of exposure and absence of important biases) with some suggestion of an exposure-response relationship.

   This consideration in effect rules out the use of a null study (i.e., a study reporting no association between TCDD and the health endpoint of interest) in the quantitative dose-response assessment used to derive an RfD. Theoretically, a no-observed-adverse-effect level (NOAEL) can be identified from a null study and used to derive an RfD; that is, such a study could provide a "free-standing NOAEL" that could serve as a basis for an RfD after appropriate uncertainty factors were applied. However, a "free-standing NOAEL" from a study in which no adverse effects have been observed is not usually chosen for RfD derivation when other available studies demonstrate lowest-observed-adverse-effect levels (LOAELs). The large and comprehensive database available to assess quantitative TCDD dose response provides many positive studies that are considered stronger candidates for derivation of an RfD than free-standing NOAEL studies. [However, null studies are used by EPA to discuss the biological significance of the critical endpoint(s) used as the basis for deriving an RfD.]
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 of 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 limit the possibility of not detecting an association that might be present. Similar considerations regarding sample size and statistical precision and power apply to other study designs such as case-control studies.

In addition to these five study considerations, three specific study inclusion criteria were used to select studies for further evaluation and potential TCDD quantitative dose-response assessment:

1. The study is published in the peer-reviewed scientific literature and provides an appropriate discussion of data collection and analysis methods, as well as sufficient detail to allow consideration of its strengths and limitations.
2. The exposure is primarily to TCDD, rather than DLCs, and can be quantified so that dose-response relationships can be assessed for non-fatal adverse endpoints.[3] Because all epidemiologic cohorts have background exposures to DLCs, in which TCDD is a minor component, only those studies for which TCDD exposure is well above background will qualify for dose-response modeling. To the extent to which background DLC exposure becomes more significant with respect to TCDD exposure, limited quantitative assessment of DLC background exposures may be necessary.
3. The effective dose and oral exposure must be quantifiable. The timing of the measurement of health endpoints (i.e., the response) also must be consistent with current biological understanding of the endpoint and its progression.

   For cancer endpoints, EPA assumes that cumulative TCDD dose estimates are toxicologically relevant measures. Thus, cancer studies must provide information about long-term TCDD exposure levels. Further, for measures of cancer occurrence or death, sufficient follow-up is needed to allow for examination of latency between the end of effective exposure and cancer detection or death.

   For noncancer endpoints, exposure estimates and analysis must allow for examination of issues of latency and other issues regarding the appropriate time window of exposure relevant for specific endpoints. That is, there must be sufficient information, either in the study or elsewhere, to allow for the identification of a biologically-relevant critical exposure window of susceptibility. A biologically-relevant critical exposure window of susceptibility ("critical exposure window" or

[3] EPA does not base RfDs on frank effects, such as mortality.

"critical window") is an exposure period during some specific life stage over which an individual is particularly susceptible to the agent (e.g., TCDD) for a particular health endpoint. In utero and early lifetime exposures are often identified as critical exposure windows for many defects in anatomical and physiological processes under development during those periods. Critical exposure windows can be identified either through conceptual understanding of the timing of the affected biological process, such as a susceptible life-stage during which the effect is manifested, or empirically, when such critical windows are evident from the results of an epidemiological study. An example of the latter is the greater effect of early exposure to TCDD for boys under 10 years of age on later semen quality than on boys aged 10−17 years at the time of exposure).[4] Identifying such critical windows is important for TCDD in the practical sense of defining a reasonable duration over which to average internal exposures that vary greatly from an initial high peak exposure to a much lower terminal exposure, as is the case for virtually all epidemiologic studies under consideration for TCDD. EPA considers the internal exposures following the actual TCDD exposure incident to be relevant for averaging because of the relatively slow elimination of TCDD and the possibility that these concentrations could still be affecting the processes leading to the adverse health outcome.

Those studies that satisfied these three study inclusion criteria and, in addition, adequately satisfied the study quality provisions specified in the five considerations were considered to be suitable for quantitative TCDD dose-response analyses (see results in Section 2.4.1 and Appendix C).

2.3.2. Study Inclusion Criteria for TCDD In Vivo Mammalian Bioassays

This section identifies the criteria EPA applied to select nonhuman in vivo mammalian studies for defining PODs for use in TCDD dose-response modeling. These criteria are specifically developed to evaluate the TCDD literature and are not necessarily generic, however, they are based on EPA's approaches for deriving OSFs and RfDs from bioassay data (see Text Box 2-1). EPA agrees with the NAS committee regarding the utility of an oral RfD and the need for reevaluation of the OSF for TCDD, specifically in light of data that have been published since the 2003 Reassessment was released. RfDs and OSFs are generally derived using data sets that demonstrate the occurrence of adverse effects, or their precursors, in the low-dose range for that chemical. RfDs and OSFs are derived from a health-protective perspective for chronic

[4] Mocarelli et al., (2008); for further details of this Seveso cohort study, see the study summary in Appendix C and RfD derivation in Section 4-3.

exposures. Thus, when a group of studies is available on a chemical for which a number of effects are observed at various doses across those studies, the studies using the lowest doses that show effects will typically be selected as the basis of the RfD and OSF derivations, all other considerations being equal. Studies conducted at higher doses 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.

EPA expresses RfDs and OSFs in terms of average daily doses, usually as mg/kg-day and per mg/kg-day, respectively. Thus, the study inclusion criteria for the animal bioassay data presented in this section include requirements that average daily exposures in the studies are within a low-dose range where, relative to other studies, they could be considered for development of a toxicity value. 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 be selected as the basis of the RfD and OSF derivations under current EPA guidance (see Text Box 2-1). Because EPA has identified hundreds of in vivo mammalian studies that may be considered for quantitative TCDD dose-response assessment, the development and application of these study inclusion criteria have been critical to moving the health assessment process forward.

EPA's method for applying TCDD-specific study inclusion criteria for mammalian bioassays is detailed below and in Figure 2-3. Four specific study inclusion criteria were used to select studies for further evaluation and potential TCDD quantitative dose-response analyses and identification of PODs:

1. The study is published in the peer-reviewed scientific literature.
2. The study was not conducted on a genetically-altered species
3. The lowest dose level tested is $\leq$1 μg/kg-day for cancer studies and $\leq$30 ng/kg-day for noncancer studies
4. The study design consists of orally administered TCDD-only doses.

Those studies that satisfied these four criteria (see results in Section 2.4.2 and Appendix D) were considered suitable for quantitative TCDD dose-response analysis.

In evaluating the selected in vivo animal studies, EPA considered study quality issues to ensure that the study provided important information needed to assess the relevance of the study's endpoints and to quantify the dose-response relationship. Each study needed to test a mammalian species and identify the strain, gender, and age of the tested animals. The study had to clearly document its testing protocol, including dosing frequency, duration, and timing of dose administration relative to age of the animals. For example, the control group or groups had to be well characterized and appropriate, given the testing protocol. Also, clinical and pathological examinations conducted during the study needed to be endpoint-appropriate, particularly for negative findings. EPA used the results of these study evaluations in drafting study summaries for all of the animal bioassays that met the study inclusion criteria (see Appendix D).

The criteria for dose requirements are intended to be reasonable limits that restrict the number of studies that would need to be considered while ensuring that all study/data set combinations that could be candidates for the cancer OSF or RfD were analyzed. Thus, the dose range under consideration allows for liberal ranges of NOAELs, LOAELs, and benchmark dose lower confidence bounds (BMDLs) for assessment of both cancer and noncancer effects. The dose requirements for cancer and noncancer studies were set after EPA conducted a brief review of typical dose levels in studies analyzed in the 2003 Reassessment and in some of the more recent studies found through EPA's literature search.

For cancer studies, the low-dose limit was selected liberally so as not to exclude a study that might possibly report a sensitive tumor endpoint. Given that the limit of 1 μg/kg-day is 3 orders of magnitude higher than the lowest-tested dose in one of the most sensitive animal bioassays (Kociba et al., 1978) evaluated in U.S. EPA (2003), it is virtually impossible that a slope factor derived from a study with a low dose of 1 μg/kg-day would ever be considered for the OSF reference value. Following identification of new animal cancer bioassays, no studies were eliminated based on this limit.

For noncancer studies, the identification of a low-dose limit is more complicated because of the variety of exposure protocols and endpoints and the consequent varied degree of toxicokinetic extrapolation to human equivalent exposures. However, EPA is confident that the low-dose limit of 30 ng/kg-day will not exclude any study from which a POD could be derived that would be low enough to be considered for the RfD. A preliminary screening of the literature indicated that, for all study types (e.g., acute, developmental, chronic), there are many studies

with apparent effect levels well below 30 ng/kg-day. Effects observed above 30 ng/kg-day, therefore, would have no chance of being considered as the basis for an RfD.

2.4. SUMMARY OF KEY DATA SET SELECTION FOR TCDD DOSE-RESPONSE MODELING

To meet the NAS' concerns regarding transparency and clarity in the identification of TCDD studies for dose-response assessment, EPA has developed and applied two sets of criteria for epidemiologic studies and animal bioassays. EPA collected these studies through October, 2009, including studies from the 2003 Reassessment and newer studies found via literature searches and through public submissions (see Section 2.2 and Figure 2-1). Based on these activities, a total of 1,441 studies were examined for their potential to be used in TCDD quantitative dose-response analysis. Of these, Figure 2-4 shows that 637 studies were eliminated from consideration as they were not suitable study types; these included, in vitro bioassays, review articles, PBPK modeling studies, and studies that evaluated PCBs or other dioxin--like compounds other than TCDD. Of the remaining studies, 49 were epidemiologic studies (7 studies contained both cancer and noncancer endpoints), and 755 were animal studies (4 studies contained both cancer and noncancer endpoints). These epidemiologic and animal studies were then evaluated using EPA's study inclusion criteria.

