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Final

Review and Comments on the Draft Report: State of the Art Assessment of Endocrine Disruptors Part 1 – Summary of the State of the Science

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Introduction

The draft report, *State of the Art Assessment of Endocrine Disruptors Part 1 – Summary Of The State Of The Science (SAAED)* (referred to in this document as the Report) was released on 31 January 2011 and reviewed on behalf of ECETOC to evaluate the strength of the scientific evidence presented for the major conclusions in the Report. Subsequent to our review, this draft was finalized and distributed on 29 January 2012 as Annex I to the *State Of The Art Assessment Of Endocrine Disrupters* prepared on behalf of the European Commission, DG Environment. The final Annex does not differ substantially from the draft based on a comparison of the chapters we reviewed and for which we provided detailed comments. However, for convenience sake, we have provided page number references to the final Annex Report, not the draft Report.

The SAAED Report attempts to provide a background introduction to endocrine disruption, overarching issues or emerging issues in endocrine disruption, and several independent chapters on various human health endpoints that could be impacted by endocrine disruption. The scope of this Report may have been overly ambitious and as a result falls short of meeting the objective in summarizing the state of the science.

Several general comments were identified based on our review of the Report overall and are presented below in the General Issues Section. The General Issues Section addresses a number of issues that were common throughout the Report. Also we selected and critically reviewed four of the individual chapters in detail and provided specific comments on these chapters. This allowed us to pay close attention to these parts of the voluminous Report. The chapters selected for more detailed review include:

- Chapter 3.2 Over-arching Issues/Emerging Issues: Low Dose Effects
- Chapter 4.5 Human Health Endpoints – Reproductive Health: Female Fertility and Adverse Pregnancy Outcomes
- Chapter 5.2 Human Health Endpoints – Hormonal Cancers: Prostate Cancer
- Chapter 6.1 Human Health Endpoints – Metabolism and Development: Developmental Neurotoxicity

The comments on the individual chapters serve as representative examples of the issues identified in the SAAED and demonstrate a number of concerns with the approach used to conduct this Report. First, the selective identification of literature and the failure to rely on primary literature rather than reviews limits the reliability of this assessment. Furthermore, failure to cite the most current studies relevant to the discussion is misleading and cannot reflect a summary of the state-of-the-science. Second, the frequent reference to broad chemical categories, such as organochlorine pesticides is inappropriate because individual compounds have differing mechanisms of action and may not mediate effects via endocrine disruption. Third, limited or inconsistent data are often presented and relied on to reach conclusions that endocrine disruption is of concern, when given the scant data available the conclusions should be characterized as preliminary and an area for further research. Fourth, data are frequently

presented to associate certain chemicals to endocrine disruption as a mode of action and other data are available to show that certain chemicals cause specific human health effects; however, the linkage between these two focus areas is generally lacking. The authors generally fail to address the most pertinent question about whether or not the chemicals cause the health effect through an endocrine-related mechanism. Finally, the authors adopted the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) criteria for attributing effects to endocrine disruption, but ignored the WHO causal criteria outlined in Chapter 7 of the same report that are recommended for assessing the "relationship between exposure to EDCs [endocrine disrupting compounds] and altered health outcomes." (p. 123, WHO 2002). Therefore, the conclusions reached in the SAAED about the potential for endocrine disruption can only be considered preliminary and non-specific for any given exposure.

General Issues

The SAAED document is not a comprehensive and unbiased state-of-the-science review

In the final document, Section 1.5 Weight of Evidence, was added to the Introduction. We agree that a review of the weight-of-evidence (WoE) is distinct from a strength of evidence evaluation and would concur with the definition of WoE as described by the authors:

> *"WoE requires the synthesis of 'all' the evidence and to achieve this goal the analysis of evidence across several dimensions needs to be conducted, and this includes large or small, strong or weak, old and new studies over scales ranging from human populations to cellular systems." (p. 7, SAAED 2012)*

However, despite the authors' own description stating a synthesis of all of the evidence, they suggest that their review of reviews approach was most appropriate to summarize the state-of-knowledge from a wide range of disciplines. However, this approach does not appear to be comprehensive or representative of all points of view – nor inclusive of all review articles. Given the current state of debate on many of the issues and many of the equivocal findings covered in this Report, this approach is misleading and does not represent a WoE review and should not be characterized as such.

In addition to the review of reviews, the authors have selectively included very specific non-review papers, leading to a very inconsistent presentation. Reviews that discount endocrine disruption or present data contradicting the papers cited by the SAAED authors are often ignored completely or dismissed outright in their discussions. This suggests that the SAAED Report is not particularly balanced. In some cases, the information given by the SAAED authors appears to have been pulled directly from the abstracts of papers being relied on, as more detailed information provided in the actual body of the papers has not been discussed. It is problematic when the summary information provided in the abstract is inaccurate. Specific examples of where important papers have been ignored or dismissed and the information discussed appears to be based on the study abstracts only are provided in the following chapters on specific health outcomes. Also, in certain cases the references are to websites, which are clearly not peer-reviewed materials and the quality of these data are not assessed.

Based on some of the data provided to support associations between specific health outcomes or endocrine-mediated mechanisms and exposure to certain chemicals, it appears that the SAAED authors did not consider the quality of the research reviewed in drawing their conclusions. Studies that used weak research designs (e.g., ecological and cross-sectional epidemiological studies) or included small sample sizes are given as much weight as studies with stronger designs (e.g., case-control and cohort studies) and larger sample sizes. Additionally, the SAAED authors seem to base on their opinions on positive findings in a single study when the larger universe of research available shows primarily negative findings (for example, in their

review of studies of organochlorine pesticides and prostate cancer, as discussed more fully in the response to Chapter 5.2 below). This suggests that this Report is biased in its interpretation of the data.

Finally, the SAAED Report cannot be considered a "state-of-the-science" summary specifically because it relies almost wholly on review articles. Review articles, by their very nature, capture research that has been conducted in the past. The most recent science is not covered. Therefore, to address the state of the science, data from primary research articles must be discussed. Unfortunately, the SAAED authors generally fail to address primary literature, except occasionally and in only limited circumstances.

Definitions of endocrine disruption

The SAAED authors devote a whole chapter to discussing the different definitions of endocrine disruption and endocrine disruptors that have been proposed over the years. The most currently relevant definition is that proposed by the WHO/IPCS in 2002:

> *"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." (p. 1)*

The authors then go on to describe some issues and regulatory implications associated with the use of this definition. Of particular interest are issues related to use of the terms "adverse" and "intact organism" in this definition.

Adverse effects. As discussed on page 9 of the Report, endocrine disruption is a mode of action rather than a health outcome, and changes in endocrine function may occur that do not adversely affect an organism or that remain within the physiological range of normal. Although various groups have proposed revising the definition of endocrine disruption based on the potential upstream events that can be characterized using *in vitro* assay systems, governmental bodies cannot regulate chemicals based on their modes of action alone. As with carcinogens and reproductive toxicants, specific adverse health outcomes must be shown. Once a specific health outcome is proven, then it may be possible to take mode of action data into consideration in the risk assessment process. Therefore, for regulatory purposes, the definition of an endocrine disruptor must make specific reference to a compound's potential to cause adversity.

Intact organism. From a regulatory perspective, the definition of an endocrine disruptor relies on the demonstration of adverse effects in an intact organism. The SAAED authors suggest that this definition translates into a requirement to show a positive finding in an *in vivo* assay system. Although true, this requirement does not preclude the use of *in vitro* assays and alternative whole animal systems to screen for potential endocrine effects. The authors create the impression that this would be the case. In fact, the U.S. Environmental Protection Agency (EPA) is currently implementing a two-tiered screening approach— the Endocrine Disruptor Screening Program (EDSP)— to identify chemicals that may disrupt the endocrine systems of

humans and wildlife (USEPA 2011). The first tier of EDSP screening involves *in vitro* assays (of hormone receptors and steroidogenic enzyme activities) and *in vivo* assays conducted in non-intact systems (the Hershberger and uterotrophic assays); these assays are in addition to Tier 1 *in vivo* screens conducted in whole animals. Although *in vitro* studies contribute to our understanding of potential mechanisms, by definition they cannot be considered as demonstrating endocrine disruption (which must be shown in whole organisms). Nevertheless, the results of Tier 1 screening (including those of *in vitro* assays) are expected to be useful in identifying chemicals to be further tested in Tier 2 for endocrine disruption in whole animals. It is also anticipated that Tier 1 findings will allow for a more hypothesis-driven approach to Tier 2 testing (USEPA 2011). Therefore, the emphasis on effects in intact organisms does not exclude the use of simpler test systems, as suggested by the authors.

Chemical classes discussed are too broad

The SAAED authors generally attribute effects to broad chemical classes throughout the Report. This type of treatment is inappropriate as the chemicals in these broad classes may not be structurally similar and do not all work by the same mechanism of action. For example, in Chapter 3 of the SAAED Report, the authors list "pesticides" as an example of an endocrine disruptor that affects the estrogen receptor, the androgen receptor, and the thyroid receptor. Pesticides are a very large class of chemicals that act on numerous target species and by various different mechanisms of action. Only a small subset of pesticides will actually disrupt the function of a nuclear receptor, and any single pesticide may disrupt the function of only one of these receptor types (if it affects any at all). Therefore, to be more accurate specific pesticides exposures need to be identified. This issue extends beyond pesticides, however, to other broad chemical classes such as pharmaceuticals, environmental contaminants, solvents, and organochlorine compounds. Additionally, the lack of specificity regarding chemicals discussed is a problem throughout the document, affecting almost every chapter of the Report.

Focus on prostaglandins is unwarranted

Most of the SAAED Report focuses on the estrogen, androgen, and thyroid hormone pathways. This emphasis seems appropriate as most of the available research has concentrated on understanding these systems. Further, the attention of Organisation for Economic Co-operation and Development (OECD) and U.S. EPA endocrine-disruption screening efforts has been to develop assays that address the estrogen, androgen, and thyroid hormone systems. Therefore, it is unclear why the SAAED authors devote a whole chapter in the emerging issues section on prostaglandins while ignoring discussion of other key human hormones such as insulin, prolactin, growth hormone, and oxytocin. The SAAED should either provide a clear justification for focusing on prostaglandins or broaden their discussion to address additional hormones and their importance in human development.

Discussions related to critical windows of vulnerability are speculative and do not take into account dose-response

The majority of chapters on human health endpoints in the SAAED Report include a sub-section on critical windows of susceptibility or developmental vulnerability. These discussions are fairly speculative and only provide limited concrete evidence to support the existence of such critical windows. Examples of the speculative language used in these discussions include:

> *"The hypothesis that disturbance of the endocrine and paracrine regulation of meiosis could disrupt chromosome segregation suggests that…" (p. 158, SAAED 2012)*
>
> *"This could be putatively related to the fact that maternal endogenous estrogens are elevated…" (p. 210, SAAED 2012)*
>
> *"The higher estradiol levels measured in African-American women during pregnancy (Henderson et al. 1988, Potischmann et al. 2005) suggest a link with the aetiology of the disease."(p. 261, SAAED 2012)*
>
> *"…disruption of developmental mechanisms seems a plausible explanation" (p. 286, SAAED 2012)*
>
> *"Evidence suggests that the fetal environment may program the differentiating tissues of the fetus towards a metabolic syndrome phenotype. The mechanisms for this effect are not well understood, however it seems possible that…" (p. 334, SAAED 2012)*
>
> *"There is also a potential role for epigenetics in the fetal origin of metabolic conditions…" (p. 334, SAAED 2012)*

In addition, these discussions generally fail to consider the importance of dose-response. Rather, they largely assume that *any* exposure during the critical period will result in an adverse effect. This, however, is not the case. As an example, McLachlan and colleagues showed that the effects of gestational DES exposure on female fertility were related to dose, with complete sterility in mice evident at doses of 100 µg/kg/day, but only minimal effects on reproductive capacity observed at 0.01 µg/kg/day (McLachlan 1979, McLachlan et al. 1982). To properly illustrate existing sensitivities during these critical periods, the SAAED authors must add further discussion on the importance of dose in mediating observed responses.

It should also be noted that, in the attribution table at the end of each chapter, the entry related to differential sensitivity does not always correlate with the text discussion of critical windows of sensitivity and developmental vulnerability. For example, in the chapter on testis cancer, there is no discussion of developmental vulnerability; however, the attribution table at the end of this chapter claims that exposures *in utero* and during puberty are critical. Additionally, in the chapter on thyroid cancer, the discussion on developmental vulnerability claims, "(t)here is no

evidence that thyroid cancer originates from hormonal disruption during fetal development," and only cites a single reference for children being vulnerable to radiation-induced thyroid changes. Nevertheless, the table at the end of this chapter indicates that, "(c)hildren and women of child bearing age are more affected." This disconnect between the text and table needs to be rectified.

WHO/IPCS criteria used for attribution of effects to endocrine disruption

For each of the human health endpoints reviewed in the SAAED, the authors relied on a list of principles developed in the WHO/IPCS *Global Assessment of Endocrine Disrupting Compounds* (EDC) (WHO 2002) as the basis for attributing health endpoints discussed to endocrine disruption. In the conclusion section of each SAAED Human Health Endpoints chapter, a table with these eight principles is presented as a summary of the state-of-the-science. This is an incomplete application of the principles as described in the WHO/IPCS document. It does not represent the state-of-the-science. As noted by the WHO/IPCS, these principles are useful in understanding and identifying a potential endocrine mode of action, but they fail to address the weight-of-the-evidence for observed effects and whether these effects are relevant at environmental exposure levels.

The principles used in the SAAED (2012) were identified by the WHO/IPCS "for defining cause-and-effect relationships" (p. 32) and were considered appropriate in context of reviewing data, particularly laboratory data. The WHO/IPCS further stated that "a collective weight-of-evidence approach is needed to classify the conditions under which the exposure is 'endocrine disruptive'" (p.32). In other words, experimental animal data and mechanistic data are critical in the identification of an endocrine disrupting mechanism of action, but these data need to be considered further in light of the potential exposures experienced by humans and wildlife.

The WHO/IPCS propose that, "to create an objective and unbiased assessment of the hypothesis that chemicals with endocrine activity may be having adverse effects on laboratory animals, wildlife populations, and humans, all of the relevant information needs to be considered in an organized and structured manner" (p. 123). The framework they present for doing so is a weight-of-evidence assessment based on criteria developed to assess causation including Hill (1965). The WHO/IPCS criteria include:

- temporality,
- strength of the association,
- consistency of the observation,
- biological plausibility, and
- evidence of recovery.

