

Original article:

HYPOTHESIS-DRIVEN WEIGHT OF EVIDENCE EVALUATION INDICATES ETHYLBENZENE LACKS ENDOCRINE DISRUPTION POTENTIAL BY EATS PATHWAYS

Christopher J. Borgert , PhD

Applied Pharmacology and Toxicology Inc, Gainesville FL, 32605 and
University of Florida College of Veterinary Medicine, Dept. Physiological Sciences,
Gainesville FL, 32610. Tel.: +1 352-219-8551

<https://dx.doi.org/10.17179/excli2024-7822>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>).

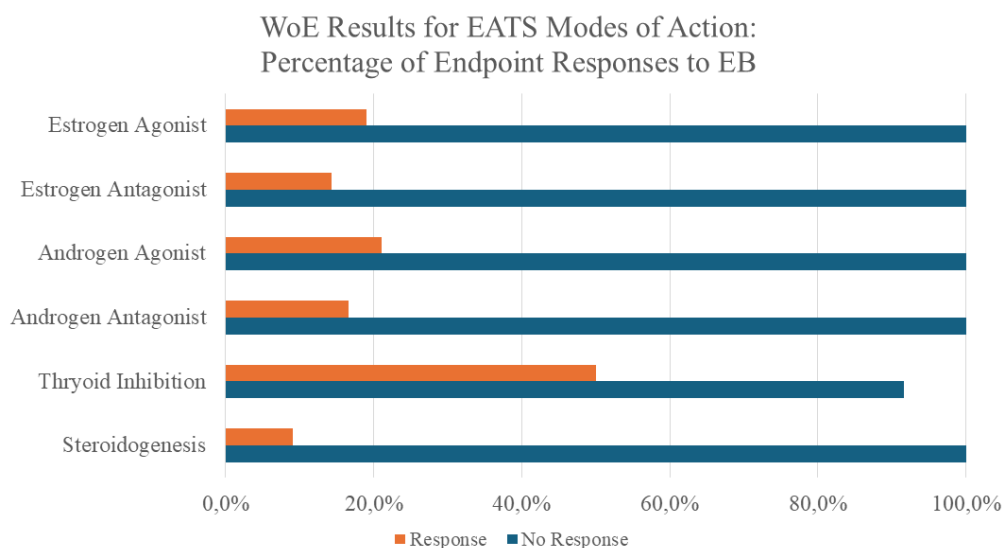


Figure 1: Graphical abstract

ABSTRACT

Ethylbenzene (EB) was placed on List 2 for Tier 1 endocrine screening in the U.S. EPA's two-tiered Endocrine Disruptor Screening Program (EDSP) and was scheduled for evaluation under TSCA. Results of toxicology studies on EB were used to evaluate estrogen, androgen, thyroid, and steroidogenic (EATS) endpoints by a Weight of Evidence (WoE) methodology, as required by U.S. EPA and OECD guidelines for evaluating a chemical's endocrine disruptive potential. The WoE method involved problem formulation, systematic literature search and selection, data quality evaluation, relevance weighting of endpoint data, and application of specific interpretive criteria. Data on EB were sufficient to assess its effects on endpoints that would be expected to respond to chemicals that operate via EATS modes of action (MoAs) in various screening assays (Tier 1) and toxicity tests (Tier 2) that evaluate reproduction, development, and sub-chronic and chronic toxicity. In those studies, EB produced a pattern of responses inconsistent with the responses that would be expected for hormones and chemicals known to operate via EATS MoAs. Endocrine-sensitive endpoints that respond to EB administration generally do so only at dose levels above its kinetic maximum dose, indicating a lack of relevance to potential effects at lower dose levels in either the test species or humans. This comprehensive WoE evaluation demonstrates that EB lacks the potential to exhibit endocrine disruptive properties and cannot be deemed an endocrine disruptor or potential endocrine disruptor. Because this WoE evaluation was based largely on Tier 2-level studies of the type considered by the U.S. EPA and OECD to be more definitive than results of Tier 1 EDSP screening results, no additional useful

information would be obtained by subjecting EB to further endocrine screening. As such, further endocrine screening of EB would be unjustified from animal welfare perspectives. This analysis supports a regulatory decision to halt further testing of EB for endocrine disruption unless unique and compelling data to the contrary arise.

Keywords: Ethylbenzene, endocrine disruptor, data quality, mode of action, weight of evidence, estrogen agonist, estrogen antagonist, androgen agonist, androgen antagonist, thyroid inhibition, steroidogenesis

Abbreviations

AMA	Amphibian Metamorphosis Assay
ARBA	Androgen Receptor Binding Assay
ATSDR	Agency for Toxic Substances and Disease Registry
CASRN	Chemical Abstracts Service Registry Number
CCL3	Third Contaminant Candidate List
CompTox	Computational Toxicology Dashboard
DART	Developmental and Reproductive Toxicology
EATS	Estrogen, Androgen, Thyroid, and Steroidogenesis
EDSP	Endocrine Disruptor Screening Program
EDSP21	Endocrine Disruptor Screening Program for the 21st Century Dashboard
ERBA	Estrogen Receptor Binding Assay
ERTA	Estrogen Receptor Transactivation Assay
EU	European Union
FSTRA	Fish Short-Term Reproduction Assay
HSDB	Hazardous Substances Data Bank
IC ₂₅	Inhibitory Concentration 25 %
IPCS	International Programme on Chemical Safety
KMD	Kinetically-derived maximum dose
LABC	Levator ani bulbocavernosus muscle
LOEC	Lowest observable effect concentration
NOEC	No observable effect concentration
MoA(s)	Mode(s) of Action
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OSRI	Other Scientifically Relevant Information
RfD(s)	Reference Dose(s)
TSCA	Toxic Substances Control Act
TSH	Thyroid Stimulating Hormone
T4	Thyroxin
U.S. EPA	United States Environmental Protection Agency
WEEL(s)	Workplace Environmental Exposure Limit(s)
WHO	World Health Organization
WoE	Weight of Evidence

EPA [June 14, 2013, 78 FR 35922] as described previously (U.S. EPA, 2009a; Borgert 2023a). Depending on the results of Tier 1 screening, Tier 2 testing may be required (U.S. EPA, 2011). Tier 2 test results are used for human and ecological risk assessments (Borgert, 2023a).

The Endocrine Disruptor Screening Program (EDSP) differs from regulatory programs in the European Union (e.g., European Parliament 2006, 2012) in critical ways. The U.S. EPA regulates on risk rather than hazard; the EDSP does not attempt to establish a causal link between adverse effects identified in Tier 2 and endocrine modes of action (MoAs) identified in Tier 1; and the U.S. EPA does not attempt to place a special label on “endocrine disruptors,” which would require satisfying the WHO/IPCS definition (WHO/IPCS, 2002; WHO/UNEP, 2012). In contrast, regulatory programs in the European Union seek to affix qualitative “hazard” labels to chemicals for purposes of classification and labeling, irrespective of whether a causal link can be established between an endocrine MoA and adverse effects, as required to satisfy the “endocrine disruptor” definition. Due to these differences, classification and labeling within the European Union may not align with the scientific data or with determinations of the U.S. EPA and other countries that regulate based on risk.

The U.S. EPA has modified the EDSP Tier 1 screening battery since 2009 when it was first applied to 52 chemicals on List 1 (List 1). The program, often referred to as “EDSP-21” (Browne et al., 2015; Kleinstreuer et al., 2017), may be combined with the results of exposure assessment models to further increase the speed and efficiency of endocrine assessments and prioritization of

INTRODUCTION

Ethylbenzene (EB) is on the second list of pesticides and industrial chemicals (“List 2”) prioritized for Tier 1 screening by the U.S.

chemicals for endocrine screening (Rotroff et al., 2013).

The Organisation for Economic Co-operation and Development (OECD) uses a slightly different approach. Tiers 2 through 5 of its 5-tiered Conceptual Framework (OECD, 2012) includes the eleven EDSP screens and tests for potential endocrine MoAs as well as general toxicology and carcinogenicity studies that evaluate adverse effects on endpoints that could be affected by endocrine MoAs (OECD, 2012). Weight of Evidence (WoE) procedures are required by the U.S. EPA (U.S. EPA, 2011) and OECD (OECD, 2012) programs to evaluate a chemical's potential for interaction with the endocrine system. Here, EB was evaluated by a widely used WoE approach (Borgert et al., 2011a), which was deemed appropriate for evaluating the available scientific data relevant to endocrine disruption (OECD, 2012). It is important to appreciate that although this analysis bears several similarities to a systematic review, e.g., in searching and selecting literature according to previously defined criteria, it is properly designated a WoE analysis because it integrates data from diverse types of studies, weighted according to relevance and strength.