Detailed results of EPA's evaluations and study summaries are shown in Appendices C and D for the epidemiologic studies and animal bioassays, respectively. Final results in tabular form are shown in this section. Tables 2-1 and 2-2 contain the final lists of key cancer and noncancer studies, respectively, that have met EPA's study inclusion criteria for epidemiologic data. Tables 2-3 and 2-4 provide the final lists of key studies that have met EPA's study inclusion criteria for animal bioassay data for cancer and noncancer studies, respectively. Collectively, these four tables contain the final set of key studies that EPA has selected for development of noncancer and cancer dose-response assessments for TCDD.

Through this study selection process, EPA has identified a relevant group of studies that spans the possible risk analytic choices for human health protection. Each study provides important TCDD dose-response information but also is associated with limitations and uncertainties that must be considered and characterized during TCDD dose-response evaluations. EPA has benefited from this effort by greatly reducing the scope of dose-response modeling and analyses to a manageable size, and by focusing on the most important studies from the

perspective of developing cancer and noncancer toxicity values. Results of applying the study inclusion criteria showed that exposure information was a primary factor in study selection (see Figure 2-4). In the epidemiologic studies, exposure needed to be primarily to TCDD and quantifiable on an individual level. In addition, the identification of critical exposure windows and the availability of latency information in the epidemiologic studies were vital data for developing human exposure estimates. In the animal studies, dose limits were the most important criteria.

2.4.1. Key Epidemiologic Data Sets

The studies listed in Tables 2-1 and 2-2, for cancer and noncancer, respectively, are those studies that have met the epidemiologic TCDD study inclusion criteria (see Section 2.3.1). Summaries for all of the epidemiologic studies evaluated are also provided in Appendix C and are organized by epidemiologic cohort. Following a brief summary of each cohort, its associated studies are then summarized chronologically, assessed for methodological considerations relative to epidemiologic cohorts and studies, and evaluated for suitability for TCDD dose-response assessment. Further, Appendix C presents explicit details regarding whether the considerations and criteria were met (see summary Tables C-2 and C-3, followed by Tables C-4 though C-56, which provide details for each study).

The cancer epidemiologic studies on TCDD that were subjected to the study selection process include 24 peer-reviewed publications from 8 cohorts. An evaluation of these against EPA's study inclusion criteria resulted in selecting 8 studies from the NIOSH, Boehringer, BASF, Ranch Hand, and Seveso cohorts for further consideration in TCDD quantitative cancer dose-response assessment (see Table 2-1). All of these studies had serum TCDD measurements on individual study participants, used kinetic models to refine exposure estimates, and accounted for latency or appropriate exposure windows in their analyses. As shown in Figure 2-4, most of the other studies were excluded because exposures were not primarily to TCDD and not quantifiable on an individual level; many studies also failed to provide information on an appropriate latency period or window of exposure for cancer (see Table C-2). In addition, two studies (Steenland et al., 1999; Flesch-Janys et al., 1998) passed all criteria but were not selected because they were superseded by other studies on the same cohort for which an updated analysis was done [i.e., Steenland et al. (2001) and Becher et al. (1998), respectively]. The

Baccarelli et al. (2006) study also passed all of the criteria but was not selected because of an issue identified during evaluation of the study considerations (i.e., lack of an obvious adverse health endpoint). The noncancer epidemiologic studies (see Table C-3) on TCDD that were subjected to the study selection process include 32 peer-reviewed publications from 10 cohorts. An evaluation of these against EPA's study inclusion criteria resulted in selecting four studies from the Seveso cohort for further consideration in TCDD quantitative noncancer dose-response assessment (see Table 2-2). The 4 Seveso cohort studies passed all criteria primarily because TCDD serum levels were available for individuals in the studies, and the critical windows of exposure were identifiable for the endpoints that served as PODs [e.g., the 9 months of pregnancy for exposed mothers clearly defined the window of exposure for the fetus in Baccarelli et al. (2008)]. As shown in Figure 2-4, many of the excluded studies failed to provide enough information on expected latency for the nonfatal endpoints or failed to provide data on the critical period of exposure to quantitatively estimate an oral human dose. A number of studies also had exposures that were not primarily to TCDD. One study, Baccarelli et al. (2005), passed all criteria but was excluded because the health endpoint, chloracne, is considered to be an outcome associated with high TCDD exposures; thus this study was not considered further in RfD derivation. The Warner et al. (2004) study also passed all criteria but was not selected because EPA could not assess the biological significance of this finding and could not establish a LOAEL for this effect (i.e., it did not satisfy one of the study considerations).

2.4.2. Key Animal Bioassay Data Sets

The studies listed in Tables 2-3 and 2-4, for cancer and noncancer, respectively, are those studies that have met the in vivo animal bioassay TCDD study inclusion criteria (see Section 2.3.2 and Figure 2-3). Appendix D provides study summaries, is organized by reproductive studies, developmental studies, and general toxicity studies (subdivided by duration), and summarizes the experimental protocol, the results, and the NOAELs and LOAELs EPA has identified for each study. The doses shown in Tables 2-3 and 2-4 are expressed as average daily administered intakes in units of nanograms per kilogram body weight per day

(ng/kg-day), adjusted for continuous exposure when necessary.[5] Tables D-1 and D-2 present the results of the study selection evaluations for the studies that met and did not meet the study inclusion criteria, respectively.

A total of eight animal cancer bioassays were available for evaluation using EPA's study inclusion criteria (see Section 2.3.2 and Figure 2-3). Table 2-3 presents the 6 studies that met these criteria and are considered suitable for quantitative TCDD dose-response modeling. As shown in Figure 2-4, only 2 of the available cancer bioassays did not meet EPA's study inclusion criteria (and are not summarized in Appendix D). These include Eastin et al. (1998) (genetically altered mouse strain) and Rao et al. (1988) (intraperitoneal injection instead of oral route of exposure).

A total of 751 animal bioassays on a noncancer endpoint were available for evaluation using EPA's study inclusion criteria (see Section 2.3.2 and Figure 2-3). As shown in Figure 2-4, 673 of the available noncancer studies were excluded based on one or more of the following reasons: (1) 66 studies used genetically-altered animals; (2) 370 studies had a lowest tested dose that was too high (i.e., greater than 30 ng/kg-day); (3) 142 studies tested chemicals that were not TCDD only or used an unspecified TCDD dose; and (4) 135 studies employed a nonoral dosing method. Table D-2 of Appendix D shows these studies and identifies the study inclusion criteria that were not met. For many studies, more than one reason for exclusion was found and identified. Conversely, in some cases, at least one identified criterion was not met, and, given the study was then excluded based on that one criterion, not all of the other criteria for exclusion were further evaluated and articulated. Tables 2-4 and D-1 of Appendix D present the 78 studies that were selected as key data sets for TCDD noncancer dose-response analyses.

In Section 4, additional evaluations are made to determine which study/endpoint data sets are the most appropriate for development of the RfD for TCDD. For further consideration in the RfD derivation process, only the toxicologically-relevant endpoints from the studies in Table 2-4 are carried forward to Section 4 (see Section 4.2.1 and Appendix H for details on study/endpoint combinations not used in RfD derivation for this reason). For some entries in Table 2-4, there are several publications from the peer-reviewed literature shown in the same row of the table. In these cases, the publications are grouped together because they are based on the same noncancer

[5] Standard EPA guidance was applied for adjustment of intermittent gavage protocols and dietary exposures as indicated in each specific study description in Appendix D.