Although dose-response is not specifically identified as an independent criterion, based on the illustrative examples provided, dose-response is considered within the other criteria. These

criteria are collectively used to determine the overall strength-of-evidence[1], which is an "evaluation regarding the relationship between an outcome of concern and exposure to a substance and whether or not these associations involve endocrine-mediated mechanisms" (p. 124). The authors of the SAAED did not apply this framework or any other weight-of-the-evidence criteria to evaluate the data on human health endpoints. Most importantly, the SAAED authors did not evaluate the potential endocrine mechanism proposed for each particular health endpoint on a chemical-specific basis. In the WHO/IPCS (2002) report, several illustrative examples are provided and the hypotheses presented are chemical-specific, for example:

> *"HYPOTHESIS: Impaired neurobehavioral development in children is related to endocrine disruption mediated by exposure to PCBs." (p. 125)*
>
> *"HYPOTHESIS: TBT [Tributyltin] originating from antifouling paints used to treat boat hulls induces a form of pseudohermaphroditism (termed imposex) in female gastropods by an endocrine-disrupting mechanism." (p. 126)*
>
> *"HYPOTHESIS: Eggshell thinning caused by exposure to DDE results in cracked or broken eggs and other adverse reproductive effects through an endocrine-mediated mechanism." (p. 127)*

Therefore, the conclusions may describe potential endocrine disrupting mechanisms for these endpoints, but do not characterize casual relationships between exposures and the effects.

[1] WHO/IPCS use both "weight-of-evidence" and "strength-of-evidence" to describe this framework.

Comments on Chapter 3.2: Low-Dose Effects

The chapter on low dose effects makes a number of points with strong language, but weak and unbalanced support for their positions. The chapter relies heavily on very controversial bisphenol A (BPA) data and interpretations, without proper thought to the full weight-of-the-evidence. The authors overstate the evidence supporting low dose effects. The low dose hypothesis remains insufficiently tested and unproven, but it is treated as if it were an accepted fact. The evidence, even for BPA is weak at best, and other examples have not been provided.

One significant error early in the Report is the authors' misunderstanding or casual misstatement of how scientists see dose and response. They state that "classically, it was assumed that dose and response are monotonic." In fact, most instances of chemical toxicity show different phases of non-response, response and even reversal. A sigmoid curve often describes the most basic dose response curve rather than a linear or monotonic response. Furthermore, induction of metabolism can profoundly alter that dose response curve such that the response may accelerate, stop or even reverse. Many biological effects, including some adverse effects, result from ligand-receptor interactions. Such effects often require some proportion of receptor occupancy, which implies that a threshold can be part of the dose-response curve.

The authors did a good job of trying to describe the meaning of "low dose" and to describe how the NTP peer review of this issue was conducted. That particular meeting actually served to confuse this issue by describing a low dose effect of BPA as any response observed at level below the current no-observed-adverse-effect-level (NOAEL) of 5 mg/kg/day under EPA's standard toxicity protocols. This means various target organ toxicity studies, and mechanism of action studies that are intended to increase the sensitivity of an assessment will demonstrate "low dose" effects. Each and every adverse effect has a precursor event that can be observed at a lower dose with the proper technology, so the NTP's definition of "low dose" is not particular useful. In the end, the NTP meeting on low dose failed to resolve the debate.

The definition of low dose continues to contribute to confusion and controversy. Creative interpretation has allowed investigators to claim low dose effects of BPA, while it was originally linked directly with a U-shaped dose response curve where organ weight changes were reported at dose levels below those previously shown to represent a NOAEL for such effects. If replicated, such an observation could profoundly affect risk assessment and risk management of BPA. Such replication still does not exist for BPA low dose effects, though it has been attempted repeatedly. However another important issue is the definition of replication. Some have interpreted replication in quite generous terms, and they have claimed that their work has been replicated by others even if the replicating study is in another organ, another species, even another phylum. Most scientists consider replication to be required in the same species and even the same test system at similar dose levels. The SAAED authors appear to ascribe to the former definition.

One of the issues related to low-dose effects currently being discussed revolves around the assumptions regulatory authorities make regarding the presence or absence of a threshold. It is

critical to understand that part of this debate relates to the development of regulatory standards - not that effects have been or could have been observed at these low dose levels. While it may be appropriate to assume linearity in the low dose range in order to protect public health, this approach should be adopted only when data are lacking on the dose-response or mechanism of action. The SAAED authors discuss this complex issue only briefly (pp. 71-72) and cite a single publication, White et al. (2009), describing the discussion from a workshop on estimating dose-response relationships in the low-dose range. This is an example of the incomplete characterization of the issue and a failure to acknowledge the state of on-going debate. In 2004, Conolly and Lutz (2004) state that "[t]here is much debate over the shape of the dose-response curve in the low-dose zone in the fields of toxicology and human health risk assessment." Other recent publications discuss alternative approaches for evaluation of dose response, including the review by Rhomberg et al. (2011) and a series of articles from an ILSI working group (Julien et al. 2009, Boobis et al. 2009, Buchanan et al. 2009, Taylor et al. 2009, Ross et al. 2009) – none of these were included in the SAAED Report.

The SAAED authors refer to a mode of action approach for low-dose extrapolation based on the single workshop (White et al. 2009), but do not mention three inference models that were also discussed. The authors give the impression that this approach is more fully developed than warranted. The categorical mode of action approach is described by White et al. (2009) as "conceptual" and that other categories and models "could be developed." Furthermore, two of the proposed categories are mis-characterized in the SAAED:

SAAED 2012 (p. 72): *"(b) low dose irreversible (e.g., mutagens)"*
vs. White et al. (2009): *"small numbers of generally irreversible events (e.g., mutagens)."*

SAAED 2012 (p. 72): *"(c) chronic, cumulative, irreversible events (e.g., neuronal loss in Parkinson's disease"*
vs. White et al. (2009): *"large numbers of chronic cumulative, irreversible events (e.g., ., neuronal loss leading to Parkinsonism."*

Based on these proposed categories, the authors of the SAAED make a presumptuous leap to applying this hypothetical approach to endocrine disruptors by stating that "it becomes imperative to examine whether there is evidence for mode of action categories 'low dose irreversible' or 'chronic cumulative, irreversible.' Again, they ignore the original, proposed categories that are differentiated based on the number of events as well as irreversibility.

The SAAED states that the "existence of dose thresholds cannot be proven or ruled out by experimental approaches." However, even White et al. (2009) – cited in the SAAED Report – acknowledge that an actual or practical threshold may exist for an individual, but they question the applicability of a threshold to a large human population based on variability and background diseases and exposures. In contrast, Rhomberg (2009) disagrees with the epidemiological evidence cited by White et al. (2009) regarding the lack of a thresholds in human populations; Rhomberg (2009) states that the range of exposures is too narrow and exposure measurements are too crude to effectively assess the presence or absence of a threshold. We note that the SAAED Report does not even mention these differences in scientific opinion.

As summarized in Julien et al. (2009), the term "threshold" as defined by regulatory authorities is "commonly interpreted to mean a dose below which no adverse effect is observed or expected." Although it may not be possible to prove or disprove the existence of a real dose threshold, practical dose thresholds for specific adverse effects can be demonstrated. Furthermore, it is important to distinguish the difference between the observed effect and the underlying processes involved in the mode of action resulting in that observed effect. For example, while no threshold can be establish for the binding of an individual receptor, the biological system is dynamic and can modulate responses or initiate repair such that an adverse effect does not occur. Boobis et al. (2009) – one of the ILSI working group publications not included in the SAAED – outline the key events for endocrine disruption and provide an example where a threshold exists for estrogens and luteinizing hormone in stimulating ovulation. Evidence is presented by Boobis et al. (2009) to show that some of the steps in this process are not linear, in other words a threshold exists. A second example is discussed to address the question of whether or not "a single molecule of an exogenous ligand is sufficient to drive the response from one that is physiological to one that is adverse?" The authors state that it is not possible to determine thresholds on a morphological basis, but that some data suggest that a threshold may exist at the gene expression level and conclude:

> *"While the methodology is still not sensitive enough to definitively demonstrate a threshold, it may be sufficiently sensitive to eliminate the difference between real thresholds and the pragmatic thresholds that represent ambient environmental exposure levels." (p. 704,Boobis et al. 2009)*

Overstating the evidence in favor of "low dose effects" is a significant issue in the Report. While the SAAED authors claim that their Report is based largely on review articles, they cite some very specific *in vitro* studies as support for low dose effects. One study, Gualtieri et al. (miscited as 2010 instead of 2011), is an *in vitro* study rather than a review and it deals with the induction of glutathione (GSH) by BPA. The study basically states that high *in vitro* doses are deleterious to Sertoli cells; intermediate dose levels induce protective GSH and no harm is observed; and lower dose levels do not induce GSH. This is described as an explanation for non-linear responses *in vitro*. Cell viability was affected, however, only at the highest dose level and GSH was induced at the next two levels, so no unusual dose-response curve was observed in this case.

Another example in the Report was the *in vitro* study by Bouskine et al. (2009). The chart shown as Figure 3 (p. 66) in the SAAED describes "an inverse U-shape does response curve." However, the error bars are quite large and all 5 dose levels are shown as statistically significantly different from controls, so this does not appear to represent a U-shaped dose-response curve at all.

The tendency to ignore or rationalize away the substantial evidence in opposition to the low dose finding, however, is the most serious flaw in this chapter. They ignore key articles published before their December 2011 deadline. For example, some very important studies conducted by the U.S. EPA testing potential low dose effects of BPA were published in 2008 and 2009 (Howdeshell et al., 2008; Ryan et al., 2010). These studies compared BPA to ethinyl estradiol in male and female rats and showed a lack of a response for BPA. Those papers were

reviewed and commented on by Dr. Richard Sharpe in 2010 (Sharpe, 2010). Dr. Sharpe even commented on Dr. Kortenkamp's 2007 paper on BPA's potential addition to other estrogens (Kortenkamp, 2007). It seems unlikely that these papers would have been missed by SAAED authors. The imbalance in this chapter and its citations unfortunately undercut the credibility of the Report.

For example, they rely on a non-reviewed website citation by one of the key advocates of the low dose hypothesis (Vom Saal) to support the statement "It is indeed true that study outcomes from sources funded by chemical corporations still contradict the vast amount of government funded studies." This highly-charged Report is uncritically cited and treated as unchallenged. However, the authors fail to note that Vom Saal is obscuring that the specific findings in his own studies, which have not been replicated by either government or industry findings, despite the use of many more animals and many more dose levels than he used in his laboratory. Studies by the Japanese government (Ema et al. 2001), U.S. government (Ryan et al. 2010, Howdeshell et al. 2008) and industry (Tyl et al. 2002, Tyl et al. 2008) all have failed to replicate Vom Saal's preliminary observations. The current state-of-the-science on the BPA low-dose controversy was well summarized by Dr. Richard Sharpe (2010) in a short note in the journal *Toxicological Sciences*. Sharpe's article, which is not cited in the "state-of-the-art" Report, puts in place the major issues on the low dose hypothesis and BPA as he reviews many points that have been ignored entirely by the authors of this paper.

The SAAED authors tend to cite various *in vitro* studies that have limitations on their ability to predict animal or human effects. The literature is very clear that oral exposure of BPA is rapidly conjugated and that the conjugated form is estrogenically inactive (Doerge et al. 2010). This helps explain why oral studies are typically negative for adverse effects on the reproductive system. *In vivo* studies by routes other than oral tend to show substantially greater hormonal activity than oral studies when compared on a mg/kg/d basis.

A published review by Dr. Willhite at the California Department of Toxic Substances Control (Willhite et al. 2008), appears to have also been overlooked by the authors as well. This is a very detailed review that addresses the strengths and weakness of many BPA studies available at the time that Willhite's review was done. This paper should be considered and cited by the authors.

The low dose effects chapter speaks to how BPA "may pose cancer risks and affect neurobehavioral endpoints." Various highly specialized studies, including some *in vitro* studies, have been cited in support of risks of prostate cancer, breast cancer and neurotoxicity. It completely fails to contend with the reality that definitive long term animal studies have not observed cancer after chronic BPA exposure. This raises significant doubt about the predictive value of these *in vitro* studies on modes of action. It is suggested that the authors consider and discuss the contrary scientific information to provide scientific balance. They should consider reviews done by various international organizations that universally describe BPA as not a human carcinogen (EU RAR 2003, EU RAR 2008, EFSA 2010, NTP CERHR 2008, FDA 2010, MAK 2011, Hengstler 2011).

Comments on Chapter 4.5: Female Fertility and Adverse Pregnancy Outcomes

This chapter purports to focus on female fertility and pregnancy outcomes and EDCs. To meet this goal the chapter should be more focused and shorter. There are some very clear examples of EDCs affecting female fertility and those should be the focus of this chapter.

Specific sections should be dropped from the chapter and, if needed, moved to other chapters in the documents. For example, the sections on birth defects and sex ratio are not especially relevant because they are not directly relevant to female fertility and would be better addressed separately. Other sections, such as Smoking and pregnancy or Environmental contaminants, indicate that the evidence does not support a conclusion that low level exposures to EDCs are likely to affect female fertility or they do not operate through an EDC mode of action. Furthermore, smoking, lead and the very general category of "pesticides" do not necessarily acting through an endocrine mechanism.

Evidence for a role of chemical exposures in female fertility and pregnancy outcomes

Table 20 (p. 186) in the SAAED describes various human epidemiology studies on diverse endpoints related to pregnancy outcomes. The potential association or lack of association is noted, but no specific information on the basis for these observations is provided. The table lacks even a hypothesis regarding an endocrine mode of action for any of the substances on the list and for those specific studies.

The authors spent significant time and effort creating Tables 20 and 21, but they really illustrate that more must be known about the modes of action before one associates a biological change with an EDC. Additionally, if there are no data to demonstrate a relationship between a chemical and an effect, there is no justification for assessing a mode of action. For example, most of the studies in Table 21 show no association between the "relevant chemicals" and sex ratio. Therefore, it is difficult to understand why this area deserves such attention if it is nearly universally negative and should be excluded from this Report.

One example of a substance and exposure that clearly affects female fertility is prenatal exposure to diethylstilbestrol (DES) and its effects on offspring fertility. This could have been used to greater advantage in the SAAED Report as a framework for understanding endocrine disruption on female fertility. One could have compared or contrasted DES with other estrogenic substances such as oral contraceptives or certain phytoestrogens. This could have been a very instructive chapter describing the importance of timing, or dose and of bioavailability. One function of this document could have been to lay the groundwork of better educating scientists and regulators on the biological issues related to EDCs, and their similarities to other toxicants in risk management decisions.

Critical Windows of Susceptibility

This section could have more useful and powerful by comparing different responses to estrogens at various life stages. Instead, the authors speak to two conflicting studies on hypermethylation of the HOX gene observed in a subcutaneous exposure to DES and BPA in one study but not another. For a review of reviews, this discussion fell deeply into detail that does not illuminate the issue.

Do current experimental approaches capture relevant endpoints/mechanisms?

The discussion on whether current experimental approaches capture relevant endpoints seems to miss the mark. They spend a paragraph on the uterotrophic assay. This is a sound method for measuring estrogenic activity *in vivo*, but cannot be considered a fertility test. There are various OECD testing protocols that do evaluate potential effects on fertility. Single and multigeneration studies and the Extended One-Generation Reproductive Toxicity Study are all capable of measuring effects on fertility at sufficient dose levels. Other studies can describe how fertility is affected.

Section Conclusions

As previously discussed, the principles used by the SAAED authors to attribute effects on female fertility and pregnancy outcome to endocrine disruption are inappropriate for the types of data being discussed. Each of the principles evaluated in the conclusions is discussed below. The authors cite all but one of the criteria as having been met, but this is misleading because the answers are quite specific to DES. The review of the criteria recited below also fails to consider that the assessment should be performed on a chemical by chemical basis, otherwise we are only learning whether or not it is biological plausible whether or not some agent might affect female fertility. In the case of DES and oral contraceptives, we believe we already know the answer, and yes, the effects of these compounds can be attributed to endocrine disruption.