The U.S. EPA developed List 2, which includes 109 chemicals, through application of exposure criteria and a public comment process, as it did for List 1 (U.S. EPA 2009b; Borgert, 2023a). The TSCA reform bill (U.S. Public Law 114-182, June 22, 2016) includes components of the EDSP in existing programs for industrial chemicals. The U.S. EPA recently released a prioritized plan for the EDSP to comply with requirements of the Federal Food, Drug and Cosmetics Act (U.S. EPA, 2023) that will comprise a combination of Tier 1 screening assays, EDSP-21 bioactivity screening results, and more definitive studies as deemed necessary to make regulatory determinations of the potential for a chemical to interact with estrogen, androgen, thyroid, or steroidogenic pathways.

SCOPE AND PURPOSE

Irrespective of the regulatory program under which a chemical is evaluated, it is anticipated that there will be an opportunity for manufacturers and importers to submit “other scientifically-relevant information” (OSRI) as was the case for the test orders released for List 1 chemicals in 2009 and included in the Agency's near-term strategy (U.S. EPA, 2023; Borgert, 2023a). This report constitutes an assessment of all scientifically-relevant data available regarding the potential for EB to act via EATS pathways and produce adverse effects via those MoAs. It was conducted consistent with the WoE methodology of Borgert et al. (2011a, 2014) as was used previously to evaluate styrene (Borgert, 2023a).

ORGANIZATION OF THE EVALUATION

This evaluation first outlines the WoE methodology used to evaluate EB, including a description of the literature search and selection criteria, the literature evaluation, and the data compilation. Following the description of methods is a summary of “ToxRTool” evaluations of the studies that met selection criteria, and a summary of EDSP-21 / ToxCast™ results. Appendix A (at the end of this article) lists the details of the ToxRTool evaluations of each study, by number, e.g., [1], so that endpoint responses can be tracked by study number throughout the evaluation. The numbers are used in the next section to identify the studies from which data were extracted to evaluate the six EATS hypotheses, along with a concise explanation of the endpoint responses relevant to each hypothesis. The numbers also identify the studies corresponding to each endpoint response, which are listed by hypothesis in Supplementary Tables 1–6, and a summary of the results is provided in Supplementary Table 7. Additional information about this WoE analysis is found in Supplemental Materials A–C.

METHODS

Literature search and selection

Literature search

The literature search strategy was conducted using the same search terms published previously (Borgert, 2023a), except that the CASRN (Ethylbenzene / 100-41-4) was used instead of chemical identifiers for styrene.

Literature and data selection

The literature identified by the search strategy was initially triaged for separation into three categories according to whether the studies were Apparently Relevant, Possibly Relevant, or Apparently Not Relevant, as published previously (Borgert, 2023a). In June of 2024, a literature search update was conducted, and 56 additional studies were evaluated. A comprehensive list of literature evaluated is provided in Supplemental Material A. Only publications meeting the inclusion criteria published previously (Borgert, 2023a) were considered for the WoE evaluation. No studies were excluded because of low data quality however, data quality was considered in evaluating the overall WoE for each hypothesis.

The goals of data selection for this WoE evaluation were identical to those described previously for styrene (Borgert, 2023a). The criteria used were broad to ensure that the WoE evaluation included all of the data that might inform EATS MoA; consequently, these may be too broad to be useful for other purposes. For example, even though some routes of exposure are more relevant than others for risk assessment, route of exposure was not an exclusion or inclusion criterion for this WoE. Studies were not excluded based solely on the use of excessively high doses even though endocrine MoAs may be obscured by systemic toxicity at high doses (Marty et al., 2018; Slikker et al., 2004; Borgert et al., 2021). Problems inherent to the use of high doses have been discussed previously (Borgert et al., 2021) and are germane to WoE evaluations. This evaluation followed the logic and criteria applied previously (Borgert, 2023a).

The homeostatic role of the endocrine system and the fact that healthy physiological functioning is supported by wide ranges of normal endocrine organ weights and hormone levels creates a situation whereby values may be statistically significant compared to concurrent controls within a particular study, yet not indicative of adverse effects because they are within normal ranges based on historical control data for the test species. These factors and the broad inclusion criteria applied tend to bias this evaluation toward a false positive conclusion. Hence, this WoE methodology could reach only provisional conclusions that a chemical exhibits potential endocrine activity. On the other hand, this approach to data selection results in a high level of confidence in negative conclusions regarding the possibility that EB exhibits EATS activity. Few data were excluded by this literature search and data selection strategy, and the data gaps that exist did not prohibit a valid WoE determination.

Consideration of the kinetically-derived maximum dose for EB

For purposes of this endocrine WoE evaluation, endpoint responses were not excluded or discounted based on dose-response. The conservatism of including endpoints regardless of the dose at which they respond is underscored by a consideration of the kinetically-derived maximum dose (KMD) for EB, as defined previously (Borgert et al., 2021). The ability to identify a KMD for a chemical indicates that above a certain dose, a fundamental change occurs in the organism's ability to process a chemical, i.e., to absorb, distribute, detoxify, and eliminate it. Doses above the KMD often produce toxicity that is qualitatively different from toxicity produced by doses below it (Borgert et al., 2021). In practice, the KMD is estimated based on a range that represents our uncertainty about the precise location of the KMD (Burgoon et al., 2022). Using published kinetic data on EB, a KMD was recently identified (Burgoon et al., 2023). The KMD range in rats was estimated to be 8-17 mg/L venous EB, and in humans,

from 10-18 mg/L venous EB. These blood concentration ranges correspond to an inhalation concentration of approximately 200 ppm EB (Burgoon et al., 2023).

It is important to appreciate that endpoints responding only at exposure levels above 200 ppm EB are unreliable for MoA analysis and cannot be used to infer any specific MoA for EB, including an endocrine MoA. For endpoint responses observable only above the KMD, the MoA involves, and may solely depend upon, high-dose dependent changes in the kinetics of EB that do not confound endpoint responses that occur at doses well below the KMD. Although all endpoint responses were tabulated and used to evaluate the overall pattern of responses for each endocrine hypothesis, the paucity of endpoints that respond below the KMD for EB or 200 ppm underscores that the conclusions reached are highly conservative.

Of the dozens of endpoints evaluated among thirteen studies included in this WoE, only four endpoints, two in each of two studies, showed responses to EB exposure below the KMD. A fifth endpoint from a third study might be considered, but is dubious. Those included:

1. The percentage of dead or resorbed fetuses was increased in all EB-exposed groups in rats (138, 276 and 553 ppm); however, there was no significant difference in the percentage of dead or resorbed fetuses in EB-exposed mice or rabbits compared with controls (Ungváry and Tátrai, 1985).
2. The mean age of acquisition of vaginal patency was reduced in all exposed groups (25, 100 and 500 ppm EB) compared to the concurrent control group in F₁ female rat offspring; similar differences were not observed in the F₂ female pups. All mean values were comparable to the historical control mean value, and therefore, the authors felt these differences were not biologically important (Faber et al., 2006).
3. The percentage of weight-retarded male and female fetuses was significantly greater in rats exposed to EB at a concentration of 553 ppm and in female rabbit

fetuses at 115 ppm compared with controls, but there was no significant difference in mean fetal weights in mice exposed to EB 3-4 hours/day intermittently at 115 ppm (Ungváry and Tátrai, 1985).

4. Absolute and relative thyroid weights were increased (approximately 18–20 % and statistically significant) in the F₀ males exposed to 100 and 500 ppm, but these increases were not observed in the F₁ male group or in females exposed to the same EB concentrations (Faber et al., 2006).
5. Statistically non-significant trends were observed in the incidences of thyroid follicular cell hyperplasia in mice in both males (control: 21:50; 75 ppm: 21:50; 250 ppm: 29:50) and females (18:50, 23:50, 25:50). Relative to chamber controls, statistically significant increased incidences were observed only in 750 ppm males (32:50) and females (35:50). There were no significant differences between control and exposed rat thyroids upon histopathological examination (NTP, 1999).

Literature evaluation & data compilation

Data quality assessment

Data used for testing the MoA hypotheses were subjected to a quality evaluation according to their primary, secondary, and tertiary validity as described previously (Borgert, 2023a; Supplemental Material in Borgert et al., 2011a) consistent with international (e.g., OECD; U.S. EPA) toxicological guidelines, as described (Borgert et al., 2016). Klimisch et al. (1997) criteria were applied using the ToxRTTool scoring system created by the European Center for the Validation of Alternative Methods (Schneider et al. 2009) and causal relationships were evaluated consistent with the U.S. EPA's guidance on data quality assessment (U.S. EPA 2011) and concepts published previously (Borgert, 2023a; Borgert et al., 2011a). Supplemental Material B contains a brief description of each endpoint used to evaluate each hypothesis, similar to that published previously for styrene (Borgert, 2023a).