animal bioassay. Additionally, in Table 2-4, the noncancer adverse effects in the animal studies listed under the heading, "endpoints examined," are presented as general categories of effects, such as "developmental effects," "liver effects," or "thyroid function." In Section 4, more detailed descriptors of the specific endpoints associated with such adverse health effects are articulated and evaluated to develop PODs for the derivation of an oral RfD for TCDD. Final candidate study/endpoint data sets are selected in Section 4 based on factors such as toxicological relevance of the endpoints, dose-response modeling results, and POD comparisons across studies, as illustrated in Figures 4-1 and 4-3 for epidemiologic and toxicological data, respectively.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Akhtar et al. (2004)	Mortality and incidence for all cancers and for site-specific cancers including prostate and melanoma	Vietnam 1962−1971	Ranch Hand (RH) cohort including 1,196 U.S. military males exposed by spraying Agent Orange during Vietnam war in Southeast Asia (SEA); comparison (C) cohort matched by age, race, and military occupation.	Cumulative serum lipid concentrations (CSLC) of TCDD based on serum levels collected from veterans in 1987, 1992, 1997, and a first-order kinetic model with a 7.6-year half-life. CSLC estimates for 1,009 RH cohort and 1,429 C cohort veterans.	CSLC (ppt-years) RH and C ≤2 yrs in SEA: All site Comparison ≤10 Low >10-118.5 High >118.5 Continuous (Log TCDD) Melanoma Comparison ≤10 Low >10-118.5 High >118.5 Continuous (Log TCDD) Prostate Comparison ≤10 Low >10-118.5 High >118.5 Continuous (Log TCDD)	 No.,% 34, 5.9 28, 9.8 22, 14.6 15, 8.6 No., % 3, 0.5 4, 1.4 4, 2.7 3, 1.7 No., % 7, 1.2 10, 3.5 6, 4.0 5, 2.9	 RR (95% CI) 1.0 1.44 (0.82−2.53) 2.23 (1.24−4.00) 2.02 (1.03−3.95) 1.24 (1.01−1.53) *p* = 0.04 1.0 2.99 (0.53-16.8) 7.42 (1.34-41.04) 7.51 (1.12-50.21) 2.24 (1.29-3.89) *p* = 0.004 1.0 1.5 (0.51-4.40) 2.17 (0.68-6.87) 6.04 (1.48-24.61) 1.48 (0.93-2.35)* *p* = 0.10	Adjusted for age at tour, military occupation, smoking, skin reaction to sun exposure, eye color, number of years in SEA. Also stratified analyses by year of tour of duty. Restricted to ≤2 years in SEA, white Air Force veterans, 0% and 100% time in Vietnam for RH and C Cohorts, respectively.	Used multiplicative Poisson regression models to compare cancer incidence and cancer mortality with national rates and proportional hazards models to contrast cohorts with regard to cancer incidence.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Becher et al. (1998)	Mortality from all cancers combined	Hamburg, Germany, production period was 1950−1984, and mortality follow-up extended through 1992	Boehringer cohort including approximately 1,189 workers employed in the production of herbicides.	CSLC of TCDD based on area under curve (in µg/kg years); back-extrapolation to date of last employment took into account age and percentage body fat; half-life value was 7.2 years.	Categorical exposures (Cox model) 0− <1 1− <4 4− <8 8− <16 16− <64 64+	124	RR (95% CI) 1.0 1.12 (0.70−1.80) 1.42 (0.70−2.85) 1.77 (0.81−3.86) 1.63 (0.73−3.64) 2.19 (0.76−6.29) *p* = 0.03	Available: year of entry, age of entry, duration of employment, birth cohort, β-HCH; TEQ other than TCDD.	Included in U.S. EPA (2003). A large number of models were fitted. These included models for 5 different latency intervals (0, 5, 10, 15, and 20 years), as well as multiplicative, additive, and power models, and different offset variables (person years and expected deaths).
					Continuous exposure TCDD (µg/kg years)	124	β = 0.0089, *p* = 0.0047	Available: year of entry, age of entry, duration of employment, birth cohort, β-HCH; TEQ other than TCDD.	

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Cheng et al. (2006)	Mortality from all cancers	USA, 1942–1993	NIOSH cohort including 3,538 occupationally exposed male workers at 8 plants in the United States; 256 cancer deaths.	CSLC of TCDD based on work histories, job-exposure matrix, and concentration and age-dependent two-compartment model of elimination kinetics.	No exposure categories provided	256 cancer deaths	The slope ( ) was 3.3×10^{-6} for lag of 15 years excluding upper 5% of TCDD exposures. The slopes ranged two orders of magnitude depending on modeling assumption.	Available: age, year of birth, and race. Risks adjusted for: year of birth, age, and race. Indirectly examined other potential confounders such as smoking and other occupational exposures.	Confounding by smoking was considered indirectly by analysis of smoking-related and smoking-unrelated cancers. Other occupational exposures were considered indirectly by repeated analyses removing one plant at a time. Based on indirect evaluation, there was no clear evidence of confounding.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Collins et al. (2009)	Mortality from all cancers and specific cancer types	Midland, MI, USA. Follow-up period: 1942–2003. Serum collection period: 2004–2005	Subset of NIOSH cohort including 1,615 occupationally exposed male workers at 1 plant in the United States; 177 cancer deaths.	CSLC of TCDD based on work histories, job-exposure matrix, and concentration and age-dependent two-compartment model of elimination kinetics. Serum samples were obtained from 280 former workers collected during 2004−2005.	Part per billion-year estimates of cumulative TCDD exposure	177 cancer deaths	The slope of a proportional hazards regression model for fatal soft tissue sarcoma was 0.05872 (95% CI not provided but for Chi-square $p = 0.0060$) for every 1-part per billion-year increase in cumulative exposure of TCDD. Slope estimates for all fatal cancers (0.00161, $p = 0.78$), fatal lung (-0.00173, $p = 0.89$), fatal prostate (0.01294, $p = 0.30$), fatal leukemias (-0.12822, $p = 0.34$), and fatal non-Hodgkin lymphomas (0.01081, $p = 0.68$) were not statistically significant.	Hazard ratios adjusted for age, year of birth, and hire year. Stratified analyses used to examine potential impact of pentachlorophenol exposure on mortality.	Confounding by smoking was not considered directly due to a lack of data. Relatively long follow-up period (average = 36 years). Potential outcome misclassification for soft tissue sarcoma due to potential inaccuracies on death certificates. Data analyzed from one plant reduces heterogeneity associated with multiplant analyses. More serum samples ($n = 280$) analyzed than used to derive TCDD estimates for other NIOSH cohort analyses.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Michalek and Pavuk (2008)	Cancer incidence, all sites combined	Vietnam 1962−1971	RH cohort including 1,196 U.S. military males exposed by spraying Agent Orange during Vietnam war in Southeast Asia (SEA); C cohort matched by age, race, and military occupation.	CSLC of TCDD based on serum levels collected from veterans in 1987, 1992, 1997, 2002, and a first-order kinetic model with a 7.6-year half-life. CSLC estimates for 986 RH cohort and 1,597 C cohort veterans.	CSLC (ppt-years) Results stratified by ≤1968, ≥30 days pre-1967, ≤2 yrs in SEA: Comparison ≤10 Low >10-91 High >91	Continuous ex-posure: Log (TCDD) No.,% 67, 12.6 Cate-gorical TCDD No., % 30, 11.2 10, 8.3 12, 24.5 15, 16.1	1.4 (1.1-1.7) $p = 0.005$ RR (95% CI) 1.0 0.5 (0.2−1.1) 1.7 (0.8−3.5) 2.2 (1.1−4.4).	Cox regression proportional hazards models adjusted for year of birth, eye color, race, smoking, body mass index at the qualifying tour, military occupation, and skin reaction to sun exposure. Also stratified analyses by years of service in SEA, days of herbicide spraying, calendar period of service.	Without stratification, there was no significant increase in the risk of cancer with log(TCDD) in the combined cohort.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Ott and Zober (1996)	Mortality and incidence for all cancers combined, as well as for specific cancer sites	Ludwig-shafen, Germany, 1954−1992	BASF cohort, 243 men exposed from accidental release that occurred in 1953 during production of trichlorophenol, or who were involved in clean-up activities.	CSLC of TCDD expressed in µg/kg based on TCDD half-life of 5.1−8.9 years, Cox regression model.	Internal comparisons based on continuous measure of TCDD. External comparisons exposure categories (for malignant neoplasms): <0.1, 0.1−0.99 1.0−1.99 >2 µg/kg	*Internal cohort analysis* 31 All cancer deaths 47 All incident cancers *External cohort analyses* Deaths 8 8 8 7	RR (95% CI) 1.22 (95% CI: 1.00−1.50) 1.11 (95% CI: 0.91−1.35) SMR (95% CI) 0.8 (0.4−1.6) 1.2 (0.5−2.3) 1.4 (0.6−2.7) 2.0 (0.8−4.0)	Available: age, BMI, smoking status, and history of occupational exposure to aromatic amines and asbestos.	Included in U.S. EPA (2003) Positive associations noted for digestive cancer, but not for respiratory cancer. Association between TCDD and increased SMRs found only among current smokers. Last published account of this cohort.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Steenland et al. (2001)	Mortality from all cancers	USA, 1942–1993	NIOSH cohort including 3,538 male workers, 256 cancer deaths.	CSLC of TCDD based on work histories, job-exposure matrix, and a simple one-compartment, first-order pharmacokinetic elimination model with 8.7-year half-life.	CSLC (ppt-years) <335 335−520 520−1,212 1,212−2,896 2,896−7,568 7,568−20,455 20,455	 64 29 22 30 31 32 48	RR (95% CI) 1.00 1.26 (0.79−2.00) 1.02 (0.62−1.65) 1.43 (0.91−2.25) 1.46 (0.93−2.30) 1.82 (1.18−2,82) 1.62 (1.03−2,56)	Available: date of birth and age. Adjusted for date of birth, and age was used as time scale in Cox model.	Included in U.S. EPA (2003)

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Warner et al. (2002)	Breast cancer incidence	Italy 1976–1998	981 women from Zones A and B with available archive serum samples, 15 breast cancer cases.	CSLC of TCDD (ppt) collected between 1976 and 1981. For most samples collected after 1977, serum TCDD levels were back-extrapolated using a first-order kinetic model with a 9-year half-life.	Categorical <20 ppt 20.1−44 ppt 44.1−100 ppt >100 ppt Continuous (Log_{10}TCDD)	Cases 1 2 7 5 15	RR (95% CI) 1.0 1.0 (0.1−10.8) 4.5 (0.6−36.8) 3.3 (0.4−28.0) p = 0.07 2.1 (1.0−4.6)	Available: gravidity, parity, age at first pregnancy, age at last pregnancy, lactation, family history of breast cancer, age at menarche, current body mass index, oral contraceptive use, menarcheal status at explosion, menopause status at diagnosis, height, smoking, alcohol consumption. Adjusted for age, which was used as time scale in Cox model; other covariates were evaluated but were not identified as confounders.	Included in U.S. EPA (2003)

Table 2-2. Epidemiologic studies selected for TCDD noncancer dose-response modeling

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Alaluusua et al. (2004)	Dental defects	Seveso, Italy, Dental exams administered in 2001 among those exposed to TCDD in 1976	65 subjects <9.5 years old at time of Seveso explosion and residing in Zones ABR (i.e., the most heavily contaminated area in decreasing order); 130 subjects recruited from the non-ABR region (i.e. the unexposed).	Serum TCDD (ng/kg) from 1976 samples for those who resided in Zones ABR; no serum levels for non-ABR residents (unexposed). TCDD exposure represent levels as of 1976 (after accident).	Non-ABR Zone 31−226 ng/kg 238−592 ng/kg 700−26,000 ng/kg <5 years of age at time of accident Non-ABR Zone or 31−226 ng/kg serum TCDD 238−26,000 ng/kg serum TCDD	10 1 5 9 25	Dental defect % 26% 10% 45% 60% *p*-value = 0.016 33% *p*-value = 0.0009 Odds Ratios (95% CI) (among those <5 years of age at time of accident) 1.0 2.4 (1.3−4.5) *p*-value = 0.007	Available: medical history, age, sex, education, smoking.	Dose-response pattern observed with dental defects in the ABR zone; however, the control population had a much higher prevalence of dental defects (26%) than those in the lowest exposure group (10%). Also assessed hypodontia and other dental and oral aberrations, but these were too rare to allow modeling by ABR zone.