Ability to isolate the response to endocrine sensitive tissues in an intact whole organism. The authors of the SAAED say this criterion is met, and in a general manner that makes sense. Their evidence summary is, however, insufficient. They noted, without references, that "many adverse pregnancy outcomes are implantation disorders." What does that have to do with whether or not female fertility meets the criterion that it can be isolated to endocrine sensitive tissues in an intact organism?

Analysis of the response at multiple levels of biological organization – from phenotypic expression to physiology, cell biology, and ultimately molecular biology. The authors correctly note the impact of prenatal DES exposure on uterine abnormalities. However, they then jump to the conflicting data on the HOX gene. There are outstanding studies on DES and ligand-receptor interactions, on cellular responses, on molecular mechanisms of action that make this

response inadequate. These cellular and biochemical studies are supplemented by numerous whole animal fertility studies that provide a much stronger basis for this criteria, as it relates to DES.

Direct measurement of altered hormone action (gene induction or repression), hormone release, hormone metabolism, or hormone interactions under the experimental regime in which the toxicologic outcome was manifest. Their response cites their earlier response. This could be sufficient if their earlier response had been sufficient.

Dose-response observations that indicate the perturbance of the endocrine system is a critical response of the organism, and not the secondary result of general systemic toxicity. This one is listed as "evidence unclear" and it does not match well with the other criteria. If one looks at the animal data on DES going all the back to Shah and McLachlan (1978), there is a dose response for effects on fertility in prenatally-exposed CD-1 mice. The estrogenic activity of DES does relate to the adverse effects. Other studies on humans indicate a minimal dose for the observation of adverse effects (Cuhna et al. 1999) on female fertility. For DES this appears to be met, but not necessarily for other substances.

Ability to compare the resulting phenotypes with outcomes from exposure to known pharmacological manipulations. They cite the literature on DES specifically. This criterion is a bit confusing as there is a divergence between the intended pharmacological activity and the observed toxicological activity.

Indication that there is differential sensitivity of certain lifestages in which dysregulation of a particular endocrine system is known to have adverse health consequences. As they cited, this is quite true for DES.

Ability to restore the phenotype or toxicologic outcome via pharmacological manipulations that counter the presumed mode of action on the endocrine system in the intact organism. This is an interesting observation and the reference should have been cited. It is not clear from the comment whether they are talking about progestagens acting in opposition to DES or some other effect. This should be clarified.

Supporting data on endocrine activity from in vitro binding, transcriptional activation, or cellular response studies. If they are talking about DES, it is true that many strong *in vitro*, transcriptional activation and cellular response studies have been published in this area.

In conclusion, the authors missed an excellent opportunity to use the rich literature on the prenatal human and animal studies on DES to illustrate the plausibility of an endocrine mode of action adversely affecting fertility and pregnancy outcome.

Comments on Chapter 5.2 Prostate Cancer

Natural History

Several risk factors for prostate cancer are identified, including age, race, and family history. Internal exposure to androgens is also described as a "known risk factor," but no reference is provided. Although androgens have been occasionally associated with the risk of prostate cancer, cancer textbooks, governmental organizations, and even references cited in the SAAED do not characterize androgens as a risk factor (DeVita et al. 2010, NCI 2008, Roddam et al. 2008, Key 2002). In fact, in these particular references, the evidence for the role of androgens in the etiology of prostate cancer is described as conflicting. Other factors have also been identified as potentially affecting the risk for prostate cancer and could be important in the evaluation of endocrine disrupting mechanisms of action; examples include phytoestrogens, number of sexual partners, and obesity.

In the discussion of the natural history of prostate cancer, the authors also discuss the trends in incidence rates around the world. They acknowledge that the introduction of prostate-specific antigen (PSA) testing has played a role in the perceived increased incidence, but they infer that a continued increase in incidence must be due to environmental exposures. In particular, they note that in the U.S. "rates have increased in the last decade" and that "since 1998 rates are slowly rising." However, an increase in prostate cancer incidence in the U.S. since 1998 is not supported by the literature cited (Jemal et al. 2010) in the SAEED for this statement. Jemal et al. (2010) conducted a time trend analysis on the incidence and mortality for several cancer sites, including prostate cancer. This analysis presented in Table 5 of Jemal et al. (2010) shows an increase of 2.4% in prostate cancer incidence between 1995 and 2000, but a decrease of 2.4% in 2000 to 2006. Jemal et al. (2010) state:

> *"The decrease in prostate cancer incidence rates (by 2.4% per year from 2000-2006) may reflect recent stabilization of prostate-specific antigen testing, resulting in decreased detection or a reduced number of undiagnosed cases."*

The trends in prostate incidence are not linked to any specific chemical exposures or endocrine disruptor modes of action. Therefore, this information does not contribute to an understanding of endocrine disruption and should not be included in the SAAED Report.

Evidence for an endocrine mechanism in prostate cancer

The discussion of the basis for an endocrine mechanism for prostate cancer is overall theoretical and relies on scant data. Qualifying language is used in many of the statements in this section:

> *"These observations formed the basis of the hypothesis…"*

> *"Attempts to demonstrate the androgen hypothesis of prostate carcinogenesis empirically by relating blood androgen levels to cancer risk have run into difficulties."*
>
> *"Several lines of evidence support the idea that prostate cancer risk"*
>
> *"Estrogens are thought to be involved in the aetiology of prostate cancer"*
>
> *"estrogenic stimulation of the prostate may lead to the activation of growth"*

Limited data are provided in the SAAED to demonstrate an endocrine mechanism for the induction of prostate cancer. While the presumption that androgens are involved in prostate cancer seems obvious and is supported by the extreme circumstance in which castrated men do not develop prostate cancer, the specific role of androgens and estrogens in the development of prostate cancer must be understood before endocrine disrupting mechanisms for increasing the risk of prostate cancer can be evaluated for exogenous compounds.

In particular, the authors of the SAAED place little weight on the negative evidence for a relationship between serum androgen levels and risk for prostate cancer, as demonstrated in a meta-analysis by Roddam et al. (2008). This is despite the fact that the findings of Roddam et al. (2008) are supported in a recent prospective, nested case-control study that stated:

> *"Serum concentrations of testosterone, DHT, SHBG, 3α-diol G, IGF-I, IGF-II, IGFBP-1, and IGFBP-3 were not associated with risk of prostate cancer. Tests for trend of quartiles of serum concentrations also did not show any association. Results were relatively unchanged for men with advanced prostate cancer and their matched controls." (Gill et al. 2010)*

These negative findings are countered by statements in the SAAED that,

> *"the relationship between serum testosterone levels and concentrations of DHT within the prostate is complex. Measurements of serum androgen levels do not reflect the androgen exposures of target cells within the prostate at times critical for the aetiology of the disease, and it may therefore not be feasible to capture androgen exposure of the prostate with currently available technology." (p. 250, SAAED 2012)*

If current technologies and data are insufficient to evaluate the etiology of prostate cancer, then the examination of the potential role of endocrine disruption in prostate cancer by exogenous compounds remains theoretical.

Evidence for a role of chemical exposures in prostate cancer through an endocrine disruption mechanism

The authors of the SAAED do not believe that the development and extensive reliance on the PSA test alone can account for the increased incidence and trends of prostate cancer around the world. They posit that environmental factors are important in contributing to prostate cancer. Evidence for an endocrine disruption mechanism of action for prostate cancer is presented based on information from pesticide applicators and pesticide manufacture, as well as data for specific chemicals.

Pesticide applicators and manufacture

As noted above in the general comments on the SAAED Report, it is inappropriate to consider broad categories of chemicals collectively as endocrine disruptors without consideration of a common mechanism of action. Although the authors acknowledge that the epidemiological studies of pesticide application and pesticide manufacture are limited in the identification of specific chemicals associated with prostate cancer, they present data on these general exposures as supporting evidence for endocrine disruption. The studies of pesticide applicators and manufacturers are not sufficient to identify specific chemicals and exposures that may be involved in prostate cancer, much less provide information on an endocrine mode of action. In fact, the two meta-analyses cited in the SAAED Report state that:

> *"The data available from the individual studies do not provide sufficient exposure information for firm conclusions to be drawn about pesticide exposure as the cause for prostate cancer, independently from other factors." (p. 559, Van Maele-Fabry and Willems 2004)*

> *"The present evaluation indicates that none of the individual studies reviewed allow drawing definitive conclusions on the risk of prostate cancer in pesticide manufacturing workers." (p. 369, Van Maele-Fabry et al. 2006)*

Additionally, in the meta-analysis of pesticide manufacturing workers (Van Maele-Fabry et al. 2006), only exposure to phenoxy herbicides was found to have a statistically significant increased risk for prostate cancer, which Van Maele-Fabry et al. suggest is due to contamination with dioxins and furans. The SAAED Report relies solely on this meta-analysis as evidence for increased risk of prostate cancer in pesticide manufacturing workers. Despite mentioning the potential confounding with dioxins and furans with phenoxy herbicide exposure, they state that this study "points to pesticide exposure as a risk factor for prostate cancer, and highlights phenoxy herbicides as specifically linked with this cancer." This statement is contradictory to the available data and the conclusions of the authors of the meta-analysis.

The methodological limitations of the individual pesticide worker studies must also be considered in the interpretation of reported statistically significant results. For example, the Agricultural Health Study (AHS) collected data from farmers on pesticide use based on a self-

administered questionnaire without quantitative measures of exposure to these pesticides. The reliance on self-administered questionnaires for establishing exposure can be affected by recall bias. The SAEED cites the report by Alavanja et al. (2003) who conducted an analysis of the AHS, where for more than half of the pesticides (28 of 50 compounds), data were only collected on a use basis of ever/never, and therefore, Alavanja et al. note:

> *"…lacking an exposure-response pattern with individual pesticides suggests that the relation with chlorinated pesticides could be due to other exposures not identified in this analysis." (p. 810)*

Another limitation in the AHS is how various risk factors for prostate cancer were controlled for in the analysis. Most of the analyses controlled for family history and age; although age was controlled for as a categorical variable (5-year age groups) rather than as a continuous variable. Also, race, a well-established risk factor for prostate cancer, was not included. Other suspected risk factors, such as obesity measured by body mass index (BMI) (Hsing et al. 2007), were not included in the analysis. BMI has been included as a variable in other studies investigating various pesticide exposures and the risk for prostate cancer (Sawada et al. 2010, Xu et al. 2010).

These general pesticide industry studies should only be considered for the identification of potential areas of concern and topics for further research. They do not provide sufficient data on the specific chemicals and specific exposures to address a causal relationship between these exposures and the outcome of interest, much less provide information on whether or not these chemicals are operating by an endocrine disrupting mechanism of action.

Specific Pesticides

Methyl bromide and organophosphates are presented as specific compounds that have been demonstrated to increase the risk of prostate cancer. Although the heading highlights organophosphate compounds, two of the seven pesticides considered in this section are not part of this class of compounds – permethrin is a pyrethroid compound and butylate is a thiocarbamate. The support for linking all of these pesticides is based on the AHS only but as discussed above, this study provides limited data for assessing the relationship between pesticide exposures and prostate cancer. A single study that reports an association between an exposure and an outcome is not sufficient for establishing a causative link. Additionally, no mechanistic data are provided to show that, even if any of these compounds induces prostate cancer, that the tumor induction occurs via an endocrine disrupting mechanism of action. Therefore, this discussion does not meet the minimum data requirements for consideration in a state-of-the-art review.

Organochlorine pesticides are also presented as specific compounds associated with an increased risk of prostate cancer. Seven studies are cited as support for an association between organochlorine pesticides and prostate cancer, but the data are inconsistent and the overall the weight-of-evidence does not suggest an association, much less a causal relationship. Despite the fact that six of these studies evaluated body burden levels of organochlorine compounds (e.g., blood, adipose tissue concentrations) and the risk of prostate cancer, statistically significant

associations were typically only observed for a single compound in a single study (see Table 1 below). The only exception is for oxychlordane, which was associated with a statistically significant OR in two of the five studies that evaluated this compound (Ritchie et al. 2003, Xu et al. 2010). These two studies do not constitute a positive observation from a weight-of-the-evidence perspective and the individual studies have limitations that raise questions about whether there is a true association between oxychlordane levels and prostate cancer. The statistically significant finding in Ritchie et al. (2003) was seen only in the 2nd tertile, and therefore, is not consistent with a typical dose-response relationship. The finding in Xu et al. (2010) was statistically significant when age-adjusted, but was no longer statistically significant when additional variables were included the result. Further, Xu et al. (2010) is a cross-sectional study based on the NHANES survey, which as a result of the study design, cannot exclude the impact of prostate cancer on serum lipid concentrations because serum measurements were made after the cancer diagnosis. Finally, no data are presented on an endocrine disrupting MOA for these compounds.

Table 1: Studies Comparing Body Burden of Organochlorine Pesticides with Incidence of Prostate Cancer

Compound	Aronson et al. (2010)	Multigner et al. (2010)	Sawada et al. (2010)	Xu et al. (2010)	Hardell et al. (2006)[1]	Ritchie et al. (2003)
Tissue, body burden	blood	plasma	plasma	serum	adipose	serum
Chlordanes	—	—	—	—	NS/*	—
Chlordecone	—	◊	—	—	—	—
o,p'-DDT	—	—	NS	—	—	—
p,p'-DDT	NS	—	NS	—	—	—
p,p'-DDE	NS	—	NS	NS	NS/*	NS
Dieldrin	—	—	—	‡	—	NS
HCB	NS	—	NS	—	NS/*	—
B-HCH	NS	—	NS	‡	—	—
Heptachlor epoxide	—	—	—	NS	NS/NS	NS
Mirex	NS	—	NS	—	—	—
trans-Nonachlor	NS	—	NS	†	—	NS
cis-Nonachlor	—	—	NS	—	—	—
Oxychlordane	NS	—	NS	†	NS/NS	Δ

[1] Statistically significant difference in adipose concentration for cases compared to controls based on low/high PSA level, ≤16.5 and >16.5 ng/mL, respectively

NS Not a statistically significant difference

* Statistically significant difference in adipose concentration of pesticide for cases with PSA levels >16.5

◊ Statistically significant adjusted OR (age, plasma lipid, waist-to-hip ratio, history of prostate cancer screening) for 4th quartile of plasma pesticide concentration

‡ Statistically significant age-adjusted and adjusted (age, race, ethnicity, BMI, education, smoking, data cycle, and marital status), OR for 4th quartile of serum pesticide concentration

† Statistically significant age-adjusted OR for cases with 3rd and 4th quartile of serum pesticide concentration

Δ Statistically significant adjusted OR (age, BMI, history of prostatitis) for 2nd tertile of serum pesticide concentration

Environmental Chemicals

Polychlorinated biphenyls (PCBs) are presented as a category of compounds that are associated with prostate cancer based on a small selection of individual studies. In contrast to other sections, where reviews and meta-analyses were cited, a recent weight-of-the-evidence review of the potential human cancer risks from exposure to PCBs was not included in this discussion (Golden and Kimbrough 2009). Golden and Kimbrough (2009) reviewed the full spectrum of studies involving occupational and environmental exposure to PCBs, including the five pre-2009 articles cited in the SAAED, and state that:

> *"Given that these studies involved workers subject to high-dose occupational exposure to PCBs, it is biologically implausible that the background PCB-related prostate-cancer cases in the incidence studies by Ritchie et al. (2003, 2005) or Hardell et al. (2006) [cited by SAAED] were etiologically associated with exposure to PCBs." (p. 302)*

In their weight-of evidence assessment, Golden and Kimbrough (2009) also note that:

> *"the animal data suggest that it is not biologically plausible that PCBs are a risk factor for prostate cancer." (p. 302)*

Golden and Kimbrough (2009) conclude that:

> "*Consequently, the WoE [weight-of-the-evidence] suggests that exposure to PCBs is not associated with increased risk of prostate cancer." (p. 303)*

Cadmium exposure is also described as being linked with prostate cancer, but the SAAED authors acknowledge that the evidence is not consistent, that "most positive studies indicate weak associations" and that "[o]n the basis of the available epidemiological evidence, it cannot be judged with certainty whether environmental exposure to cadmium is linked with prostate cancer." Given this lack of uncertainty of an association between cadmium exposure and prostate cancer, it is premature to be evaluating a potential endocrine disrupting mechanism of action.

