ToxStrategies

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To whom it may concern:

On May 21, 2010, the U.S. Environmental Protection Agency (EPA) released a draft document titled *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments* (2010 Draft Report). This document represents the EPA's response to the 2006 National Academy of Science (NAS) Evaluation of EPA's 2003 Dioxin Reassessment. The Federal Registry Notice announcing the release of the 2010 Draft Report for public comment originally defined a 90-day public comment period, designating August 19, 2010 as the deadline for such (75 FR 29610). As a part of the peer review process, the EPA convened an independent SAB panel, and held a public teleconference on June 24 and a public meeting on July 13-15, 2010 with the SAB (75 FR 28805). ToxStrategies, Inc. submitted an initial set of comments on the 2010 Draft Report for the SAB panel's consideration on July 9, 2010. Given the short period of time afforded to both the pubic and the SAB for review of the EPA's 1,800-page document, and in response to numerous requests regarding the 90-day comment period, the EPA subsequently lengthened the public comment period to 120 days, extending the deadline to September 20, 2010 (75 FR 35800).

Since submitting our initial round of comments to the SAB more than two months ago, we have drafted a more thorough set of comments regarding the EPA's 2010 Draft Report on behalf of Tierra Solutions, Inc. These comments are attached for the benefit of the SAB panel as they continue their review of the 2010 Draft Report. We appreciate the opportunity to comment on this important step in the EPA's effort to provide the public with a scientifically sound and defensible risk assessment for dioxin.

Sincerely,

Dr. Mark Harris
Principal Health Scientist
ToxStrategies, Inc.
(281) 712-2062

Comments on "EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments"

SEPTEMBER 20, 2010

ToxStrategies

Innovative solutions
Sound science

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PREPARED FOR:
Tierra Solutions, Inc.
2 Tower Center Boulevard, Floor 10
East Brunswick, NJ 08816
(732) 247-3400

PREPARED BY:
ToxStrategies, Inc.
23501 Cinco Ranch Blvd.
Suite G265
Katy, TX 77494
(281) 712-2062

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Executive Summary

ToxStrategies reviewed the draft document titled "EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments" (2010 Draft Report). The massive 1,850-page Draft Report serves as the Environmental Protection Agency's (EPA's) response to some of the recommendations made by the National Academy of Sciences (NAS) related to the dose-response assessment for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) described in EPA's 2003 Dioxin Reassessment. Our comments are focused on developing robust and meaningful toxicity criteria based on the best science available and using guidelines and procedures set forth by the EPA. We commend EPA for their major efforts to conduct additional analyses and respond to the NAS committee. However, we have identified a number of issues in this document that should be carefully evaluated by the SAB and revisited by the EPA prior to finalizing the 2010 Draft Report.

After reviewing the 2010 Draft Report, we identified nine general areas on which the EPA should focus their attention as they strive to meet their NAS-mandated mission of providing "clear, comprehensive, and transparent" analysis of dioxin risk. First, it was apparent that the EPA did not sufficiently address several key recommendations made by the NAS Dioxin Panel following their review of the EPA's Dioxin Reassessment. Key NAS recommendations which the EPA has not sufficiently addressed include: 1) evaluation of a nonlinear dose-response relationship for TCDD cancer risk based on data supporting a nonlinear mode of action; 2) characterization of the uncertainty associated with the TCDD toxic equivalency factors (TEFs) and their application in risk assessment; 3) evaluation of the impacts that variability and uncertainty have on toxicity values; and 4) updating the dioxin exposure assessment presented in the 2003 Dioxin Reassessment. As the NAS Dioxin Panel clearly stated in their 2006 Evaluation of the 2003 Dioxin Reassessment, these are issues that should be addressed by the EPA in an effort to present a complete, thorough, and transparent risk analysis of TCDD.

Second, the EPA utilized a physiologically-based pharmacokinetic (PBPK) model that was not peer-reviewed and was not validated. By simply modifying a version from the literature, the EPA failed to adhere to its own guidance on selection and application of PBPK models (i.e., their 2006 Guidelines on PBPK Model Selection in Risk Assessments report). Nor did the EPA provide an adequate uncertainty analysis on key parameters used in its model.

Third, we found many shortcomings and significant limitations associated with the underlying study used to derive a TCDD Reference Dose (RfD) (Mocarelli et al., 2008). The list of specific concerns that need to be addressed by the EPA include: Questionable relevance of the critical effect (EPA made assumptions about the statistically uncharacterized population below the screening level, no dose response relationship was demonstrated, and biological plausibility of clinical screening guidelines in the context of TCDD was not adequately justified); EPA relied on data that were insufficient to determine that effects were related to TCDD exposure; no justification for the appropriateness of relying on analysis involving a high-level dioxin exposure event to derive an RfD; and failure to consider significant presence of non-TCDD Toxic

Equivalency (TEQ) and increased elimination rate of TCDD in children, both of which led EPA to over-estimate non-cancer hazard. While the RfD comments are generally focused on the Mocarelli et al. (2008) study, several of these issues are also applicable to the "co-principal" study, Baccarelli et al. (2008), that was also considered by the EPA.

The remaining six areas of comment are in regard to the underpinnings of the EPA's proposed oral cancer slope factor (OSF). With this in mind, our fourth general comment was on confounding chemical exposures experienced by the National Institutes of Occupational Safety and Health (NIOSH) worker cohort. The EPA failed to acknowledge what was reported in the original NIOSH Dioxin Registry Reports for each of the chemical plants on this topic, which is clear evidence that these workers had the opportunity to be exposed to more than 35 known or potential human carcinogens in addition to TCDD (including benzene, ethylene oxide, acetaldehyde). In addition, the EPA excluded the Ranch Hand cancer analyses due to the confounding exposures of 2,4-dichlorophenoxyacetic acid (2,4-D), yet failed to address the same confounding exposure documented within the NIOSH cohort on which the EPA's proposed TCDD OSF is based.

Fifth, EPA's proposed OSF was derived using the Cheng et al. (2006) analysis of the NIOSH cohort based on their exposures to TCDD. These exposure estimates were derived using a job exposure matrix (JEM) rather than actual measured serum TCDD values for the majority of workers in the cohort. The resulting exposure estimates were used in highly quantitative mathematical models by the EPA to derive the OSF. The JEM relies upon qualitative parameters based on subjective judgment. As a result, the exposure estimates were not quantitative and thus have limited application in the mathematical models used by the EPA to derive an OSF. This major limitation is acknowledged by the original authors in the peer-reviewed literature but was not recognized by the EPA.

Sixth, the rationale for adhering to a low-dose linear model as presented by the EPA in the 2010 Draft Report is not sufficient to support the decision to use a linear approach. This is especially true with regard to their invocation of several smaller non-consensus hypotheses (e.g., the impact of reactive oxygen species, the role of dietary and endogenous aryl hydrocarbon receptor (AhR) agonists in "additivity to background," use of non-receptor-based chemicals to support the use of a linear, population-based statistical modeling) while down-playing evidence of nonlinearity (receptor-based chemicals affect pathways in a nonlinear fashion). We found that, collectively, these arguments ignored the weight-of-the-evidence for a nonlinear mechanism present in the published literature; and since only sparse evidence was provided regarding the biological plausibility of the non-consensus hypotheses, a rather incoherent defense was made on behalf of a low-dose linear model.

Our seventh comment addressed the fact that, although the EPA clearly acknowledged key events in the mode of action (MOA) for TCDD-induced carcinogenesis in the 2010 Draft Report, the EPA did not fully develop an MOA, nor was there adequate evidence regarding a lack of data on an MOA for TCDD provided. Furthermore, it appeared that the EPA misinterpreted and/or ignored their own technical guidelines and frameworks

regarding the analysis of such. To demonstrate that such evidence exists to develop a defensible MOA for TCDD, herein we provide a thorough, evidence-based description of an AhR-mediated MOA for TCDD-induced lung and liver carcinogenesis using genomic data that illustrate the common involvement of proliferative and anti-apoptotic mechanisms in these two seemingly disparate tumor types.

Our eighth comment highlights the fact that the EPA has again chosen to evaluate cancer risk using a linear model rather than nonlinear model in spite of comments presented by three prestigious scientific advisory panels that have reviewed the EPA's dioxin reassessment over the years. All three scientific advisory panels identified the linear modeling assumption as a specific point of concern and recommended that nonlinear approaches be incorporated into the assessment. This recommendation was founded on data demonstrating clear nonlinear dose-response relationships. Given the EPA's limited rationale for selecting a linear model, and clear recommendations from multiple scientific panels that have reviewed the assessment, the EPA's history of insisting on low-dose linearity for TCDD associated risk appears to have less to do with the available science and more to do with a policy decision.

Finally, our ninth general comment on the 2010 Draft Report is regarding the implications of EPA's proposed toxicological benchmarks. When these proposed benchmarks are used in typical risk assessment calculations, the results indicate that current levels of TCDD in breast milk, foodstuffs, and soil may pose unacceptable health risks. Furthermore, these proposed benchmarks suggest costly reevaluation and reopening of numerous federal and state dioxin sites that have already been previously addressed. However, these implications were not addressed by the EPA. These issues clearly have great impact on public perception of health and may result in unwarranted and/or unnecessary concerns or actions.

In conclusion, we recognize that members of the Scientific Advisory Board (SAB) have undertaken a monumental task in agreeing to review and critique the EPA's 2010 Draft Report, as this document represents four years worth of the EPA's effort and includes a tremendous amount of information and analysis. There were three qualities emphasized by the 2006 NAS Dioxin Panel in their evaluation of the 2003 Dioxin Reassessment that the EPA reiterated in the 2010 Draft Report: transparency, thoroughness, and clarity. The comments provided herein were developed with this in mind. Therefore, we submit our comments anticipating that they prove useful to the SAB panel as it prepares its final review of the 2010 Draft Report and the EPA as it seeks to complete its long anticipated dioxin risk assessment.

1.0 EPA did not sufficiently respond to NAS Recommendations

Issue: In its 2010 Draft Report, the EPA did not sufficiently address several key recommendations made by the NAS Dioxin Panel following their review of the EPA's Dioxin Reassessment[1]*. Key NAS recommendations which the EPA has not sufficiently addressed include: 1) evaluation of a nonlinear dose-response relationship for TCDD cancer risk based on data supporting a nonlinear mode of action; 2) characterization of the uncertainty associated with the TCDD TEFs and their application in risk assessment; 3) evaluation of the impacts that variability and uncertainty have on toxicity values; and 4) updating the dioxin exposure assessment presented in the 2003 Dioxin Reassessment.*

Suggested Improvement: As the NAS Dioxin Panel clearly stated, the EPA should seriously commit to addressing the issues highlighted in the 2006 NAS Recommendations in an effort to present a complete, thorough, and transparent analysis of TCDD.

1.1 The NAS recommended use of a nonlinear dose response for TCDD based on scientific mode of action data supporting a nonlinear approach

In its 2006 evaluation of EPA's Dioxin Reassessment, the NAS Dioxin Panel concluded that the scientific evidence for TCDD MOA was "adequate to favor the use of a nonlinear model that would include a threshold response over the use of the default linear assumption" (NAS 2006, pg. 85). To support their position on MOA, the NAS Dioxin Panel provided the EPA with four major lines of evidence:

1. TCDD, other dioxins, and dioxin-like compounds (DLCs) are not directly genotoxic

2. Receptor-mediated agents have sublinear dose-response relationships

3. Evidence that liver tumors are secondary to hepatotoxicity

4. Bioassay evidence of nonlinearity

The EPA presented several "illustrative RfDs" in their 2010 Draft Report, but cited a lack of evidence to support an MOA as the rationale for not using such in the derivation of toxicity values. However, the EPA did not adequately address or refute the four major lines of evidence supporting a nonlinear MOA outlined by the 2006 NAS Dioxin Panel. Rather than evaluating the scientific data supporting the MOA, the EPA focused on extremely limited (and insufficient) data to support its assertion that a linear approach was more appropriate than a nonlinear approach. Collectively, the reasoning is not

[1] Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment, 2006 (referred to as the 2006 NAS Dioxin Panel Report)

biologically plausible, is not supported by available data, and is not in line with the NAS Recommendations. We encourage the SAB and EPA to refer to Section 7.4 of this comments document, where we provide a thorough, evidence-based description of an AhR-mediated MOA for TCDD-induced lung and liver carcinogenesis using genomic data that illustrate the common involvement of proliferative and anti-apoptotic mechanisms in these two seemingly disparate tumor types.

We support the NAS recommendations regarding a nonlinear MOA based on available data and thus strongly recommend the EPA revisit the issue of TCDD nonlinear dose-response and include a much more comprehensive evaluation of the evidence in this document and for the final Dioxin Risk Assessment.

1.2 The NAS recommended that several issues related to TEFs and their application to risk assessment be evaluated

Importantly, we would like to emphasize that we consider the current World Health Organization (WHO) TEF approach to be the best method for addressing the health risk of environmental mixtures of dioxin-like compounds, and therefore agree with the EPA's current policy to adopt the 2005 WHO TEFs in its risk assessments. [And, as a matter of consistency, we have stated as much in our public comments in response to EPA's release of its 2009 Recommended TEFs for Human Health Risk Assessments of Dioxin and Dioxin-like Compounds (EPA, 2009)].

However, the NAS specifically recommended that the EPA conduct further evaluation on nine issues related to the use of TEFs in risk assessment. The EPA did not address many of these recommendations:

1. Address the need for better uncertainty analysis of TEF values, and consider the derivation and adoption of 'consensus probability density functions' for the dioxin-like congeners.
2. In the event that EPA adopts body burdens as a dose metric, a separate set of body burden TEFs should be developed and applied for this evaluation, or that the appropriateness of intake TEFs for body burden TEQs be scientifically justified. Without these corrected values, the overall TEQs estimated by use of intake TEFs could be inaccurate.
3. EPA should acknowledge the possibility that AhR antagonists present in a complex mixture could affect the magnitude and overall toxic effects produced by a calculated amount of total TEQs present in a given sample even if they do not affect the TEQ calculations.
4. If significant differences in the relative potencies (REPs) of DLCs are found between humans and other species, then adjustments should be made in the TEFs, and these should be acknowledged in the Reassessment.
5. The EPA should consider how synthetic and naturally occurring non-DLC AhR agonists would affect the current TEF/TEQ approach for potency estimates.

The EPA has indicated that the finalized Dioxin Risk Assessment will be a compilation of the original 2003 Dioxin Reassessment and the finalized version of the 2010 Draft Report. In recognition of the importance of the issues related to TCDD TEFs raised by the NAS Dioxin Panel regarding EPA's Dioxin Reassessment, we urge the SAB to encourage the EPA to address these recommendations, as they are important to the evaluation of risk associated with exposures to TCDD.

1.3 The NAS recommended a comprehensive evaluation of the impact of variability and uncertainty on risk estimates

Though the 2010 Draft Report recognizes a number of areas of uncertainty related to the current derivation of cancer and non-cancer risk estimates, the EPA did not conduct a "clear evaluation of the impacts [of uncertainty] on the risk estimates" as recommended by the NAS (Chapters 2 and 7 of the 2006 NAS Dioxin Panel Report):

1. The 2003 Dioxin Reassessment did not adequately address uncertainty and variability that result from the numerous decisions the EPA made in deriving point estimates of cancer risk in the comprehensive risk assessment.
2. The EPA should provide distributions that provide clear insights about the uncertainty in the risk assessments, along with discussion about the key contributors to the uncertainty.

To underscore the importance of the second recommendation listed above, the NAS provided several specific prescriptions regarding the quantitative evaluation of the cancer risk assessment that were not addressed by the EPA (NAS 2006, pg. 92):

> *"EPA could have estimated the corresponding risks and reported the impact of [alternative assumptions or methodologies] on the risk assessment results. The potential impacts of four sources of uncertainty are [:]*
>
> - *The full range of plausible parameter values for the dose-response functions used to characterize the dose-response relationship for the three occupational cohort studies selected by EPA (Ott and Zober 1996; Becher et al. 1998; Steenland et al. 2001).*
> - *Use of other points of departure, not just the ED01 (or LED01), to develop a CSF.*
> - *Alternative dose-response functional forms as well as goodness of fit of all models, especially at low doses.*
> - *Uncertainty introduced by estimation of historical occupational exposures.*

The uncertainty section was a particularly focused point of concern raised by several SAB members during the SAB panel's July 13-15, 2010 public meeting (it was also a primary topic of concern in public comments). In response to the SAB, the EPA stated that it was not feasible to perform a comprehensive quantitative analysis for the cancer and non-cancer toxicity values it developed. We concur with the SAB that this response is completely inadequate. By not comprehensively evaluating the variability and

uncertainty, the EPA ignores both the NAS recommendations as well as the current SAB recommendations. Thus, it is strongly recommended that the EPA conduct uncertainty analyses to understand the impact on the draft toxicological values as well as the impact of such on risk assessment.