Table 2-2. Epidemiologic studies selected for TCDD noncancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Baccarelli et al. (2008)	b-TSH measured 72 hours after birth from a heel pick (routine screening for all newborns in the region)	Italy, 1976; children, 1994–2005	*Population-based study:* 1,041 singletons (56 from Zone A, 425 from Zone B, and 533 from reference) born between Jan. 1, 1994-June 30, 2005. *Plasma dioxin study:* 51 children born to 38 women of fertile age who were part of the Seveso Chloracne Study.	Based on zone of residence, estimated mean values from a previous study. Maternal plasma TCDD levels estimated at the date of delivery using a first-order pharmacokinetic model and elimination rate estimated in Seveso women (half-life = 9.8 years).	*Population-based study:* Reference Zone A Zone B *Plasma dioxin study:* Continuous maternal plasma TCDD	 533 births 56 births 425 births	*Population-based study* Geometric Mean b-TSH (log-transformed) Reference: 0.98 (95% CI: 0.90−1.08) Zone B: 1.66 (95% CI: 1.19−2.31) Zone A: 1.35 (95% CI: 1.22−1.49) Association between neonatal b-TSH with plasma TCDD: adjusted = 0.75 ($p < 0.001$)	Available: gender, birth weight, birth order, maternal age at delivery, hospital, type of delivery. There was limited evidence of confounding, so mean TSH results presented here are unadjusted.	An association with serum TCDD levels of mothers was found with b-TSH among the 51 births in the plasma dioxin study.

Table 2-2. Epidemiologic studies selected for TCDD noncancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Eskenazi et al. (2002b)	Menstrual cycle characteristics: menstrual cycle length.	Seveso, Italy, follow-up interview conducted in 1996-1997 of women exposed to TCDD in the 1976 accident	Women who were <40 years from Zones A or B in 1976.	Serum TCDD (ng/kg) from 1976 samples. TCDD exposure level was back-extrapolated to 1976 using the Filser or the first-order kinetic models.	Interquartile range was 64−322 ppt TCDD examined as continuous measure (per 10-fold increase in serum levels).		Lengthening of the menstrual cycle by 0.93 days (95% CI: -0.01, 1.86)	Interview data: medical history, personal habits, work history, reproductive history, age, smoking, body mass index, alcohol and coffee consumption, exercise, illness, abdominal surgeries.	A positive association between menstrual cycle length and serum TCDD was found among women who were premenarcheal at the time of accident (n = 134).

Table 2-2. Epidemiologic studies selected for TCDD noncancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Mocarelli et al. (2008)	Sperm conc. (million/mL) Progressive motility (%) Serum E_2 (pmol/L)	Italy, 1976, 1998	Among the 257 exposed (from Zone A), men 1−26 in 1976 with serum levels <2000 ppt in 1976, 135 (53%) were included. Among the 372 nonexposed invitees, 184 (49%) men aged 1−26 in 1976 were included.	Serum TCDD (in ppt) from 1976−1977 samples (for exposed men); background values were assumed for unexposed men based on serum analysis of residents in uncontaminated areas.	Median serum TCDD levels (in ppt) by quartile for men aged 1−9 in 1976 (68; 142; 345; 733 ppt)		Men exposed between the ages 1−9 had reduced semen quality 22 years later. Reduced sperm quality included decreases in sperm count (p = 0.025), progressive sperm motility (p = 0.001), and total number of motile sperm (p = 0.01) relative to the comparison group.	Available: age, abstinence time, smoking status, education, alcohol use, maternal smoking during pregnancy, employment status, BMI, chronic exposure to solvents and other toxic substances. Adjusted for smoking status, organic solvents, age at time of tests, BMI, alcohol use, education, employment status, and abstinence (days) for sperm data. Hormone data not adjusted for education level, employment status, and abstinence time.	Results stratified by timing of exposure (1−9 yrs old vs. 10−17 yrs old in 1976).

Table 2-3. Animal bioassays selected for cancer dose-response modeling

Reference	Species/strain	Sex exposure route/duration	*n*	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)
Della Porta et al. (1987)	Mouse/ $B6C3F_1$	Male/female Oral gavage once per week; 52 weeks	~40 to 50 in each dose group including controls	0, 351, and 714	Females and males: hepatocellular adenomas and carcinomas	Liver: adenomas and carcinomas in females and carcinomas in males (using incidental tumor statistical test)
Kociba et al. (1978); Goodman and Sauer (1992)	Rat/Sprague-Dawley	Male/female Oral-lifetime feeding; 2 years	50 each (86 each in vehicle control group)	0, 1, 10, or 100	Females: liver, lung, oral cavity Males: adrenal, oral cavity, tongue	Adrenal cortex: adenoma Liver: hepatocellular adenoma(s) or carcinoma(s); hyperplastic nodules Lung: keratinizing squamous cell carcinoma Oral cavity: stratified squamous cell carcinoma of hard palate or nasal turbinates Tongue: stratified squamous cell carcinoma
NTP (1982c)	Mouse/ $B6C3F_1$	Male/female Oral-gavage twice per week; 104 weeks	50 each (75 each in vehicle control group)	0, 1.4, 7.1, or 71 for males; 0, 5.7, 28.6, or 286 for females	Females: hematopoietic system, liver, subcutaneous tissue, thyroid Males: liver, lung	Hematopoietic system: lymphoma or leukemia Liver: hepatocellular adenoma or carcinoma Lung: alveolar/bronchiolar adenoma or carcinoma Subcutaneous tissue: fibrosarcoma Thyroid: follicular-cell adenoma
NTP (1982c)	Rat/Osborne-Mendel	Male/female Oral-gavage twice per week; 104 weeks	50 each (75 each in vehicle control group)	0, 1.4, 7.1, or 71	Females: adrenal, liver, subcutaneous tissue, thyroid Males: adrenal, liver, thyroid	Adrenal: cortical adenoma, or carcinoma or adenoma, NOS Liver: neoplastic nodule or hepatocellular carcinoma Subcutaneous tissue: fibrosarcoma Liver: neoplastic nodule or hepatocellular carcinoma Thyroid: follicular-cell adenoma or carcinoma

Table 2-3. Animal bioassays selected for cancer dose-response modeling (continued)

Reference	Species/strain	Sex exposure route/duration	*n*	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)
NTP (2006a)	Rat/Harlan Sprague-Dawley	Female Oral-gavage 5 days per week; 2 years	53 or 54	0, 2.14, 7.14, 15.7, 32.9, or 71.4	Liver Lung Oral mucosa Pancreas	Liver: hepatocellular adenoma Liver: cholangiocarcinoma Lung: cystic keratinizing epithelioma Oral mucosa: squamous cell carcinoma Pancreas: adenoma or carcinoma
Toth et al. (1979)	Mouse/ Outbred Swiss/H/Riop	Male Gastric intubation once per week; 1 year	43 or 44 (vehicle control group = 38)	0, 1, 100, or 1,000	Liver	Liver: tumors

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Reproductive toxicity studies									
Bowman et al.(1989a; 1989b); Schantz and Bowman (1989); Schantz et al. (1992; 1986)	Monkey/ Rhesus	Daily dietary exposure in female monkeys (3.5−4 years)	F (F0, F1, F2, F3)	3 to 7 (F1)	0, 0.12, or 0.67	None	0.12	Reproductive and developmental effects	Neurobehavioral effects (e.g., discrimination-reversal learning affected)
Franc et al. (2001)	Rat/Sprague-Dawley, Long-Evans, Han/Wistar	Biweekly oral gavage (22 weeks)	Female	8	0, 10, 30 or 100	10	30	Body weight, relative liver weight, relative thymus weight	Increased relative liver weight in Sprague-Dawley and Long-Evans Rats; Increased relative thymus weight in Sprague-Dawley, Han/Wistar, and Long-Evans Rats
Hochstein et al (2001)	Mink	Daily dietary exposure (132 days)	F	12	0.03 (control), 0.8, 2.65, 9, or 70	None	2.65	Reproductive effects	Reduced kit survival
Hutt et al. (2008)	Rat/Sprague-Dawley	Oral gavage (GDs 14 and 21, postpartum days 7 and 14), (Pups: once per week for 3 months)	Female (F0 and F1)	3 (F0 and F1)	0 or 7.14	None	7.14	Developmental effects	Lower proportion of morphologically normal pre-implantation embryos during compaction stage