The literature search strategy used here attempted to identify all data that might be informative regarding an endocrine mechanism of action underlying outcomes of EB exposure. Because of its broad and exhaustive nature, the literature search identified several studies that were speculative or severely limited, and could not be ranked for relevance consistent with the method (Borgert et al., 2011a, 2014; Borgert, 2023a). Eight such studies (Gong et al., 2018, 2023; Harrath et al., 2022; Lei et al., 2023; Nakhjirgan et al., 20199; Rouget et al., 2021; Werder et al., 2019, 2020) were not used in this evaluation, but are referenced as per the rationale described previously (Borgert, 2023a), as explained briefly in Supplemental Materials C.

Weight-of-evidence methodology

The methodology used for this WoE analysis was designed to be broadly applicable within any hypothesis-testing paradigm that can be tested based on objective data (Borgert et al., 2011a). This hypothesis-driven framework was initially applied to endpoints measured by the U.S. EPA's EDSP Tier 1 screening assays and involved hypotheses related to interactions with specific endocrine MoAs (i.e., EATS pathways). The methodology requires weighting the importance of the data with respect to their mechanistic relevance for each hypothesized MoA. For these endocrine WoE evaluations, endpoints were categorized according to three ranks, and endpoint responses to the chemical were then interpreted by an algorithm that sequentially considered them in order of their importance to the hypothesis. The rankings have been described previously (Borgert et al., 2014), and have been adapted to accommodate endpoints assessed in long-term toxicity tests, consistent with previously published data and approaches (Afarinesh et al., 2020; Andrews et al., 2002; Biegel et al., 1998; Delclos et al., 2009; Borgert, 2023a; Mihaich and Borgert, 2018; Mihaich et al., 2017; Neal et al., 2017; NTP, 2010). Because endocrine screening assays do not determine whether a chemical produces adverse effects by the endocrine

mechanism probed, they cannot determine whether a chemical possesses endocrine disruptive properties.

The relevance rankings and their use in evaluating endpoints was applied here as described previously (Borgert, 2023a). The rationale and complexities are fully described therein and are not repeated here. Many endpoints evaluated by this WoE method are relevant to more than one hypothesis, consistent with prior literature on endocrine screening methods (EDSTAC, 1998; U.S. EPA, 2011; Borgert et al., 2011a, b). It is important to recognize that the relevance rankings are specific to each hypothesis, and that the relevance ranking for an endpoint may differ across hypotheses. Because EB has been subjected to extensive toxicity testing, but not to the EDSP Tier 1 screening battery, there are several data gaps for Rank 1 endpoints measured following EB exposure. However, due to the large amount of data from more definitive toxicity studies, and the fact that a lack of response in endocrine-sensitive endpoints is more informative than a response (Borgert et al., 2011a, 2014; Borgert, 2023a), the data gap does not diminish this WoE evaluation.

Like styrene (Borgert, 2023a), the magnitude of the responses produced by EB was not informative regarding EB's potency via an endocrine pathway because the data available for this WoE evaluation comprise mostly apical endpoints that can be affected by various MoAs other than EATS. Statistically significant non-monotonicity was not discounted, but mechanistic interpretations would be extremely tenuous if based on apparent "trends" that occur within the normal biological range for hormone-sensitive endpoints, since those endpoints can fluctuate for numerous reasons. A more complete discussion of these issues was published previously (Borgert, 2023a).

RESULTS – DATA SELECTION, DATA QUALITY EVALUATION, AND HIGH-THROUGHPUT SCREENING

Thirteen studies provided data useful for determining whether EB can operate through EATS MoAs (Appendix A). In Supplement-

ary Tables 1–6, “Assay” (second column from the left) means the general type of toxicological study in which the endpoint was measured. Similar endpoints are measured in different types of studies, but the conditions of those studies are often different. Therefore, the same endpoint from different types of studies may be included within the same MoA table and in different MoA tables depending on its relevance for evaluating the various endocrine MoA(s) that can affect it. This is important for understanding the discussion of Supplementary Tables 1–6. Of the thirteen studies, only Ungváry and Tátrai (1985) [11] differed significantly from guideline regulatory toxicology studies. Details are provided in Appendix A.

ToxRTool summary

The Toxicological data Reliability Tool known as ToxRTool (Schneider et al., 2009) was applied to the thirteen studies used here. Twelve of the thirteen studies used in this evaluation [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13¹] met all ToxRTool reliability criteria (21 for *in vivo* and 18 for *in vitro* studies). One publication (Mellert et al., 2007; [3]) lacks a description of histological findings, but those findings were made available via the original study report, and thus, this study merits a score of 21 for the WoE evaluation. ToxRTool evaluation summaries are found in Appendix A, listed by study number as described above. An explanation of deficiencies for studies that did not meet all criteria is included.

No studies were eliminated from consideration or discounted based on ToxRTool results; however, ToxRTool informed the overall quality of the data selected for the WoE and the interpretation of conflicting results between studies. Because few studies reported a response to EB and ToxRTool scores

were similar regardless of whether an effect was reported, interpretations were unaffected.

Results for Ethylbenzene in U.S. EPA’s ToxCast™ high throughput and EDSP 21 assays

Results available for the U.S. EPA’s CompTox Chemicals Dashboard and the National Toxicology Program’s [NTP] Integrated Chemical Environment [ICE]) show that EB produced no reliable activity in the Tox21 suite of high-throughput *in vitro* and *in silico* assays. Those included assays for potential agonism or antagonism via estrogen receptors, androgen receptors, thyroid hormone receptors, thyroid stimulating hormone receptors, and assays for aromatase inhibition. However, these results are unreliable due to the lack of EB detectable in the wells according to the Tox21 Program (QC Grade: FNS – no sample detected (biological activity unreliable), per ICE.

Although the U.S. EPA’s CompTox Chemicals Dashboard returns a single active hit call for the RXR receptor (not an endocrine endpoint) via the assay TOX21 RXRBLA Agonist_ratio, the Tox21 Program noted that no EB results are trustworthy due to quality concerns. Data quality flags were not available through the U.S. EPA’s Chemicals Dashboard, but are provided in NTP’s ICE knowledgebase. More information on the chemical QC can be found at <https://ice.ntp.niehs.nih.gov/DA-TASETDESCRIPTION?section=cHTS> and in this download file from ICE (<https://ice.ntp.niehs.nih.gov/downloads/MOA/ChemicalQC.xlsx>).

Both U.S. EPA’s Chemicals Dashboard and ICE were accessed February 22, 2024.

The following charts were downloaded from the Integrated Chemical Environment (ICE) showing data quality flags for ToxCast™ / Tox12 results (Figure 2).

¹ These references are marked with an exponent in the bibliography.

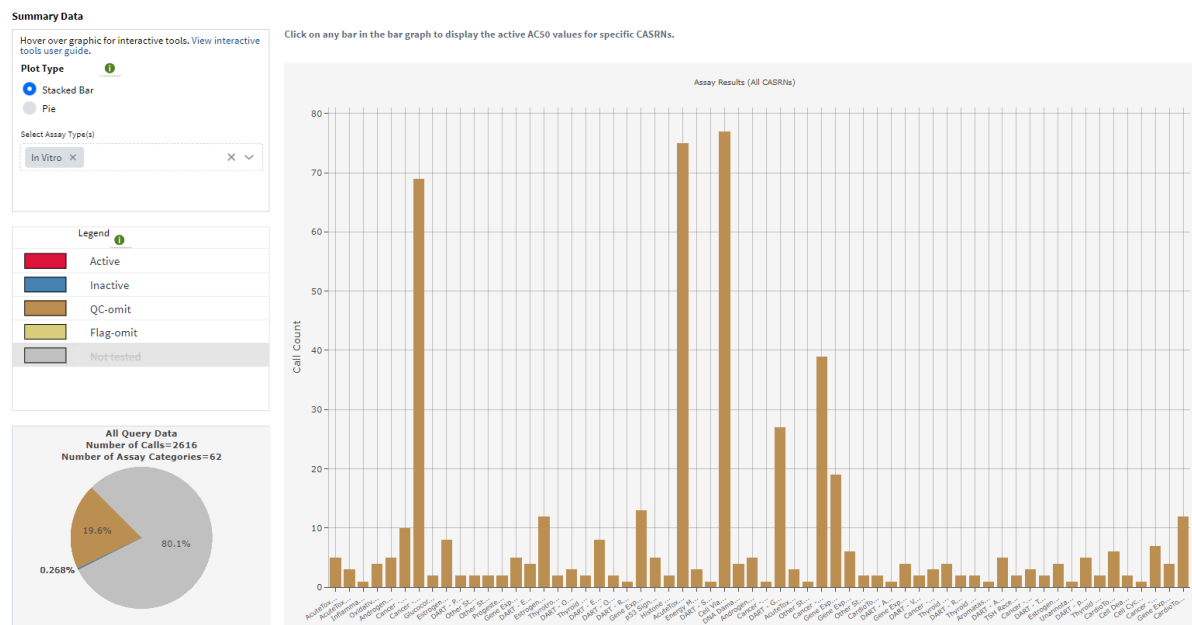


Figure 2: ToxCast / Tox21 data summary from the Integrated Chemical Environment (ICE) Database showing interpretable results (Active / Inactive) and uninterpretable results (QC Omit and Flag Omit) for ethylbenzene