1.4 The NAS recommended that the EPA update the exposure analysis

The NAS Dioxin Panel recommended that the EPA make significant efforts to improve the dioxin exposure assessment component of the 2003 Dioxin Reassessment. The EPA stated that it did not address these issues in the 2010 Draft Report: "*this document does not address the NAS recommendations pertaining to the assessment of human exposures to TCDD and other dioxins.*" The EPA instead defered to a recent study published by Lorber et al. (2009), in which the authors provide updated estimates of dose and body burden of DLCs, analyze the impact of measurements below the detection limits, and analyze temporal trends in DLC body burden levels over the past two decades. As published, the Lorber study does an exemplary job in calculating and characterizing recent DLC body burdens, but it only addresses one of the eight recommendations. Thus the EPA has still not addressed several important NAS recommendations (NAS, 2006; pgs 74-75), including:

1. EPA should use a top-down approach that attempts to account for observed levels and consider what emissions would be required to account for these levels to complement the bottom-up approach addressed in the 2003 Dioxin Reassessment.
2. EPA should add some discussion in the exposure chapter about what factors (such as diet, activities, and location) tend to increase or decrease intake of DLCs.
3. EPA should construct their reports so that information in the summary emissions inventory table of Part III of the 2003 Dioxin Reassessment can be more clearly and more easily traced back to the source chapters that provide background information.
4. EPA should evaluate the impact on early emission-inventory estimates (1987, 1995) of sources added in more recent assessments (2005) so that the overall percentage declines reflect all sources.
5. EPA should define a strategy for "intelligent" collection of samples and reanalysis of archived samples to answer a number of remaining questions about exposure trends and to fill in some important data gaps.
6. EPA should create a congener-specific and active database of typical concentrations for the whole range of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs) included in the WHO TEF list. This recommendation applies to work separate from the Reassessment. The database should be based on a compendium of all available data and be updated on a regular basis with new data as they are published in the peer-reviewed literature.

7. EPA should explicitly characterize the variability of population exposures to PCBs, especially given the number of sites with increased levels of PCBs in the environment and anticipating that those levels could result in higher contributions of the dioxin-like PCB fraction to total TEQ exposure (e.g., through local fish consumption). EPA should estimate the magnitude of the ratio of high-end to median and mean exposure, the factors (e.g., proximity to sources, geographic region, and eating habits) that give rise to high-end exposure, and the relative uncertainty with which high-end exposures can be estimated.

Clearly, the EPA should address these issues before issuing the final Dioxin Risk Assessment.

2.0 The EPA ignored its own guidance when selecting a PBPK model to derive dose estimates and toxicological benchmarks

Issue: The EPA utilized a PBPK model that was not peer-reviewed and was not validated. By simply modifying a version from the literature, the EPA failed to adhere to its own guidance on selection and application of PBPK models (Guidelines on PBPK Model Selection in Risk Assessments, 2006).

Suggested Improvement: The EPA should follow its own policy guidelines and have the modified Emond model peer-reviewed by an independent panel. In addition, the EPA should provide an adequate uncertainty analysis on key parameters used in its model.

We commend EPA for incorporating toxicokinetics into its dose response assessment. However, we echo the concerns raised by several public commentors and SAB member Dr. Harvey Clewell during the July 13-15, 2010 public meeting. Importantly, the EPA failed to adhere to its own guidance on selection and application of PBPK models because it used a non-peer-reviewed and unvalidated PBPK model. Rather than using a PBPK model directly from the literature (several of which were available), the EPA modified a PBPK model published by Emond et al. (2006). In doing so, the EPA failed to abide by its own 2006 guidance on the issue of PBPK model selection, which specifically states that the selection and use of such models in risk assessment "must have been peer-reviewed and evaluated for its quality and predictive capacity" (EPA 2006, pg. 4-1). Selection of a model that was not peer-reviewed is also inconsistent with criteria set out for the selection of studies to derive toxicological benchmarks, which also strictly states that studies must be peer reviewed. Thus, we encourage the SAB to suggest that EPA select a model from the literature or to publish (i.e., peer review) the modified PBPK model before it is used to derive toxicological values.

Further, one of the specific issues associated with the selection and application of a PBPK model that was not based on a peer-reviewed publication is the Hill coefficient value (a specific parameter in the model). This parameter plays an important role in the mechanics of the PBPK model, as it defines the slope of the dose-response relationship between CYP1A2 induction and TCDD dose. However, this value is subject to significant variability (Wang et al., 1997), and thus it is essential that the EPA provide discussion of the implications of the Hill coefficient value used in the model, as well as a quantitative evaluation of its variability.

3.0 The EPA needs to address the wealth of uncertainty associated with the underlying data as well as the quantitative interpretation of such in the derivation of an RfD

Issue: There are many shortcomings and significant limitations associated with the underlying study (Mocarelli et al., 2008) used to derive a TCDD Reference Dose (RfD). Specific concerns that need to be addressed by the EPA include:

1. *The EPA determined a critical effect based on data that (a) were not actually reported by the study authors, (b) were not clinically significant, (c) did not demonstrate a dose response relationship, and (d) were not supported by a discussion on biological plausibility.*

2. *The EPA relied on data that were insufficient to determine that effects were related to TCDD exposure.*

3. *The EPA did not sufficiently address the appropriateness of using a high-level dioxin exposure event (that is considerably atypical relative to the experience of the general population) to derive an RfD.*

4. *EPA needs to account for the over-estimate of risk due to exposures to other dioxin-like chemicals.*

5. *EPA needs to account for the over-estimate of risk due to the increased elimination rate of TCDD in children.*

These comments are focused on the key study used to derive an RfD (Mocarelli et al. 2008). However, several of these issues are also applicable to the "co-principal" study, Baccarelli et al. (2008), that was also considered by the EPA.

Suggested Improvement: Given that one of the SAB members is a co-author on both of the co-principal studies used by the EPA in their RfD derivation process, the EPA should request the appropriate, unpublished data necessary to conduct key analyses that elucidate: 1) the significance of the number of exposed individuals with sperm counts below the screening level, 2) the blood TCDD levels of those with low sperm counts, 3) and the geographical and other important demographic factors associated with the control group. The EPA must also defend the use of high TCDD exposure event studies, elucidate the biological plausibility of the endpoints in question, and – if the EPA is still convinced that these studies provide the best information for their analysis – derive RfDs that take into account the non-TCDD TEQ levels present in both the exposed and control populations.

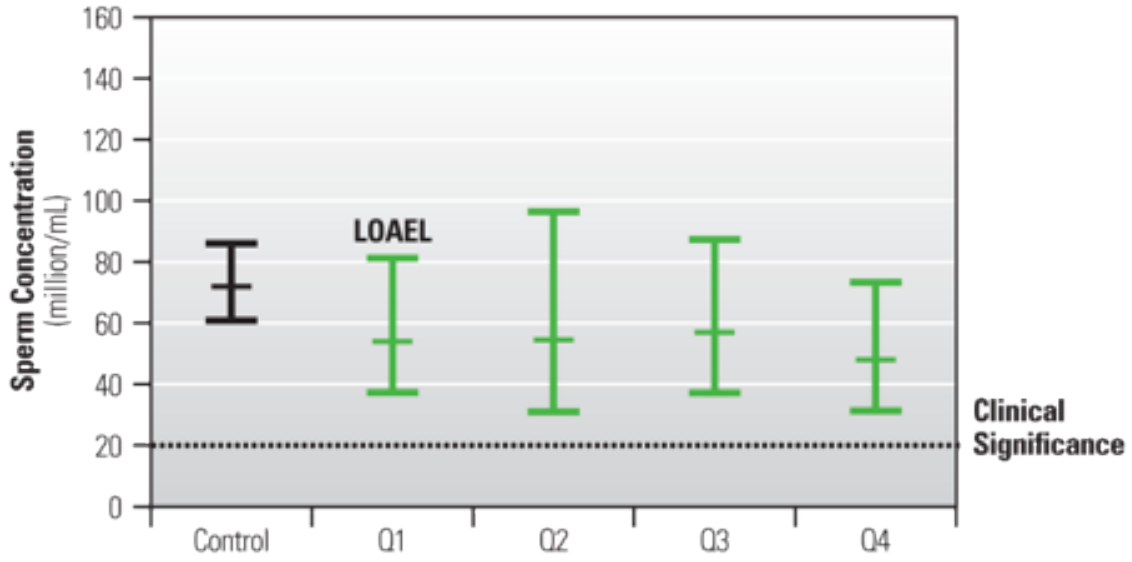
3.1 The EPA determined a critical effect based on data that (a) were not actually reported by the study authors, (b) were not clinically significant, (c) did not demonstrate a dose response relationship, and (d) were not supported by a discussion on biological plausibility.

The EPA relied on decreased sperm concentrations measured in Seveso males who were 1-9 years of age at the time of the Seveso incident as a critical effect when deriving an RfD. The EPA correctly noted that the actual values used to make this determination (unadjusted mean: 53.6 million/ml, SD: 21.8–131.8 million/ml) do not fall below the clinical level of concern (20 million/ml). However, the EPA explains its selection of this endpoint of concern (even though the study authors do not) by claiming that there must be individuals within this group whose sperm concentrations fall below the low-end standard deviation value of 21.8 million (and therefore may have sperm concentrations that would be of clinical concern). Not only was the EPA unable to verify this (it was unable to obtain the original data even though the primary author is an SAB member), but it also had no information regarding the actual TCDD concentrations in the persons that may have had sperm concentrations below the low end of the standard deviation (e.g., these persons could have had high or low serum concentrations of TCDD). Thus, in determining the critical effect, the EPA had no information to verify that the persons with the potentially low values were associated with higher exposures to TCDD.

In addition, the EPA fails to comment on the low-end standard deviation value for the control group (31.7 million/ml), which is also near the level of clinical significance. Using their reasoning, this would also suggest that a fraction of the control population has sperm concentrations of clinical concern (and therefore may not be different than the exposure group). The EPA needs to address these issues and provide a more scientifically robust rationale, as well as a statistical evaluation, to support their decision in selecting this endpoint and dataset.

Separately, the EPA used data adjusted for confounders (rather than the unadjusted data used to rationalize the selection of the endpoint as critical) provided by the study authors to calculate an RfD. The original study authors conducted an analysis based on adjusted data and compared sperm concentrations by TCDD serum concentration quartile (see Figure 3-1) to the control group (note: the study did not evaluate TCDD serum concentrations in the control group). The EPA then selected the median serum TCDD concentration (68 ppt) in the first quartile as the lowest observable adverse effect level (LOAEL), which was further evaluated in a PBPK model to determine the point of departure for the RfD derivation. It is important to note the lack of dose response for this effect (and for most other effects evaluated), as well as the lack of clinical significance for all groups (including control).

Figure 3-1. Sperm concentration (adjusted mean and 95% confidence interval) for exposed men 1-9 years old in 1976 and sampled for sperm endpoints in 1998. (Adapted from Figure 3A from Mocarelli et al., 2008.)



Given the lack of clinical significance and dose-response relationship, the EPA needs to provide a weight-of-the-evidence assessment of the biological plausibility of the endpoint of concern, drawing from both the human and animal data. Related to such, it is suggested that the EPA provide comment on the inconsistent findings of the Mocarelli et al. (2008) study. Although sperm concentration and motility counts are significantly reduced for the youngest of the Seveso age group relative to control, there is no significant reduction in total sperm count or testosterone levels. Further, the study reports significantly elevated sperm concentrations and motility counts for the Seveso pubescent males (10-17 years old) present at the time of the incident relative to controls. Collectively, there are a number of scientific and quantitative issues that need to be addressed regarding the underlying data used to derive an RfD.

3.2 The EPA relied on data that were insufficient to determine that effects were related to TCDD exposure

Mocarelli et al. (2008) determined statistical significance by comparing to the control group. However, it seems there is a significant amount of uncertainty given that the reported demographics of the control population were different than the exposure groups, and because the study authors had no information on the TCDD levels in the control group. It is very difficult to understand how the EPA can derive an RfD using a dataset in which neither the study authors nor the EPA can confirm that the findings were exposure-related. It should also be noted that Mocarelli et al. (2008) do not present information on the geographic origins of the control group. Several studies to date have demonstrated that sperm counts can vary dramatically from city to city and among different geographic

regions (Fisch et al., 1996a; Swan et al., 2003; reviewed in Safe et al., 2000). For example, Fisch et al. (1996b) conducted a literature review of geographical sperm concentration data and reported a high degree of variability within the United States, with mean values ranging from 52.9 million/ml in Iowa to 134 million/ml in New York. Thus, this is an important factor that could have clearly impacted the analysis, yet was not considered by the study authors or the EPA.

3.3 The EPA did not sufficiently address the appropriateness of using a high-level dioxin exposure event (that is considerably atypical relative to the experience of the general population) to derive an RfD

All four of the key epidemiological studies identified by the EPA for derivation of an RfD were based on the Seveso population. The 1976 Seveso exposure incident involved high-level exposures that are atypical of exposures experienced by the general population. Since the exposure levels experienced by those nearest the explosion far exceed what is observed in the general population, it is not clear that adverse effects described by Mocarelli et al. (2008) (as well as effects described in the other studies) would be expected to occur in the general population experiencing chronic, low-level background exposures in the absence of the initial high exposure event.

This point is highlighted by the scientific data available on neonatal hypothyroidism, the primary endpoint of concern in the co-principal study considered by the EPA for deriving the RfD. In this study, Baccarelli et al. (2008) identified a relationship between TCDD exposures and neonatal hypothyroidism. However, several other studies have shown that background dioxin exposures are not associated with neonatal hypothyroidism. Both Giacomini et al. (2006) and Goodman et al. (2010) reviewed prior studies that looked at this possible association and found there was no consistent evidence that supported a relationship between low-level exposure to dioxins and neonatal thyroid hormone dysfunction.

Therefore, the EPA must address the relevance of the high-exposure Seveso studies in the context of the endpoint of concern it selected (i.e., sperm concentrations).

3.4 EPA needs to account for the over-estimate of risk due to exposures to other DLCs

Although the Seveso population was primarily exposed to high levels of TCDD, studies have clearly documented exposures to other DLCs that significantly contribute to total TEQ in this population. The EPA did not account for these exposures when evaluating dose response. In a report on blood levels in the Seveso population (also cited by Mocarelli et al., 2008), Eskenazi et al. (2004) reported that average total TEQ levels were 100 ppt in areas of Seveso outside of the dioxin exposure zones, of which TCDD accounted for 20%. Thus, these data indicate that the average local level of non-TCDD TEQ at the time of the incident was approximately 80 ppt. The EPA used a value of 68 ppt based on TCDD as a point of departure (POD) from Mocarelli et al. (2008); the POD

for the co-principal study, Baccarelli et al. (2008) was 39 ppt. Thus, the EPA did not account for non-TCDD TEQ when evaluating the dose-response relationship with endpoints of concern. This failure to account for non-TCDD TEQ underestimated the total TEQ POD dose by ~55% for RfD derived by EPA from the Mocarelli et al. (2008) study (and ~40% when the Baccarelli et al. 2008 study is considered). The EPA clearly needs to address the role of other dioxin-like chemicals contributing to the total TEQ in their quantitative analysis of an RfD.

3.5 EPA needs to account for the over-estimate of risk due to the increased elimination rate of TCDD in children

Dose estimates for the Mocarelli et al. (2008) study were derived using a PBPK model based on Emond et al. (2006). However, this model fails to account for the known increased elimination rate of TCDD in children (Clewell et al., 2004; Kerger et al., 2006). The failure to account for such is particularly concerning given that the EPA acknowledges the importance of the differential elimination rates throughout the 2010 Draft Report (EPA 2010, pgs. 2-30, 3-15, 3-16). Since the Mocarelli et al. (2008) study focused on the impact of high-level dioxin exposure on male children and their sperm quantity/quality as adults, the use of the selected PBPK model, whose dose predictive ability is optimized according to adult human kinetics (EPA 2010, pg. 3-41, ln. 17-19), resulted in an underestimation of the TCDD intake dose. This underestimated intake dose led to an underestimated tissue concentration that was identified by the EPA as the point of departure for RfD derivation, which ultimately led to an overestimation of non-cancer risk. The EPA clearly needs to address the role of differential elimination rates in children in their quantitative analysis of an RfD.