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Reproductive toxicity studies (continued)									
Ikeda et al. (2005)	Rat/ Holtzman	Corn oil gavage (initial loading dose followed by weekly dose during mating, pregnancy, and lactation–about 10 weeks)	F (F0) F and M (F1 and F2)	12 (F0) Not specified (F1 and F2)	0 or 16.5	None	16.5 (maternal exposure)	Reproductive and developmental effects	Decreased development of the ventral prostrate (F1), decreased sex ratio (percentage of males) (F2)
Ishihara et al. (2007)	Mouse/ICR	Sesame oil gavage (initial loading dose followed by weekly doses for 5 weeks)	M (F0)	42 or 43	0, 0.095, or 950	0.1	100	Reproductive effects	Decreased male/female sex ratio (percentage of males) (F1)
Latchoumy-candane and Mathur (2002) and related Latchoumy-candane et al. (2003, 2002a; 2002b)	Rat/Wistar albino	Olive oil gavage (daily for 45 days)	M	6	0, 1, 10, or 100	None	1	Reproductive effects	Reduced sperm production, decreased reproductive organ weights

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Reproductive toxicity studies (continued)									
Murray et al. (1979)	Rat/Sprague-Dawley	Daily dietary exposure (3 generations)	F and M, (F0) F and M, (F1 and F2)	10–32 (F0) 22 (F1) 28 (F2)	0, 1, 10, or 100	1	10	Reproductive and developmental effects	Decrease in fertility, decrease in the number of live pups, decrease in gestational survival; decrease in postnatal survival, decreased postnatal body weight in one or more generations
Shi et al. (2007)	Rat/Sprague-Dawley	Maternal corn oil gavage (weekly on GDs 14 and 21; PNDs 7 and 14) Offspring corn oil gavage (weekly for 11 months)	F (F0) F (F1)	3 (F0) 10 (F1)	0, 0.14, 0.71, 7.14, or 28.6	0.14	0.71	Reproductive effects	Decrease serum estradiol levels (F1)
Yang et al. (2000)	Rhesus monkey/ Cynomolgus	Fed gelatin capsules (5 days/week for 12 months)	F	6 (treatment) 5 (controls)	0, 0.71, 3.57, or 17.86	17.86	None	Endometriosis effects	Increased endometrial implant survival, increased maximum and minimum implant diameters, growth regulatory cytokine dysregulation

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Developmental toxicity studies									
Amin et al. (2000)	Rat/Harlan Sprague-Dawley	Corn oil gavage (GDs 10−16)	F (F0)	80−88 (F1)	0, 25, or 100	None	25	Developmental effects	Decreased preference in the consumption of 0.25% saccharin solution (F1)
Bell et al. (2007b)	Rat/CRL:WI (Han)	Maternal daily dietary exposure for an estimated 20 weeks (12 weeks prior to mating through parturition)	F (F0) M (F1)	65 (F0 treatments) 75 (F0 controls) at study initiation; following interim sacrifice ~30 animals were allowed to litter; F1 on PND 21 was ~7	0, 2.4, 8, or 46	None	2.4 (maternal exposure)	Reproductive and developmental effects	Delayed BPS (F1)
Franczak et al. (2006)	Rat/Sprague-Dawley	Maternal corn oil gavage (GDs 14 and 21; PNDs 7 and 14) Offspring corn oil gavage (weekly for 8 months)	F (F0 and F1)	2 or 3 (F0) 7 (F1)	0, 7.14, or 28.6	None	7.14	Developmental effects	Decreased serum estradiol levels (F1)

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Developmental toxicity studies (continued)									
Hojo et al. (2002) and related Zareba et al. (2002)	Rat/Sprague-Dawley	Maternal single corn oil gavage (GD 8) Offspring exposed during gestation and lactation (35 days)	F (F0) F and M (F1)	12 (F0) 50 or 60 (F1)	0, 20, 60, or 180	None	20 (maternal exposure)	Developmental effects	Abrogation of sexually dimorphic neuro-behavioral responses (F1)
Kattainen et al. (2001)	Rat/ Han/Wistar and Long-Evans	Maternal single corn oil gavage (GD 15)	F (F0) F and M (F1)	4 to 8 (F0) 3F/3M per treatment group (F1)	0, 30, 100, 300, or 1,000	None	30 (maternal exposure)	Developmental effects	Reduced mesiodistal length of the lower third molar (F1)
Keller et al. (2008a; 2008b; 2007)	Mouse/ C57BL/6J, BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J	Maternal single corn oil gavage (GD 13)	F (F0) F and M (F1a, b, c)	Dams not specified (F0); 23−36 (F1a); 4−5 (F1b); 107−110 (F1c)	0, 10, 100, or 1,000	None	10 (maternal exposure)	Developmental effects	Variation in M1 morphology in C57BL/10J males and females (F1a); decreased mandible shape and size in C3H/HeJ males (F1b); variation in molar shape in C3H/HeJ males (F1c) (2008a; 2008b; 2007)

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Developmental toxicity studies (continued)									
Kuchiiwa et al. (2002)	Mouse/ddY	Maternal olive oil gavage (weekly for 8 weeks prior to mating)	F (F0) M (F1)	7 (F0) 3 (F1 immuno-cytochemical analysis) 6 (F1 cell number count)	0, 0.7, or 70	None	0.7 (LOEL) (maternal exposure)	Neurotoxicity	Decreased serotonin-immunoreactive neurons in raphe nuclei of male offspring (F1)
Li et al. (2006)	Mouse/NIH (pregnant and pseudo-pregnant)	Maternal sesame oil gavage daily for 8 days (GDs 1−8)	F	10	0, 2, 50, or 100	None	2	Developmental effects	Decreased progesterone and increased serum estradiol levels
Markowski et al. (2001)	Rat/Holtzman	Maternal single olive oil gavage (GD 18)	F (F0 and F1)	4−7 (F0 and F1)	0, 20, 60, or 180	None	20 (maternal exposure)	Behavioral effects	Decreased training responses (F1)
Miettinen et al. (2006)	Rat/Line C	Maternal single corn oil gavage (GD 15)	F (F0) F and M (F1)	24−32 (treatment) 12−48 (controls)	0, 30, 100, 300, or 1,000	None	30 (maternal exposure)	Developmental effects	Increase in dental caries (F1)
Nohara et al. (2000)	Rat/ Holtzman	Maternal single corn oil gavage (GD 15)	F (F0) M (F1)	Not specified (F0) 5 males and 3 females (F1)	0, 12.5, 50, 200, or 800	800 (maternal exposure)	None	Immunotoxicity	Decreased spleen cellularity (F1)
Ohsako et al. (2001)	Rat/ Holtzman	Maternal single corn oil gavage (GD 15)	F (F0) M (F1)	6 (F0) 5 males and 3 females (F1)	0, 12.5, 50, 200, or 800	12.5 (maternal exposure)	50 (maternal exposure)	Developmental effects	Decreased anogenital distance (F1)

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Developmental toxicity studies (continued)									
Schantz et al. (1996)	Rat/Harlan Sprague-Dawley	Maternal corn oil gavage (GDs 10−16	F(F0)	~4 (F0); 80−88 (F1)	0, 25, or 100	None	None	Developmental effects	Facilitatory effect on radial arm maze learning (F1)
Seo et al. (1995)	Rat/Sprague-Dawley	Maternal corn oil gavage (GDs 10−16)	F and M (F1)	~15 (F0); 5−9 (F1)	0, 25, or 100	25	100	Developmental effects	Decreased thymus weight
Simanainen et al. (2004a)	Rat/TCDD-resistant Han/Wistar bred with TCDD-sensitive Long-Evans	Maternal corn oil gavage (GDs 15)	F (F0) M (F1)	5−8 (F0)	0, 30, 100, 300, or 1,000	100	300	Reproductive effects	Reduction in daily sperm production and cauda epididymal sperm reserves
Sparschu et al. (1971)	Rat/Sprague-Dawley	Maternal corn oil gavage (GDs 6-15)	F (F0)	31 (controls) 10-14 (F0)	0, 30, 125, 500, 2,000, or 8,000	50	125	Maternal toxicity; Developmental effects	Decreased body weight in dams and male fetuses; fetal intestinal hemorrhage and subcutaneous edema
Smith et al. (1976)	Mouse/CF-1	Maternal corn oil gavage (GDs 6-15)	F (F0)	14-41 (F0)	0, 1.0, 10, 100, 1,000, or 3,000	1,000 (maternal) 100 (fetal)	3,000 (maternal) 1,000 (fetal)	Teratogenic and developmental effects	Increased relative liver weight (F0 dams); increased incidence of cleft palate (fetuses)

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Developmental toxicity studies (continued)									
Sugita-Konishi et al. (2003)	Mouse/C57/6 NCji	Maternal drinking water exposure (daily for 17-day lactational period)	F (F0) F and M (F1)	8 (F0) Not specified (F1)	0, 1.14, or 11.3	1.14 (NOEL) (maternal exposure)	11.3 (LOEL) (maternal exposure)	Immunotoxicity	Increased susceptibility to *Listeria* (F1 males and females); increase in thymic CD4+ cells (F1 males); decreased spleen weight (F1 males)
Acute toxicity studies									
Burleson et al. (1996)	Mouse/B6C3 F_1	Corn oil gavage (single exposure)	F	20	0, 1, 5, 10, 50, 100, or 6,000	5	10	Immunotoxicity	Increased mortality from influenza infection 7 days after a single TCDD exposure
Crofton et al. (2005)	Rat/Long-Evans	Corn oil gavage (4 consecutive days)	F	14, 6, 12, 6, 6, 6, 6, 6, 6, and 4, respectively, in control and treated groups	0, 0.1, 3, 10, 30, 100, 300, 1,000, 3,000, or 10,000	30	100	Thyroid effects	Reduction in serum T4 levels
Kitchin and Woods (1979)	Rat/Sprague-Dawley	Corn oil gavage (single dose)	F	4 (treated); 9 (control)	0, 0.6, 2, 4, 20, 60, 200, 600, 2,000, 5,000, or 20,000	0.6 (NOEL)	2 (LOEL)	Enzyme induction	Increased benzo(a)pyrene hydroxylase (BPH)