RESULTS – ENDOCRINE WoE EVALUATION FOR EB

Evaluation of estrogen agonist MoA

Rank 1 endpoints for the estrogen agonist MoA were not measured following exposure to EB (Supplementary Table 1). For this analysis, responses to EB were available for 20 out of 53 potential Rank 2 endpoints that are relevant for evaluating the estrogen agonist MoA among five repeat dose toxicity studies [1, 3, 9, 10], five developmental toxicity studies [4, 5, 6, 11, 13], and one reproductive toxicity study [2]. One study [2] included both reproductive and developmental endpoints, and one study [13] included both repeat dose and developmental endpoints; endpoints were counted under a single category to avoid overcounting. The analysis indicated that none of the 20 endpoints exhibited consistent responses to ethylbenzene exposure, with 16 endpoints remaining unchanged across all studies in which they were measured. Rank 2 endpoints that failed to respond in any study in which they were measured included testes atrophy and weight and histopathology of epididymides, ovaries, uterus, and vagina in

repeat dose toxicity studies; number of corpora lutea and pre-implantation loss in developmental toxicity studies; and fertility, gestational length, number of implantations, litter size, mating index, ovarian follicle count in offspring, epididymal sperm counts, and time to mating in a reproductive toxicity study. Gross pathology, a Rank 3 endpoint, was unaffected in one repeat dose toxicity study.

Only four endpoints responded to EB in the direction expected for the estrogen agonist MoA, but none were consistently changed across studies (Supplementary Table 1). Epididymis weight was decreased in mice but not in rats in one repeat dose toxicity study [9]. Moreover, this occurred only at the highest exposure concentration tested (1000 ppm) and was not considered biologically significant by the study authors since spermatid counts, sperm motility, and caudal weight were normal. In this study, other Rank 2 endpoints for the estrogen agonist MoA were unchanged in either species, including testes weight and histopathology of the epididymis, uterus, and ovaries. Hence, in this study, EB did not produce a pattern of responses

consistent with activity via the estrogen agonist MoA.

Supplementary Table 1 also shows that in developmental toxicity studies, post-implantation loss was unchanged in rats in three studies [4, 5, 6], was increased in rabbits but not rats in one study [13], and in rats but not in rabbits or mice in another [11]. The lack of consistency of the response of this endpoint and its occurrence at high doses suggests that, where observed, post-implantation losses were due to general toxicity rather than to changes in endocrine function. In the reproduction study [2], time to vaginal patency was decreased in the F₁ generation of female pups for all exposed groups (25, 100 and 500 ppm EB) relative to the concurrent controls, however, the differences are unlikely to be biologically significant because the mean values were comparable to the historical control mean value [2]. No change in time to vaginal patency was observed in the F₂ offspring [2]. That study [2] found no change in ovarian follicle counts or gestational length. Thus, the slight but statistically significant decrease in time to vaginal patency in one reproduction study [2] provides no evidence of activity via the estrogen agonist MoA. In the reproduction arm of the same study, estrous cyclicity was slightly reduced at the highest exposure concentration (500 ppm) in the parental (F₀), but not in the F₁ generation, however the difference is unlikely to be biologically significant because all females in the exposure group cycled normally and the cycle length was within normal values for the strain of rat used in the study [2]. This also fails to provide evidence of activity via the estrogen agonist MoA.

In summary, even if the few positive responses in Rank 2 endpoints were to be considered biologically significant, the endpoints that responded to EB are inconsistent with the response pattern expected of a chemical that acts via an estrogen agonist MoA. Supplementary Table 1 shows that most Rank 2 endpoints measured for the estrogen agonist MoA were consistently unaffected by EB, including histopathology of the vagina, uterus, ovaries, epididymides, as well as ovary weights in

repeat dose toxicity studies. In reproductive toxicity studies, endpoints unresponsive to EB include gestational length, ovary weight, estrous cyclicity, time to mating, gross pathology and histopathology of vagina and prostate, and time to vaginal patency and ovarian follicle count in offspring.

Although thirty-three of a possible fifty-three endpoints relevant to the estrogen agonist MoA were not measured in the available studies, the high degree of consistency among those endpoints that failed to respond to EB is sufficient evidence to conclude that EB lacks the potential to act via this endocrine MoA. Not only is the pattern of endpoint responses elicited by EB inconsistent with activity via the estrogen agonist MoA, but several additional observations strengthen the conclusion. A very low proportion of endpoints responded to EB in any study and the magnitude of response was small when observed. Although statistically significant, some endpoint responses were unlikely to be biologically significant because they were within the normal range for the rodent test strain. Together with the lack of consistency of any of those responses across studies, these observations confer very high confidence in the conclusion that EB lacks the potential to act as an estrogen agonist.

Evaluation of estrogen antagonist MoA

Responses to EB were not measured in any Rank 1 endpoints for the estrogen antagonist MoA (Supplementary Table 2). Thirteen of a possible twenty-six endpoints of Rank 2 relevance for the estrogen antagonist MoA were evaluated among four repeat dose toxicity studies [1, 3, 9, 10], two developmental toxicity studies [5, 13] and one reproductive toxicity study [2] conducted with EB. Among those studies, most Rank 2 endpoints measured for the estrogen antagonist MoA were unaffected by EB. In two developmental toxicity studies, the number of corpora lutea were unchanged. Additionally, no relevant endpoints, including testes weight and histopathology of testes, epididymis, prostate,

seminal vesicle, and ovaries were altered in the four repeat dose toxicity studies.

Supplementary Table 2 also shows that in a reproductive toxicity study, time to mating, litter size, epididymal sperm count, and fertility were unaffected, but two endpoints were altered in one of the two generations evaluated. Time to vaginal patency was increased in the F₁, but not in the F₂ generation. Estrous cyclicity was slightly reduced at the highest exposure concentration (500 ppm) in the parental, but not in the F₁ generation, however the difference is unlikely to be biologically significant because all females in the exposure group cycled normally and the cycle length was within normal values for the strain of rat used in the study [2].

In summary, the available data provide sufficient evidence to conclude that EB lacks the potential to act as an antagonist in the estrogen pathway because the pattern of endpoints that responded to administration of EB is inconsistent with the pattern of responses expected of a chemical with estrogen antagonist MoA. That conclusion is strengthened by the lack of replication of responses across studies, confounding due to other MoAs operative at the excessively high doses required to elicit responses when they were observed, and the fact that many of the endpoints responded only at doses exceeding the KMD for EB. Finally, although statistical significance of the responses was established in comparison to concurrent controls, the biological significance was not typically established by a formal comparison to historical control values. Thus, there is very high confidence in the conclusion that EB does not act as an estrogen antagonist.

Evaluation of androgen agonist MoA

Responses to EB were not measured in any Rank 1 endpoints for the androgen agonist MoA (Supplementary Table 3). Eighteen of a possible forty-seven Rank 2 endpoints relevant for androgen agonism were measured among four repeat dose toxicity studies [1, 3, 9, 10], four developmental toxicity studies [4, 5, 6, 13], and one reproductive toxicity

study [2] conducted with EB. No endpoints were altered by EB in repeat dose toxicity studies in which sperm count, testes weight, and histopathology of testes and ovary were evaluated. EB slightly reduced litter size in rabbits at the highest dose administered in one developmental toxicity study [13] but had no effect on rats or on any other endpoint in rabbits in that study. EB also did not alter any endpoints relevant to an androgen agonist MoA in four other developmental toxicity studies in which litter size, sex ratio, and number of implantations were measured.

Supplementary Table 3 also shows that in a reproductive toxicity study [2], no change was observed in prostate weights of male offspring, sperm counts, sex ratio, time to mating, fertility, mating index, litter size, or number of implantations. Three endpoints were different from controls, but the authors of the study considered the changes too small to be biologically meaningful. Time to vaginal patency was increased in F₁ females in all exposure groups compared with the control group, however, mean values were comparable to historical controls and thus, the change was not considered to be biologically important. No change in time to vaginal patency was observed in the F₂ females. Estrous cyclicity for the F₀ was reduced compared to the F₀ control group in EB-exposed animals, however, this change was biologically insignificant because all females in this group were cycling normally, and their mean estrous cycle length (4.0 ± 0.3 days versus 4.4 ± 0.8 days) was within the 4-5 day range for estrous cycles normally exhibited by this strain of rat. Mean estrous cycle length did not differ between control and experimental F₁ offspring. Time to balano-preputial separation (PND 44.7 ± 2.0) was unaffected in F₂ treatment groups. It was reduced in the F₁ offspring only at the highest exposure concentration (500 ppm) compared to concurrent F₁ controls (PND 43.5 ± 2.2), however, this mean value was close to the F₂ controls (PND 45.3) and to historical control values from the conducting laboratory (PND 44.7), and, therefore, was not

considered by the authors to be biologically meaningful.