4.0 The EPA should address the role of confounding exposures to carcinogenic compounds in the cohort from the epidemiological study used to derive the OSF

Issue: In addition to the TCDD-contaminated products to which the NIOSH cohort was exposed, workers were clearly exposed to several other carcinogenic compounds (e.g., benzene, ethylene oxide, acetaldehyde, etc.). EPA excluded the Ranch Hand cancer analyses due to the confounding exposures of 2,4-dichlorophenoxyacetic acid (2,4-D), yet failed to address the same confounding exposure documented within the NIOSH cohort on which the EPA's proposed TCDD oral cancer slope factor (OSF) is based.

Suggested Improvement: The EPA needs to justify the use of the NIOSH analyses that are based on exposure cohorts clearly exposed to several carcinogenic chemicals in addition to TCDD, especially since their chosen endpoint of concern is "total cancers." Furthermore, the EPA must provide a clear and consistent explanation for selective exclusion of studies based on confounding exposures.

The EPA derived an updated oral cancer slope factor (OSF) using the analyses of the NIOSH occupational exposure subcohort reported by Cheng et al. (2006). Initially, the NIOSH study identified 12 U.S. chemical plants where workers were potentially exposed to process materials contaminated with TCDD. NIOSH prepared a Dioxin Registry Report for each of the 12 plants, which provided information on the chemical processes involved at each plant. As illustrated in Table 4-1, these reports clearly demonstrate that workers at the eight plants identified by NIOSH to have the most TCDD exposure data also had the opportunity to be exposed to more than 35 carcinogens (Marlow et al., 1984, 1986, 1989, 1990, 1991a, 1991b, 1997; Piacitelli et al., 1990).

Table 4-1. Known or potential human carcinogens present at the eight plants studied by NIOSH where workers had the opportunity to be exposed to more than 35 carcinogens.

Known or Potential Human Carcinogens Identified at Chemical Plants Studied by NIOSH		
Benzene	Monochlorophenol	2,5-Dichlorophenol
Ethylene oxide	Diamine	Ethyl acrylate
ortho-toluidine	Dichlorodiphenyltrichloroethane	N-nitrosomorpholine
para-aminobiphenyl	Dioxane	p-Dichlorobenzene
Sulfuric acid	Ethylene dichloride	Aldrin
N-nitrosodimethylamine	Hexachlorobenzene	Dieldrin
polychlorinated biphenyls	Methylene chloride	aniline
petroleum polymer resins	Pentachlorophenol	hexachlorobutadiene
trichloroethylene	2-Chloroallyl diethyldithiocarbamate	parathion
Carbon tetrachloride	2,3,4,6-Tetrachlorophenol	n-Butyl benzyl phthalate
Nitrobenzene	2,4-Dichlorophenol	o-Toluene
2,4,6-Trichlorophenol	2,4,5-Trichlorophenol	2,5-Dichlorophenol
Acetaldehyde	Monochlorophenol	Ethyl acrylate

Serum TCDD levels were assessed in former employees from only one of the eight plants included in the final NIOSH cohort assessment (Plant 1) (Piacitelli et al., 2000; Marlow et al., 1986). Several investigators have used these analytical serum results to study the correlation between serum TCDD levels and reconstructed exposure estimates (Steenland et al., 1999, 2001; Aylward et al., 2005; Cheng et al., 2006). Since much focus has been on this particular subcohort at Plant 1, it is imperative to understand all of the chemical exposures these individuals likely experienced, as well as the potential confounding nature of such on carcinogenic outcomes. As illustrated in Table 4-2, the Plant 1 employees were not only exposed to TCDD-tainted 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), but had the opportunity to be additionally exposed to benzene, sulfuric acid, 2,4-dichlorophenol, 2,4,6-trichlorophenol, acetaldehyde, dichlorodiphenyltrichloroethane (DDT), and hexachlorobenzene.[2] All of these compounds are known, probable, or possible human carcinogens according to EPA and the International Agency for Research on Cancer (IARC).

Table 4-2. Confounding exposures at Plant 1: Known chemical inventories and carcinogens.

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
71-43-2	Benzene	A	1
7664-93-9	Sulfuric acid*	--	1
120-83-2	2,4-Dichlorophenol (DCP)	--	2B
88-06-2	2,4,6-Trichlorophenol	B2	2B
75-07-0	Acetaldehyde	B2	2B
50-29-3	Dichlorodiphenyltrichloroethane (DDT)	B2	2B
118-74-1	Hexachlorobenzene	B2	2B
67-63-0	Isopropyl alcohol	--	3
7647-01-0	Muriatic acid	--	3
8014-95-7	Oleum	--	3
108-95-2	Phenol	DI	3
108-90-7	Monochlorobenzene	D	--
77-55-6	1,1,1-Trichloroacetaldehyde	--	--
95-94-3	1,2,4,5-Tetrachlorobenzene (TCB)	--	--
104-76-7	2-Ethylhexanol	--	--
93-76-5	2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	--	--
N.I.	2,4,5-T amine	--	--

[2] See Appendix A for confounding exposures tables for the other seven plants represented in the NIOSH cohort.

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
N.I.	2,4,5-T ester	--	--
N.I.	2,5-Dichlorophenyl-p-chlorobenzene sulfonate	--	--
80-07-9	4,4'-Dichlorodiphenylsulfone	--	--
64-19-7	Acetic acid	--	--
108-24-7	Acetic anhydride	--	--
98-09-9	Benzensulfonyl chloride	--	--
39332-55-3	Butoxyethoxypropanol	--	--
35296-72-1	Butyl alcohol	--	--
7782-50-5	Chlorine	--	--
7790-94-5	Chlorosulfonic acid	--	--
124-40-3	Dimethylamine (DMA)	--	--
58-89-9	Lindane	--	--
67-56-1	Methanol	--	--
79-11-8	Monochloroacetic acid (MCA)	--	--
N.I.	N-oleyl-1,3-propylenediamine amine salts	--	--
54-11-5	Nicotine	--	--
65-30-5	Nicotine sulfates	--	--
121-60-8	P-acetylaminobenzene sulfonyl chloride	--	--
138-41-0	P-chlorobenzenesulfonamide	--	--
98-60-2	P-chlorobenzenesulfonyl chloride	--	--
80-33-1	P-chlorophenyl-p-chlorobenzene sulfonate (Ovex)	--	--
98-68-0	P-methoxybenzene sulfonyl chloride	--	--
136-32-3	Sodium 2,4,5-trichlorophenate (NaTCP)	--	--
1310-73-2	Sodium hydroxide	--	--
12408-10-5	Tetrachlorobenzene	--	--
121-44-8	Triethylamine	--	--

†EPA Classifications: A = human carcinogen, B2 = probable human carcinogen - animal data, DI = inadequate data, D = not classifiable, -- = chemical not addressed
‡IARC Classification: 1 = human carcinogen, 2B = possibly carcinogenic to humans, 3 = not classifiable, -- = chemical not addressed
*Included in IARC's monogaph for "acid mists, strong inorganic"
N.I. – CAS registry number not identified.

This is of particular concern given that the endpoint used to derive the OSF was "all cancers." Fingerhut et al. (1991) noted the following:

> *"Two observations argue against a carcinogenic effect of TCDD. First, there was not a significant linear trend of increasing mortality with increasing duration of exposure to products contaminated with TCDD (Table 4). However, our use of duration of exposure may have misclassified the cumulative dose of some workers. In addition, a dose-response relation is generally viewed as strong evidence for an association when it is present, but as fairly weak evidence against an association when it is absent. Second, our study did not directly assess the effect of exposure to TCDD alone. The workers were exposed concurrently to the chlorophenols and phenoxy herbicides that were contaminated with TCDD. In addition, they may have been exposed to numerous other chemicals while employed at the plants."*

It is also important to note that the EPA was inconsistent in its exclusion criteria regarding cancer cohorts. The EPA excluded the Ranch Hand cancer analyses (Akhtar et al., 2004; Mickalek and Pavuk, 2008) due to confounding exposures to 2,4-dichlorophenoxyacetic acid (2,4-D), while basing its proposed TCDD OSF on the NIOSH cohort where the same confounding exposure was present at three of the eight chemical plants (Plants 3, 4, and 9; Marlow et al., 1990, 1991a, 1991b). Agent Orange, to which the Ranch Hand cohort was exposed, was comprised of 2,4-D and 2,4,5-T in equal measure. The EPA stated that it considered 2,4-D to be a potential carcinogen (EPA 2010, pg. 2-65 to 2-67), although no supporting references were provided. Regardless, the EPA rationalized that it could not conduct a quantitative dose-response analysis on these studies because it would have been "impossible" to distinguish the cancer effects of 2,4-D and TCDD-tainted 2,4,5-T. Thus it is unclear why the EPA has taken an inconsistent approach given the well-documented exposures to 2,4-D in the NIOSH cohort Plants 3, 4, and 9. Using this rationale, these confounding exposures should have excluded the entire cohort from EPA's quantitative analysis, or at the very least the EPA should have evaluated the NIOSH cohort in the absence of these plants where confounding exposures were occurring.

Thus, it seems essential that the EPA address the potential confounding nature of exposures to these many other carcinogenic compounds, as well as address the issue of inconsistently using the confounding presence of 2,4-D as an exclusion criterion.

5.0 The EPA should evaluate the impact of the qualitative exposure estimates used in the derivation of the OSF.

Issue: The EPA derived an OSF using the Cheng et al. (2006) analysis of the NIOSH cohort based on their exposures to TCDD. These exposures were estimates derived using a job exposure matrix (JEM) rather than actual measured serum TCDD values for the majority of workers in the cohort. The resulting estimates of exposure were used in highly quantitative mathematical models by the EPA to derive the OSF. The JEM relies upon qualitative parameters that incorporate subjective judgment. Therefore, the resulting exposure estimates were not quantitative and thus have limited application in the mathematical models used by the EPA to derive an OSF. This major limitation is acknowledged by original authors in the peer-reviewed literature but was not recognized by the EPA.

Suggested Improvement: The EPA should acknowledge the subjective nature of the exposure estimates and, at a minimum, conduct an analysis to address the uncertainty in the underlying data and explain the potential impact of such on the resulting OSF.

5.1 The study relied upon by the EPA utilized a job exposure matrix that is inherently dependent on subjective, qualitative exposure factors (that are even recognized as such by study authors)

The EPA derived an OSF using the analyses of the NIOSH occupational exposure subcohort reported by Cheng et al. (2006). The NIOSH subcohort consisted of 3,538 workers from eight U.S. chemical plants, and of these, serum samples from only 170 workers from one plant taken in 1987-1988 were used, more than two decades after initial TCDD exposures. It should be noted that the original NIOSH cohort analysis presented by Fingerhut et al. (1991) included 5,172 workers from 12 chemical plants, as well as serum TCDD level data from 253 workers from two of the 12 plants. However, subsequent analyses (Steenland et al., 1999, 2001) reduced the cohort and serum pool to the numbers used in Cheng et al. (2006) for various reasons (e.g., wrong gender, incomplete worker records, insufficient information on exposure, data quality concerns). The EPA's OSF derivation, therefore, relied on an analysis that incorporated only 69% of the original NIOSH cohort, and only 67% of the original cohort serum data. To be absolutely clear – Steenland et al. (2001) and Cheng et al. (2006) had only 170 TCDD serum samples from a single plant – the other 3,538 TCDD serum values were simply estimated using a qualitative JEM. Thus, EPA should not think that there is some huge jump in certainty/quality from Fingerhut et al. (1991) to these later analyses.

In an effort to estimate TCDD exposures, the NIOSH investigators developed a JEM that assigned a relative TCDD exposure score to each of the workers (Piacitelli et al., 2000)

(Figure 5-1). However, the job exposure matrix incorporates qualitative and subjective factors that, in turn, render subsequent exposure calculations, such as those presented by Cheng et al. (2006), as qualitative estimates. Further shortcomings include: (1) NIOSH assumed each job description involved the same degree of worker contact with the process materials regardless of the plant site NIOSH investigated, and (2) little to no explanation of how NIOSH derived the fractions of daily exposure it applied to each exposure score algorithm. The authors of Cheng et al. (2006) specifically noted a number of these limitations in an earlier publication (Aylward et al., 2005):

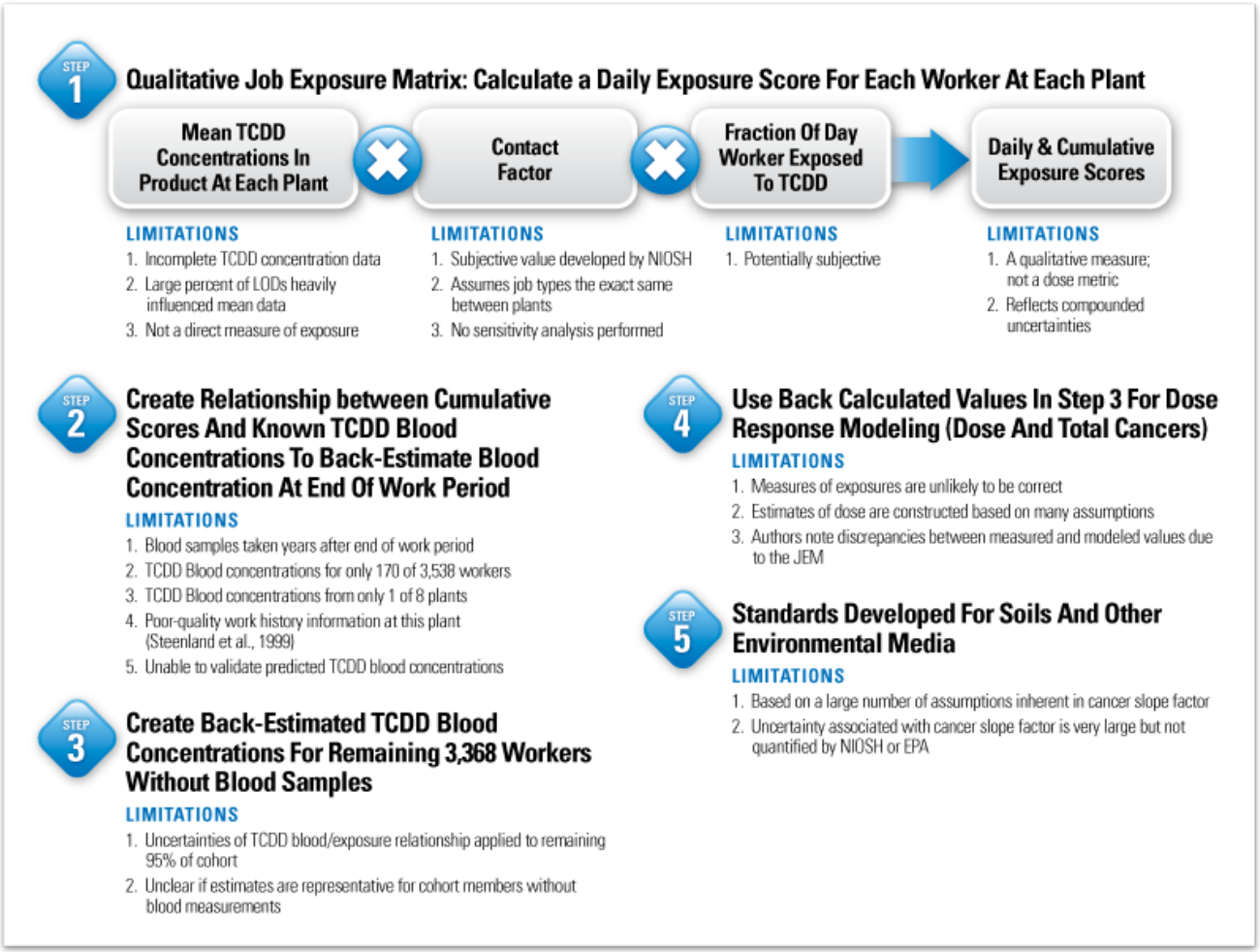
> *"…the job-exposure matrix constructed by NIOSH researchers necessarily relied on limited sampling data over time, and on subjective judgments on contact time, contact factor, and relative exposure potential for jobs at 12 different manufacturing facilities over a period of decades (including numerous process changes) (Piacitelli et al., 2000). The parameter for contact factor assigned by Piacitelli et al. (2000) varied among jobs by 150-fold (from 0.01 to 1.5), and the total exposure score assigned to individual jobs varied by a factor of more than 1,000,000. Furthermore, the dose-rate regressions presented here and in Steenland et al. (2001) for this cohort are based solely on data for a small subcohort of individuals with measured serum lipid TCDD concentrations sampled in 1987–1988. These individuals were drawn from a single plant out of the 12 originally included in the NIOSH cohort (only eight plants were included in the exposure reconstruction effort by NIOSH). Thus, the results of the dose-rate regression for these individuals may or may not be representative of the exposures of cohort members from other plants."* — Aylward et al. (2005).