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Acute toxicity studies (continued)									
Li et al. (1997)	Rat/Sprague-Dawley	Corn oil dose via oral gastric intubation (single dose)	F	10	0, 3, 10, 30, 100, 300, 1,000, 3,000, 10,000, or 30,000	3	10	Hormonal effects	Increased serum FSH (1997)
Lucier et al. (1986)	Rat/Sprague-Dawley	Corn oil gavage or TCDD-contaminated soil (single dose)	F	6	0, 15, 40, 100, 200, 500, 1,000, 2,000, or 5,000 in corn oil 0, 15, 44, 100, 220, 500, 1,100, 2,000, or 5,500 in contaminated soil	None	15 (LOEL)	Enzyme induction	Induction of aryl hydrocarbon hydroxylase (at low dose in both treatment protocols)
Nohara et al. (2002)	Mouse/ B6C3F$_1$, BALB/c, C57BL/6N and DBA2	Corn oil gavage (single dose)	M, F	10−40	0, 5, 20, 100, or 500	500	None	Mortality and body-weight changes	No increased mortality of virus-infected mice or treatment-related changes in body weight
Simanainen et al. (2002)	Rat/TCDD-resistant Han/Wistar bred; TCDD-sensitive Long-Evans	Corn oil gavage (single dose)	M, F	9−11	30–100,000	100	300	General toxicological endpoints, organ weights, dental defects	Reduction in serum T4 levels

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Acute toxicity studies (continued)									
Simanainen et al. (2003)	Rat/TCDD-resistant Han/Wistar bred with TCDD-sensitive Long-Evans	Corn oil gavage (single dose)	M, F	5−6	Line A: 30−3,000,000 Line B: 30−1,000,000 Line C: 30−100,000	100	300	General toxicological endpoints, organ weights, dental defects	Decreased thymus weight
Smialowicz et al. (2004)	Mouse/ C57BL/6N CYP1A2 (+/+) wild-type	Corn oil gavage (single dose)	F	Not specified	0, 30, 100, 300, 1,000, 3,000, or 10,000	300	1,000	Immunotoxicity	Decreased antibody response to SRBCs
Vanden Heuvel et al. (1994)	Rat/Sprague-Dawley	Corn oil gavage (single dose)	F	5−15	0, 0.05, 0.1, 1, 10, 100, 1,000, or 10,000	0.1 (NOEL)	1 (LOEL)	Liver effects	Increase in hepatic EROD activity and CYP1A1 mRNA levels

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Acute toxicity studies (continued)									
Weber et al. (1995)	Inbred Mouse/ C57BL/6	Corn oil gavage (single dose on Day 0) Sacrificed on Day 8	M	4-7	0, 30, 100, 300, 1,000, 3,000, 9,400, 37,500, 75,000, 100,000, 133,00, or 235,000	1,000	3,000	Hepatic and renal enzyme and hormone alterations; liver and kidney weight	Increased relative liver weight
	Inbred Mouse/ DBA/2	Corn oil gavage (two doses on Days -1 and 0) Sacrificed on Day 8	M	4-7	0, 1,000, 10,000, 97,500, 375,000, 1,500,000, 1,950,000, or 3,295,000	10,000	97,500		
Subchronic toxicity studies									
Chu et al. (2001)	Rat/Sprague-Dawley	Corn oil gavage (daily for 28 days)	F	5	0, 2.5, 25, 250, or 1,000	250	1,000	Body- and organ-weight changes	Decreased body weight, increased relative liver weight and related biochemical changes, decreased relative thymus weight
Chu et al. (2007)	Rat/Sprague-Dawley	Corn oil gavage (daily for 28 days)	F	5	0, 2.5, 25, 250, or 1,000	2.5	25	Liver effects	Alterations in thyroid, thymus, and liver histopathology

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Subchronic toxicity studies (continued)									
DeCaprio et al. (1986)	Guinea pig/ Hartley	Daily dietary exposure (90 days)	M, F	10/sex	0, 0.12, 0.61, 4.9, or 26 (males); 0, 0.12, 0.68, 4.86, or 31 (females)	0.61	4.9	Body- and organ-weight changes	Decreased body weight (male and females); increased relative liver weights (males); decreased relative thymus weight (males)
DeVito et al. (1994)	Mice/B6C3F_1	Corn oil gavage (5 days/week for 13 weeks)	F	5	0, 1.07, 3.21, 10.7, 32.1, or 107	None	1.07 (LOEL)	Body- and organ-weight changes; enzyme induction	Increased EROD, ACOH and phosphotyrosyl proteins at all doses
Fattore et al. (2000)	Rat/Iva:SIV 50-Sprague-Dawley	Daily dietary exposure (13 weeks)	M, F	6	0, 20, 200, or 2,000	None	20	Liver effects	Reduced hepatic vitamin A levels
		Daily dietary exposure (13 weeks)	M, F	6	0 or 200				
		Daily dietary exposure (13 weeks)	M, F	6	0, 200, or 1,000				
		Daily dietary exposure (13 weeks, 26, and 39 weeks)	F	6	0 or 100				

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Subchronic toxicity studies (continued)									
Fox et al. (1993)	Rat/Sprague-Dawley	Gavage loading/ maintenance doses (every 4 days for 14 days)	M, F	6	0, 0.55, 307, or 1,607	0.57	327	Body- and liver-weight changes; hepatic cell proliferation	Increased absolute and relative liver weight
Hassoun et al. (1998)	Mouse/ $B6C3F_1$	Corn oil gavage (5 days/week for 13 weeks)	F	Not specified	0, 0.32, 1.07, 10.7, or 107	None	0.32 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses
Hassoun et al. (2000)	Rat/Harlan Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	F	6	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14 (LOEL)	Liver and brain effects	Induction of biomarkers of oxidative stress at all doses in liver and brain
Hassoun et al. (2003)	Rat/Harlan Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	F	12	0, 7.14, 15.7, or 32.9	None	7.14 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Subchronic toxicity studies (continued)									
Kociba et al. (1976)	Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	M, F	12	0, 0.71, 7.14, 71.4, or 714	7.14	71.4	Liver effects, body-weight changes, and hematologic and clinical effects	Reduced body weight and food consumption, slight liver degeneration, lymphoid depletion, increased urinary porphyrins and delta aminolevulinic acid, increased serum alkaline phosphatase and bilirubin
Mally and Chipman (2002)	Rat/F344	Corn oil gavage (2 days/week for 28 days)	F	3	0, 0.71, 7.14, or 71.4	None	0.71 (LOEL)	Clinical signs and histopathology	Decreased Cx32 plaque number and area in the liver
Slezak et al. (2000)	Mouse/ $B6C3F_1$	Corn oil gavage (5 days/week for 13 weeks)	F	Not specified	0, 0.11, 0.32, 1.07, 10.7, or 107.14	1.07 (NOEL)	10.7 (LOEL)	Liver, lung, kidney, and spleen effects	Increased hepatic superoxide anion
Smialowicz et al. (2008)	Mouse/ $B6C3F_1$	Corn oil gavage (5 days/week for 13 weeks)	F	8−15	0, 1.07, 10.7, 107, or 321	None	1.07	Immunotoxicity and organ weight	Reduced antibody response to SRBC, increased relative liver weight
Van Birgelen et al. (1995a; 1995b)	Rat/Sprague-Dawley	TCDD in diet (13 weeks)	F	8	0, 14, 26, 47, 320, or 1,024	None	14	Multiple end-points	Decreased absolute and relative thymus weights, decreased liver retinoid levels