No endpoint relevant for evaluating the androgen agonist MoA consistently responded to EB in any study or across studies, and most endpoints failed to respond in any study in which they were measured. Although several endpoints were not measured that would have provided relevant information, there is sufficient evidence available to conclude that EB produces a pattern of endpoint responses inconsistent with activity via the androgen agonist MoA. Thus, there is high confidence that EB does not act as an androgen agonist.

Evaluation of androgen antagonist MoA

Responses to EB were not measured in any Rank 1 endpoints for the androgen antagonist MoA (Supplementary Table 4). Seventeen of forty-five Rank 2 endpoints for the androgen antagonist MoA were measured among four repeat dose toxicity studies [1, 3, 9, 10] and one reproductive toxicity study [2] conducted with EB. Histopathology of the epididymis, ovary, prostate, seminal vesicles, testes, and uterus was unaffected by EB exposure in repeat dose toxicity studies. In the single repeat dose toxicity study in which it was measured [9], epididymis weight was reduced in mice, but not in rats. The decrease occurred only at the highest dose and was not considered to be biologically significant since epididymis histopathology, spermatid counts, sperm motility, and caudal weight were unchanged.

Supplementary Table 4 also shows that EB did not affect sperm count, sperm motility, prostate weight, gross pathology, time to mating, fertility, and litter size in a reproductive toxicity study [2]. EB had no effect on time to balano-preputial separation (PND 44.7 ± 2.0) in F₂ treatment groups. EB reduced the time to balano-preputial separation in F₁ offspring, but only at the highest exposure concentration (500 ppm) compared to concurrent F₁ controls (PND 43.5 ± 2.2). The reduced mean value, however, was close to the F₂ controls (PND 45.3) and to historical

control values from the conducting laboratory (PND 44.7), and, therefore, was not considered by the authors to be biologically meaningful. Estrous cyclicity for the F₀ was reduced compared to the F₀ control group in EB-exposed animals. However, this change was biologically insignificant because all females in this group were cycling normally and their mean estrous cycle length (4.0 ± 0.3 days versus 4.4 ± 0.8 days) was within the 4–5-day range for estrous cycles normally exhibited by this strain of rat. Mean estrous cycle length did not differ between control and experimental F₁ offspring.

No endpoint relevant for evaluating the androgen antagonist MoA consistently responded to EB in any study or across studies, and most endpoints failed to respond in any study in which they were measured. Although twenty-eight Rank 2 endpoints (of 45 total) were not measured (Supplementary Table 4), the consistency of negative responses among the seventeen endpoints that were measured shows that the pattern of endpoint responses elicited by EB is inconsistent with activity via the androgen antagonist MoA. Thus, there is high confidence that EB does not act via the androgen antagonist MoA.

Evaluation of thyroid inhibition MoA

EB was not evaluated in Rank 1 endpoints for the thyroid inhibition MoA, as shown in Supplementary Table 5. Effects of EB were measured in six of a possible twenty-one Rank 2 endpoints for the thyroid inhibition MoA among four repeat dose toxicity studies [1, 3, 9, 10], five developmental toxicity studies [4, 5, 6, 11, 13], and a reproductive toxicity study [2]. EB produced mixed results among Rank 2 endpoints in repeat dose and developmental toxicity studies, but the positive responses are likely to be caused by systemic toxicity rather than via a thyroid MoA. Six of a possible twenty-one Rank 3 endpoints were measured among six repeat dose toxicity studies [3, 6, 7, 8, 9, 10], the reproductive toxicity [2] and a developmental neurotoxicity [12] study. This number of Rank 3

results provide useful corroboration of Rank 2 results.

Among the Rank 2 endpoints measured, only thyroid follicular cell histopathology and thyroid weight reflect responses within the thyroid gland itself (Supplementary Table 5). Thyroid follicular cell histopathology was unchanged in rats in four [1, 3, 9, 10] repeat dose toxicity studies, unchanged in rabbits in one [10] study, and unchanged in mice in two [9, 10] studies. In one repeat dose toxicity study [1], a positive trend in follicular cell hyperplasia was identified as statistically significant with chronic exposure of mice but not rats, but only at the highest exposure concentration of 750 ppm. Liver weight, a Rank 3 endpoint for potential thyroid activity, was not measured in this chronic toxicity and carcinogenicity study [1], however, data summary tables and text from the report [1] clearly state that in addition to thyroid gland follicular cell hyperplasia, EB exposure increased the incidence of syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis, and hyperplasia of renal tubules and of the pituitary *pars distalis*, at the highest dose administered to mice. Therefore, it is likely that the thyroid follicular cell hyperplasia observed in mice in this study was caused by a general non-specific toxicity that leads to hyperplastic changes in many organs and tissues, rather than through a MoA involving the thyroid hormone pathway or feedback system. It is well established that many non-endocrine MoAs can produce changes in endocrine endpoints (Marty et al., 2018). Therefore, it is highly unlikely that this endpoint response reflects a potential for thyroidal activity of EB.

Absolute and relative thyroid weights were 18–20 % higher than concurrent controls in F₀ males exposed to 100 and 500 ppm EB in a reproductive toxicity study [2], but these increases were not replicated in the F₁ male group or among female animals [Supplementary Table 5, Rank 2]. Because this increase occurred only in F₁ males, the authors attributed it to normal biological variation and not to EB exposure. Histopathological

examination of the thyroid tissue was conducted but pathology was not reported, implying that pathologic changes were not observed. Therefore, it is unlikely that the increased thyroid weights were produced by a thyroid MoA. Pup growth and survival, also Rank 2 endpoints for thyroid inhibition, were not affected by EB in this study, further supporting the interpretation that EB exerted no thyroid activity in this study.

Liver weight increase, a Rank 3 endpoint for the thyroid inhibition MoA when measured in repeat dose and reproductive toxicity studies (Supplementary Table 5), was observed with sub-chronic or short-term administration in mice and rats [9, 10], rats [3, 6, 7] and mice [8], but not in rabbits [10]. Three of these studies also evaluated histopathology of the thyroid gland, but observed no changes [3, 9, 10]. The liver weight increases observed in these studies occurred at the highest doses tested, were reversible in all three studies and were considered adaptive on two studies [6, 10]. In mice, the liver weight changes occurred secondary to regio-specific liver enzyme induction [8]. Thus, there is no evidence suggesting that EB increases liver weights via a thyroidal or thyroid hormone-related MoA. Other Rank 3 endpoints were unchanged by EB exposure in a developmental toxicity study [12], including auditory startle response, motor activity, learning and memory, and brain morphometry.

Fetal weight and fetal survival (Rank 2) were reduced in rats and rabbits in some developmental toxicity studies with EB, but not in others. Fetal weights were decreased in rats at exposure levels of 1,000 ppm and greater secondary to reduced maternal weight gain and/or food consumption in some studies [4, 5, 6] but not in rats or rabbits in another study [13]. In rats and rabbits but not in mice, exposure to 552 ppm EB decreased fetal weights [11], however, 552 ppm and lower concentrations also resulted in aborted fetuses. Therefore, this effect is likely to have been the result of systemic toxicity rather than to a thyroid MoA. Fetal survival was slightly reduced in rabbits [11, 13] at high doses that may have

produce maternal toxicity, but not in rats [4, 5, 6, 11, 13]. Thus, this consistency of negative responses among Rank 3 endpoints corroborates that EB lacks the potential for activity via the thyroid MoA.

In summary, although Supplementary Table 5 shows that some data gaps exist, the data available from repeat dose, developmental, and reproductive toxicity studies are sufficient to allow evaluation of EB's potential to act via a thyroid MoA. Where changes were elicited by EB in endpoints relevant to these hypotheses, these were observed inconsistently across studies, were within the normal ranges for the test species, and occurred at high doses. Effects on thyroid-relevant endpoints occurred secondary to high-dose, generalized liver toxicity. Therefore, the available data indicate that EB lacks the potential to cause effects via thyroid inhibition and there is high confidence in this interpretation.

Evaluation of steroidogenic enzymes MoA

Of a possible 37 endpoints relevant for assessing the potential to act via a steroidogenic MoA, eleven were measured following exposure to EB (Supplementary Table 6). Of those ten endpoints, nine are Rank 2 endpoints and one is Rank 3. Only estrous cyclicity (Rank 2) responded to EB in a reproductive toxicity study in rats [2]. Although estrous cyclicity was slightly reduced at the highest exposure concentration (500 ppm) in the parental, but not in the F₁ generation, the difference is unlikely to be biologically significant because all females in the exposure group cycled normally and the cycle length was within normal values for the strain of rat used in the study [2]. All other Rank 2 and 3 endpoints relevant for assessing activity via the steroidogenic MoA for which data were available were unaffected by EB in repeat dose toxicity studies, including histopathology of the ovaries, uteri and testes [1, 3, 9, 10] and gross pathology [3]. Sex ratio was unchanged in a developmental toxicity study [5] and sperm count, fertility, mating index, sex ratio, and number of live births were unchanged in a reproductive toxicity study [2]. Although several data

gaps exist for the steroidogenic pathway (Supplementary Table 6), there was a consistent lack of response among the eleven endpoints that were measured. This is sufficient to provide a clear indication that EB lacks the potential to cause effects by a steroidogenic MoA.