Additionally, the creators of the JEM lacked key information about the TCDD concentration in various products. For example, the TCDD concentrations for 2,4,5-T operators at Plant 1 from Feb 1951-Aug 1967 was assumed to be 18.6 mg/g based on samples collected in 1965 and 1966; whether those samples are reflective of the entire time period is not known.

5.2 The qualitative nature of the estimates is associated with a great deal of uncertainty

Given the qualitative nature of important elements of the exposure matrix upon which the Cheng et al. (2006) analyses relies, the EPA's updated OSF is, by extension, itself a qualitative estimate that contains a great deal of uncertainty derived from the subjective judgments applied to the exposure scores. Figure 5-1 overviews how these limitations clearly impact the resulting OSF. The EPA does not address these important limitations, nor does it account for such in their quantitative analysis, despite their significant potential for impacting the resulting OSF. Thus it is suggested that quantitative sensitivity analyses be conducted to determine the impact of the underlying subjective, qualitative parameters on their assessment.

Figure 5-1. Dose estimation from epidemiology studies for the EPA OSF derivation: Process and limitations.



6.0 Additional rationale is needed to support the decision to use linear extrapolation to derive an OSF — the rationale provided in the 2010 Draft Report was insufficient

Issue: The rationale in the 2010 Draft Report is not sufficient to support the decision to use a linear approach. Collectively, the reasoning is not biologically plausible and is not supported by the wealth of data available (on the contrary, it suggests the use of nonlinear extrapolation).

Suggested Improvement: If the EPA insists on relying on non-consensus hypotheses as the rationale for supporting the use of low-dose linear versus non-linear approach to deriving toxicological benchmarks for TCDD, it must present a more thorough review of the evidence. The EPA must also consult their 2003 Dioxin Reassessment and clarify inconsistencies in positions on certain issues that were presented in the current 2010 Draft Report (e.g., the impact of dietary and endogenous AhR agonists on TCDD toxicity).

EPA provides a discussion of possible biological mechanisms related to TCDD that support the "appropriateness" of low-dose linear extrapolations. However, there are a number of limitations to these arguments.

6.1 The EPA suggests that a linear response in reactive oxygen species (ROS) may support the use of linear extrapolation, but does not provide actual evidence of such

In both the 2003 Dioxin Reassessment and the 2010 Draft Report, the EPA readily acknowledged that TCDD is a nongenotoxic and nonmutagenic compound (EPA 2010, pgs. 5-16, 5-64; EPA 2003, pgs. 1-64, 6-1, 6-9). But the EPA suggested in its recent 2010 Draft Report that if TCDD generated ROS at low doses, it would support their application of a low-dose linear cancer model similar to what would be used for directly DNA reactive agents, and thus negate the distinction of nongenotoxicity. This implies that any compound responsible for generating ROS at low doses would be indirectly genotoxic and therefore would necessarily promote carcinogenic activity in a linear dose response fashion. Aside from the practical matter that such a hypothesis insinuates an exponentially larger group of chemical, physical, and biological agents being classified as "carcinogenic," it is unclear how the EPA could support this assertion as it is made without citation or the support of any evidence. The mere induction of oxidative markers does not in itself support the use of a low-dose, one-hit dose response model since background and low dose ROS induce adaptive cellular responses capable of preventing and removing potential DNA damage (Feinendegen, 2002). Thus, the induction of such adaptive response activities supports the application of a low-dose, nonlinear dose response model (Trosko, 1998). Also, interspecies variability in susceptibility to ROS

(e.g., rodents are more susceptible to ROS than primates) indicates that simple low dose generation of ROS in laboratory animal models other than primates cannot simply be extrapolated to humans (Klaunig et al., 1998), adding an additional layer of complexity to TCDD-induced ROS generation that would need to be considered were the EPA to seriously consider this an element in TCDD dose-response.

6.2 The EPA suggests that statistical modeling for other compounds supports linear extrapolation on a population basis but does not provide sufficient evidence of such for receptor-mediated compounds such as TCDD

The EPA also relies greatly on White et al. (2009) for supporting the mathematical basis of the low-dose linearity for TCDD dose response. These authors reported the general consensus (though there were exceptions) of a workshop held in 2007 on low-dose extrapolation in which participants felt that low-dose linear extrapolation was the most appropriate extrapolation method for both cancer and noncancer endpoints. This approach was based on the notion that the dose-response for a population will be linear due to interindividual variability, background disease, and background exposure. The authors cite examples of particulate matter, ozone, lead, environmental tobacco smoke (ETS), and radon as supporting low-dose linearity at the population level. However, the applicability of the MOAs for these chemicals to those that act through receptor-mediated mechanism (e.g., TCDD) was not addressed in White et al. (2009) or the 2010 Draft Report. A thorough consideration of a) the validity of low-dose linearity for the aforementioned chemicals, and b) the relevance of the examples discussed in the workshop to chemicals with receptor-mediated MOAs is needed in order for the EPA to rely on the concepts presented at the workshop (rather than on their own guidance and/or the many other publications on this topic in the peer review literature).

It is of interest to note that White and colleagues cite Lutz et al. (2005) as a key publication for supporting low-dose linear approaches. Yet White et al. do not appear to consider statements by Lutz such as,

> *"Strongly sublinear (up-bent) curves and apparent thresholds may allow for a rejection of the linear-no threshold (LNT) default assumption and for a discussion of threshold doses and safety factors to derive tolerable exposure levels. This appears to be appropriate if mechanistic considerations can explain the threshold-like shape of the dose–response curve."*

In an earlier publication, Lutz (1998) specifically cited TCDD as an example of a compound that exhibited J-shaped tendencies in liver tumor formation in rodents as well as in initiation/promotion studies with phenobarbital (and thus was non-linear). Based on the rationale expressed in White et al. (2009), the "linearization" of population dose-response curves would be a universal phenomenon for all toxicants. This theory is not supported by the scientific community (Rhomberg et al. 2009). These issues need to be further considered by the EPA.

6.3 The EPA provides an incomplete rationale for suggesting that additivity impacts receptor mediation involved in carcinogenic responses

The 2010 Draft Report also cites Crump et al. (1976) in support of a linear low-dose extrapolation based on the concept of "additivity." EPA argues that since AhR activity exists at some background level (due to endogenous, natural, and dioxin-like AhR ligands), the additional stimulation by TCDD adds to background responses and thus supports a linear model. But here the EPA contradicts itself: it assumes the AhR-mediated process for carcinogenicity is equivalent for induction by TCDD and endogenous/natural AhR agonists, while also recognizing that AhR can be selectively activated. The existence of AhR ligands that can activate certain — but not all — aspects of AhR-mediated pathways associated with TCDD argues against the EPA's assertion of background additivity in this case. A review of the literature indicates there is much more evidence that the AhR can be selectively modulated than EPA suggests. In addition to Fretland et al. (2004), several studies have demonstrated selective AhR modulation in both in vitro and in vivo model systems (Chen et al., 1995; Fritz et al., 2009; McDougal et al., 2001; Murray et al., 2010a,b).

The phenomenon of selective receptor modulation has been reported for several other nuclear receptors and is not an exception to the rule (Gronemeyer et al., 2004). Rather, it is a widely observed phenomenon, reported extensively by pharmacologists studying diverse classes of receptors, that subtle differences in chemical structures will induce – upon binding – differences in receptor conformational change that translate into a limited set of receptor signaling responses unique to each compound (reviewed in Urban et al., 2007). This suggests that a "spectrum" of receptor conformations is likely, each one influencing the receptor's ability to interact with the proteins that moderate the downstream events associated with full agonists. The reality of this phenomenon argues against EPA's assumption that the activity elicited by all AhR agonists is fundamentally congruent and therefore additive in nature. Thus, it is suggested that the EPA review the additional studies cited here, as well as others available in the literature, and revisit the theory of additivity in their support of a linear model.

6.4 The EPA contradicts its position in the 2003 Dioxin Reassessment by suggesting that additivity to background AhR activity generated by endogenous and natural AhR ligands will impact receptor mediation involved in carcinogenic responses

As mentioned above, in an effort to provide support for a low-dose linear model for TCDD, the EPA puts forth the controversial idea that exposure to exogenous dioxins and dioxin-like compounds adds to a constitutive (or background) level of AhR activity that is modulated by natural and endogenous AhR ligands, thereby pushing the AhR activity to a level that would hypothetically exceed a low-dose threshold for toxicity. This is a direct contradiction of the position the EPA has taken on natural and endogenous ligands in the past. In fact, in the 2003 Dioxin Reassessment – to which it is presumed the 2010

Draft Report will be added upon its finalization – the EPA stated that there is insufficient data to predict how such ligands would influence the AhR-based TCDD TEF approach:

> *"Presently, there are several limitations in our understanding of the importance of naturally occurring dioxin-like compounds vs. the dioxin-like compounds included in the TEF methodology. First is the limited data available on the dioxin-like toxicities of the natural ligands. In addition, there is a lack of data on the interactions between these classes of chemicals. Few if any mixtures of natural AhR ligands and PCDDs or PCDFs examining a toxic response have been published. Many of the natural AhR ligands have multiple mechanisms of action that presently cannot be accounted for in the TEF methodology. For example, I-3-C has anticarcinogenic properties in tumor promotion studies, and these effects may or may not be mediated through AhR mechanisms (Manson et al., 1998). The lack of data and the role of non-AhR mechanisms in the biological effects of these chemicals prohibit a definitive conclusion on the role of natural vs. anthropogenic dioxins in human health risk assessment. Though it is important to address these issues, the available data do not lend themselves to an appropriate quantitative assessment of these issues." - EPA 2003, Part 2, pg. 9-21*

Previously, the EPA highlighted the fact that natural and endogenous ligands are not persistent and therefore not present for a physiologically relevant amount of time to impact AhR activity in the presence of exogenous dioxins and dioxin-like compounds:

> *"One of the most significant differences between the industrial Ah receptor ligands (i.e. dioxins) and the natural Ah receptor ligands is the persistence of the dioxins in biological systems. Because of their long half-lives, dioxins provide a persistent activation of the Ah receptor. In contrast, the natural ligands are rapidly metabolized and the activation of the Ah receptor is short-lived […] Most of the studies examining the relative potency of the natural ligands are based on in vitro or short-term in vivo studies. The estimates of the relative potencies of these chemicals is [sic] greatly exaggerated in these short-term assays because of the bioaccumulative nature of TCDD. The relative potencies of the natural ligands would best be estimated following long term. These data are unavailable and thus the estimates of the relative potencies of these chemicals is [sic] unreliable." - EPA 2003, Part 2, pgs. 9-21 to 9-22*

But recently Connor et al. (2008) demonstrated that background PCDDs, PCDFs, and DL-PCBs are responsible for only <1% of the mean total AhR activity measured in human serum, with the remaining >99% attributable to endogenous, dietary, or anthropogenic AhR agonists. In addition, individuals fed a 4-day diet with specified amounts of cruciferous vegetables (e.g., cabbage, broccoli, Brussels sprouts, and kale) - known to contain dietary AhR ligands or their precursors – had mean human serum AhR activity that was elevated an additional 67% above "background." Moreover, study participants that ingested a daily supplement of indole-3-carbinol (I3C, a dietary AhR agonist) for an additional 2-day period had serum blood AhR activity three times greater

than total background, or almost 5,000 times the serum TEQ associated with background PCDDs, PCDFs, and DL-PCBs. So while it is true that many dietary AhR receptor agonists do not have the persistent chemical qualities associated with environmental dioxins and dioxin-like compounds, it is obvious that the continuous dietary intake of dietary AhR agonists (an expectedly common scenario among individuals with restricted diets such as vegetarians) induces a "background" AhR activity level so high as to render moot any discussion of "additivity to background" in the context of TCDD low-dose linear modeling. It is clear that the EPA is inconsistent in its own dioxin risk assessment on the issue of additivity to background where natural and endogenous AhR ligands are concerned.

6.5 The EPA did not address commonly-accepted principles regarding thresholds for receptor-mediated responses

Given that receptor-mediated responses are generally regarded as having a threshold, the EPA failed to adequately address the non-linear nature of receptor-mediated dose response. Basic receptor theory dictates that most receptor-based effectors are not activated in a linear fashion. As indicated by the EPA, this concept was originally put forth by Stephenson et al. (1956), who was the first to formulate a receptor binding model (based on the availability of spare receptors) that accounted for the observed discordance between ligand binding affinities and activating doses. The issue of non-linear receptor-based thresholds regarding TCDD has been put forth to the EPA on several prior occasions [e.g., in a letter to the editor of Risk Analysis signed by 20 pharmacologists, Byrd et al. (1998) identify the need for EPA to focus its TCDD risk assessment on the nonlinear consequences of AhR activation]. To date, these issues have not been adequately addressed by the EPA, especially in light of the many comments on such from previous SAB panels.

6.6 EPA ignores that other major organizations (such as the WHO) evaluate TCDD using a nonlinear, threshold-based approach

EPA failed to acknowledge the several scientific bodies and regulatory agencies that have recognized the threshold nature of TCDD dose response relationship. The Joint Exposure Committee on Food Additives from the World Health Organization (JECFA/WHO) noted that TCDD kinetics were nonlinear at concentrations that induce CYP proteins in the livers of rats and mice. This observation is of particular interest given that CYP1A2 induction is a critical mechanism of the Emond et al. (2006) model EPA used to derive their proposed TCDD toxicological benchmarks. JECFA derived a tolerable daily intake (TDI) for TCDD of 2.3 pg/kg-day with the support of a nonlinear power model (JECFA, 2001). Similarly, both the United Kingdom's Food Standards Agency's Committee on Toxicity and the European Commission's Scientific Committee on Food have recommended TDIs of 2 pg TEQ/kg BW (UK FSA/COT, 2001; EC, 2001). These values are protective of both cancer and non-cancer effects.

The European branch of the International Life Sciences Institute (ILSI) also recognized the threshold nature of TCDD's MOA in a published review of the thresholds of toxicological concern (TTC) method for establishing de minimus acceptable intake values for substances that are present at low levels in food. The Institute commented that linearized dose-response extrapolation for establishing risk estimates is inappropriate for nongenotoxic compounds like TCDD (Kroes et al., 2004).

Additionally, the National Toxicology Program (NTP) reported a non-linear dose-response relationship between TCDD concentration and all neoplasms observed in the most comprehensive chronic TCDD rodent bioassays to date (Walker et al., 2006). Specifically, Hill shape parameters of approximately 1.0 indicate a linear relationship; in the case of the NTP bioassay, modeling of the tumor data resulted in Hill shape estimates greater than 2.0, a clear indication of nonlinearity.

It is clear that both scientific and regulatory bodies recognize the threshold nature of TCDD's biological activity. If the EPA continues to adhere to a linearized model for TCDD dose-response, it should at the very least acknowledge its unique position on this issue.

7.0 The EPA should evaluate the mode of action (MOA) when determining the extrapolation approach (nonlinear or linear) for carcinogenic effects of TCDD

Issue: EPA clearly acknowledged key events in the MOA for TCDD-induced carcinogenesis, and particularly the role of AhR activation. Despite the robust data available, the EPA did not fully develop a MOA and did not provide an adequate explanation of why it elected not to do so. Further, the EPA appeared to misinterpret and/or ignore their own technical guidelines and frameworks regarding analysis of such. Herein we provide the SAB and EPA with toxicogenomic evidence for a TCDD-elicited proliferative and anti-apoptotic mode of action mediated by AhR activation that leads to liver and lung carcinogenesis. The MOA demonstrates that TCDD elicits differential gene expression associated with proliferative pathways in a species-, tissue- and cell-specific manner. Compelling MOA level evidence is presented that extends beyond AhR binding to help explain the proliferative and anti-apoptotic effects of TCDD in the liver and lung.

Suggested Improvement: The EPA must be consistent in its treatment/acknowledgement of TCDD MOA in keeping with the NAS recommendation "that EPA more thoroughly justify and communicate its approaches to dose-response modeling for health effects and make its criteria for selection of key data sets more transparent" (NAS, 2006). In addition, given the volume of studies that touch on TCDD MOA, it is imperative that the EPA put forth the necessary effort to present its own science-based TCDD MOA, or else adopt or modify the TCDD MOA presented below or another of similar scientific rigor and explanation.