Finally, arsenic is mentioned as an environmental chemical that may influence the risk of prostate cancer. The discussion is limited to a review of the evidence for arsenic causing prostate cancer with no discussion of the mechanism of action. Further, the data available for assessing a causal relationship between arsenic and prostate cancer is exaggerated in the SAAED Report. Arsenic is described as being "strongly associated with prostate cancer" based on authoritative reviews by Benbrahim-Tallaa and Waalkes (2008) and Schuhmacher-Wolz et al. (2009). Although this suggests a consensus on a review of all studies, in fact, the Schuhmacher-Wolz et al. (2009) review mentions prostate cancer only once and specifically references the other review article, Benbrahim-Tallaa and Waalkes (2008), in this statement. Additionally, it is important to note that the Taiwan and Australian studies that investigated

arsenic exposure and prostate cancer employed ecological and cross-sectional study designs (Chen and Wang 1990, Hinwood et al. 1999). These types of study designs evaluate exposure and cancer incidence or mortality simultaneously. Consequently, exposure and disease can be mischaracterized and these studies cannot be relied on for demonstrating causal relationships. In addition, information on dose-response relationships are conflicting; for example no dose-response was seen in Utah (Lewis et al. 1999), but a dose-response trend was observed in Taiwan (Wu et al. 1989). Because arsenic has not been shown to cause prostate cancer and no data are provided for an endocrine disrupting mechanism of action, the discussion of arsenic as a potential endocrine disruptor should be excluded.

Endocrine disrupting properties of chemicals

As noted above, no specific information on endocrine disrupting mechanisms of action is provided for any of the compounds reviewed in the early part of this section of the SAAED Report. As an introduction to this discussion, the authors acknowledge that, "[t]he precise mechanisms by which the chemicals demonstrated in epidemiological studies as being related to prostate cancer induce the carcinogenic process remain to be resolved." Furthermore, in discussing the information available for various compounds or categories of chemicals, the statements are nebulous (e.g., "likely to be relevant," "might indirectly disturb"). Overall, the scant data provided are insufficient to demonstrate an endocrine disrupting mechanism of action and do not represent a summary of the state-of-the science. For example, they state that organochlorine pesticides are associated with increased prostate cancer risk and have "estrogen-like activities," citing a single reference. This reference, Soto et al. (1995), is a description of the *in vitro* assay that measures the proliferation of the breast cancer cell line, MCF-7, called the E-Screen. This assay has been proposed as a screening tool used for the identification of potential estrogenic compounds. This assay is based on the measurement of MCF-7 cell proliferation. Therefore unless the E-Screen is modified to include co-administration of an known anti-estrogenic compound, it cannot provide any information on the mechanism of proliferation. Positive results from the standard assay as referenced in Soto et al. (1995) could be the result of non-estrogenic activity. Furthermore, it is important to note that of the three organochlorine pesticides identified in the SAAED as having estrogenic activity – trans-nonachlor was not even tested and chlordane was found to be nonestrogenic:

> *"**Nonestrogenic** compounds (natural and synthetic progestagens, glucocorticoids, and pesticide derivatives such as mirex, chlordane-α isomer, **chlordane**, and heptachlor) **did not affect the proliferation of MCF-7 cells."** (p. 116, Soto et al. 1995; emphasis added)*

Evidence of developmental vulnerability in prostate cancer

Epidemiological and experimental animal data are presented as evidence for a developmental vulnerability in prostate cancer. The review of epidemiological data is speculative and described as "several lines of evidence that support the notion that estrogen exposure during morphogenesis can profoundly alter the developmental trajectory of the [prostate] gland, sensitizing it to hyperplasia and cancer later in life" (p. 261, SAAED 2012).

The data relied on in this section do not include the most current information on this topic. Specifically, prostatic squamous cell metaplasia in men exposed to DES *in utero* is presented as evidence for a developmental vulnerability. The authors of the SAAED note that these men "can therefore be expected to experience higher prostate cancer risks, but thus far, epidemiological studies of DES and prostate cancer have not established increased risks (Giusti et al. 1995)." Although newer studies may also lack sufficient follow-up to exclude an increased risk, two newer publications continue to confirm the lack of increased prostate cancer risk in men exposed *in utero* to DES based on the observations of benign prostatic hyperplasia (Palmer et al. 2009) or cancer incidence (Strohsnitter et al. 2001). Failure to cite the most current studies relevant to the discussion is misleading and cannot reflect a summary of the state-of-the-science.

The discussion of experimental animal data is limited to the general vulnerability of the prostate gland, but do not present data on the development of prostate cancer, the outcome of interest. Overall, the discussion is focused on the impact of perinatal exposure to estradiol or androgens and the impact on prostate development. While these may reflect potential developmental vulnerabilities for prostate development, more specific information on the chemicals that can affect such changes and whether these changes actually result in cancer, not just pre-neoplastic lesions, is critical for an evaluation of endocrine disruption.

Only one chemical is mentioned in the discussion of developmental vulnerability for prostate cancer, BPA. It is important to note that there is no evidence that chronic exposure to BPA has resulted in prostate cancer in any experimental animal model (Willhite et al. 2008). The one article cited for evidence of prostatic lesions from BPA (Ho et al. 2006), in fact, does not report neoplastic lesions from the exposure to BPA alone. Only when neonatal exposure to BPA was followed by 16 weeks of exposure to estradiol+testosterone during adulthood was an increased incidence of prostate intraepithelial neoplasia (PINs)[2], proliferation, and apoptosis observed. Regardless, this study is methodologically limited and should not be the sole basis for assessing potential endocrine disrupting activity. Some of the limitation include: the small numbers of animals per group (5-7), the use of a single dose level (10 µg/kg), and subcutaneous administration of BPA, which is not biologically relevant to human oral exposures.

Do experimental tools exist for the study of prostate cancer?

The authors of the SAAED describe the animal models for prostate cancer and identify several limitations in extrapolating experimental findings to humans. They cite Bostwick et al. (2004), as the basis for stating that there are more than ten animal models for prostate cancer – yet, Bostwick et al. do not review any animal or cell culture models in detail and ten models are not described. They present only the categories of experimental models available: 1) rodent models, 2) transgenic mouse models, 3) mouse prostate reconstitution models, 4) severe combined immunodeficiency syndrome mouse, and 5) canine model. The other two models summarized

[2] Although this term includes the word "neoplasia" this finding is not considered cancer. Only high-grade PINs are thought to be the histologic precursors of invasive carcinoma and may proceed the cancer by 10 years.

in Bostwick et al., xenograft models and cell culture models, cannot be considered animal models.

The Noble rat model is specifically presented as a good model for the study of hormone-induced prostate cancer, but the SAAED authors acknowledge that this strain has not been widely used for the study of prostate cancers induced by chemicals. While the Noble rat model appears to have been predominately used to evaluate prostatic inflammation and carcinogenesis in the scientific literature, it is not clear how well this model can help to understand human prostate cancer; therefore, additional information should be presented for recommending this particular strain.

Section Conclusions

The conclusions for this section, as in all sections on human health endpoints, are based on a series of questions outlined by the WHO/IPSC for attributing an effect to endocrine disruption. As discussed above in the general comments, this approach is useful in understanding and identifying a potential endocrine mode of action, but does not demonstrate that exposure to a particular chemical results in endocrine disruption that ultimately causes prostate cancer. In the conclusion, each question was posed generically for prostate cancer and was deemed to be met. However, in addressing the question about a specific exposure these questions need to be considered independently for each compound of interest. Below we discuss the limitations of the summary of evidence provided for each question.

Ability to isolate the response to endocrine sensitive tissues in an intact whole organism. The evidence cited, that "chronic exposure to testosterone and estradiol induces prostate cancers in the Noble rat" does not demonstrate that an exogenous compound can induce prostate cancer. It is not clear why the authors highlight this particular animal model since they state earlier in this section, "[t]his rat strain has not been widely used for the study of prostate cancers induced by chemicals." Elsewhere in the chapter examples are given where a compound is associated with prostate cancer or mechanisms of action are proposed for certain compounds, but there is a failure to link the exposure with prostate cancer via an endocrine-mediated mechanism of action in an intact organism.

Analysis of the response at multiple levels of biological organization—from phenotypic expression to physiology, cell biology, and ultimately molecular biology. This type of systematic evaluation was only attempted for BPA based on a single study, Ho et al. (2006) that has a number of methodological limitations (discussed above). Most importantly, none of the large, chronic cancer bioassays administering BPA have demonstrated an increased risk for prostate cancer (Willhite et al. 2008). The development of molecular responses to explain a phenotypic expression that has not been demonstrated is meaningless and this criterion has not been met.

Direct measurement of altered hormone action (gene induction or repression), hormone release, hormone metabolism, or hormone interactions under the experimental regime in which the toxicologic outcome was manifest. No specific evidence was provided to respond to this question, the table refers only to the previous two responses, which do not provide information on measurement of altered hormone action. In the discussion of specific chemicals, the authors only speculate that compounds with androgenic or estrogenic activity "are likely to be relevant" or that "pesticides might indirectly disturb the normal hormonal balance." In addition, potential molecular mechanisms are presented in the section on developmental vulnerability, but no data are presented on compound-induced changes in hormones associated with prostate cancer. Therefore, insufficient data were provided in this section to respond to this question and this criterion has not been met.

Dose–response observations that indicate the perturbance of the endocrine system is a critical response of the organism, and not the secondary result of general systemic toxicity. Again, no specific evidence was provided to respond to this question. Given the lack of data in this chapter to demonstrate perturbation of the endocrine system by any specific compounds, obviously dose-response information are also lacking; therefore, this criterion has not be met.

Ability to compare resulting phenotypes with outcomes from exposures to known pharmacological manipulations. For the chemicals discussed in this chapter, insufficient data are provide to demonstrate a causal link between exposure and a phenotypic response of prostate cancer, much less show the impact of pharmacological manipulations. The evidence provided in the SAAED refers to the fact that exposure to BPA can induce prostatic dysplasia. First of all, prostatic dysplasia is not prostate cancer, the outcome of interest. Secondly, based on the citation provided in this chapter (Ho et al. 2006), prostatic dysplasia was only induced with the additional administration of testosterone+estradiol. This criterion cannot be considered to be met.

Indication that there is differential sensitivity of certain lifestages in which dysregulation of a particular endocrine system is known to have adverse health consequences. Only one experimental study was provided to demonstrate the developmental vulnerability of the prostate from BPA exposure (Ho et al. 2006); this study as described above has numerous methodological limitations and cannot be the basis of meeting this criterion. Examples of epidemiological data are provided, but these only "suggest" possible impacts of endogenous hormonal changes or do not support an increased risk for prostate cancer (e.g., male exposure to DES *in utero*). Therefore, this criterion has not met.

Ability to restore the phenotype or toxicologic outcome via pharmacological manipulations that counter the presumed mode of action on the endocrine system in the intact organism. The only evidence presented is the effects of androgen withdrawal through castration – this does not provide any evidence for a chemical inducing endocrine disruption leading to prostate cancer. Thus, this criterion cannot be met.

Supporting data on endocrine activity from in vitro binding, transcriptional activation, or cellular response studies. The authors of the SAAED state that this criterion is not applicable, despite multiple references to *in vitro* studies and their discussion on developmental vulnerability that outlines the impact of various steroid receptors on hormone signaling that they postulate "predisposes the rat prostate to hyperplasia and sensitizes it to cancerous lesions later in life (Ho et al. 2006)." While sufficient data are not presented in this chapter to meet this criterion, these types of data are useful in understanding and evaluating potential endocrine disrupting mechanisms of action. Therefore, this criterion cannot be dismissed as not applicable.

In conclusion, these questions do not address the weight-of-the-evidence for a particular compound causing prostate cancer, whether the exposure induces prostate cancer via an endocrine-mediated mechanism, and if this mechanism is relevant at environmental exposure levels. Until all of these pieces of data are available, the state-of-the-science is speculative, at best.

Chapter 6.1 Developmental Neurotoxicity

In chapter 6.1 of the SAAED Report, the authors attempt to show that endocrine disruption plays a role in chemically-mediated developmental neurotoxicity. Although data exist to support a role of the endocrine system in neurodevelopment, specific data linking endocrine-mediated mechanisms to chemically-induced developmental neurotoxicity (DNT) are lacking. For individual chemicals, information may exist to support possible endocrine disruption or alterations in neurodevelopment; however, data that support actions in both areas (endocrine and neurological) generally do not exist.

Natural History of Developmental Neurotoxicity

In this section, the authors attempt to set the scene for a discussion of DNT and the potential role of endocrine disruptors in the etiology of various neurodevelopmental disorders. In doing so, they take an alarmist view suggesting that many neurodevelopmental disorders are highly prevalent within the human population or are on the increase. However, the authors generally fail to cite primary literature to back up their claims, instead referring to various review articles. They also allude to the potential role of endocrine disruptors in the development of these disorders. Without providing actual data to support these statements, however, they are purely speculative at this point.

To address incidence trends, the authors discuss four neurodevelopmental disorders in particular: autism, attention deficit disorder (ADD), cerebral palsy (CP), and neural tube defects (NTDs). As there is little to no evidence that exposure to endocrine disruptors plays a role in the development of these disorders, the relevance of this discussion to overall chapter is unclear.

Autism. The authors suggest that the incidence of autism has increased dramatically in recent years and they cite a number of studies to support this contention. It is important to note, however, that the recent review by Fombronne (2009), which they also cite in their discussion, presents an excellent case showing that expanded diagnostic criteria, improved case ascertainment, increased awareness, and the greater availability of services for those diagnosed with autism and other pervasive developmental disorders (PDD) likely accounts for the increases that have been observed over time. For example, Fombronne indicates that improved case ascertainment likely played a role in the increased incidence of autism observed in California, as children previously diagnosed as having mental retardation were now classified as PDD. He also shows that cross sectional surveys of children from the same geographic area and age range done at approximately the same time found highly different prevalence rates (a 6-fold variation in UK studies and a 14-fold variation in US studies) based on the use of different case identification methods across studies. Additionally, two studies of children in the UK (one from 1992-1995 and the other from 1996-1998) used identical rigorous methods for case identification and showed no increased prevalence over time. Other examples to support the major role of changing diagnostics and increased awareness are provided in Fombronne's review. Therefore, while "other factors cannot be ruled out" as the SAAED authors contend,

the upward trend that has been observed over time most likely is not due to a true increase in the incidence of autism and related disorders.