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Subchronic toxicity studies (continued)									
Vos et al. (1973)	Guinea pig/ Hartley	Corn oil gavage (weekly for 8 weeks)	F	10	0, 1.14, 5.71, 28.6, or 143	1.14	5.71	Immunotoxicity	Decreased total leukocytes and lymphocyte count, decreased absolute thymus and weight, increase in primary serum tetanus antitoxin
White et al. (1986)	Mouse/ $B6C3F_1$	Corn oil gavage (daily for 14 days)	F	6−8	0, 10, 50, 100, 500, 1,000, or 2,000	None	10	Immunotoxicity	Reduction of serum complement activity
Chronic toxicity studies									
Cantoni et al. (1981)	Rat/CD-COBS	Corn oil gavage (weekly for 45 weeks)	F	4	0, 1.43, 14.3, or 143	None	1.43	Hepatic porphyria	Increased urinary porphyrin excretion
Croutch et al. (2005)	Rat/Sprague-Dawley	Loading/ maintenance dose (every 3 days for different durations up to 128 days)	F	5	0, 0.85, 3.4, 13.6, 54.3, or 217 (28-day duration)	54.3 (28-day duration)	217 (28-day duration)	Body-weight changes and changes in PEPCK activity and IGF-I levels	Decreased body weight, decreased PEPCK activity, and reduced IGF-I levels
Hassoun et al. (2002)	Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 30 weeks)	F	6	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Chronic toxicity studies (continued)									
Hong et al. (1989)	Rhesus monkeys.	Daily dietary (4 years)	F	7-8	0, 0.12, or 0.67	None	None	Immunotoxic effects	None
Kociba et al. (1978)	Rat/Sprague-Dawley	Daily dietary exposure (2 years)	M, F	50	0, 1, 10, or 100	1	10	Multiple endpoints measured	Increased urinary porphyrins, hepatocellular nodules, and focal alveolar hyperplasia
Maronpot et al. (1993)	Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 3.5, 10.7, 35, or 125	10.7	35	Body- and organ-weight changes, clinical chemistry, hepatocellular proliferation	Increased relative liver weight
NTP (1982c)	Mouse/ $B6C3F_1$; Rat/Osborne Mendel	Corn oil gavage (2 days/week for 104 weeks)	M, F	50	0, 1.4, 7.1, or 71 for rats and male mice; 0, 5.7, 28.6, or 286 for female mice	None	1.4	Liver and body-weight changes	Increased incidences of liver lesions in mice (males and females)
NTP (2006a)	Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 105 weeks)	F	53	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14	Liver and lung effects	Increased absolute and relative liver weights, increased incidence of hepatocellular hypertrophy, increased incidence of alveolar to bronchiolar epithelial metaplasia

Table 2-4. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Chronic toxicity studies (continued)									
Sewall et al. (1993)	Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 3.5, 10.7, 35, or 125	None	3.5 (LOEL)	EGFR kinetics and auto-phosphorylation, hepatocellular proliferation	Decrease in EGFR maximum binding capacity
Sewall et al. (1995)	Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 0.1, 0.35, 1, 3.5, 10.7, 35, or 125	10.7	35	Thyroid function	Decreased serum T^4 levels
Toth et al. (1979)	Mouse/Swiss/ H/Riop	Sunflower oil gavage (weekly for 1 year)	M	38−44	0, 1, 100, or 1,000	None	1	Skin effects	Dermal amyloidosis and skin lesions
Tritscher et al. (1992)	Rat/Sprague-Dawley	Initiated with i.p. injection of diethylnitrosamine (175 mg/kg) or saline, followed 2 weeks later by biweekly TCDD in corn oil gavage (30 weeks)	F	At least 9 per group	3.5, 10.7, 35.7, or 125	None	None	CYP induction	None

ND = not determined.

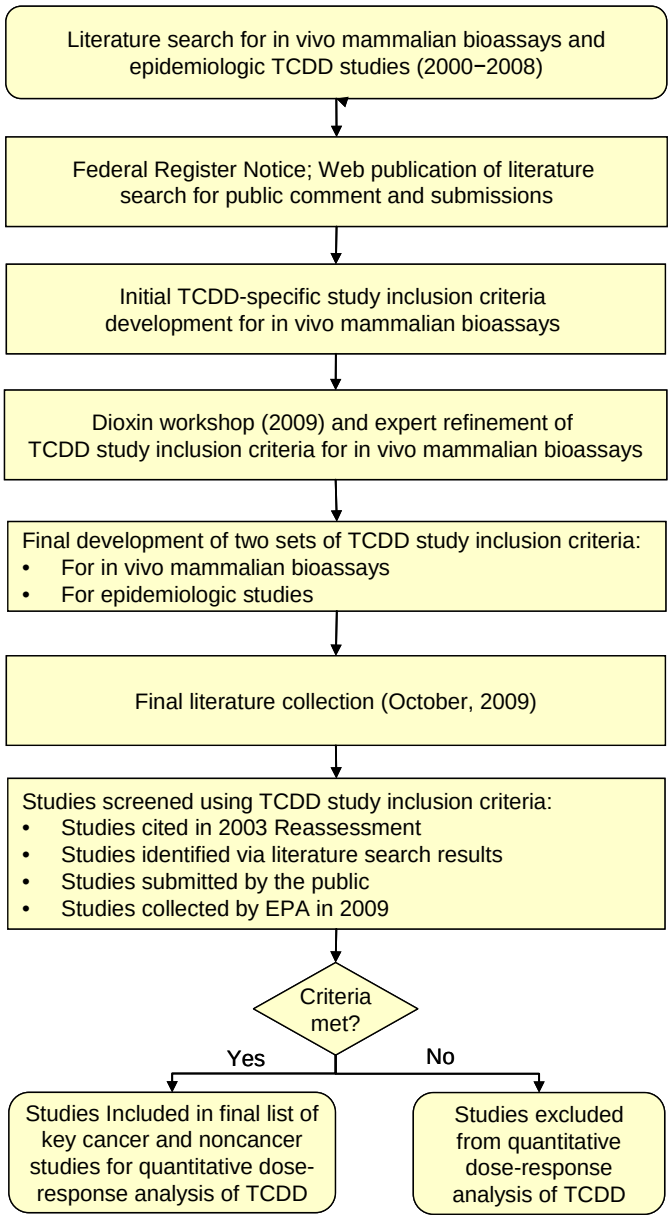


Figure 2-1. EPA's process to select and identify in vivo mammalian and epidemiologic studies for use in the dose-response analysis of TCDD.
EPA first conducted a literature search to identify studies published since the 2003 Reassessment. Results were published, and additional study submissions were accepted from the public. Next, EPA developed TCDD-specific study inclusion criteria for in vivo mammalian studies and held a Dioxin Workshop where these criteria were discussed and refined. Third, EPA developed two final sets of study inclusion criteria, one for in vivo mammalian studies and another for epidemiologic studies. Finally, EPA applied these two sets of criteria to all studies from the literature search, public submissions, 2003 Reassessment, and additional studies identified by EPA after the Dioxin Workshop through October 2009. The studies that met these criteria formed a list of key studies for EPA's consideration in TCDD dose-response assessment.

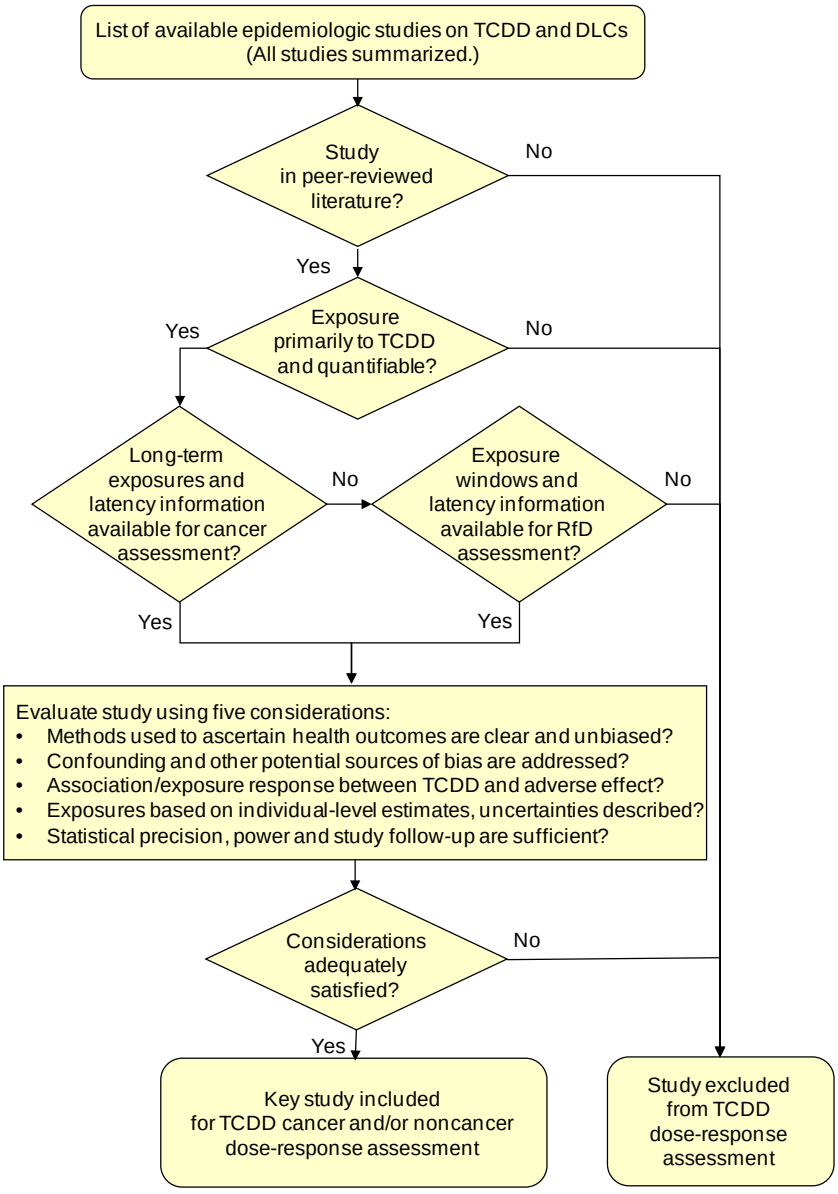


Figure 2-2. EPA's selection process to evaluate available epidemiologic studies using study inclusion criteria and other epidemiologic considerations for use in the dose-response analysis of TCDD.