7.1 The EPA is inconsistent in its use of the evidence supporting an MOA for TCDD.

In Section 5 of the 2010 Draft Report, the EPA stated the evidence for AhR-mediation of TCDD toxicity across species was a sound enough MOA to support the biological plausibility of TCDD being a "known human carcinogen." As the EPA pointed out, biological plausibility was one of the seven points of evidence for causality that the 2005 Cancer Guidelines identified as essential for a compound to be classified as a "known human carcinogen" (EPA 2010, pg. 5-9). This was in stark contrast to how the EPA handled the AhR-based MOA in the context of dose-response modeling, wherein it embraced an overly strict interpretation of the 2005 Cancer Guideline recommendations for characterizing a threshold MOA. Although the 2006 NAS Dioxin Panel provided EPA with a sound, consensus-based foundation for TCDD threshold MOA (see comments on NAS recommendations above in Section 1.2), EPA used the remainder of Section 5 to selectively cite studies, or to propose non-consensus hypotheses, that contradicted a nonlinear MOA in order to support its application of a linear dose-

response model. In the end, the EPA presented a very confused position on TCDD MOA, and in doing so fell short of satisfying the general NAS recommendations it cited: namely, present a transparent, thorough, and justified dose-response analysis. Arguably this confusion could have been avoided had the EPA followed its own framework on assessing carcinogenic MOA:

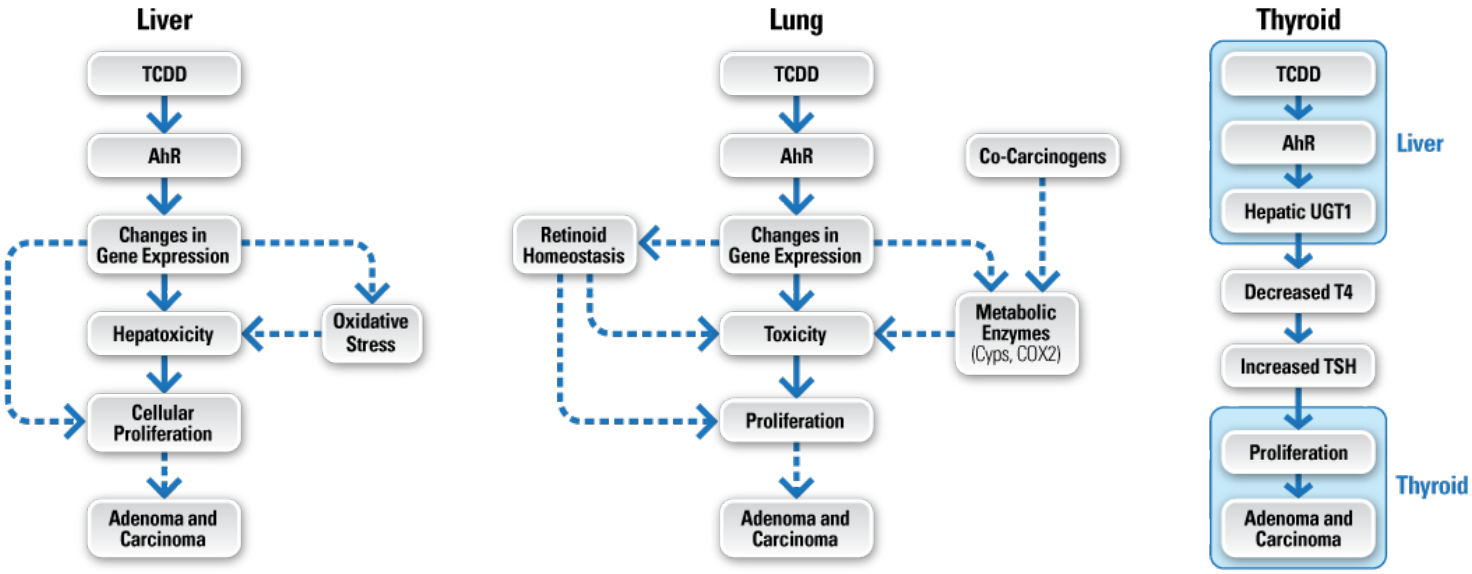
> *"All pertinent studies are reviewed in analyzing a mode of action, and an overall weighing of evidence is performed, laying out the strengths, weaknesses, and uncertainties of the case as well as potential alternative positions and rationales. Identifying data gaps and research needs is also part of the assessment…It is not generally expected that the complete sequence will be known at the molecular level. Instead, empirical observations made at different levels of biological organization—biochemical, cellular, physiological, tissue, organ, and system—are analyzed." - EPA, 2005, pg. 2-41*

7.2 Sufficient data are available to develop an MOA

Although the EPA denied sufficient evidence for a MOA for TCDD, events in the MOA were clearly discussed throughout the 2010 Draft Report.

In this regard, the EPA has demonstrated a generalized MOA for multiple tumor types that share some common key events and themes in their carcinogenic processes (see Figures 5-1 and 5-2 in the 2010 Draft Report; Figure 5-2 copied here (See Figure 7-1)). The EPA further stated "a picture is emerging wherein TCDD is considered a "receptor-mediated carcinogen" in laboratory animals…acting in a manner similar to peroxisome proliferators, phorbol esters, or estrogen" (p. 5–11). For liver, lung, and thyroid tumors, the first key event involves interaction between TCDD and AhR. In both the liver and lung hypothesized MOAs, the next key event is changes in gene expression. The penultimate key event in the three tumor types appears to be cell proliferation ultimately leading to adenomas and carcinomas. This suggests the EPA recognized a generalized MOA for three tumor types associated with TCDD in laboratory animals.

Figure 7-1. TCDD's hypothesized modes of action in site-specific carcinogenesis. See text for details. In each instance, the solid arrows depict pathways that are well established and are associated with low uncertainty. The dashed arrows represent connections that are less established and are associated with higher uncertainty. (Adapted from Figure 5-2 of EPA's 2010 Draft Report.)



Despite the voluminous data available characterizing key events in MOA (and particularly the role of the AhR), the EPA attempts to inject uncertainty in the TCDD MOA by citing that "non-AhR mediated carcinogenic effects are possible." The EPA only provides one reference in support of this conclusion, and in doing so, did not accurately convey the authors' findings. Fernandez-Salguero et al. (1996) used AhR-null mice to demonstrate that toxicity of TCDD was in fact AhR mediated. At an incredibly high dose (2000 µg/kg), some of the AhR-null mice demonstrated occasional scattered hepatocellular necrosis or pulmonary vaculitis, an observation that was not evaluated statistically nor confirmed in later studies. Yet the EPA characterize these same observations as "several minor lesions including scattered necrosis and vaculitis in the liver and lungs," later claiming that these observations were "consistent." The authors concluded "these results conclusively demonstrate that essentially the in vivo effects of TCDD are AhR-mediated." In stark contrast to these findings, the EPA concluded that AhR-independent carcinogenic effects of TCDD were possible. The more logical conclusion is that the occasional yet minimal toxicities observed in AhR-null mice administered a very high TCDD dose represent non-specific, high-dose effects and are irrelevant to any discussion of TCDD's MOA.

It is of particular interest to note that the EPA has a rather inconsistent approach in their discussions related to AhR-mediated events in TCDD MOA; though the EPA considers the TCDD AhR MOA to be insufficient to support the nonlinear modeling of TCDD cancer risk, it relies on this MOA in several areas in its 2010 Draft Report, most notably using the TCDD AhR MOA as evidence in support of the biological plausibility of tumor causality in humans. The document also cites evidence that constitutively activated AhR can also induce cancer; however, it is not made clear how this refutes the MOA that TCDD activation of the AhR can cause cancer at certain doses. Moreover, it has recently been reported that low levels of AhR activation may act as a tumor suppressor (Fan et al., 2010); thus if EPA's intent is to suggest that all AhR activation is pro-carcinogenetic, there is evidence to the contrary.

7.3 Data suitable for the EPA framework on developing a MOA are readily available

Given the amount of literature on TCDD (over 7,000 citations in PubMed), there would appear to be sufficient data to suggest that the MOA for at least some TCDD-induced tumors can be plausibly hypothesized and their relevance to humans evaluated. The EPA should have identified tumors from animal studies, applied their own MOA framework, and examined human relevance through consideration of available epidemiological data. It is not clear why the EPA did not consider these data as supporting a general MOA for TCDD-induced tumors. It would seem that understanding exact events between AhR activation and cell proliferation would constitute a level of mechanistic detail that rarely exists for chemicals. At the very least, examining these tumors through the MOA Framework would allow for a more transparent assessment of these tumors and their potential relevance to humans.

For the EPA to suggest that the available data equate to an unknown MOA, places TCDD together with numerous less- or unstudied chemicals for which there may truly be limited or no data beyond evidence for carcinogenicity. In this regard, a full MOA evaluation and the identification of data gaps would allow for a more appropriate evaluation.

7.4 Toxicogenomic Arguments for a TCDD-Elicited Proliferative and Anti-Apoptotic Mode of Action Mediated by the AhR that Leads to Liver and Lung Carcinogenesis

TCDD is not directly mutagenic or genotoxic, but classified as a potent hepatic tumor promoter in rodents (Pitot et al., 1980) with negligible tumor-initiating potential (Maronpot et al., 1993). Review of TCDD-mediated differential gene expression provides compelling evidence for proliferation and cell death leading to carcinogenesis. Comparative examination of global gene expression across species facilitates the development of a descriptive AhR network for the proliferative effects of TCDD. Such models are limited by a number of factors including (i) incomplete functional annotation for all differentially expressed genes, (ii) the lack of complementary proteomic, metabolomic and enzymatic activity data, and (iii) the inability to place all gene expression changes into context. However, the integration of complementary dioxin response element (DRE), chromatin immunoprecipitation (ChIP), and histopathology data support model development for TCDD-elicited proliferation and suppression of cell death leading to hepatocarcinogenesis and neoplastic changes in the lung. Although it may not be possible to propose a detailed "mechanism of action," there is compelling evidence for a mode of action that goes beyond TCDD binding to the AhR. Importantly, the EPA recognizes that "[a]t least some information bearing on mode of action…is present for most agents undergoing assessment of carcinogenicity, even though certainty about exact molecular mechanisms may be rare" (EPA, 2005). In this regard, the vast database for TCDD, including available toxicogenomic data, suggest that a plausible mode (if not mechanism) of action can be posited for some tumors induced by TCDD.

7.4.1 Toxicogenomic Effects of TCDD in the Liver

TCDD elicits a cascade of differential gene expression responses linked to changes in protein levels, metabolite levels, enzymatic activities and histological lesions. These gene expression changes are mediated by the AhR and have been associated with a number of responses including xenobiotic metabolism, oxidative stress, fat metabolism and transport, and cell proliferation. Therefore it is possible to develop a descriptive MOA model for AhR-mediated effects via the integration of complementary data sets (i.e., computational DRE searches, gene expression, metabolomic, molecular, and histopathological).

Recent whole genome in vivo gene expression studies identified the differential expression of ~2400 hepatic genes (|fold change|>1.5, P1(t) >0.9), in immature, ovariectomized C57BL/6 mice across time (2-168 h) following a single oral gavage of 30 µg/kg TCDD (Dere et al., submitted-a; Sun et al., 2004; Boverhof et al., 2005).

Approximately 1600 of these hepatic genes exhibited dose-dependent expression at 24 h (Boverhof et al., 2005; Kopec et al., 2010; Kopec et al., 2003). This included ~160 differentially expressed transcription factors. For example, Myc, a transcription factor that serves multiple roles in cell proliferation (Grandori et al., 2000), exhibited significant and sustained induction from 4-168 h and sigmoidal dose-dependent induction at 24 h (Dere et al., submitted-a; Sun et al., 2004; Boverhof et al., 2005; Kopec et al., 2010; Kopec et al., 2003). Computational searches identified 4 putative functional DREs in its proximal promoter region, and a region of AhR enrichment in hepatic genome-wide ChIP-Chip assays (Table 7-1) (Dere et al., submitted-a,b; Sun et al., 2004; Dere et al., 2008). Collectively, these data suggest that TCDD induction of Myc gene expression in the liver is mediated by the AhR.

Myc regulates gene expression associated with cell proliferation, apoptosis, growth, and metabolism (Dang et al., 1999). In the TCDD microarray data set, there were several Myc-regulated genes that were differentially expressed. For example, early response 3 (Ier3) was induced by TCDD which is regulated by Myc. Ier3 is under the precise control of multiple transcriptional regulators including p53 and Nf-κB, and transgenic mice studies indicate Ier3 is anti-apoptotic (Wu et al., 2003). In addition, Myc represses genes involved in growth arrest such as growth arrest specific 1, Gas1 (Lee et al., 1997), which was down-regulated in the TCDD microarray data set. Moreover, TCDD induced Tnf and Tnfaip2 expression, members of the tumor necrosis factor (TNF)-signaling pathway that activate Nf-κB-regulated cell proliferation (Fabregat et al., 2007). Casp9, which when activated leads to apoptosis (Green and Reed, 1998), was also found to be repressed. Furthermore, activation of the anti-apoptotic Bcl2 family members prevents permeabilization of the outer mitochondrial membrane, inhibiting cell death (Green and Reed, 1998; Budinger et al., 2002). TCDD also induced the pro-survival gene, Bcl2l11, which may contribute to sustained cell growth.

Nrf2 is a transcription factor that regulates oxidative stress, apoptosis, and cell survival (Kensler et al., 2007). It was induced by TCDD between 2-168 h and exhibited sigmoidal dose-responsive expression at 24 h (Dere et al., submitted-a; Sun et al, 2004; Boverhof et al., 2005; Kopec et al., 2010; Kopec et al., 2003). Oxidative stress contributes to cell death and results in Nrf2 activation, leading to induction of antioxidants that protect against apoptosis (Kensler et al., 2007). TCDD induced Nrf2-regulated genes with oxidoreductase and glutathione conjugation functions (e.g., Nqo1, Ugdh, Gstp1, and Gstm1) limiting cell death (Rangasamy et al., 2004; Yeager et al., 2009). Computational analysis of the Nrf2 promoter identified DREs and a region of AhR enrichment in genome-wide ChIP-Chip assays (Table 7-1) (Dere et al., submitted-a,b; Sun et al., 2004; Boverhof et al., 2005; Kopec et al., 2010; Kopec et al., 2003; Dere et al., 2008), consistent with reports of AhR-Nrf2 cross-talk (Rangasamy et al., 2004; Yeager et al., 2009). These Nrf2-regulated changes are also consistent with TCDD inducing proliferation and inhibiting apoptosis.

Table 7-1. Pro-proliferation and anti-apoptotic gene expression changes induced by TCDD in the mouse and rat containing putative DREs and exhibiting regions of AhR enrichment in ChIP-chip assays.

		MOUSE		
Gene Name	**Gene Symbol**	**Gene Expression**	**Dioxin Response Elements***	**AhR enrichment[a]**
Myelocytomatosis oncogene	Myc	↑	4	+
Growth arrest specific 1	Gas1	↓	12	-
Immediate early response 3	Ier3	↑	3	+
Tumor necrosis factor	Tnf	↑	2	-
Tumor necrosis factor, alpha-induced protein 2	Tnfaip2	↑	5	+
Caspase 9	Casp9	↓	5	+
BCL2-like 11 (apoptosis facilitator)	Bcl2l11	↑	4	+
NAD(P)H dehydrogenase, quinone 1	Nqo1	↑	3	+
UDP-glucose dehydrogenase	Ugdh	↑	10	+
Glutathione S-transferase, pi 1	Gstp1	↑	2	+
Glutathione S-transferase, mu 1	Gstm1	↑	1	+
Nuclear factor, erythroid derived 2, like 2	Nrf2	↑	1	+
Notch gene homolog 1 (Drosophila)	Notch1	↑	3	+
Hairy and enhancer of split 1 (Drosophila)	Hes1	↑	7	+

		RAT	
Gene Name	**Gene Symbol**	**Gene Expression**	**Dioxin Response Elements***
Jun oncogene	Jun	↑	2
p21 protein (Cdc42/Rac)-activated kinase 1	Pak1	↑	4
Sphingosine kinase 1	Sphk1	↑	7
BCL2-associated X protein	Bax	↓	7
Caspase 3	Casp3	↓	2

*Computational identification of putative DREs (-10,000 to +1,000 relative to the transcriptional start site) with a matrix similarity score of at least 0.80.

[a]Significant (+) AhR enriched regions identified using chromatin immunoprecipitation.