In summary, the available data provide sufficient and strong evidence that EB lacks the potential to disrupt steroidogenesis because the pattern of endpoint responses elicited by EB is inconsistent with this MoA. Although data is lacking for several endpoints that are relevant for evaluating the steroidogenesis MoA, there is high confidence in that conclusion due to the consistency of negative responses across studies for the endpoints measured. Therefore, it is unlikely that conducting evaluations of these missing endpoints would reveal positive findings. Even if a few positive findings were identified, it is unlikely this would shift the weight-of-evidence to support a potential for endocrine-mediated adverse effects.

DISCUSSION

This Weight of Evidence (WoE) evaluation was carried out using an established methodology (Borgert et al., 2011a, b) that incorporates essential components for an unbiased, transparent, and thorough analysis of the potential for EB to act through EATS MoAs. Key elements of this evaluation include precise problem formulation, a systematic literature search and selection process based on defined criteria, assessment of data quality using published methodologies, consistent criteria for weighting data relevance, and interpretation of findings in alignment with expected response patterns produced by chemicals and hormones that operate via these MoAs. The methodology employed in data selection yielded a dataset adequate for a comprehensive WoE evaluation. While acknowledging certain data gaps, there was sufficient information available to evaluate the impact of EB on most endpoints linked to EATS MoAs in the domains of reproductive toxicity, de-

developmental toxicity, and repeat dose toxicity studies.

Several conceptual and methodological limitations apply to WoE evaluations. A critical appraisal of WoE methods described several deficiencies that are important to avoid when developing a WoE framework (Krimsky, 2005). Krimsky asserts that many WoE methodologies fall short of their intended objectives, which include improving the clarity and transparency of evaluations, enhancing the consistency of regulatory decisions, and elucidating the foundational assumptions that underpin these methodologies. He highlights that the epistemic basis of WoE approaches is often left ambiguous, leading to diminished clarity and consistency, and potentially compromising scientific integrity and validity. According to Krimsky (2005), the *a priori* assumptions regarding the value of various evidentiary modalities tend to rely on expert judgments rather than empirical evidence, a situation that may decrease scientific objectivity. Frequently, the rationale behind expert evaluations concerning the relative quality and significance of different types of evidence is inadequately articulated. Nonetheless, these expert judgments are subsequently utilized to arrive at binary or ternary conclusions — “yes,” “no,” or “maybe” — which necessitate distilling complex, detailed biological data into simplistic dichotomous or triadic variables that lack both contextual nuance and clarity regarding their derivation methods. In summary, Krimsky contends that the conclusions drawn from WoE are often the product of a scientifically opaque process characterized by a lack of transparency and an overreliance on subjective assessment.

Rhomberg et al. (2013) reviewed WoE methods and offered recommendations on best practices. The extent to which the methodology employed here avoids the criticisms of Krimsky (2005) and achieves the practice recommendations of Rhomberg et al. (2013) can be evaluated against the epistemic foundation of our method, which has been described previously (Borgert et al., 2011 Supplemental Materials). To summarize, we

discussed three levels of validity targeted by the WoE methodology used here.

Primary validity: minimal epistemic status

To ensure the validity and reliability of WoE determinations, it is essential to assess the overall quality of the data involved in the evaluation process. Initially, one must consider the minimal epistemic status, referred to as 'primary validity' of the data (Borgert et al., 2011a). This entails a thorough examination of each study's results against the fundamental principles of scientific validity as articulated in various commentaries and editorials by Dr. Gio B. Gori, the former Deputy Director of the Division of Cancer Cause and Prevention at the National Cancer Institute (Gori 1999, 2001, 2002, 2009a, 2009b, 2010).

Dr. Gori emphasizes that for data to be regarded as established scientific facts, they must conform to three critical criteria that underpin the fundamental principles of scientific inquiry: First, the identity and authenticity of scientific measurements must be verifiable within a specific range of precision. Second, such measurements and observations must be free from confounding influences that could compromise their accuracy and precision. Third, these measurements and observations must be replicable by independent researchers.

These three principles are universally acknowledged as the minimum requirements for valid regulatory science in the United States (Subcommittee on Energy and Environment, 2010; Subcommittee on Health, 2010). These principles offer a clear and definitive standard against which all data should be evaluated for their suitability in WoE assessments.

Although disarmingly simple, these three tenets are critically important and powerfully discriminative. To demonstrate the application of tenets, the production of vitellogenin in male fish serves as a prominent example of putative environmental endocrine disruption. The initial step is to ascertain that the study accurately measures what it claims to within a specified range of precision. This funda-

mental principle inherently promotes a distinction between the measurement itself, and the interpretations attributed to it. In this case, the variable of interest is vitellogenin, typically quantified in blood plasma, though it may also be detected in other tissues such as the liver. Vitellogenin, a dimeric glycolipoprophosphoprotein, functions as the egg yolk precursor protein across all oviparous vertebrates and can be quantified using several methodologies (e.g., Alda and Barceló, 2001; Wheeler et al., 2005; Wu et al., 2006), each subject to defined margins of error.

Despite its utility as a biomarker, causal relationships between vitellogenin levels and reproductive impairment, or effects on populations, remain unverified. In the absence of such causal links, the presence of vitellogenin in male fish cannot be conclusively associated with "endocrine disruption" (Mills et al., 2003; Mills and Chichester, 2005). Establishing such causality necessitates additional experimental evidence derived from counterfactual study designs. Furthermore, the experimental conditions during which the measurements are taken can be difficult to adequately control. Apart from the methodological controls pertinent to specific analytical techniques, assessments of plasma vitellogenin in male fish must account for baseline levels of the protein within the study population, as well as the influence of viruses known to impact plasma vitellogenin concentrations in both male and female fish (Trubiroha et al., 2010). Additional variables may also need consideration depending on whether the study occurs in a controlled laboratory setting or in the field.

Lastly, it is essential to evaluate whether measurements have been replicated by independent laboratories, which entails diverse investigators utilizing different instruments and personnel. As shown by the cited literature, the assessment of plasma vitellogenin in male fish is generally reproducible in independent laboratories, provided that appropriate experimental and methodological controls and consistent study designs are employed.

Secondary validity: data reliability and transparency

WoE evaluations must prioritize the reliability of reported data, which we have referred to as 'secondary validity' (Borgert et al., 2011a). According to Klimisch et al. (1997), reliability is defined by the transparency and comprehensiveness of data reporting. In the context of *in vivo* studies, they recommend placing more emphasis on studies that provide extensive details regarding the test species, the test substances (including purity and origin), the number of animals studied, the extent of investigations conducted per animal (e.g., clinical chemistry, organ weights, hematology, histopathology), observations of changes or lesions, and relevant control and historical control groups. Additionally, important factors such as test conditions, route of administration, dosage schedule, and dose concentration (along with analytical verification) should be meticulously documented.

For *in vitro* studies, Klimisch et al. assert that greater weight should be given to those that articulate similar particulars concerning the test substances, alongside information pertinent to *in vitro* assays. This includes details about the test system and method, positive and negative controls, potential interferences with the methodology, and data on secondary effects that could sway results (e.g., solubility, impurities, pH fluctuations, osmolarity). Moreover, they emphasize the necessity of similar information in ecotoxicity studies, as well as details regarding the life stages of the animal subjects.

Given the potential for confounding factors in endocrine activity studies, additional elements may be warranted for inclusion in Klimisch's framework. Such considerations could encompass the composition of diets, the materials used for water bottles and cages, bedding, stressors like handling and manipulation, and any other variables that might influence hormonal systems. Furthermore, it is crucial to provide insights into the mathematical and statistical algorithms employed in analyzing the data.

Klimisch et al. (1997) assert that since studies performed under Good Laboratory Practices (GLP) in compliance with regulatory guidelines, referred to as "guideline studies," must document all pertinent information, they should serve as reference standards for assessing reliability. For guideline studies to serve as reference standards, they should either undergo rigorous validation processes, such as those mandated by ICCVAM or ECVAM², or be extensively utilized to ensure their performance is well-documented. Klimisch et al. do not limit their highest reliability rating (code) to guideline studies, but acknowledge that any study that adequately reports on these parameters should be prioritized over studies that do not, regardless of adherence to regulatory guidelines or GLP. Research characterized by enhanced rigor, transparency, and accessibility of documented data should be seen as more credible regardless of the origin or setting of the study (Schreider et al., 2010). The implementation of uniform, objective criteria, as outlined by Schneider et al. (2009), establishes a scientifically robust foundation for allocating suitable weights to all pertinent toxicity studies, regardless of whether they are GLP or non-GLP. The advantages of GLP have been examined in other sources (Borgert et al., 2016).