Attention Deficit Disorder (ADD). The authors correctly state that the incidence of ADD likely has not increased over time; rather, changes in diagnostics, methods of case ascertainment, awareness and the availability of resources probably accounts for the upward trend. With regard to the papers cited on the topic of environmental risk factors (Aguiar et al. 2010, Eubig et al. 2010), it is important to note that these two papers are by the same group of researchers and only the latter study (Eubig et al. 2010) actually addresses the potential role of environmental risk factors in ADD. Further, this work is still very preliminary. Although lead and PCBs are highlighted as specific chemicals that may identify risk factors or mechanism for ADD, currently epidemiologic data supporting an association between lead or PCB exposure and ADD are lacking. Additionally, although ADD rates have likely been stable over time, lead exposure in the US has gone down, as seen from the NHANES data (Pirkle et al. 1994). This would suggest that if lead contributed to the incidence of ADD, rates would be going down; however, such data that support a link between exposure to lead and ADD do not exist.

Cerebral Palsy (CP). This section relies primarily on a review by Blair and Watson (2006) and could benefit from the discussion of data from additional studies. Again, it is important to note that rates of CP have not been increasing in recent years. If exposures to endocrine disrupting chemicals have been increasing over time, as the authors contend, the stability of CP over time would argue against a role of endocrine disruption in its etiology.

Neural Tube Defects (NTDs). As mentioned in the Report, NTDs have decreased substantially since folic acid supplementation was first introduced into practice. Although Sever (1995) suggests that more attention should be paid to the possible role of chemical exposures in the etiology of NTDs, he discusses large chemical classes (pesticides, organic solvents, agricultural pesticides, etc.) rather than specific chemicals, although the chemicals in these large classes are unlikely to act via the same mechanisms. Additionally, the data he presents in his review article are extremely limited and require further confirmation. Until confirmed through multiple large epidemiological investigations and mechanistic data from animal studies, a potential role of environmental exposures in NTDs should be considered hypothetical at best.

Finally, there appears to be a general disconnect between the more severe neurodevelopmental disorders that are presented in this section of the Report and the types of minor alterations that are discussed in the following sections as evidence for an endocrine mechanism in DNT.

Evidence for Endocrine Mechanisms in Developmental Neurotoxicity

On page 298, the authors cite a single study (Ahmed et al. 2008) to support the statement that "interactions between brain development and thyroid hormone have been exhaustively reviewed;" additional references on this topic are available and should be cited. It should be

further noted that many of the fetal mechanisms for neurodevelopmental disorders related to endocrine disruption, as given at the bottom of page 289, are still only hypothetical at this point.

Thyroid disruption. In their introduction to this chapter of the SAAED Report, the authors indicate that thyroid disruption is the endocrine-related mechanism with greatest support for a role in DNT. With that said, they provide scant primary data in the subsection on thyroid disruption (section 6.1.2.1). Further, they elaborate on hypothyroidism and its link with mental retardation, but fail to discuss the potential role of *hyper*thyroidism in neurodevelopmental disorders. This latter topic should be developed in some detail in order to be complete. With regard to hypothyroidism, the authors state that the incidence of congenital hypothyroidism is increasing. A recent workshop sponsored by the Centers for Disease Control and Prevention (CDC) addressed this perceived upwards trend (Shapira et al. 2010). Workshop participants agreed that, until diagnostic criteria are standardized for assessing transient versus congenital hypothyroidism, an increased incidence cannot be assumed.

It is important to note that, although the thyroid develops and functions in a manner generally similar between rodents and humans, there are important distinctions that may make findings from rat studies irrelevant to understanding outcomes in humans. Many of these differences are outlined in two of the major citations relied upon by the SAAED authors (Howdeshell 2002, Ahmed et al. 2008) and include:

- The thyroid gland begins developing relatively early in gestation (by the third week) in humans, but not until gestational day (GD) 9 in rats, when gestation is almost half-way complete;
- The hypothalamus in humans appears by the 5th week of gestation, while in the rat it does not appear until GD 12.5;
- Vasculature connecting the hypothalamus to the pituitary is present around 11.5 weeks of gestation and continues to mature through late gestation in humans, but does not fully mature in rats until 5-6 weeks <u>after</u> birth;
- Thyroid stimulating hormone (TSH) reaches peak serum levels sometime between gestational weeks 22 and 30 in humans, decreasing slightly thereafter until birth, while in the rat TSH does not peak until 10 to 12 days <u>after</u> birth;
- The human fetal pituitary can response to thyroxin-releasing hormone (TRH) as early as gestational week 25, while the hypothalamic-pituitary-thyroid axis of rats does not respond to TRH until two weeks <u>after</u> birth;
- In rats, maternal iodide deficiency (even that which is borderline) reduces the availability of T4 to the fetus; and
- In humans, proper levels of free thyroid hormone can be maintained during pregnancy via increased peripheral metabolism and enhanced thyroid hormone binding to serum proteins. Therefore, children with congenital hypothyroidism that cannot synthesize thyroid hormones may be born with low-normal concentrations of thyroid hormone due to compensation by the maternal thyroid system.

Overall, humans are born with a fully mature thyroid system, while the thyroid system of rats does not reach maturity until around 4 weeks after birth. Additionally, the human maternal

system can compensate for fetal thyroid deficiencies until birth; the same does not appear to be true for the rat. In sum, the differences in thyroid development between humans and rats mean that the thyroid system of rats is 1) more susceptible to alteration than that of humans, and 2) less capable of compensating for possible alterations in function. The EPA, in its assessment of thyroid follicular cell tumors for human relevance, also concludes that the rat thyroid shows much greater sensitivity to perturbation versus that of humans (USEPA 1998). Many of the studies showing a link between altered thyroid function and neurodevelopmental effects have been conducted in rats. Therefore, additional research must be done to show that the findings in rats are relevant to humans.

Much of the evidence for chemicals modulating thyroid function comes from rat studies, but the lack of data in humans brings into question the importance of some of these findings for human health. This is particularly true for the over 150 chemicals adopted from Howdeshell (2002) and referred to on page 299 of the SAAED report. For example, although perchlorate has been shown to block iodine uptake into the thyroid, a cross-sectional health study of iodine deficient women found that perchlorate exposure during the first trimester of pregnancy was not associated with alterations in thyroid function (Pearce et al. 2010, Brent 2010).

Sex Hormone Disruption. The authors rely on a review by Watson et al. (2000) for much of their discussion about the role of estrogens in neuro-behavior without providing any supporting data. This section needs to be developed further with specific examples, including information on doses, duration of exposure and species. Moreover, the relevance of these examples to humans needs to be demonstrated or discussed.

Much of the information regarding neuro-steroids and neural plasticity is not discussed in enough detail to provide the reader with a clear picture of the data. For example, the authors claim that the paper by Kawato (2004) shows that BPA and DES, "acutely modulate the local synthesis of estrogen in the embryonic and neonatal rat hippocampus and thereby modulate synaptic plasticity." First, this paper is actually a review and not primary experimental data. Second, although data are presented to show effects of BPA exposure on estradiol synthesis, similar data for DES are not provided. Third, the reported effects of BPA on calcium signaling in rat hippocampal neurons and BPA and DES on long-term potentiation in rat hippocampal slices are based on *in vitro* studies. Further study is needed to show that this type of mechanistic work is actually relevant to the *in vivo* circumstance. Fourth, these slices were purportedly derived from 4- and 12-week old rats; therefore, embryonic rat tissues were not evaluated. Therefore, the information provided in the actual paper does not fully support the description provided by the authors of the SAAED Report.

Neuroendocrine Disruption. In this section of the Report, the SAAED authors indicate that there may be "crosstalk" between the AhR pathway and other hormonal receptor pathways, including those of estrogen, progestin, androgen, glucocorticoid and thyroid hormones. However, the authors fail to include any supporting data to support these claims. More information is required, including specific data regarding the doses at which such crosstalk may occur.

Evidence for a Role of Chemical Exposures in Developmental Neurotoxicity Through an Endocrine Disruption Mechanism.

This section of the document relies primarily on a review article by Grandjean and Landrigan (2006) for epidemiological and mechanistic evidence of DNT (as noted at the top of page 303). However, this paper does not specifically identify and address compounds that act through endocrine disruption; in fact, the paper only mentions endocrine disruption once, as a potential mechanism through which PCBs may alter neurodevelopment. Further, the data are scarce to support either neurodevelopmental effects for some chemicals (e.g., brominated flame retardants, perchlorate, and BPA) or endocrine-related mechanisms of action for the other chemicals (e.g., lead, mercury and arsenic). Consequently, a solid case for neurodevelopmental toxicity in humans mediated through endocrine-related mechanisms is lacking.

Organochlorine pollutants (PCBs and Dioxins). Although prenatal PCB exposures have been reported to be associated with neurodevelopmental effects in humans, the evidence is not consistent. In fact, some studies have reported no adverse effects associated with environmentally relevant PCB exposures. For example, Vermier et al. (2005) found no dose-related effects between serum PCB concentrations and performance on a neurobehavioral evaluation battery in a cohort of Flemish children. Likewise, Wilhelm et al. (2008) found no associations between PCB (and PCDD/F) exposures and adverse effects on neurological examinations at 2 weeks and 18 months of age or on mental and motor development evaluations at 12 and 18 months of age in the Duisburg birth cohort. In conducting follow-up on a cohort of children exposed to PCBs perinatally, Winneke et al. (2005) found that although these children showed cognitive deficits on the Kaufman Assessment Battery for Children at 30 and 42 months of age, no adverse effects were found when the test was administered at 72 months (6 years of age). Winneke et al. interpreted these findings to suggest that PCBs may induce transient delays in cognitive development only. In another study, Gray et al. (2005) reported that third trimester serum PCB concentrations were associated with slightly increased, rather than decreased, IQ scores in children at 7 years of age. The possibility that environmentally relevant PCB exposures may have no effect on neurodevelopment or that effects of PCB exposure may be transient in nature are not discussed in the SAAED Report. To provide a balanced review, these topics should be expanded upon further.

Although the Report authors mention the papers by Ross (2004) and Arisawa et al. (2005) and acknowledge that these reviews find no consistent evidence to associate PCB exposures with endocrine disruption, they completely discount these conclusions. Instead, they focus on limited data suggesting that PCBs might alter thyroid function. It is important to note, however, that an adverse effect on the thyroid due to environmental PCB (or PCDD/F) exposures is not an established fact. Not only did the study by Wilhelm et al. (2008) find no association between PCB (PCDD/F) exposure and neurodevelopment, they also found no decrease in thyroid hormone in relation to maternal blood and milk concentrations of these chemicals. Similarly, a review of multiple PCB studies looking at birth weight, immune and thyroid parameters found that thyroid function was “within the normal range” in the various studies (Kimbrough and Krouskas, 2001), suggesting no effect on thyroid function. From these data, Kimbrough and Krouskas concluded that studies of children's cohorts "do not provide solid evidence that

environmental exposures to PCBs and related chemicals affect the neurobehavioral development of infants and children." As reported in the review by Arisawa (2005), "[i]n humans, data about the association between exposure to dioxin-related compounds and thyroid function have been inconsistent for both adults and infants, though the blood levels of T4 free T4, and TSH are generally with the normal range even in heavily exposed subjects." These types of findings make the connection between organochlorine compounds, thyroid function and neurodevelopmental effects unclear.

Brominated flame retardants. While the SAAED Report authors discuss at length the possibility that brominated flame retardants may interact with the thyroid system, they have not adequately addressed whether these compounds actually cause neurodevelopmental effects in humans or if such effects may be mediated through actions on the thyroid. Although various animal studies have suggested neurological impairments as a result of perinatal exposure to polybrominated diphenyl ethers (PBDEs), as detailed in the review by Williams and DeSesso (2010), these studies are generally inconsistent in the parameters reportedly affected, the direction and pattern of the changes observed, permanency of the findings, whether a dose-response is observed, and if gender-related differences in responses exist. Further, guideline-compliant DNT studies of PBDE209, tetrabromoBPA (TBBPA), and hexabromocyclododecane (HBCD) have found no adverse effects on neurodevelopment (Jacobi et al. 2009; Ema et al. 2008, Schroeder 2002); these studies involved exposures that were of longer duration and orders of magnitude greater than those of other studies showing potential effects of the nervous system.

The Report authors also fail to mention that the epidemiological data to support an association between PBDE exposure and neurodevelopmental effects are extremely limited. To our knowledge, only two studies have been published on this topic. The SAAED authors mention the study by Herbstman et al. (2010), but they fail to acknowledge that this study is in consistent with the only other epidemiologic study to address the neurobehavioral effects of PBDE exposure (Roze et al. 2009). More specifically, Herbstman et al. (2010) reported statistically significant effects on the Wechsler Preschool and Primary Scale of Intelligence, Revised Edition (WPPSI-R) with prenatal PBDE exposure, but Roze et al. (2009) did not. Due to the inconsistency in both epidemiological data and animal data on this issue, it is not possible to conclude that perinatal PBDE exposure is associated with neurodevelopmental effects in people.

Perchlorate. It is unclear why perchlorate is listed in this chapter because, as the SAAED authors themselves state, perchlorate is not a known human neurotoxicant. Further, data suggesting that perchlorate causes neurodevelopmental effects does not exist. In the absence of such data in humans or animals, a discussion of perchlorate does not belong in this chapter.

Pesticides. As previously discussed, pesticides is an extremely large chemical grouping. Grandjean and Landrigan (2006) indicate in their review that over 600 different pesticides are registered for use. Pesticides are most commonly grouped according to the target phyla upon which they act: insecticides, herbicides, fungicides, rodenticides, and fumigants; but even within these groupings, pesticides may act by very different mechanisms of action. Insecticides represent the 2nd most commonly used type of pesticide worldwide and almost all insecticides in

use today act by targeting the nervous system of target organisms (Costa, 2008).[3] Because the nervous system of insects shares many similarities with that of other species, including humans, insecticides tend to show low species-specificity. Therefore, it should come as no surprise that many insecticides (e.g., organophosphates, which target the enzyme acetylcholinesterase) can cause neurotoxicity and DNT in humans. However, these adverse outcomes are mediated through direct action on the nervous system and do not involve the endocrine system. Therefore, for the purposes of discussing pesticides as potential endocrine disruptors, it does not make sense to collectively refer to pesticides. Rather, the SAAED authors should either discuss specific pesticides or specific chemical classes of pesticides that work through similar mechanisms of action.