Notch1 is a transcription factor induced by TCDD that determines cell fate by regulating proliferation, differentiation, and apoptosis (Wakabayashi et al., 2010a). Like Myc and Nrf2, Notch1 was induced by TCDD (2-168 h), exhibited a sigmoidal dose-response at 24 h, and contains DREs in its proximal promoter with significant AhR enrichment (Table 7-1) (Dere et al., submitted-a,b; Sun et al, 2004; Dere et al., 2008). Hes1, a Notch1 target gene and transcriptional regulator, was induced by TCDD in the liver (Lee et al., 2005). Notch1 is also regulated by Nrf2 and the Nrf2-Notch1 signaling pathway to facilitate cell growth and differentiation during liver regeneration (Wakabayashi et al., 2010b).

Data also indicate that while TCDD may activate genes differentially across species, the apical results are similar. For example, cell proliferation and apoptotic pathways were both activated by TCDD in mice and rats, yet the expression of different genes was elicited by TCDD in a species-specific manner (Boverhof et al., 2006). Unlike the induction of Myc in C57BL/6 mice, TCDD induced the proto-oncogene Jun, leading to AP-1-mediated gene transcription and cell proliferation (Roberts et al., 1997). TCDD also induced p21-activated kinase (Pak1), which regulates cell growth and morphogenesis, as well as hyperplasia in breast tumors, possibly via activation of Nf-κB (Balasenthil et al., 2004; Sells and Chernoff, 1997). Moreover, Sphk1, which regulates the synthesis of sphingosine-1-phosphate, a signaling molecule involved in cell growth, proliferation, survival, and morphogenesis, was induced by TCDD (Maceyka et al., 2008; Allende et al., 2004). Over-expression of Sphk1 inhibits Casp3, leading to marked cell proliferation by promoting the G1 to S phase transition (Olivera et al., 1999). TCDD suppressed pro-apoptotic Casp3 and Bax expression, which would inhibit apoptosis in the rat (Fabregat et al., 2007; Green and Reed, 1998).

These hepatic gene expression changes are consistent with TCDD inducing proliferation and inhibiting apoptosis in the liver. For example, morphological determination of apoptosis revealed a significant decrease in the incidence of cell death in rat liver and normal human mammary epithelial MCF10A cells following TCDD treatment (Stinchcombe et al., 1995; Schrenk et al., 2004; Park and Matsumura, 2006). In addition, increases in hepatic cell proliferation quantified by microscopic evaluation of bromodeoxyuridine incorporation were detected in TCDD-exposed rodents (Maronpot et al., 1993; Lucier et al., 1991; Tritscher et al., 1995; Mitchell et al., 2010; NTP, 2006). However, a more comprehensive description of the mechanisms involved in TCDD-elicited cell proliferation is limited by incomplete functional annotation for all differentially expressed genes, the lack of complementary proteomic, metabolomic and enzymatic activity data, and the inability to place all gene expression changes into context.

7.4.2 TCDD-Elicited Proliferative and Anti-Apoptotic Pathways in the Lung

There are only a few reports describing the toxicogenomic effects of TCDD in the lung, limiting the development of a more detailed MOA. Consequently, proliferation and

apoptotic pathways activated by TCDD in other models were investigated to determine their potential relevance in the lung.

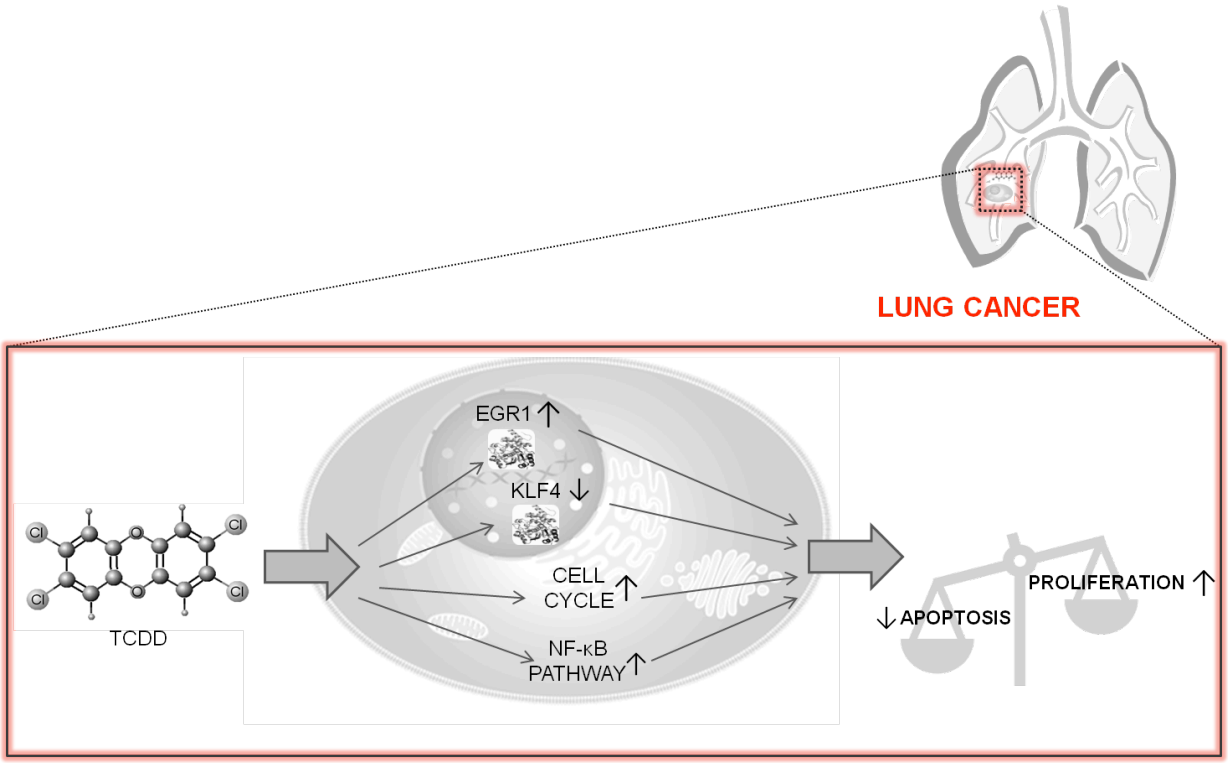
Gene expression analysis of TCDD-treated human peripheral lung epithelial (HPL1A) and adenocarcinomic alveolar basal epithelial (A549) cells identified alterations in signaling pathways associated with pulmonary disease and lung cancer (Martinez et al., 2002, 2004). Exposure to 0.1-10 nM TCDD induced expression of several cell cycle genes (CDKN1A, MDM4, MAD2L2 in HPLA1 cells; CDK6, CDC25A, MCM5, TFDP2 in A549 cells). These changes were consistent with alterations in the G1 phase, although they may be mediated through different effector molecules.

In addition, TCDD significantly down regulated KLF4, a transcription factor that inhibits DNA synthesis (Martinez et al., 2002; Shields et al., 1996; Dang et al., 2001), and represses the expression of SPARC, an extracellular matrix protein that promotes tumor development and metastasis (Zhou et al., 2010). KLF4 suppression by TCDD has been reported in malignant and nonmalignant human airway epithelial cells (Martinez et al., 2002, 2004).

EGR1 is linked to cell growth and cell cycle progression in prostate cancer cells (Virolle et al., 2003). TCDD induced EGR1 in A549 and HPL1A cells (Martinez et al., 2002, 2004). Consequently, proliferative pathways activated by EGR1 in prostate cancer cells have been extrapolated to A549 and HPL1A cells. For example, EGR1 in C2 prostate cancer cells suppressed CDKN2D that inhibits CDK4 and CDK6 cyclin-dependent kinase expression (Virolle et al., 2003). In contrast, TCDD induced CDK6 in A549 adenocarcinomic alveolar basal epithelial cells, consistent with cell growth (Martinez et al., 2002). EGR1 also down regulated NFKBIA in C2 cells, which inhibits proliferation and sensitizes cells to apoptosis via inhibition of Nf-κB pathway (Virolle et al., 2003; Huang et al., 2001). However, TCDD induced BLC3 that activates Nf-κB (Martinez et al., 2002). These examples suggest that the induction of EGR1 by TCDD facilitates proliferation in the lung by suppressing inhibitory genes.

Studies in mouse lung fibroblasts report TCDD suppressed TGF-β signaling that inhibits apoptosis (Henry et al., 2010) and leads to cell proliferation. More specifically, 27 of 82 genes associated with TGF-β signaling were down regulated including Tgfb1, Tgfb2, Tgfbr1, Id1–4, Mapk3, Bmp 4 and 6. This is consistent with reported interactions between AhR and TGF-β in cell culture and in vivo in the presence and absence of xenobiotic ligand (Vogel et al., 1994; Dohr and Abel, 1997; Vogel et al., 1997). Furthermore the induction of Tgfβi, Tnfrsf19 and Traf5 implicates TNF- and TGF-mediated cell growth and survival pathways that affect inflammation, differentiation and mucin secretion (Henry et al., 2010). Figure 7-2 shows a plausible MOA for TCDD-induced lung tumors in rodents.

Figure 7-2. Proposed mode of action of the proliferative effects of TCDD in the lung. Gene expression changes and pathways altered by TCDD that may contribute to proliferation leading to neoplastic lesions in the lung are indicated.



7.4.3 Summary

TCDD elicits differential gene expression associated with proliferative pathways in a species-, tissue-, and cell-specific manner. Despite annotation limitations, the examples discussed indicate that TCDD elicits the differential expression of different complements of genes in different cells, tissues, and species in order to elicit proliferation. Although detailed molecular networks describing the regulation of these genes in any one cell, tissue, or species have yet to be determined, disruption of their associated pathways has been implicated in the etiology of effects elicited by TCDD. Furthermore, computational studies suggest these gene expression responses involve putative DREs and are regulated by the AhR (Dere et al., submitted-a,b; Sun et al., 2004; Dere et al., 2008; Sartor et al., 2009). Overall, compelling MOA-level evidence is presented that extends beyond AhR binding to partially explain the proliferative and anti-apoptotic effects of TCDD in the liver and lung.

8.0 The EPA should reconsider their selection of a linear extrapolation approach to evaluate cancer risk given that three previous scientific panels have recommended nonlinear approaches.

Issue: The EPA chose to evaluate cancer risk using a linear model rather than a nonlinear model. However, three prestigious scientific advisory panels asked to review the EPA's dioxin reassessment all identified the linear modeling assumption as a specific point of concern and recommended that nonlinear approaches be incorporated into the assessment. This recommendation was founded on data demonstrating clear nonlinear dose-response relationships.

Suggested Improvement: Given the EPA's lack of rationale for selecting a linear model, and clear recommendations from multiple scientific panels that have reviewed the assessment, the EPA should put forth an effort on a low-dose nonlinear quantitative approach to deriving a TCDD cancer slope factor that is at least equal to what they presented for preferred linear analysis.

During the EPA's ongoing dioxin reassessment process, three scientific panels have identified the linear modeling assumption as a specific point of concern and clearly stated that alternate, non-linear approaches should at the very least be identified and discussed in the context of the available epidemiological, pharmacokinetic modeling, and bioassay data.

8.1 The 1995 SAB identified the EPA's reliance on a linear model as a major deficiency

In the review report issued in 1995, the SAB stated that the major deficiency of the EPA's 2003 Dioxin Reassessment was "its reliance on the standard EPA default assumption of a linear non-threshold model for carcinogenic risk," and suggested using available data to construct an alternate model that would better fit minimal responses to low levels of environmental exposure (SAB, 1995). The report also urged the EPA to further examine fundamental principles of receptor theory, saying that dioxin is a cancer promoter, not initiator, which acts via the AhR, which exhibits a U-shaped dose response curve. The report further noted that all other agencies that have evaluated the same toxicological and epidemiologic dioxin data have incorporated some type of threshold approach in their risk evaluations.

8.2 The 2001 SAB stated that non-linearity better describes the receptor mediated response

The report issued by the 2001 SAB review panel further addressed the importance of

identifying and evaluating possible alternatives to linear modeling, again bringing to attention that non-linearity better describes a receptor mediated response that could potentially follow very strict thresholds. Additionally, this panel pointed out that some of the epidemiological carcinogenicity data appear to be non-linear and fitting these data with a linear model results in much higher risk estimates. The report stated, that,“ given the current questions about how much more regulatory action is appropriate for dioxin, there is a legitimate need to also include “best estimates” of the cancer risk, and even a “lower” risk estimate that is not solely reliant on a linear model” (SAB, 2001). Consistent with the 1995 review, the 2001 panel concluded that, at the very minimum, other modeling approaches should be addressed and that there appear to be sufficient data that would support the use of a non-linear modeling approach, especially given what we know about the receptor-mediated mode of action of dioxin.

8.3 The 2006 NAS thought that the decision to rely on a linear model lacked scientific support

The final review report submitted by the NAS in 2006 again highlighted the need to explore non-linear dose-response models, stating that, “EPA’s decision to rely solely on a default linear model lacked adequate scientific support,” and, “the committee unanimously agrees that the current weight of evidence on TCDD, other dioxins, and DLCs carcinogenicity favors the use of nonlinear methods for extrapolation below the point of departure (POD) of mathematically modeled human or animal data” (NAS, 2006). The committee summarized four major areas where the scientific evidence supports the use of a nonlinear over a linear model: (1) TCDD, other dioxins, and DLCs are not directly genotoxic, and act as tumor promoters, which exhibit nonlinear dose-response relationships; (2) adverse effects of dioxin exposure are receptor mediated, which have been shown to exhibit more complex dose-response curves, and the EPA has concluded in previous assessments of receptor-mediated agents that a nonlinear model at low doses is appropriate; (3) liver tumors, which the EPA used in the reassessment to evaluate response, are secondary to hepatotoxicity, which raises concern for using tumor data to extrapolate at low doses; and (4) there is clear evidence of a nonlinear, sigmoidal dose-response relationship in recent bioassay data. As discussed in previous review reports, this committee also recommended that the EPA include risk estimates based on nonlinear models in addition to linear approaches for comparison purposes, and discuss the strengths and weaknesses of each method.

9.0 The EPA did not consider the impact of the proposed oral cancer slope factor or RfD

Issue: The EPA has proposed toxicological benchmarks, when used in typical risk assessment calculations, that indicate current levels of TCDD in breast milk, foodstuffs, and soil may pose unacceptable health risks. Furthermore, these proposed benchmarks suggest costly reevaluation and reopening of numerous federal and state dioxin superfund sites. However, these implications were not addressed by the EPA. These issues clearly will have a great impact on the public's perception of health and may result in unwarranted and/or unnecessary concerns or actions.

Suggested Improvement: The draft toxicological benchmarks will have significant impacts on public health policy and various regulatory actions (e.g., Superfund, RCRA, and state environmental programs). The EPA needs to explain to the public the significance of the proposed toxicological benchmarks and the significant cost to the United States if these toxicological benchmarks are utilized by the EPA. For example, will use of these toxicological benchmarks lead to the U.S. government discouraging mothers from breast feeding an infant, and how will the EPA and other U.S. government agencies deal with concerns about the safety of the U.S. food supply? Finally, the EPA should provide a detailed uncertainty analysis of the proposed toxicological benchmarks.

9.1 EPA does not address the major issue that the current intake of TCDD from breast milk far exceeds the RfD proposed in the 2010 Draft Report

Dioxin intake for a breastfeeding infant was compared to the draft RfD. EPA scientists reported high-end, typical background concentrations in human breast milk of 242 pg TEQ/kg-day (Lorber and Phillips, 2002). The draft RfD, a daily intake that the EPA suggests is likely to be without an appreciable risk of deleterious effects during a life time, is 0.7 pg/kg-day. Thus, use of the draft RfD yields an unacceptable hazard for nursing infants when compared to typical background concentrations of dioxins in breast milk. This is particularly concerning, as mothers may opt to not breastfeed their infants and forgo the nutritional benefits to the baby if they believe there are dangerously high dioxin levels present in human breast milk. The EPA clearly needs to address the public significance of this issue.

9.2 The draft toxicological values suggest that the U.S. food supply is unsafe for human consumption, though the EPA does not address how the public should deal with such.

Using generic equations, risk-based concentrations (RBC) ("safe" levels based on the proposed toxicological values) for foodstuffs that contain dioxin (i.e., beef, milk, and fish) were determined and compared to average dioxin concentrations reported in these

media by EPA scientists (Lorber et al., 2009). Exposure scenarios, ingestion rates, and other parameters were based on those applied by the EPA when reporting intakes associated with these foodstuffs (Lorber et al., 2009). As shown in Table 9-1, the results indicate that risk based concentrations for beef, milk, and fish, derived using the OSF were all below their respective average background concentrations at cancer risk levels of 1×10^{-6} and 1×10^{-5}. This yields the conclusion that average beef, milk, and fish available in the U.S. for human consumption may contain unacceptable levels of dioxin, which may lead to widespread, unnecessary concern regarding the safety of the U.S. food supply. However, because of the large and unquantified uncertainty surrounding the proposed toxicological benchmarks, the real human health risk associated with TCDD in the food supply and environmental media may actually be below levels of concern.