While the U.S. EPA's endocrine screening battery includes guideline studies, certain guidelines, like the uterotrophic assay, have undergone rigorous validation programs, whereas others inadequately fulfill the necessary sensitivity and specificity to reliably distinguish between non-endocrine active agents and active ones. Moreover, while their application in the pharmaceutical sector confirms that the binding assays and the uterotrophic and Hershberger assays effectively identify chemicals with significant hormonal activity, it is yet to be established whether these EDSP assays can differentiate weakly hormonal chemicals *in vivo* from false positives, which are substances that generate a signal in the

assay but do not exhibit the anticipated hormonal activity *in vivo*. The Tier 1 EDSP cannot be regarded as equally reliable as previous guideline studies until further validation data are obtained (Borgert et al., 2011). The U.S. EPA issued testing orders for the screening battery on 67 pesticide compounds, for which substantial reproductive and developmental toxicity data already exist. The initial screening phase may be seen as a validation step, contingent upon the clarity of the data, as the predictive value of the screenings can be assessed by comparing them to the outcomes of definitive guideline studies for these substances. In several cases, the need to revise performance criteria was identified (e.g., Schapaugh et al. 2015).

Tertiary validity: relevance and probative power of study design and causality

To establish that a chemical acts via a particular endocrine mode of action to produce adverse effects, it is insufficient to rely solely on the correlation between a Tier 1 screening result and an effect observed in Tier 2 testing. The evidential strength of the study design must first be assessed, a characteristic that may be referred to as 'tertiary validity'. Establishing a general causal link between the putative change in endocrine function and a negative outcome is crucial. In this context, an initial demonstration of biological plausibility is necessary, but is itself insufficient to determine a causal mechanistic link. Due to extensive knowledge of the procedures for inducing effects in each Tier 1 EDSP assay, affirmative results might inform the formulation of a preliminary hypothesis concerning the likely mechanism of action. Due to the uncertainty surrounding these steps, it is fitting to characterize this as a "working hypothesis of the mode of action." This will enable the formulation of plausible working hypotheses on the agent's impact on critical processes. After formulating working hypotheses, the causal linkages among the suggested mechanistic

² Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and European Centre for Validation of Alternative Methods (ECVAM)

processes must be validated by counterfactual experimental procedures. Counterfactual concepts and methodologies for determining causality in pharmacology, toxicology, and epidemiology have been integrated into the assessment of studies utilized in this WoE; however, significant uncertainty persists due to the various issues associated with the necessity of employing excessively high and irrelevant dose levels in toxicology studies (Borgert et al., 2021).

No statistical analysis of literature on EB was conducted to control for publication bias. Tools such as Egger's regression test and funnel plots are commonly employed in systematic reviews and meta-analyses to account for the possibility that negative studies may go unpublished, or that small sample sizes produced false-positives, which would leave the impression that only positive or expected results were obtained. However, this WoE evaluation is not a meta-analysis or a meta-review. Rather, it is a WoE analysis, as required by both the OECD³ and the U.S. EPA⁴ for the evaluation of endocrine disruptive potential, and was conducted by a well-established WoE methodology. Much of the data used in this WoE evaluation were from guideline toxicology studies commissioned by the industrial producers of EB in response to regulatory requirements. By statute, the results of such studies must be reported to regulatory agencies irrespective of whether the results are considered “positive” or “negative,” or whether the data are also published in journals. For toxicology studies in general, publication bias towards positive results is the greater risk (McCarty et al., 2012). The possibility of unpublished negative results for EB are a moot issue here because the extant data yield a negative result, notwithstanding. Thus, publication bias towards the negative, as would be addressed by Egger's regressions tests and funnel plots, would not have affected the conclusions of this WoE evaluation.

The assessment of data quality involved the careful selection of studies that fulfilled the inclusion criteria and provided endpoint data that could be ranked by relevance in accordance with the WoE methodology utilized. The studies included underwent evaluation using the established ToxRTool scoring system. In the WoE evaluation, only a single study [11] received a score below 21 (see Appendix A). The study's results were categorized based on test species, distinguishing between responses and non-responses for each endpoint. The literature assessed in this WoE evaluation of EB demonstrates adequate quality, allowing for a credible determination with a reasonable level of certainty that the findings were not skewed towards a false-negative conclusion. The conclusions of this evaluation are supported by the systematic parameters applied in the literature and data selection process, the limited data gaps identified in the existing literature, and the objective methodology employed in this WoE assessment, resulting in a high degree of confidence in the findings. Supplementary Table 7 presents a comprehensive overview of the results pertaining to endpoint responses across all six mechanisms of action assessed.

The WoE methodology utilized in this evaluation is distinctive as it ranks the evaluated endpoints based on their significance for testing each hypothesis. Optimally, relevance rankings would rely on empirical evidence that is adequate to compute both positive and negative predictive values. In the absence of such evidence, the rankings used herein represent the interpretations made by an expert panel, grounded in empirical observations of endpoint responses to established positive and negative controls for each mechanism of action (Borgert et al., 2014). The rankings vary mainly in terms of the specificity and sensitivity of the endpoints related to the hypothesis being examined. The assessment of specificity was based on two criteria: first, the extent to which the endpoint accurately repre-

³ OECD (2018), Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment, OECD Publishing, Paris. <https://doi.org/10.1787/9789264304741-en>

⁴ EPA US. (2011). 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*.

sents a response of the physiological system to the mechanism of action (MoA) being evaluated; and second, the extent to which the endpoint can respond to MoAs that differ from the one under investigation, particularly those that are non-endocrine in nature. This is a significant factor to consider in any assessment of endocrine mechanisms of action, as the endocrine system plays a crucial homeostatic role in nearly every system it regulates, including primary biological functions like growth, development, reproduction, and metabolism. As a result, endocrine pathways influence and are influenced by various non-endocrine physiological and biochemical processes. Identifying direct and indirect effects fundamentally depends on comprehending the specificity of the response.

This WoE methodology addresses specificity by requiring an evaluation of the response patterns associated with each MoA. Patterns that deviate from the expected responses of known effectors and inhibitors are interpreted as unlikely to signify activity through that pathway. It is theoretically possible for an agonist or antagonist of a specific hormonal pathway to demonstrate a novel pattern of endpoint responses, aligning with the “selective response modifier” concept of hormone receptor interactions. However, a limited number of response types have been identified across various hormone receptor systems, each corresponding to ligand-receptor affinities, potencies, and the tissue distribution of hormone receptors (see Borgert et al., 2018 and references therein). Assessment approaches that do not evaluate this fundamental aspect of hormonal action, such as the key characteristic approach proposed by La Merrill et al. (2020), have little utility for identifying potential endocrine disruptors (Borgert, 2023b).

It is reasonable to consider the methodology's effectiveness for a chemical that elicits responses in more endpoints pertinent to a specific hormonal mechanism of action compared to EB, especially in Rank 1 and Rank 2 endpoints. The response patterns anticipated for each mechanism of action necessitate

detailed examination, as do the possible influences of competing mechanisms on the endpoints, including hormonal mechanisms beyond the one being evaluated. While there exists potential for ligand-receptor interactions among various hormonal receptors, receptor specificity remains a significant characteristic of the endocrine system. For example, although androgens can bind and activate estrogen receptors at high concentrations, they do not function as potent estrogens, and therefore, it is unlikely for a chemical to demonstrate action by both modes concurrently. When responses arise from different hormonal MoAs, it is essential to consider the possibility of systemic toxicity as a common underlying factor.

A thorough examination of response weightings, as suggested by Borgert et al. (2014), is likely essential for evaluation of chemicals that produce responses in many endpoints, despite the limited discussion here due to the absence of a relevant response pattern to EB. The application of response weighting to elucidate the endocrine-disruptive potential of a chemical exhibiting weak responses in Rank 1 and Rank 2 endpoints has been documented for octamethylcyclotetrasiloxane (Borgert and Burgoon, 2025; Borgert et al., 2018; Matthews, 2021). Response weighting pertains to mechanistic potency, a core principle of receptor, enzyme, and transport kinetics relevant to the interactions between biological macromolecules and all small molecules, regardless of their endogenous or exogenous nature.

The data concerning the potential endocrine activity of EB assessed in this study indicate that it does not exhibit a pattern of results aligned with EATS MoAs and demonstrates minimal or negligible potential for interaction with EATS pathways. The lack of potential for EB to operate through EATS MoAs is evidenced by the inconsistent responses observed in endpoints pertinent to each EATS hypothesis, as well as the absence of a response pattern suggestive of any MoA. Supplementary Tables 1 to 6 present a range of results concerning endpoints associated

with potential EATS activity. Endpoint responses are rarely reported in isolation across studies; when they are, such responses typically occur at high doses, which are complicated by systemic toxicity and other MoAs. The endpoint response patterns to EB, as indicated in Supplementary Tables 1–6, do not align with EATS activity. The absence of a discernible pattern suggesting an endocrine response among the MoAs assessed is significant. While some variability among endocrine modulators is anticipated due to the potential for selective endocrine response modifiers (e.g., Kuiper et al. 1999), even selective responses should not be random or inconsistent with an endocrine mechanism, unlike the responses noted with EB.