With the exception of references to general pesticides or pesticide mixtures (terms that are too broad to be useful for the purposes of this discussion), all of the pesticides discussed in this section are insecticides – either organophosphates or organochlorines. Although organophosphates have been implicated in DNT, they are not considered endocrine disruptors, and therefore, are not relevant to this chapter. Organochlorines, such as DDT, have been associated with endocrine disruption; however, these compounds also directly target the nervous system by interfering with sodium ion channels at the nerve axon (Costa 2008) and it is this latter mechanism of action that likely plays a large role in any associated neurotoxic effects. In order to make a connection between endocrine disruption and the possible neurodevelopmental effects of organochlorine exposure, the SAAED authors need to provide mechanistic data from animal studies showing that endocrine disruption, and not direct action on the nervous system, is the underlying culprit in any neurodevelopmental outcomes.

The SAAED authors refer readers to a paper by Boas et al. (2006) for a review of thyroid effects due to pesticides. However, pesticides are only addressed specifically in three paragraphs of this review; thus, this paper is not considered an adequate reference to address the hypothesis that pesticides may target the thyroid system. Finally, with regard to endocrine disruption, organochlorines such as DDT have most commonly been associated with agonist/antagonist activities at the estrogen and androgen receptors (Costa 2008). The SAAED authors have not addressed this aspect at all, much less mentioned this fact.

BPA. The SAAED authors provide no data in this section to support a role of BPA exposures in neurodevelopmental toxicity. In fact, the only data that they do discuss is a GLP- and guideline-compliant DNT study that showed no evidence of DNT in rats at doses as high as 2,250 ppm in the diet (Stump et al. 2010). In order to propose BPA as a potential developmental neurotoxicant, the SAAED authors must include primary data that show adverse neurodevelopmental outcomes due to BPA exposure. In the absence of such data, discussion of BPA does not belong in this chapter.

[3] A few insecticides target cuticle synthesis or act by other mechanisms that are less understood, but which do not involve the nervous system.

BPA is another example of a chemical for which the SAAED authors fail to address the more commonly associated endocrine effect – that is, its estrogenic activity. In the interest of being complete, this aspect of BPA's endocrine activity should at least be mentioned.

Perfluorinated chemicals. As support for the potential neurodevelopmental effects related to perfluorinated chemicals (PFCs) exposure, the SAAED authors cite a cross-sectional study by Hoffman et al. (2010) in which children with higher serum levels of PFCs (based on NHANES data) has slightly increased odds ratios for ADHD based on parental reports (not confirmed by medical records). A more recent cross-sectional study examined parental reports of ADHD in a population of children 5-18 years of age living in an area of West Virginia with PFOA-contaminated groundwater (Stein and Savitz 2011). This latter population generally had higher serum concentrations of PFCs (mean PFOA concentration of 66.3 ng/mL) than the children of the former study (median PFOA concentration of 4.4 ng/mL). Despite this, a reduction in ADHD (rather than an increase) was seen at the highest PFOA exposure levels in this study. The data regarding PFHxS were suggestive of a potential relationship, but as the serum concentrations of PFHxS were relatively low, this finding requires further investigation. Both of these studies are of a cross-sectional study design, which limit the evaluation of the relationship between an exposure and a disease outcome because they are assessed at the same point in time. Regardless, these preliminary data are conflicting regarding a possible relationship between exposure to PFCs and ADHD.

The SAAED authors fail to discuss a couple of other epidemiology studies examining the association of PFC exposures with neurodevelopmental disorders (Fei et al. 2008, 2011). These cohort studies are more reliable for the evaluation of a potential association compared to the cross-sectional studies discussed above and involve the measurement of PFOA and PFOS concentration in maternal blood drawn during the first trimester. Such measurements are thought to be better indicators of exposure during the critical neurodevelopmental period than serum concentrations measured at 5+ years of age. Fei et al. (2008) evaluated Apgar scores and parental reports of developmental milestones at 6 and 18 months of age in children from the Danish National Birth Cohort. Fei et al. (2011) was a follow-up study that examined parental responses on the Strengths and Difficulties Questionnaire (reported to be "a validated tool to screen for hyperactivity and attention problems among children") and the Developmental Coordination Disorder Questionnaire for children of 7 years of age. These studies found that PFOA or PFOS blood concentrations were not associated with differences in Apgar scores at birth, latency to reach developmental milestones, or behavioral or motor coordination problems in childhood.

Although the data are rather limited, as a whole the epidemiological studies do not suggest that an association exists between PFC exposures and adverse neurodevelopmental outcomes in children. In order to provide a balanced and unbiased discussion, the SAAED authors must include the additional studies reviewed herein.

Another example of a biased presentation of the data to support an endocrine-related mechanism can be found in the discussion of thyroid effects. The SAAED authors include discussion of a study by Melzer et al. (2010) in which higher serum concentrations or PFOA and PFOS were

associated with adult thyroid disease. However, they fail to report on another study that involved higher PFC exposures but found no association with thyroid disease (Emmett et al., 2006), although this study was specifically discussed in Melzer et al. (2010).

Phthalates. As support for a role of phthalates in neurodevelopmental disorders, the SAAED authors cite a couple of epidemiology studies (Engel et al. 2010, Cho et al. 2010). With regard to the paper by Engel et al. (2010), it is important to note that this study was relatively small (n = 188). Further, the 95% confidence intervals around their point estimates of scores on the Behavioral Assessment System for Children-Parent Rating Scales (BASC-PRS) were very large, indicating the large uncertainty in these reported values. Interestingly, these same investigators had previously reported that exposure to the lower molecular weight phthalates was associated with *improved* motor performance in boys (Engel et al., 2009). Across studies, statistically significant changes in neurobehavioral endpoints have varied depending on whether researchers have looked at high or low molecular weight phthalates and the specific phthalate metabolites examined. Obviously, more research is needed to confirm the findings of these preliminary investigations.

With regard to endocrine actions, the SAAED authors primarily rely on a nonspecific review by Boas et al. (2009) to discuss possible actions on the thyroid. Primary data and references specific to phthalates must be added to fully support this discussion. Finally, the phthalates represent another example of a chemical class about which the SAAED authors have not addressed the more commonly associated endocrine effect – in this case, their anti-androgenic activity. In the interest of being complete, this aspect of the endocrine activity of phthalates should at least be mentioned.

UV filter agents. This section provides yet another example of where the authors have proposed particular compounds (in this case, UV filter agents) as affecting neurodevelopment without providing any data to support this claim. In fact, data addressing the endocrine and neurodevelopmental effects of UV filter agents are relatively sparse. The SAAED authors must provide primary data supporting a potential role of UV filters in neurodevelopmental toxicity; otherwise, this category of compounds should be removed from discussion in this chapter.

Finally, the SAAED Report authors only provide one reference in this section (Boas et al. 2009), which is a general review and not specific to UV filter agents. More reference material should be cited in this section to support the inclusion of UV filter agents.

Lead. The potential neurodevelopmental effects of lead are well known and these are adequately reviewed in the Report. Although the mechanism(s) by which lead mediates its effects on brain development have not been firmly established, oxidative stress, disruption of calcium ion signaling and alteration of neurotransmission appear to play a role (Marchetti 2003, Toscano and Guilarte 2005, Sanders et al. 2009). The authors suggest that lead may be acting through thyroid disruption; however, there is very little data available to support this claim or to suggest that lead is not acting on the nervous system directly. Further, the data presented in the Report regarding potential thyroid effects are conflicting. For example, the study of Singh et al. (2000) finds no effects on T3 and T4, but increased TSH levels with occupational lead exposure,

while the study by Dundar et al. (2006) reports reduced T4 levels, but no effects on T3 or TSH. Abdelouhab et al. (2008), in contrast, reported reduced TSH in females only, but no effects on T3 or T4 in males or females due to lead exposure from fish consumption. Finally, the authors fail to present a balanced discussion on this topic. More specifically, they do not mention any of the studies that fail to find a connection between lead exposure and thyroid effects. For example, Refowitz (1984) found no association between blood lead levels and T4 in a cross-sectional study of workers with long-term lead exposure and Erfurth et al. (2001) found no differences in thyroid hormone levels or thyrotropin among active or retired lead smelter workers and a referent population, although lead concentrations were significantly different among the three groups. Similarly, Siegel et al. (1989) found no relationship between blood lead levels and T4 in children at an outpatient pediatric clinic. Based on the data available, it cannot be definitively concluded that lead adversely affects thyroid function or that it causes neurodevelopmental toxicity via an endocrine-mediated mechanism of action. Consequently, a discussion of lead neurotoxicity does not belong in this chapter.

Mercury, methyl mercury. First, it should be noted that mercury comes in many different forms (elemental, inorganic, and organic); of these, methyl mercury is the form most commonly associated with neurodevelopmental effects. Further, as with lead, the effects of methyl mercury exposure on brain development have been established and are well known. Evidence suggests that methyl mercury targets the nervous system directly through oxidative stress, alterations in calcium ion signaling, and possible effects on astrocytes (Atchison and Hare 1994, Shanker et al. 2003, Ceccatelli et al. 2010, Farina et al 2011). The SAAED authors, in contrast, suggest multiple mechanisms by which mercury could disrupt the endocrine system as relevant to the issue of methyl mercury neurotoxicity (it should be noted that simple accumulation of mercury in the endocrine system is not a mechanism of action, as purported by the authors). However, what is missing from this discussion is primary data to support these proposed mechanisms as well as dose-response information to characterize these responses. Further information is needed to clarify whether the doses at which endocrine disruption occur are similar or higher than those at which DNT is observed. If endocrine-related changes are only observed at doses higher than those at which DNT is seen, then it is unlikely that endocrine-disruption plays a role in the neurodevelopmental effects of mercury.

Arsenic. Again, the authors implicate endocrine disruption as a mechanism by which the neurodevelopmental effects of a metal (arsenic, in this case) are mediated without clear data to support this contention. In fact, no primary data are actually presented related to endocrine disruption; rather, the only reference they cite is a single review article (Iavicoli et al. 2009) that speculates about several potential endocrine-related mechanisms. In the absence of data to support an endocrine-mediated mechanism of action, discussion of arsenic does not belong in this chapter.

Evidence of Developmental Vulnerability in Developmental Neurotoxicity

The purpose of this section is not clear because, as the SAAED authors themselves report, "(B)y definition, DNT as a toxic endpoint is intimately linked to development." The pertinent

question is not whether developmental vulnerability exists for DNT, but rather, whether DNT is mediated through effects on the endocrine system during development.

The SAAED authors refer to a review by Landrigan (2010) to show a possible environmental contribution to the development of autism. Unfortunately, the Landrigan paper is not well-supported; that is, few citations are provided to show that the environmental factors discussed therein (i.e., thalidomide, misoprostal, valproic acid, rubella infection, and chlorpyrifos) have any connection to autism. Further, of the various environmental factors listed, the only one of even peripheral relevance to the SAAED Report is chlorpyrifos. However, chlorpyrifos has not been shown to cause autism and has not been shown to be an endocrine disruptor.

As support for a role of chlorpyrifos in autism, Landrigan (2010) references a study from the Slotkin laboratory in which rats treated postnatally with chlorpyrifos showed altered performance in various maze apparatuses (Levin et al. 2001). These altered behaviors are not indications of autism. In fact, the behaviors seen in animals developmentally exposed to chlorpyrifos are inconsistent with those that would be expected in autism (i.e., impaired social interactions, deficits in communications, and repetitive or stereotyped behaviors). Instead of impaired social interactions, developmental chlorpyrifos treatment is associated with no change or increased social responses in mice (Ricceri et al. 2003, Venerosi et al. 2006, 2008). Developmental chlorpyrifos treatment has also been observed to have no or equivocal effects on pup ultrasonic communications (Ricerri et al. 2003, Venerosi et al. 2009), to not affect or to increase maternal responses to pup vocalizations (Ricerri et al. 2006, Venerosi et al. 2008), and to increase vocalizations during social play (Venerosi et al. 2006). These responses are in contrast to expected deficits in communication. Finally, instead of increasing repetitive or stereotypes behaviors, most studies show no effect of developmental chlorpyrifos exposure on grooming, rearing, and wall-rearing (Ricceri et al. 2006, Dam et al. 2000). In summary, the data from studies of developmental chlorpyrifos exposure are not congruent with what one would expect to see if chlorpyrifos caused autism.

The SAAED authors further suggest that neurodevelopmental effects of perinatal chlorpyrifos exposure occurs in the absence of detectable cholinesterase inhibition. This has not been shown to be true. Studies suggesting noncholinergic mechanisms of action have been primarily generated in the Slotkin laboratory. Most of these studies, however, have not measured cholinesterase activity. Further, the few that have (Song et al. 1997, Dam et al. 2000) show that cholinesterase inhibition is occurring at the same doses for which changes in other measured parameters are reported; these data are supported by work from another laboratory (Carr and Nail 2008). Therefore, no data exist to support the claim that neurodevelopmental effects can occur at chlorpyrifos doses that do not cause cholinesterase inhibition.

Do Experimental Tools Exist for the Study of Developmental Neurotoxicity, and Are Assays Applicable to, and Adequate for, the Assessment of Chemicals?

Guidelines exist for the *in vivo* evaluation of DNT (OECD TG 426). Further, as noted, the history and performance of the DNT study was reviewed in support of finalizing these guidelines (Makris et al. 2009). However, the SAAED authors mischaracterize the results of this review, suggesting that it expresses significant criticism of DNT study methods. The conclusions of the review by Makris et al. (2009) are as follows:

> *"The OECD DNT guideline represents the best available science for assessing the potential for DNT in human health risk assessment, and data generated with this protocol are relevant and reliable for the assessment of these end points. The test methods used have been subjected to an extensive history of international validation, peer review, and evaluation, which is contained in the public record. The reproducibility, reliability, and sensitivity of these methods have been demonstrated using a wide variety of test substances, in accordance with OECD guidance on the validation and international acceptance of new or updated test methods for hazard characterization. Multiple independent, expert scientific peer reviews affirm these conclusions." (p. 17)*

Therefore, while the review paper suggests room for appropriate revisions to the test method as future experience warrants, it also expresses full support for the existing DNT guidelines.

A number of assays that could be used to identify chemicals that may interfere with thyroid function are listed in this section of the Report. As noted in the original paper from which this list was taken (Howdeshell 2002), none of these assays is considered adequate to serve as a first tier screening assay. Further, as already discussed in more detail above, humans and rats differ considerably in their thyroid development and function. As a result, humans are less susceptible to thyroid alterations than rats and are more able of compensate for possible alterations in function. Therefore, the results of assays of thyroid function in rats, as proposed in this section of the Report, may not be relevant to humans.

Section Conclusions

As previously discussed, the principles used to by the SAAED authors to attribute DNT (and other health outcomes) to endocrine disruption are inappropriate for the types of data being discussed. These principles are meant to be applied to an evaluation of laboratory data, particularly that from mechanistic studies. They do not apply to epidemiological data or for conducting a weight-of-evidence evaluation of causality. Further, the pertinent question to be addressed is not whether alterations in endocrine function during the developmental period might cause neurotoxicity, but rather, whether evidence exists to show that chemicals causing DNT do so through an endocrine-related mechanism. Each of the principles evaluated in the conclusions is discussed below.

Ability to isolate the response to endocrine sensitive tissues in an intact whole organism. Although some data are available to associate certain chemicals to endocrine disruption and other data are available to show that certain chemicals cause neurodevelopmental effects, the linkage between these two focus areas is generally lacking. For many of the chemicals discussed in this chapter, data exist to only support one or the other—and for a few of the chemicals, the data are sparse in both areas. Because specific linkages between endocrine disruption and neurodevelopmental effects have not been shown for any of the chemicals discussed in this chapter, this criterion has not been met.