Table 9-1. Comparison of dioxin risk-based concentrations (RBCs) to average concentrations observed in foods.[a,b]

Media	RBC Based on OSF[c]			RBC Based on RfD[d] (pg/g)	Average Media Concentration[e] (pg TEQ/g)
	Risk Level: 1E-06 (pg/g)	Risk Level: 1E-05 (pg/g)	Risk Level: 1E-04 (pg/g)		
Beef	0.003	0.03	0.34	1.03	0.142
Milk	0.001	0.01	0.10	0.29	0.017
Fish	0.011	0.11	1.10	3.30	0.94

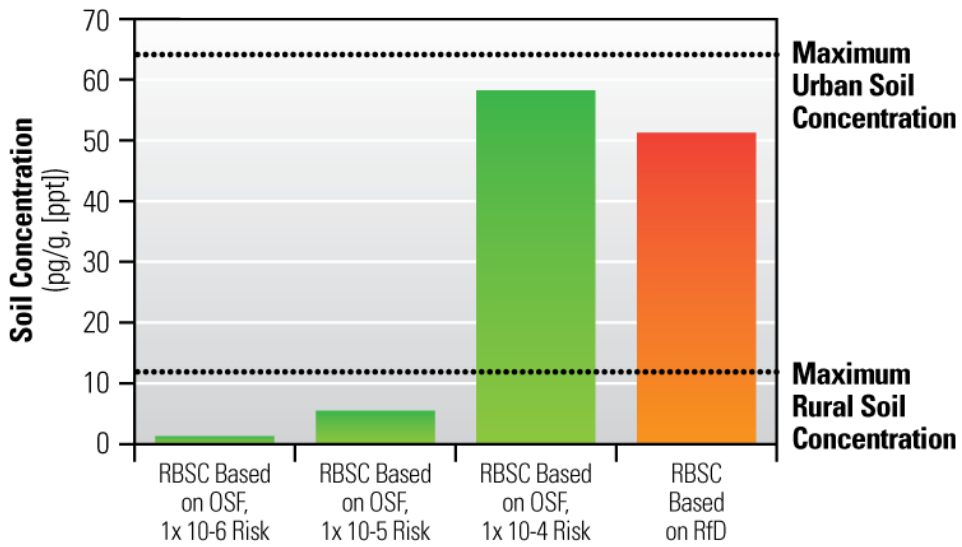
Notes:
[a]All scenarios are based on adult exposures.
[b]Shaded cells indicate calculated RBCs below the respective average media concentrations.
[c]EPA (2010)-recommended OSF of 1×10^{-6} $(mg/kg\text{-}day)^{-1}$ [based on Cheng et al. (2006)]
[d]EPA (2010)-recommended RfD of 7×10^{-10} mg/kg-day [based on Mocarelli et al. (2008)]
[e]As reported by Lorber et al. (2009)

Definitions:
OSF = oral slope factor
pg/g = picogram per gram
RBC = risk-based concentration
RfD = oral reference dose
TEQ = toxicity equivalent

9.3 Use of the draft toxicological values indicates that soils in typical urban areas are contaminated with unacceptable levels; the EPA needs to address the potential concern and unnecessary soil cleanup efforts that would result

Using the same equations and exposure assumptions employed by the EPA in calculating soil risk-based screening concentrations (RBSCs) for dioxin in EPA (2009), we calculated soil-based RBSCs using both the OSF (at three different risk levels: 1×10^{-6}, 1×10^{-5}, and 1×10^{-4}) and the RfD. The results were then compared to maximum soil concentrations in the U.S. (EPA, 2007; UM, 2009). Figure 9-1 clearly demonstrates that the RBSCs based on the EPA's draft toxicological benchmarks – both cancer and non-cancer – result in levels that are below maximum concentrations measured in rural areas of the U.S. Therefore, use of these toxicity benchmarks yields the conclusion that typical soils in the U.S. may contain dioxin at unacceptable levels; such a conclusion could lead to unwarranted alarm and costly, unnecessary soil cleanup efforts.

Figure 9-1. Comparison of dioxin risk-based soil concentrations calculated using EPA 2010 proposed toxicity benchmarks with background concentrations.



The adoption of EPA's 2010 Draft Report toxicity benchmarks would also affect the soil screening and cleanup criteria developed and/or used by state and local environmental agencies across the nation. As of August 2009, 15 states had derived their own screening or cleanup level for soil dioxins. In deriving these values, some states have set their soil dioxin screening levels to what is known to be an average background level for the state (e.g., Massachusetts at 20 ppt). Another 15 states relied on either the EPA's 1998 Preliminary Remediation Goal (1 ppb) or the EPA's Regional Soil Screening Level for guidance on cleanup and remediation of soil dioxins. In general, these criteria are calculated using the EPA's 1985 TCDD OSF of 156,000 $(mg/kg\text{-}day)^{-1}$ though some states have developed their own OSF (e.g., California and Michigan). The proposed OSF is approximately an order of magnitude greater than that currently used to derive the state

risk-based soil dioxin screening and cleanup levels. Thus, if the proposed cancer slope factor were to be officially adopted, several states would find that soil dioxin levels they originally considered to be background would in fact exceed newly derived soil screening and cleanup criteria. This would place unwarranted pressure and costs on many states to remediate their soils to below background dioxin levels.

9.4 Newly drafted EPA toxicity benchmarks will necessitate the costly reevaluation and reopening of numerous Superfund sites

We conducted a survey of EPA's Superfund Information Search[3] and Record of Decision (ROD) System[4] websites to evaluate the scope of Superfund sites impacted by the toxicological benchmarks proposed in the 2010 Draft Report. Specifically, the objective was to quantify the number of Superfund sites where dioxins were identified as a "chemical of potential concern" (COPC) and to determine which sites would not be in compliance with respect to the proposed benchmarks. Results of the survey indicated that PCDDs and PCDFs are considered COPCs in the soils of 163 Superfund sites, of which currently 130 still remain on the National Priorities List (NPL). Thus, the EPA will need to reevaluate 163 sites on the basis of the proposed toxicological benchmarks.

Of these 163 sites identified, 105 sites (dating back to the 1984 ROD for Times Beach, Missouri) had soil dioxin remediation goals established in a ROD. Of these, 76 sites listed a soil dioxin remediation goal of 1 part per billion (1 ppb) or greater. This soil remediation goal is a risk-based standard derived in 1998 by EPA using an OSF of 156,000 $(mg/kg\text{-}day)^{-1}$. The proposed OSF of 1,000,000 $(mg/kg\text{-}day)^{-1}$ would result in a significantly lower remediation goal, thus suggesting that the EPA would need to reconsider the soil dioxin remediation goals of these 76 Superfund sites (EPA, 1998). Additionally, since EPA is likely to soon adopt the WHO 2006 TEFs for dioxin risk assessments (EPA, 2009), it stands to reason that the risk represented by the 12 DL-PCBs will be assessed separately from that of the other PCB congeners. A search of the Superfund Information database shows that there are 345 Superfund sites where PCBs are listed as COPCs, of which 273 of these sites are on the current NPL. Combined, this represents more than 500 Superfund sites where risk managers may be required to reevaluate whether the soil levels of PCDDs, PCDFs, and DL-PCBs pose a significant health risk.

Ultimately, the adoption of the 2010 Draft Report toxicity benchmarks by the EPA will result in considerably more stringent risk-based soil screening and cleanup criteria for DLCs, necessitating the reevaluation and re-opening of Superfund sites where DLCs were not originally considered to present an unacceptable health risk, or where considerable resources have already been spent in remediating the site to the contemporary soil dioxin cleanup levels. These actions will result in considerable remediation expenses, though it is uncertain if these actions provide any health protective benefit.

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[3] http://cfpub.epa.gov/supercpad/cursites/srchsites.cfm
[4] http://cfpub.epa.gov/superrods/index.cfm?fuseaction=main.search

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APPENDIX A: Confounding Exposures Tables for NIOSH Dioxin Registry Plants 3, 4, 7, 8, 9, 10, & 11

Table A-1. Confounding exposures at Plant 3 - Known chemical inventories and carcinogens

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
95-95-4	2,4,5-Trichlorophenol (2,4,5-TCP)	--	2B
93-72-1	Silvex (2-(2,4,5-trichlorophenoxy) propionic acid)	D	--
95-94-3	1,2,4,5-Tetrachlorobenzene (TCB)	--	--
598-78-7	2-Chloropropionic acid	--	--
94-75-7	2,4-Dichlorophenoxyacetic acid (2,4-D)	--	--
2008-39-1	2,4-D dimethylamine	--	--
N.I.	2,4-D esters	--	--
93-76-5	2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	--	--
N.I.	2,4,5-T amine	--	--
25168-15-4	2,4,5-T isooctyl ester	--	--
64-19-7	Acetic acid	--	--
N.I.	Alkyl phenols	--	--
64742-94-5	Aromatic naphtha, heavy	--	--
1194-65-6	Casoron	--	--
N.I.	Castor oil ethoxylate	--	--
N.I.	Catacarb	--	--
67-48-1	Choline chloride	--	--
123-01-3	Dodecylbenzene	--	--
N.I.	Ethylene oxide condensates	--	--
N.I.	Glycolate byproducts	--	--
26952-21-6	Isooctyl alcohol	--	--
8008-20-6	Kerosene	--	--
N.I.	Long chain alcohols	--	--
79-11-8	Monochloroacetic acid (MCA)	--	--
N.I.	MCA ester	--	--
111-87-5	Octyl alcohol	--	--
136-32-3	Sodium 2,4,5-trichlorophenate (NaTCP)	--	--
1310-73-2	Sodium hydroxide	--	--
7664-93-9	Sulfuric acid	--	--
121-44-8	Triethylamine	--	--

Table A-2. Confounding exposures at Plant 4 - Known chemical inventories and carcinogens

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
120-83-2	2,4-Dichlorophenol (DCP)	--	2B
95-95-4	2,4,5-Trichlorophenol (2,4,5-TCP)	--	2B
25167-81-1	Dichlorophenol (DCP)	--	2B
N.I.	Tetrachlorophenols (TetCP)	--	2B
25167-82-2	Trichlorophenol (TCP)	--	2B
50-29-3	Dichlorodiphenyltrichloroethane (DDT)	B2	2B
56-23-5	Carbon tetrachloride	L	2B
7647-01-0	Hydrochloric acid	--	3
121-75-5	Malathion	--	3
298-00-0	Methyl parathion	--	3
309-00-2	Aldrin	B2	3
60-57-1	Dieldrin	B2	3
108-88-3	Toluene	II	3
71-36-3	n-Butanol	D	--
93-72-1	Silvex (2-(2,4,5-trichlorophenoxy) propionic acid)	D	--
95-94-3	1,2,4,5-Tetrachlorobenzene (TCB)	--	--
598-78-7	2-Chloropropionic acid	--	--
104-76-7	2-Ethylhexanol	--	--
75-99-0	2,2-Dichloropropionic acid (Dalapon)	--	--
94-75-7	2,4-Dichlorophenoxyacetic acid (2,4-D)	--	--
1928-43-4	2,4-D 2-ethylhexyl ester	--	--
94-80-4	2,4-D butyl ester	--	--
20940-37-8	2,4-D diethylamine salt	--	--
1713-15-1	2,4-D isobutyl ester	--	--
6130-75-2	2,4,5-Trichloroanisole (TCA)	--	--
93-76-5	2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	--	--
N.I.	2,4,5-T amines and esters	--	--
1928-47-8	2,4,5-T 2-ethylhexyl ester	--	--
93-79-8	2,4,5-T butyl ester	--	--
2008-46-0	2,4,5-T ionic triethylamine salt	--	--
4938-72-1	2,4,5-T isobutyl ester	--	--
120-36-5	2-(2,4-dichlorophenoxy)propionic acid (2,4-DP)	--	--
N.I.	2,4-DP isobutyl ester	--	--
N.I.	2,4-DP butyl ester	--	--
39277-47-9	Agent orange	--	--
109-89-7	Diethylamine (DEA)	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
115-10-6	Dimethyl ether (DME)	--	--
124-40-3	Dimethylamine (DMA)	--	--
78-83-1	Isobutanol	--	--
8008-20-6	Kerosene	--	--
67-56-1	Methanol	--	--
28554-00-9	Monochloropropionic acid (MCA)	--	--
3926-62-3	MCA sodium salt (NaMCA)	--	--
N.I.	Organic contaminants including TCDD	--	--
53404-76-5	Silvex 2-ethylhexyl ester	--	--
136-32-3	Sodium 2,4,5-trichlorophenate (NaTCP)	--	--
7647-14-5	Sodium chloride	--	--
1310-73-2	Sodium hydroxide	--	--
124-41-4	Sodium methoxide	--	--
121-44-8	Triethylamine	--	--

Table A-3. Confounding exposures at Plant 7 - Known chemical inventories and carcinogens

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
7664-93-9	Sulfuric acid	--	1
8002-05-9	Petroleum polymer resins	--	2A
1336-36-3	Polychlorinated biphenyls, unspecified	B2	2A
120-83-2	2,4-Dichlorophenol	--	2B
N.I	a-Chlorophenol	--	2B
95-57-8	o-Chlorophenol	--	2B
106-48-9	p-Chlorophenol	--	2B
106-46-7	p-Dichlorobenzene	--	2B
87-86-5	Pentachlorophenol	--	2B
131-52-2	Pentachlorophenol, sodium salt	--	2B
25167-83-3	Tetrachlorophenol (TetCP)	--	2B
98-95-3	Nitrobenzene	L	2B
88-73-3	1-Chloro-2-nitrobenzene	--	3
100-00-5	1-Chloro-4-nitrobenzene	--	3
7647-01-0	Hydrochloric acid	--	3
82-68-8	Pentachloronitrobenzene	--	3
7757-83-7	Sodium sulfite	--	3
7446-09-5	Sulfur dioxide	--	3
85-68-7	N-Butyl benzyl phthalate	C	3
95-50-1	o-Dichlorobenzene	D	3
108-95-2	Phenol	D; DI	3
108-90-7	Monochlorobenzene	D	--
N.I.	((Monotrichloro) tetra-(monopotassium dichloro)) pentaisocyanurate	--	--
N.I	1-Phenol-4-sulfonic acid	--	--
99-54-7	1,2-Dichloro-4-nitrobenzene	--	--
87-90-1	1,3,5-Trichloro-s-trianzine-2,4,6 (1 H,3H,5H)-trione	--	--
N.I	111-V Intermetallics compounds	--	--
1918-16-7	2-Chloro-N-isopropylacetanilide (propachlor)	--	--
N.I	2,2'-Thiobis (4,6-di-sec-amyphenol)	--	--
N.I	2,4,5-D esters	--	--
N.I	2,4,5-T esters	--	--
836-30-6	4-Nitrodiphenylamine	--	--
80-09-1	4,4'-Sulfonyldiphenol	--	--
N.I	Alkylate, linear	--	--
A026000000	Alkylbenzenesulfonic Acid and Salts	--	--
64742-94-5	Aromatic naphtha, heavy	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
590-02-3	Butyl chloroacetate	--	--
52673-62-8	Capacitor fluid (Pyranol)	--	--
7782-50-5	Chlorine	--	--
7790-94-5	Chlorosulfonic acid	--	--
N.I	Coumarone-idene polymer resins	--	--
26444-49-5	Cresyl diphenyl phosphate	--	--
108-80-5	Cyanuric acid	--	--
108-91-8	Cyclohexylamine and derivatives	--	--
2244-21-5	Dichloro-s-triazine-2,4,6 (1 H,3H,5H) trione, potassium salt	--	--
N.I	Dichloro-s-triazine-2,4,6 (1 H,3H,5H) trione, soduim salt	--	--
101-83-7	Dicyclohexylamine	--	--
28675-17-4	Dodecylaniline	--	--
105-39-5	Ethyl chloroacetate	--	--
N.I	Lubricating oil and grease additives (unspecified)	--	--
127-68-4	M-Nitrobenzene sulfonic acid, sodium salt	--	--
7790-94-5	Monochlorosulfuric acid	--	--
N.I	N-(1,3-dimethylamyl)-N'-phenyl-p-phenylenediamine	--	--
N.I	N-(1,4-dimethylamyl)-N'-phenyl-p-phenylenediamine	--	--
101-72-4	N-isopropyl-N'-phenyl-p-phenylenediamine	--	--
3081-14-9	N,N'Bis-(1,4-dimethylpentyl)p-phenylenediamine	--	--
N.I	Nitro-dodecylbenzene	--	--
120-32-1	o-Benzyl-p-chlorophenol	--	--
88-74-4	o-Nitroaniline	--	--
25154-55-6	o-Nitrophenol	--	--
N.I	Orthochloroparatoluene sodium sulfonate	--	--
78-31-9	p-Cresyl diphenyl phosphate	--	--
100-01-6	p-Nitroaniline	--	--
100-02-7	p-Nitrophenol	--	--
156-43-4	p-Phenetidine	--	--
92-94-4	p-Terphenyl	--	--
7664-38-2	Phosphoric acids, ortho	--	--
10025-87-3	Phosphorus oxychloride	--	--
1314-80-3	Phosphorus pentasulfide	--	--
7719-12-2	Phosphorus trichloride	--	--
1310-58-3	Potassium hydroxide	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
7440-21-3	Silicon, ultra pure	--	--
1310-73-2	Sodium hydroxide	--	--
127-68-4	Sodium m-nitrobenzenesulfonate	--	--
824-78-2	Sodium p-nitrophenate	--	--
7757-82-6	Sodium sulfate	--	--
1313-82-2	Sodium sulfide	--	--
52673-62-8	Transformer fluid (Pyranol)	--	--
78-32-0	Tri-p-cresyl phosphate	--	--
1344-32-7	Trichlorobenzyl chloride	--	--
115-86-6	Triphenyl phosphate	--	--
101-02-0	Triphenyl phosphite	--	--