EPA applied its TCDD-specific epidemiologic study inclusion criteria to all studies published on TCDD and DLCs. For all peer-reviewed studies, EPA examined whether the exposures were primarily to TCDD and if the TCDD exposures could be quantified so that dose-response analyses could be conducted. Then, EPA required that the effective dose and oral exposure be estimable: (1) for cancer, information is required on long-term exposures, (2) for noncancer, information is required regarding the appropriate window of exposure that is relevant for a specific, nonfatal health endpoint, and (3) for all endpoints, the latency period between TCDD exposure and the onset of the health endpoint is needed. Finally, studies were evaluated using five considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses. Only studies meeting these criteria and adequately satisfying the considerations were included in EPA's TCDD dose-response analysis.

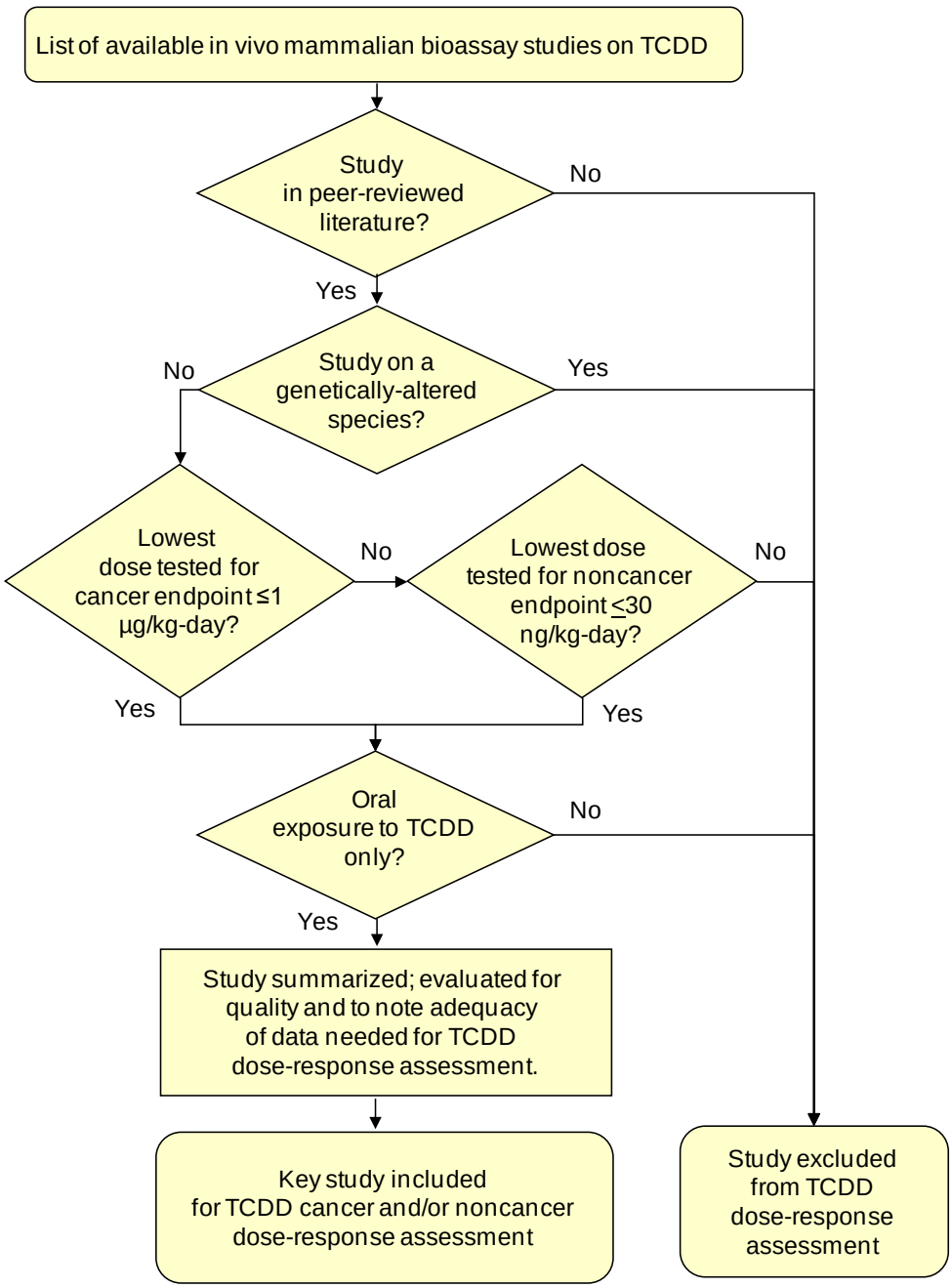
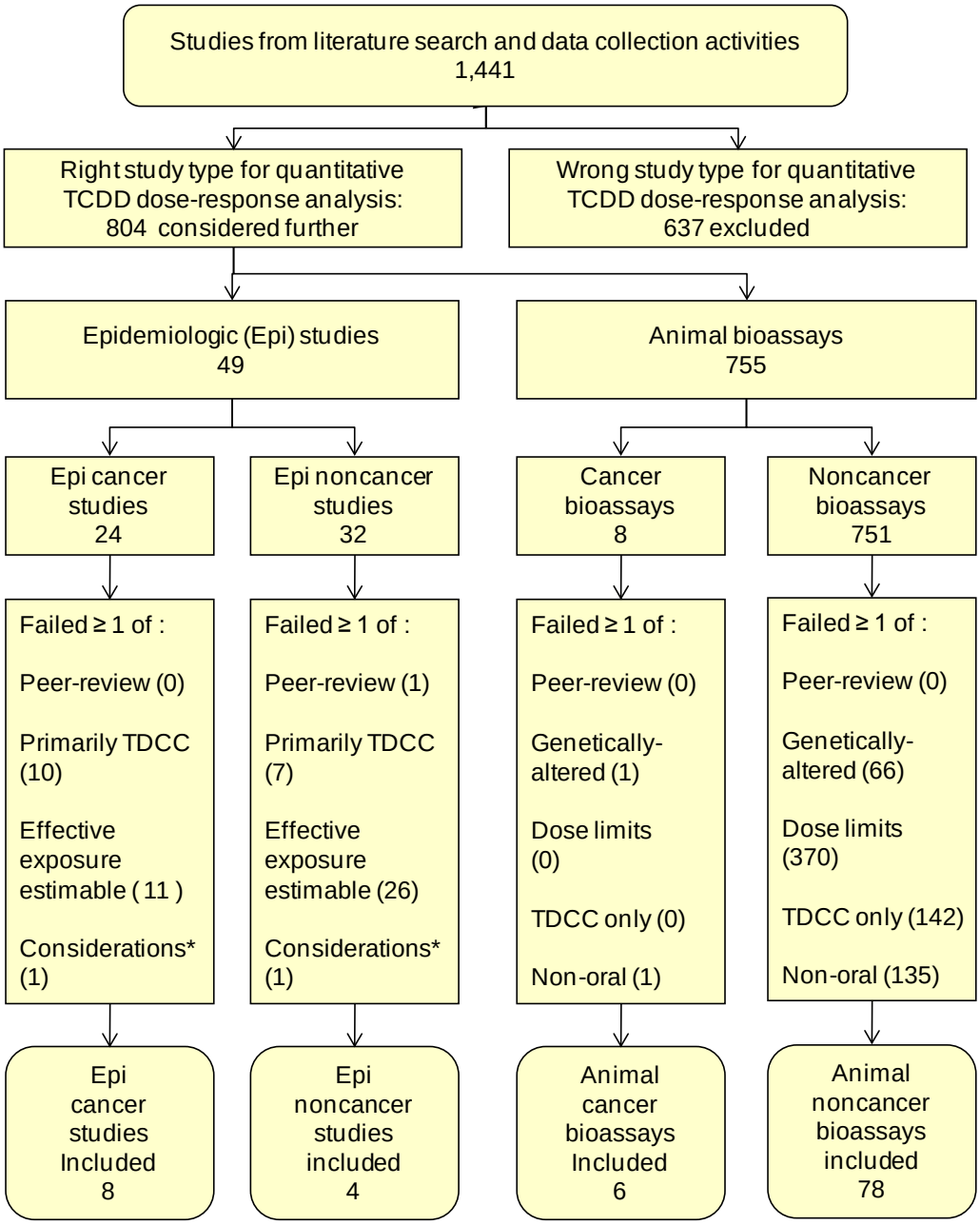


Figure 2-3. EPA's process to evaluate available animal bioassay studies using study inclusion criteria for use in the dose-response analysis of TCDD.
EPA evaluated all available in vivo mammalian bioassay studies on TCDD. Studies had to be published in the peer-reviewed literature. Studies on genetically-altered species were excluded as their relevance to human health is not known. Next, EPA applied dose requirements to each study's lowest tested average daily dose, with requirements for cancer (≤1 µg/kg-day) and noncancer (≤30 ng/kg-day) studies. EPA also required that the animals were exposed via the oral route to only TCDD. Finally, the studies were evaluated for quality and summarized to ensure providing the most relevant information for quantitative human health risk analyses. Only studies meeting all of the criteria were included in EPA's TCDD dose-response analysis.



*Indicates those studies that passed all three criteria but were not selected based on study considerations.

Figure 2-4. Results of EPA's process to select and identify in vivo mammalian and epidemiologic studies for use in the dose-response analysis of TCDD.

Criteria not met are not mutually exclusive. Four animal studies and seven epidemiologic studies contained both cancer and noncancer endpoints. Two epidemiologic cancer studies, Steenland et al. (1999) and Flesch-Janys et al. (1998), passed all criteria, but were still not selected because they were superseded by other studies on the same cohort for which an improved analysis was done. One noncancer epidemiologic study, Baccarelli et al. (2005), passed all criteria, but was excluded because the health endpoint, chloracne, is considered to be an outcome associated with high TCDD exposures.