Analysis of the response at multiple levels of biological organization – from phenotypic expression to physiology, cell biology, and ultimately molecular biology. This type of systematic evaluation has not been conducted for any of the chemicals discussed in this chapter. Changes at the molecular level have not been specifically linked to cellular biological alterations, and ultimately, to physiological changes in the whole organism. The SAAED authors concede this fact when they state that linkages from single cells to mood (or other physiological outcomes) have not been made. As such, this criterion has not been met.

Direct measurement of altered hormone action (gene induction or repression), hormone release, hormone metabolism, or hormone interactions under the experimental regime in which the toxicologic outcome was manifest. Although hypothyroidism has been shown to be associated with impaired neurodevelopment, the information provided is not specific to chemical exposures. As already discussed, specific alterations in endocrine function have not been measured in response to the chemicals discussed in this chapter *under an experimental regime in which DNT was observed.* Such a linkage is vital for chemically-mediated DNT to be attributed to endocrine function. It should be further noted that, since humans are less sensitive than rats to thyroid alterations resulting from chemical exposures, such a linkage will also need to be shown in humans to be applicable for human health risk assessment. Because this linkage has not been made for any of the chemicals discussed in this chapter, this criterion has not been met.

Dose-response observations that indicate the perturbance of the endocrine system is a critical response of the organism, and not the secondary result of general systemic toxicity. For many of the chemicals discussed in this chapter, specific data showing endocrine effects at doses lower than those causing general toxicity—or more importantly, below doses causing neurodevelopmental toxicity—have not been provided. Further, for some of the chemicals discussed (e.g., lead, mercury, and arsenic), it is unlikely that such data even exist. Based on the lack of specific dose-response data, this criterion has not been met.

Ability to compare the resulting phenotypes with outcomes from exposure to known pharmacological manipulations. For many of the chemicals discussed in this chapter, the potential endocrine effects have not been well characterized and data comparing observed endocrine changes with those induced upon pharmacologic manipulation have not been presented. Further, because causative linkages between endocrine disruption and neurodevelopmental outcomes have not been shown for the chemicals discussed in this chapter, the ability to shown parallel outcomes with pharmacologic manipulation does not exist.

Because connections cannot be made between pharmacologic manipulations and the outcomes observed upon exposure to the chemicals discussed, this criterion has not been met.

Indication that there is differential sensitivity of certain lifestages in which dysregulation of a particular endocrine system is known to have adverse health consequences. By definition, DNT occurs during development, which is therefore, the sensitive life-stage. This criterion is met.

Ability to restore the phenotype or toxicologic outcome via pharmacological manipulations that counter the presumed mode of action on the endocrine system in the intact organism. Although treatment of hypothyroidism prevents neurological impairment, data are not available to show that, for the chemicals discussed in this chapter, pharmacologic manipulations of the endocrine system can rescue an intact organism from DNT resulting from the chemical exposures. This criterion has not been met.

Supporting data on endocrine activity from in vitro binding, transcriptional activation, or cellular response studies. Various supporting data exist to suggest that disruption of endocrine activity can affect neurodevelopment. However, what is missing is data from intact organisms showing that these mechanisms are in play in the DNT resulting from chemical exposures. Thus, although this criterion is mostly met, the more pertinent information from whole organisms is lacking.

In conclusion, although data exist to support a role of the endocrine-system in neurodevelopment, data linking endocrine-mediated mechanisms to chemically induced DNT are lacking. For individual chemicals, data may exist to support possible endocrine disruption or alterations in neurodevelopment; however, data to support both actions generally do not exist. Further, to make the linkage between endocrine disruption and DNT for any particular chemical, additional mechanistic data would be required to demonstrate that disruption of the endocrine system actually caused the neurodevelopmental alterations observed.

References

Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M, Spear PA, Chan HM. 2008. Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of Quebec (Canada). Environmental Research 107:380-392.

Aguiar A, Eubig PA, Schantz SL. 2010. Attention deficit/hyperactivity disorder: A focused overview for children's environmental health researchers. Environmental Health Perspectives 118:1646-1653.

Ahmed OM, El-Gareib AW, El-Bakry AM, Abd El-Tawab SM, Ahmed RG. 2008. Thyroid hormones states and brain development interactions. International Journal of Developmental Neurosciences 26:147-209.

Alavanja MCR, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF, Knott C, Thomas K, Hoppin JA, Barker J, Coble J, Sandler DP, Blair A. 2003. Use of agricultural pesticides and prostate cancer risk in the agricultural Health study cohort. American Journal of Epidemiology 157:800-814.

Arisawa K, Takeda H, Mikasa H. 2005. Background exposure to PCDDs/PCDFs/ PCBs and its potential health effects: A review of epidemiologic studies. Journal of Medical Investigation 52:10-21.

Atchison WD, Hare MF. 1994. Mechanisms of methylmercury-induced neurotoxicity. FASEB Journal 8:622-629.

Axelstad M, Boberg J, Hougaard KS, Christiansen S, Jacobsen PR, Mandrup KR, Nellemann C, Lund SP, Hass U. 2011. Effects of pre- and postnatal exposure to the UV-filter octyl methoxycinnamate (OMC) on the reproductive, auditory and neurological development of rat offspring. Toxicology and Applied Pharmacology 250:278-290.

Benbrahim-Tallaa L, Waalkes MP. 2008. Inorganic arsenic and human prostate cancer. Environmental Health Perspectives 116:158-164.

Blair E, Watson L. 2006. Epidemiology of cerebral palsy. Seminars in Fetal and Neonatal Medicine 11:117-125.

Boas M, Feldt-Rasmussen U, Skakkebaek NE, Main KM. 2006. Environmental chemicals and thyroid function. European Journal of Endocrinology 154:599-611.

Boas M, Main KM, Feldt-Rasmussen U. 2009. Environmental chemicals and thyroid function: an update. Current Opinion in Endocrinology, Diabetes, and Obesity 16:385-391.

Boobis AR, Datson G, Preston J, Olin S. 2009. Application of key events analysis to chemical carcinogens and noncarcinogens. Critical Reviews in Food Science and Nutrition 49:690-707.

Bostwick DG, Burke HB, Djakiew D, Euling S, Ho SM, Landolph J, Morrison H, Sonawane B, Shifflett T, Waters DJ, Timms B. 2004. Human prostate cancer risk factors. Cancer 101:2371-2490.

Bouskine A, Nebout M, Brucker-Davis F, Benahmed M, Fenichel P. 2009. Low doses of bisphenol A promote human seminoma cell proliferation by activating PKA and PKG via a membrane G-protein-coupled estrogen receptor. Environmental Health Perspectives 117:1053-1058.

Brent GA. 2010. The impact of perchlorate exposure in early pregnancy: Is it safe to drink the water? Journal of Clinical Endocrinology and Metabolism 95:3154-3157.

Buchanan RL, Havelaar AH, Smith MA, Whiting RC, Julien E. 2009. The key events dose-response framework: Its potential for application to foodborne pathogenic microorganisms. Critical Reviews in Food Science and Nutrition 49:718-728.

Carr RL, Nail CA. 2008. Effect of different administration paradigms on cholinesterase inhibition following repeated chlorpyrifos exposure in late preweanling rats. Toxicological Sciences 106:186-192.

Ceccatelli S, Daré E, Moors M. 2010. Methylmercury-induced neurotoxicity and apoptosis. Chemico-Biological Interactions 188:301-308.

Center for the Evaluation of Risks to Human Reproduction (CERHR). 2008. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. http://ntp.niehs.nih.gov/ntp/ohat/bisphenol/bisphenol.pdf

Chen CJ, Wang CJ. 1990. Ecological Correlation Between Arsenic Level in Well Water and Age-Adjusted Mortality from Malignant Neoplasms. Cancer Research 50:5470-5474.

Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, Kim JW, Yoo HJ, Cho IH, Kim HW. 2010. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environmental Health Perspectives 118:1027-1032.

Conolly RB, Lutz WK. 2004. Nonmonotonic dose-response relationships: mechanistic basis, kinetic modeling, and implications for risk assessment. Toxicological Sciences 77:151-7.

Costa LG. 2008. Toxic effects of Pesticides. Chapter 22. *In:* Casarett & Doull's Toxicology. The Basic Science of Poisons. 7th Edition. Ed. CD Klaassen. The McGraw-Hill Companies, Inc. New York. pp. 883-930.

Cunha GR, Frosberg JG, Goldern R, Haney A, Iguchi T, Newbold R, Swan S, Welshons W. 1999. New approaches for estimating risk from exposure to Diethlstilbestrol. Environmental Health Perspectives 107:625-630.

Dam K, Seidler FJ, Slotkin TA. 2000. Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. Developmental Brain Research 121:179-187.

DeVita VT, Lawrence TS, Rosenberg SA. 2010. DeVita, Hellman, and Rosenberg's cancer: Principles & Practice of Oncology. 8th edition. Eds: DeVita VT, Lawrence TS, Rosenberg SA.

Doerge DR, Twaddle NC, Woodling KA, and Fisher JW. 2010. Pharmacokinetics of bisphenol A in neonatal and adult rhesus monkeys. Toxicology and Applied Pharmacology 248(1):1-11.

Doerge DR, Vanlandingham M, Twaddle NC, and Delclos KB. 2010. Lactational transfer of bisphenol A in Sprague-Dawley rats. Toxicology Letters 199(3):372-376.

Dundar B, Oktem F, Arslan MK, Delibas N, Baykal B, Arslan C, Gultepe M, Ilhan IE. 2006. The effect of long-term low dose lead exposure on thyroid function in adolescents. Environmental Research 101:140-145.

European Food Safety Authority (EFSA). 2010. Scientific Opinion on Bisphenol A. http://www.efsa.europa.eu/en/efsajournal/doc/1829.pdf

Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, and Harazono A. 2001. Rat two-generation reproductive toxicity study of bisphenol A. Reproductive Toxicology 15:505-523.

Ema, M., Fujii, S., Hirata-Koizumi, M., and Matsumoto, M. 2008. Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reproductive Toxicology 25:335–351.

Emmett EA, Zhang H, Shofer FS, Freemen D, Rodway NV, Desai C, Shaw LM. 2006. Community exposure to perfluorooctanoate: relationships between serum levels and certain health parameters. Journal of Occupational and Environmental Medicine 48:771-9.

Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, Wolff MS. 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environmental Health Perspectives 118:565-571.

Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, Miodovnik A, Wolff MS. 2009. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. Neurotoxicology 30:522-528.

Erfurth EM, Gerhardsson L, Nilsson A, Rylander L, Schütz A, Skerfving S, Börjesson J. 2001. Effects of lead on the endocrine system in lead smelter workers. Archives of Environmental Health 56:449-455.

EU Risk Assessment. 2003. http://ecb.jrc.it/documents/Existing-Chemicals/RISK_ASSESSMENT/ADDENDUM/bisphenola_add_325.pdf

EU Risk Assessment. 2003. http://ecb.jrc.it/documents/Existing-Chemicals/RISK_ASSESSMENT/ADDENDUM/bisphenola_add_325.pdf

EU Updated Risk Assessment. 2008. http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/bisphenolareport325.pdf

EU Updated Risk Assessment. 2008. http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/bisphenolareport325.pdf

Eubig PA, Aguiar A, Schantz SL. 2010. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. Environmental Health Perspectives 118:1654-1667.

Evans R, Kortemkamp A, Martin O, McKinlay R, Orton F, Rosivatz. 2011. Part 1: State of the art assessment of endocrine disrupters (SAAED). 2nd Interim Report.

Farina M, Aschner M, Rocha JB. 2011. Oxidative stress in MeHg-induced neurotoxicity. Toxicology and Applied Pharmacology 256:405-417.

Food and Drug Administration (FDA). 2010. Update on Bisphenol A for Use in Food Contact Applications: January 2010. http://www.fda.gov/NewsEvents/PublicHealthFocus/ucm197739.htm#current

Fei C, McLaughlin JK, Lipworth L, Olsen J. 2008. Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environmental Health Perspectives 116:1391-1395.

Fei C, Olsen J. 2011. Prenatal exposure to perfluorinated chemicals and behavior or coordination problems at age 7 years. Environmental Health Perspectives 119:573-578.

Fombronne E. 2009. Epidemiology of pervasive developmental disorders. Pediatric Research 65:591-598.

Gill JK, Wilkens LR, Pollak MN, Stanczyk FZ, Kolonel LN. 2010. Androgens, growth factors and risk of prostate cancer: The multiethnic cohort. Prostate 70:906-915.

Giusti RM, Iwamoto K, Hatch EE. 1995. Diethylstilbestrol Revisited - A Review of the Long-Term Health-Effects. Annals of Internal Medicine 122:778-788.

Golden R, Kimbrough R. 2009. Weight of evidence evaluation of potential human cancer risks from exposure to polychlorinated biphenyls: an update based on studies published since 2003. Critical Reviews in Toxicology 39:299-331.

Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 268:2167-2178.

Gray Jr. LE, Ryan B, Hotchkiss AK, and Crofton KM. 2010. Rebuttal of "Flawed Experimental Design Reveals the Need for Guidelines Requiring Appropriate Positive Controls in Endocrine Disruption Research" by vom Saal. Toxicological Sciences 115(2):614-620.

Gray KA, Klebanoff MA, Brock JW, Zhou H, Darden R, Needham L, Longnecker MP. 2005. *In utero* exposure to background levels of polychlorinated biphenyls and cognitive functioning among school-age children. American Journal of Epidemiology 162:17-26.

Gualtieri AF, Iwachow MA, Venara M, Rey RA, Schteingart HF. 2010. Bisphenol A effect on glutathione synthesis and recycling in testicular Sertoli cells. Journal of Endocrinological Investigation 34:102-9..

Hardell L, Andersson SO, Carlberg M, Bohr L, van Bavel B, Lindstrom G, Bjornfoth H, Ginman C. 2006. Adipose tissue concentrations of persistent organic pollutants and the risk of prostate cancer. Journal of Occupational and Environmental Medicine 48:700-707.

Hengstler JG, Foth H, Gebel T, Kramer PJ, Lilienblum W, Schweinfurth H, Völkel W, Wollin KM, and Gundert-Remy U. 2011. Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. Critical Reviews in Toxicology 41(4):263-291.

Herbstman JB, Sjödin A, Kurzon M, Lederman SA, Jones RS, Rauh V, Needham LL, Tang D, Niedzwiecki M, Wang RY, Perera F. 2010. Prenatal exposure to PBDEs and neurodevelopment. Environmental Health Perspectives 118:712-719.

Hill AB. 1965. The Environment and Disease: Association or Causation? Proceedings of the Royal Society of Medicine 58:295-300.

Hinwood AL, Jolley DJ, Sim MR. 1999. Cancer incidence and high environmental arsenic concentrations in rural populations: results of an ecological study. International Journal of Environmental Health Research 9:131-141.

Ho SM, Tang WY, de Frausto JB, Prins GS. 2006. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Research 66:5624-5632.

Hoffman K, Webster TF, Weisskopf MG, Weinberg J, Vieira VM. 2010. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environmental Health Perspectives 118:1762-1767.

Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, and Gray LE Jr. 2008. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male Long Evans hooded rat. Toxicological Sciences 102(2):371-382.

Howdeshell KL. 2002. A model of the development of the brain as a construct of the thyroid system. Environmental Health Perspectives 110 Suppl. 3:337-348.

Hsing AW, Sakoda LC, Chua SC. 2007. Obesity, metabolic syndrome, and prostate cancer. The American Journal of Clinical Nutrition 86:843S-57S.

Iavicoli I, Fontana L, Bergamaschi A. 2009. The effects of metals as endocrine disruptors. Journal of Toxicology and Environmental Health Part B. Critical Reviews 12:206-223.

Jacobi S, Silberberg H, Stedeford T, Biesemeier J, Ariano J, and Beck M. 2009. An oral developmental neurotoxicity study of decabromodiphenyl ether (Deca BDE) in rats. Presented at the 2009 Eurotox Meeting, September, Dresden, Germany.

Jemal A, Siegel R, Xu J, Ward E. 2010. Cancer Statistics, 2010. CA – A Cancer Journal for Clinicians 60:277-300.

Julien E, Boobis AR, Olin SS, The Ilsi Research Foundation Threshold Working Group. 2009. The key events dose-response framework: A cross-disciplinary mode-of-action based approach to examining dose-response and thresholds. Critical Reviews in Food Science and Nutrition 49:682-689.

Kawato S. 2004. Endocrine disrupters as disrupters of brain function: A neurosteroid viewpoint. Environmental Sciences 11:1-14.

Key TJ. 2002. Prostate cancer: Rates in Europe, dietary hypothesis, and plans for EPIC. IRAC research publications 156:197-200.

Kimbrough RD, Krouskas CA. 2001. Polychlorinated biphenyls, dibenzo-p-dioxins, and dibenzofurans and birth weight and immune and thyroid function in children. Regulatory Toxicology and Pharmacology 34:42-52.

Kortenkamp A. 2007. Ten years of mixing cocktails: A review of combination effects of endocrine disruptors. Environmental Health Perspective 115:98-105

Kortenkamp A, Evans R, Martin O, McKinlay R, Orton F, Rosivatz E. 2012. Annex 1: State of the art assessment of endocrine disruptors. Final Report. European Commission, DG Environment.

Landrigan PJ. 2010. What is autism? Exploring the environmental contribution. Current Opinion in Pediatrics 22:219-225.

Levin ED, Addy N, Nakajima A, Christopher NC, Seidler FJ, Slotkin TA. 2001. Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. Developmental Brain Research 130:83-89.

Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J, Calderon RL. 1999. Drinking water arsenic in Utah: A cohort mortality study. Environmental Health Perspectives 107:359-365.

Marchetti C. 2003. Molecular targets of lead in brain neurotoxicity. Neurotoxicity Research 5:221-236.

Makris SL, Raffaele K, Allen S, Bowers WJ, Hass U, Alleva E, Calamandrei G, Sheets L, Amcoff P, Delrue N, Crofton KM. 2009. A retrospective performance assessment of the developmental neurotoxicity study in support of OECD test guideline 426. Environmental Health Perspectives 117:17-25

Maximale Arbeitsplatzkonzentrationen (MAK). 2011. Bisphenol A . The MAK-Collection for Occupational Health and Safety. Vol 50.

McLachlan JA. 1979. Transplacental effects of diethylstilbestrol in mice. National Cancer Institute Monographs 1979:67-72.

McLachlan JA, Newbold RR, Shah HC, Hogan MD, Dixon RL. 1982. Reduced fertility in female mice exposed transplacentally to diethylstilbestrol (DES). Fertility and Sterility 38:364-371.

Melzer D, Rice N, Depledge MH, Henley WE, Galloway TS. 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. national health and nutrition examination survey. Environmental Health Perspectives 118:686-692.

National Cancer Institute (NCI.) 2008. What you need to know about Prostate Cancer. http://www.cancer.gov/cancertopics/wyntk/prostate.

Palmer JR, Herbst AL, Noller KL, Bogg DA, Troisi R, Titus-Ernstoff L, Hatch EE, Wise LA, Strohsnitter WC, Hoover RN. 2009. Urogenital abnormalities in men exposed to diethylstilbestrol in utero: a cohort study. Environmental Health 8:37.

Pearce EN, Lazarus JH, Smyth PP, He X, Dall'amico D, Parkes AB, Burns R, Smith DF, Maina A, Bestwick JP, Jooman M, Leung AM, Braverman LE. 2010. Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnancy women. Journal of Clinical Endocrinology and Metabolism 95:3207-3215.

Pirkle JL, Brody DJ, Gunter EW, Kramer RA, Paschal DC, Flegal KM, Matte TD. 1994. The decline in blood lead levels in the United States. The National Health and Nutrition Examination Surveys (NHANES). Journal of the American Medical Association (JAMA) 272:284-291.

Refowitz R. 1984. Thyroid function and lead: No clear relationship. Journal of Occupational Medicine 26:579-583.

Rhomberg LR. 2009. Linear low-dose extrapolation for noncancer responses is not generally appropriate. Environmental Health Perspectives 117: 141-142.

Rhomberg LR, Goodman JE, Haber LT, Dourson M, Andersen ME, Klaunig JE, Meek B, Price PS, McClellan RO, Cohen SM. 2011. Linear low-dose extrapolation for noncancer health effects is the exception, not the rule. Critical Reviews in Toxicology 41:1-19.

Ricceri L, Markina N, Valanzano A, Fortuna S, Cometa MF, Meneguz A, Calamandrei G. 2003. Developmental exposure to chlorpyrifos alters reactivity to environmental and social cues in adolescent mice. Toxicology and Applied Pharmacology 191:189-201.

Ricceri L, Venerosi A, Capone F, Cometa MF, Lorenzini P, Fortuna S, and Calamandrei G. 2006. Developmental neurotoxicity of organophosphorous pesticides: fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice. Toxicological Sciences 93:105-113.

Ritchie JM, Vial SL, Fuortes LJ, Guo HJ, Reedy VE, Smith EM. 2003. Organochlorines and risk of prostate cancer. Journal of Occupational and Environmental Medicine 45:692-702.

Ritchie JM, Vial SL, Fuortes LJ, Roberston LW, Guo H, Reedy VE, Smith EM. 2005. Comparison of proposed frameworks for grouping polychlorinated biphenyl congener data applied to a case-control pilot study of prostate cancer. Environmental Research 98:104-113.

Roddam AW, Allen NE, Appleby P, Key TJ. 2008. Endogenous sex hormones and prostate cancer: A collaborative analysis of 18 prospective studies. Journal of the National Cancer Institute 100:170-183.

Ross G. 2004. The public health implications of polychlorinated biphenyls (PCBs) in the environment. Ecotoxicology and Environmental Safety 59:275-291.

Ross AC, Russell RM, Miller SA, Munro IC, Rodricks JV, Yetley EA, Julien E. 2009. Application of key events dose-response analysis to nutrients: A case study with vitamin A (retinol). Critical Reviews in Food Science and Nutrition 49:708-717.

Roze E, Meijer L, Bakker A, Van Braeckel KN, Sauer PJ, Bos AF. 2009. Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. Environmental Health Perspectives 117:1953-1958.

Ryan BC, Hotchkiss AK, Crofton KM, and Gray Jr. LE. 2010. In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility and anatomy of female LE rats. Toxicological Sciences 114(1):133-148.

Sanders T, Liu Y, Buchner V, Tchounwou PB. 2009. Neurotoxic effects and biomarkers of lead exposure: A review. Reviews in Environmental Health 24:15-45.

Sawada N, Iwasaki M, Inour M, Itoch H, Sasazuki S, Yamaji T, Shimazu T, Tsugane S. 2010. Plasma organochlorines and subsequent risk of prostate cancer in Japanese men: A nested case-control study. Environmental Health Perspectives 118:659-665.

Schroeder R. 2002. An oral two generation reproductive, fertility, and developmental neurobehavioral study in tetrabromoBPA in rats. Study ID Number: 474-004. MPI Research, Inc., Mattawan, MI. Study abstract available in HPV data summary and test plan for phenol, 4,4'-isopropylidenbis[2,6-dibromo- (TetrabromoBPA, TBBPA). Prepared by American Chemistry Council Brominated Flame Retardant Industry Panel (BFRIP).

Schuhmacher-Wolz U, Dieter HH, Klein D, Schneider K. 2009. Oral exposure to inorganic arsenic: evaluation of its carcinogenic and non-carcinogenic effects. Critical Reviews in Toxicology 39:271-298.

Sever LE. 1995. Looking for causes of neural tube defects: Where does the environment fit in? Environmental Health Perspectives 103 Suppl. 6:165-171.

Shanker G, Syversen T, Aschner M. 2003. Astrocyte-mediated methylmercury neurotoxicity. Biological Trace Element Research 95:1-10.

Shapira SK, Lloyd-Puryear MA, Boyle C. 2010. Future research directions to identify causes of the increasing incidence rat of congenital hypothyroidism in the Unites States. Pediatrics 125:S64-S68.

Sharpe RM. 2010. Toxicological Highlight: Is it time to end concerns over the estrogenic effects of Bisphenol A? Toxicological Sciences 114:1-4.

Siegel M, Forsyth B, Siegel L, Cullen MR. 1989. The effect of lead on thyroid function in children. Environmental Research 49:190-195.

Singh B, Chandran V, Bandhu HK, Mittal BR, Bhattacharya A, Jindal SK, Varma S. 2000. Impact of lead exposure on pituitary-thyroid axis in humans. Biometals 13:187-192.

Song X, Seidler FJ, Saleh JL, Zhang J, Padilla S, Slotkin TA. 1997. Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenylyl cyclase signaling cascade. Toxicology and Applied Pharmacology 145:158-174.

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. 1995. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspectives 103 Suppl 7:113-122.

Stein CR, Savitz DA. 2011. Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5-18 years of age. Environmental Health Perspectives 119:1466-1471.

Strohsnitter WC, Noller KL, Hoover RN, Robboy SJ. Palmer JR, Titus-Ernstoff L, Kaufman RH, Adam E, Herbst AL, Hatch EE. 2001. Cancer risk in men exposed in utero to diethylstilbestrol. Journal of the National Cancer Institute 93:545-551.

Stump DG, Beck MJ, Radovsky A, Garman RH, Freshwater LL, Sheets LP, Marty MS, Waechter JM, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Chappelle AH, Hentges SG. 2010. Developmental neurotoxicity study of dietary BPA in Sprague-Dawley rats. Toxicological Sciences 115:167-182.

Taylor SL, Gendel SM, Houben GF, Julien E. 2009. The key events dose-response framework: A foundation for examining variability in elicitation thresholds for food allergens. Critical Reviews in Food Science and Nutrition 49:729-739.

Toscano CD, Guilarte TR. 2005. Lead neurotoxicity: From exposure to molecular effects. Brain Research Brain Research Reviews 49:529-554.

Twaddle NC, Churchwell MI, Vanlandingham M, and Doerge DR. 2010. Quantification of deuterated bisphenol A in serum, tissues, and excreta from adult Sprague-Dawley rats using liquid chromatography with tandem mass spectrometry. Rapid Communications in Mass Spectrometry 24(20):3011-3020.

Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, and Waechter JM. 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicological Sciences 68(1):121-146.

Tyl RW, Myers CB, Marr MC., Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, and Waechter JM. 2008. Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1® (Swiss) mice. Toxicological Sciences 104(2):362-384. Supplementary data in Critical Evaluation column.

U.S. Environmental Protection Agency (USEPA). 1998. Assessment of thyroid follicular cell tumors. Risk Assessment Forum, Washington DC. EPA/630/R-97/002. March 1998.

U.S. Environmental Protection Agency (USEPA). 2011. Endocrine Disruptor Screening Program. Weight-of-Evidence: Evaluating Results of EDSP Tier 1 Screening to Identify the Need for Tier 2 Testing. Office of Chemical Safety and Pollution Prevention. September 14, 2011.

Van Maele-Fabry G, Libotte V, Willems J, Lison D. 2006. Review and meta-analysis of risk estimates for prostate cancer in pesticide manufacturing workers. Cancer Causes Control 17:353- 373.

Van Maele-Fabry G, Willems JL. 2004. Prostate cancer among pesticide applicators: a meta-analysis. International Archives of Occupational and Environmental Health 77:559-570.

Venerosi A, Calamandrei G, Ricceri L. 2006. A social recognition test for female mice reveals behavioral effects of developmental chlorpyrifos exposure. Neurotoxicology and Teratology 28:466-471.

Venerosi A, Cutuli D, Colonnello V, Cardona D, Ricceri L, Calamandrei G. 2008. Neonatal exposure to chlorpyrifos affects maternal responses and maternal aggression of female mice in adulthood. Neurotoxicology and Teratology 30:468-474.

Venerosi A, Ricceri L, Scattoni ML, Calamandrei G. 2009. Prenatal chlorpyrifos exposure alters motor behavior and ultrasonic vocalization in CD-1 mouse pups. Environmental Health 8:12.

Vermier G, Viaene M, Staessen J, Hond ED, Roeis HA. 2005. Neurobehavioural investigations in adolescents exposed to environmental pollutants. Environmental Toxicology and Pharmacology 19:707-713.

Watson CS, Alyea RA, Cunningham KA, Jeng YJ. 2010. Estrogens of multiple classes and their role in mental health disease mechanisms. International Journal of Women's Health 2:153-166.

White RH, Cote I, Zeise L, Fox M, Dominici F, Burke TA, White PD, Hattis DB, Samet JM. 2009. State-of-the-Science workshop report: Issues and approaches in low-dose-response extrapolation for environmental health risk assessment. Environmental Health Perspectives 117:283 – 287.

Wilhelm M, Wittsiepe J, Lemm F, Ranft U, Krämer U, Fürst P, Röseler SC, Greshake M, Imöhl M, Eberwein G, Rauchfuss K, Kraft M, Winneke G. 2008. The Duisburg birth cohort study: Influence of the prenatal exposure to PCDD/Fs and dioxin-like PCBs in thyroid hormone status in newborns and neurodevelopment in infants until the age of 24 months. Mutation Research 659:83-92.

Willhite CC, Ball GL, McLellan CJ. 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. Journal of Toxicology and Environmental Health, Part B 11:69-146

Williams AL, DeSesso JM. 2010. The potential of selected brominated flame retardants to affect neurological development. Journal of Toxicology and Environmental Health, Part B 13:411-448.

Winneke G, Krämer U, Sucker K, Walkowiak J, Fastabend A, Heinzow B, Steingrüber HJ. 2005. PCB-related neurodevelopmental deficit may be transient: follow-up of a cohort at 6 years of age. Environmental Toxicology and Pharmacology 19:701-706.

World Health Organization (WHO). 2002. Global assessment of the state-of-the science of endocrine disruptors.

Wu MM, Kuo TL, Hwang YH, Chen CJ. 1989. Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. American Journal of Epidemiology130:1123-32.

Xu X, Dailey AB, Talbott EO, IIacqua VA, Kearney G, Asal NR. 2010. Associations of serum concentrations of organchlorine pesticides with breast cancer and prostate cancer in U.S. adults. Environmental Health Perspectives 118:60-66