Table A-4. Confounding exposures at Plant 8 - Known chemical inventories and carcinogens

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
64-17-5	Ethyl alcohol	--	1
95-53-4	Ortho-toluidine	--	1
92-67-1	Para-aminobiphenyl (PAB)	--	1
71-43-2	Benzene	A	1
79-01-6	trichloroethylene	--	2A
62-75-9	N-nitrosodimethylamine	B2	2A
59-89-2	N-nitrosomorpholine	--	2B
95-06-7	2-Chloroallyl diethyldithiocarbamate (Sulfallate)	--	2B
95-95-4	2,4,5-Trichlorophenol (2,4,5-TCP)	--	2B
140-88-5	Ethyl acrylate	--	2B
75-07-0	Acetaldehyde	B2	2B
302-01-2	Diamine	B2	2B
298-00-0	Methylparathion	--	3
99-80-9	N-methyl-N,4-dinitrosaniline	--	3
103-11-7	2-Ethylhexyl acrylate	--	3
137-26-8	Tetramethyl thiuram disulfide (Thiram)	--	3
94-36-0	Benzoyl peroxide	--	3
137-30-4	Zinc dimethyl dithiocarbamate (Ziram)	--	3
123-31-9	Hydroquinone	--	3
110-91-8	Morpholine	--	3
110-86-1	Pyridine	--	3
2303-16-4	S-2, 3-dichloroallyl diisopropylthiocarbamate (Avadex)	--	3
7757-83-7	Sodium sulfite	--	3
62-56-6	Thiourea	--	3
137-26-8	Tetramethyl thiuram disulfide (Thiram)	--	3
103-11-7	2-Ethylhexyl acrylate	--	3
62-53-3	Aniline	B2	3
107-02-8	Acrolein	DI	3
1330-20-7	Xylene	DI	3
95-48-7	o-Toluene	C	--
108-90-7	Monochlorobenzene	D	--
142-82-5	Heptane	D	--
67-64-1	Acetone	DI	--
880-09-1	1,1'-Methylenedipiperidine condensate	--	--
147-47-7	1,2-Dihydro-2,2,4-trimethylquinoline	--	--
91-53-2	1,2-Dihydro-6-ethoxy-2,2,4-trimethylquinoline	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
N.I.	1,2-Dihydro-6-dodecyl-2,2,4-trimethylquinoline	--	--
96-19-5	1,2,3-Trichloropropene	--	--
95-94-3	1,2,4,5-Tetrachlorobenzene (TCB)	--	--
64216-20-2	1,3-Bis (2-benzothiazolylmercaptomethyl) urea	--	--
102-08-9	1,3-Diphenyl-2-thiourea	--	--
102-06-7	1,3-Diphenylguanidine	--	--
118-99-0	1,3-Diphenylguanidine phthalate	--	--
2492-26-4	2-Benzothiazolethiol, sodium salt	--	--
104-76-7	2-Ethylhexanol	--	--
149-30-4	2-Mercaptobenzothiazole (Thiotaz)	--	--
120-78-5	2,2'-Dithiobis(benzothiazole)	--	--
2303-17-5	2,3,3-Trichloroallyl n,n-diisopropylthiolcarbamate	--	--
N.I.	2,4,5-T ester	--	--
6130-75-2	2,4,5-Trichloroanisole (TCA)	--	--
93-76-5	2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	--	--
79-74-3	2,5-Di (tert-amyl) hydoquinone	--	--
141-91-3	2,6-Dimethyl morpholine	--	--
85-60-9	4,4'-Butylidenebis (6-tert-butyl-m-cresol)	--	--
103-34-4	4,4'-Dithiodimorpholine	--	--
96-69-5	4,4'-Thiobis(6-tert-butyl-m-cresol) (Santonox)	--	--
88-60-8	6-Tert-butyl-m-cresol	--	--
1333-89-7	Abitol	--	--
39277-47-9	Agent orange	--	--
300-57-2	Alkylbenzene	--	--
7446-70-0	Aluminum chloride	--	--
7664-41-7	Ammonia	--	--
1762-95-4	Ammonium thiocyanate	--	--
123-72-8	Butyraldehyde	--	--
N.I.	Butyraldehyde-acetaldehyde-analine condensate	--	--
N.I.	Butyraldehyde-butylideneaniline condensate	--	--
107-92-6	Butyric acid	--	--
592-01-8	Calcium cyanide	--	--
75-15-0	Carbon disulfide	--	--
7782-50-5	Chlorine	--	--
61789-28-4	Creosote oil	--	--
2201-15-2	Cyclohexamine	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
104-72-3	Decylbenzene	--	--
1322-98-1	Decylbenzenesulfonic acid, sodium salt	--	--
N.I.	Defoamer PC 1244 and 1244	--	--
N.I.	Dibutyl-p-cresol process oil	--	--
26952-23-8	Dichloropropene	--	--
60-29-7	Diethyl ether	--	--
111-46-6	Diethylene glycol	--	--
107-39-1	Diisobutylene	--	--
N.I.	Dimer acid	--	--
124-40-3	Dimethylamine (DMA)	--	--
122-39-4	Diphenylamine	--	--
102-06-7	Diphenylguanidine	--	--
N.I.	diphenylparaphenylene	--	--
120-95-6	Di-tert-amylphenol	--	--
25155-30-0	Sodium dodecylbenzenesulfonate	--	--
N.I.	DPS	--	--
N.I.	Synthetic resin antioxidant	--	--
107-21-1	Ethylene glycol	--	--
147-47-7	Flectol A	--	--
110-17-8	Fumaric acid	--	--
74-90-8	Hydrogen cyanide	--	--
64742-94-5	Naphtha, aromatic heavy	--	--
108-25-8	Isopropyl xanthate	--	--
8008-20-6	Kerosene	--	--
64742-95-6	Naphtha, light aromatic	--	--
1305-78-8	Lime	--	--
6915-15-7	Mailc acid	--	--
149-30-4	Mercaptobenzothiazole (MBT)	--	--
67-56-1	Methanol	--	--
583-91-5	Methionine hydroxy analog	--	--
74-93-1	Methyl mercaptan	--	--
N.I.	Molten sulfur	--	--
79-11-8	Monochloroacetic acid (MCA)	--	--
N.I.	N-(2,6-dimethylmorpholine)-2-benzothiazolesulfenamide	--	--
4275-07-4	N-butyraldehyde-aniline-condensate	--	--
95-33-0	N-cyclohexyl-2-benzothiazolesulfenamide	--	--
102-77-2	N-oxydiethylene-2-benzothiazolesulfenamide	--	--
95-31-8	N-tert-Butyl-2-benzothiazolesulfenamide	--	--
139-60-6	N,N' bis (1-ethyl-3-methylpentyl) p-phenylenediamine	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
74-31-7	N,N' diphenyl-para-phenylene-dianine	--	--
103-96-8	N,N' bis (1-methylheptyl) p-phenylenediamine)	--	--
2492-26-4	Sodium 2-mercaptobenzothiazole (NaMBT)	--	--
93-69-6	o-Tolybiguanide	--	--
143-28-2	Ocenol	--	--
148465-31-0	Olifen	--	--
123-01-3	Dodecyl benzene	--	--
112-80-1	Oleic acid	--	--
N.I.	P,P' di-tert-octyl-diphenyl amine	--	--
N.I.	Paper sizing agent	--	--
30525-89-4	Paraformaldehyde	--	--
100-02-7	Paranitrophenol	--	--
156-43-4	Paraphenetidine	--	--
1314-56-3	Phosphorus pentoxide	--	--
85-44-9	Phthalic anhydride	--	--
584-08-7	Pot ash	--	--
1310-58-3	Potassium hydroxide	--	--
8050-09-7	Rosin	--	--
136-32-3	Sodium 2,4,5-trichlorophenate (NaTCP)	--	--
13560-99-1	Sodium 2,4,5-trichlorophenoxyacetate (Na 2,4,5-T)	--	--
143-33-9	Sodium cyanide (NaCN)	--	--
1310-73-2	Sodium hydroxide	--	--
7681-52-9	Sodium hypochlorite	--	--
57-11-4	Stearic acid	--	--
N.I.	Sulfohydrate	--	--
7704-34-9	Sulfur	--	--
10545-99-0	Sulfur dichloride	--	--
N.I.	Sulfur dichloride condensate	--	--
10025-67-9	Sulfur monochloride	--	--
7446-11-9	Sulfur trioxide	--	--
N.I.	Tert butyl meta cresol	--	--
75-85-4	Tert-amyl alcohol	--	--
75-64-9	Tert-butylamine	--	--
12408-10-5	Tetrachlorobenzene	--	--
60320-18-5	Tetrachloropropene	--	--
26544-38-7	Tetrapropenyl-succinic anhydride	--	--
104-15-4	Toluene sulfonic acid	--	--
1330-78-5	Tricresyl phosphate	--	--
N.I.	Triethyl-tert-amine	--	--
151-01-9	Xanthate	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
100-61-8	N-methylaniline	--	--
7631-99-4	Sodium nitrate	--	--
35175-85-0	Trichloropropene	--	--
90-41-5	Ortho aminodiphenyl	--	--
583-91-5	2, hydroxy-4-methylthio-butyric acid	--	--
106-50-3	P-phenylenediamine	--	--
N.I.	Other nitrosamines	--	--

Table A-5. Confounding exposures at Plant 9 - Known chemical inventories and carcinogens

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
75-21-8	Ethylene oxide	--	1
58-90-2	2,3,4,6-Tetrachlorophenol	--	2B
120-83-2	2,4-Dichlorophenol (DCP)	--	2B
95-95-4	2,4,5-Trichlorophenol (2,4,5-TCP)	--	2B
25167-81-1	Dichlorophenol	--	2B
25167-82-2	Trichlorophenol (TCP)	--	2B
88-06-2	2,4,6-Trichlorophenol	B2	2B
75-09-2	Methylene chloride	B2	2B
87-86-5	Pentachlorophenol (PCP)	B2	2B
7647-01-0	Hydrochloric acid	--	3
108-95-2	Phenol	DI	3
111-76-2	2-Butoxyethanol	NL	3
108-90-7	Chlorobenzene	D	--
93-72-1	Silvex (2,4,5-TP)	D	
9594-3	1,2,4,5-Tetrachlorobenzene	--	--
136-25-4	2-(2,4,5-trichlorophanoxy)-ethyl 2,2-dichloropropionate (Erbon)	--	--
75-99-0	2,2-Dichloropropionic acid (Dalapon)	--	--
94-75-7	2,4-Dichlorophenoxyacetic acid (2,4-D)	--	--
N.I.	2,4-D esters	--	--
2008-39-1	2,4-D dimethylamine salt	--	--
3599-58-4	2,4-D ethanolamine salt	--	--
6365-72-6	2,4-D isopropanolamine salt	--	--
93-76-5	2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	--	--
N.I.	2,4,5-T amines	--	--
93-79-8	2,4,5-T butyl ester	--	--
N.I.	2,4,5-T esters	--	--
2008-46-0	2,4,5-T triethylamine salt	--	--
6130-75-2	2,4,5-Trichloroanisole (2,4,5-TCA)	--	--
N.I.	Alpha-monochloropropionic acid alcohol ester	--	--
7446-70-0	Aluminum chloride	--	--
35296-72-1	Butyl alcohol	--	--
7782-50-5	Chlorine	--	--
115-10-6	Dimethyl ether (DME)	--	--
124-40-3	Dimethylamine (DMA)	--	--
23436-19-3	Dowanol PIB	--	--
141-43-5	Ethanolamine	--	--

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
26952-21-6	Isooctyl alcohol	--	--
78-96-6	Isopropanolamine	--	--
67-56-1	Methanol	--	--
2143-68-2	Methoxy	--	--
N.I.	Monochloroacetic acid (MCA) alcohol ester	--	--
3926-62-3	MCA sodium salt (NaMCA)	--	--
25167-80-0	Monochlorophenol	--	--
2524-03-0	O,O-dimethylphosphoro-chloridothioate (DMPCT)	--	--
N.I.	Propionic analogues	--	--
299-84-3	Ronnel	--	--
136-32-3	Sodium 2,4,5-trichlorophenate (NaTCP)	--	--
N.I.	Sodium alpha-mono-chloropropionate	--	--
1310-73-2	Sodium hydroxide	--	--
7681-52-9	Sodium hypochlorite	--	--
124-41-4	Sodium methylate (methoxide)	--	--
131-52-2	Sodium pentachlorophenate	--	--
121-44-8	Triethylamine	--	--

Table A-6. Confounding exposures at Plant 10 - Known chemical inventories and carcinogens

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
70-30-4	Hexachlorophene (HCP)	--	3
95-95-4	2,4,5-Trichlorophenol (2,4,5-TCP)	--	2B
583-78-8	2,5-Dichlorophenol	--	2B
25167-81-1	Dichlorophenol (DCP)	--	2B
25167-82-2	Trichlorophenol (TCP)	--	2B
123-91-1	Dioxane	B2	2B
95-94-3	1,2,4,5-Tetrachlorobenzene (TCB)	--	--
111-46-6	Diethylene glycol	--	--
107-21-1	Ethylene glycol	--	--
7647-01-0	Hyrdrogen chloride, anhydrous	--	--
7727-37-9	Nitrogen gas	--	--
136-32-3	Sodium 2,4,5-trichlorophenate (NaTCP)	--	--
7647-14-5	Sodium chloride	--	--
N.I.	Sodium ethylene glycolate	--	--
1310-73-2	Sodium hydroxide	--	--
112-27-6	Triethylene glycol (TEG)	--	--

Table A-7. Confounding exposures at Plant 11 - Known chemical inventories and carcinogens

CAS Number	Chemical	Cancer Classification	
		EPA†	IARC‡
71-43-2	Benzene	A	1
95-95-4	2,4,5-Trichlorophenol (2,4,5-TCP)	--	2B
107-06-2	Ethylene dichloride (1,2-Dichloroethane)	B2	2B
70-30-4	Hexachlorophene (HCP)	--	3
7647-01-0	Muriatic acid	--	3
8014-95-7	Oleum	--	3
95-94-3	1,2,4,5-Tetrachlorobenzene	--	--
93-76-5	2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	--	--
107-21-1	Ethylene glycol	--	--
N.I.	Filtrol (Bentonite clay and aluminum silicate)	--	--
N.I.	Other filter aids	--	--
30525-89-4	Paraformaldehyde	--	--
7647-14-5	Sodium chloride	--	--
1310-73-2	Sodium hydroxide	--	--
7664-93-9	Sulfuric acid	--	--

†EPA Classifications: A = human carcinogen, L = likely/ known human carcinogen, B2 = probable human carcinogen - animal data, C = Possible human carcinogen, DI = inadequate data, II = inadequate information, NL = not likely to be carcinogenic to humans, D = not classifiable, -- = chemical not addressed

‡IARC Classification: 1 = human carcinogen, 2A = probably carcinogenic to humans, 2B = possibly carcinogenic to humans, 3 = not classifiable, -- = chemical not addressed

N.I. – CAS registry number not identified.