The data employed in this WoE evaluation of EB are primarily from repeat dose, sub-chronic, and chronic toxicity studies, i.e., OSRI, rather than from the mechanistic screening assays included in the U.S. EPA's original EDSP Tier 1 and the lower tiers of the OECD Toolbox (U.S. EPA, 2009a; OECD, 2012). This WoE evaluation for EB indicates that further EDSP Tier Screens or OECD screening-level assays would not provide additional valuable information, as any responses observed would merely prompt the types of studies and endpoint measurements assessed in this evaluation, which demonstrate a lack of potential endocrine activity through EATS MoAs. Moreover, as only four studies examining the reproductive and developmental effects of EB were conducted prior to 2001, the dataset includes endpoints that are sensitive to adverse effects potentially arising from endocrine mechanisms. Consequently, the Tier 2 data remain relevant and comprehensive. The existing studies on reproductive, developmental, and repeat dose toxicology have evaluated the life stages deemed most pertinent for assessing the adverse effects of EB that may result from any mode of action, including endocrine disruption. Ecological effects are not expected based on the applications of EB, its physicochemical characteristics, and its environmental behavior and degradation. The limited potential for

obtaining useful information from further endocrine screening and testing of EB suggests that justifying the use of additional animals for this purpose is unwarranted.

The measurement of apical endpoints in the repeat dose, sub-chronic, and chronic reproductive toxicity studies with EB is of particular significance, as it encompasses many Rank 2 endpoints evaluated in this context. Responses in highly specific endocrine screening assays (Rank 1) are not conclusive; they suggest potential activity that requires further investigation through definitive, long-term studies. Apical endpoints, on the other hand, indicate possible adverse effects that may arise from either endocrine or non-endocrine pathways. A lack of response in apical endpoints is more informative and should be prioritized over a response. The absence of response in apical endpoints anticipated to react to endocrine modulators operating through EATS pathways rules out both an endocrine mechanism of action (MoA) and a non-endocrine MoA that indirectly influences the endocrine system. Conversely, a response suggests the potential existence of an underlying endocrine MoA for the observed effect. The significant absence of response to EB in these apical endpoints further emphasizes the strong evidence indicating that EB does not possess endocrine activity or endocrine disruptive potential.

Finally, the fact that only four endpoints responded to EB exposure at levels below the KMD for EB of 200 ppm (Burgoon et al., 2023) underscores that the conclusions reached here are highly conservative. Most endpoint responses were observable only above the KMD, making it highly likely that the MoA by which they arise involves high-dose-dependent changes in the kinetics of EB, in effect, kinetic overload of the organism. Since exposure levels below the KMD do not produce kinetic overload, responses observed only at exposure levels higher than the KMD do not portend similar effects at doses below the KMD, nor should they be used for risk assessment or for making inferences about endocrine disruptive potential.

CONCLUSIONS

Using an objective, pre-defined WoE methodology, there is high confidence in the conclusion that EB lacks the potential to produce adverse effects through (anti)estrogen, (anti)androgen, thyroid, or steroidogenic pathways. This conclusion is obligate because there is an inconsistency across studies among the few endpoints that responded to EB, but a high consistency among endpoints that did not respond to EB:

100 % failed to respond as an estrogen agonist; 19 % responded inconsistently.

100 % failed to respond as an estrogen antagonist; 14.3 % responded inconsistently.

100 % failed to respond as an androgen agonist; 21.1 % responded inconsistently.

100 % failed to respond as an androgen antagonist; 16.7 % responded inconsistently.

91 % failed to respond as a thyroid inhibitor; 50.0 % responded inconsistently.

100 % failed to respond as steroidogenesis inhibitor; 9.1 % responded inconsistently.

Given that EB demonstrates no potential to act through EATS pathways, it is biologically implausible for any adverse effects of EB to arise via these endocrine MoAs. While various endocrine mechanisms of action exist, there is insufficient evidence to support a contention that EB functions through endocrine mechanisms not assessed in this study. Consequently, EB cannot be classified as an endocrine disruptor, a potential endocrine disruptor, or as possessing endocrine disruptive properties according to an objective assessment of the available data. Thus, additional endocrine screening of EB would be an inefficient use of resources and animals, raising ethical concerns regarding animal welfare at this time. Further endocrine testing of EB is unnecessary unless new and compelling evidence for alternative endocrine mechanisms of action is presented. Although this evaluation for EB concentrated on OSRI as defined by the U.S. EPA's EDSP, the findings are also pertinent to the regulatory assessment of endocrine disruptors in the EU, which aims to identify chemicals that meet the WHO/IPCS

definition for labeling as “endocrine disruptors.”

Acknowledgments

The author thanks Kathleen C. Findlay, M.S., PharmD, for initial extraction of data, ToxRTool evaluations, and compilation of data summaries by endpoint; Susan A. Borgert, R.Ph., M.S., CPh, for compilation of data tables, manuscript formatting and proof-reading; Janice R. Ballo, M.A., MLS. for consultation on literature search strategies, conducting literature searches, and manuscript proof-reading and formatting.

Author roles

C.J. Borgert devised the methodologies used (see previous Borgert et al., publications, as cited), directed the literature searches, selected the literature, evaluated the literature, directed data compilation into data tables, directed literature summaries contained in Supplemental Materials, composed the manuscript, and responded to peer-review of the manuscript submissions.

Declaration of interest

Disclosures for this publication are identical to those for the WoE evaluation of styrene, published previously (Borgert, 2023a).

Disclosure: Funding and conflict of interest disclosures for this publication are identical to those published previously (Borgert, 2023a).

REFERENCES

- Afarinesh MR, Shafiei F, Sabzalizadeh M, Haghpanah T, Taheri M, Parsania S, et al. Effect of mild and chronic neonatal hypothyroidism on sensory information processing in a rodent model: A behavioral and electrophysiological study. *Brain Res Bull.* 2020;155:29–36.
- Alda M, Barceló D. Review of analytical methods for the determination of estrogens and progestogens in waste waters. *Fresenius J Anal Chem.* 2001;371-4:437-47.

- ^{5[13]} Andrew FD, Buschbom RL, Cannon WC, RA M, Montgomery LF, Phelp DW, et al. Teratologic assessment of ethylbenzene and 2-ethoxyethanol. US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Experimental Toxicology Branch 1981.⁶
- Andrews P, Freyberger A, Hartmann E, Eiben R, Loof I, Schmidt U, et al. Sensitive detection of the endocrine effects of the estrogen analogue ethinylestradiol using a modified enhanced subacute rat study protocol (OECD Test Guideline no. 407). *Arch Toxicol.* 2002; 76:194–202.
- Biegel LB, Flaws JA, Hirshfield AN, O'Connor JC, Elliott GS, Ladics GS, et al. 90-day feeding and one-generation reproduction study in Crl:CD BR rats with 17 beta-estradiol. *Toxicol Sci.* 1998;44:116–42.
- Borgert CJ. Hypothesis-driven weight of evidence evaluation indicates styrene lacks endocrine disruption potential. *Crit Rev Toxicol.* 2023a;53:53-68.
- Borgert CJ. Issue analysis: key characteristics approach for identifying endocrine disruptors. *Arch Toxicol.* 2023b;97:2819-22.
- Borgert CJ, Burgoon LD. Octamethylcyclotetrasiloxane (D4) lacks endocrine disruptive potential via estrogen pathways. *Arch Toxicol.* 2025. doi: 10.1007/s00204-024-03896-y.
- Borgert CJ, Mihaich EM, Ortego LS, Bentley KS, Holmes CM, Levine SL, et al. Hypothesis-driven weight of evidence framework for evaluating data within the US EPA's Endocrine Disruptor Screening Program. *Regul Toxicol Pharmacol.* 2011a;61:185–91.
- Borgert CJ, Mihaich EM, Quill TF, Marty MS, Levine SL, Becker RA. Evaluation of EPA's Tier 1 Endocrine Screening Battery and recommendations for improving the interpretation of screening results. *Regul Toxicol Pharmacol.* 2011b; 59:397–411.
- Borgert CJ, Stuchal LD, Mihaich EM, Becker RA, Bentley KS, Brausch JM, et al. Relevance weighting of Tier 1 endocrine screening endpoints by rank order. *Birth Defects Res Dev Reprod Toxicol.* 2014;101:90–113.
- Borgert CJ, Becker RA, Carlton BD, Hanson M, Kwiatkowski PL, Sue Marty M, et al. Does GLP enhance the quality of toxicological evidence for regulatory decisions? *Toxicol Sci.* 2016; 151:206–13.
- Borgert CJ, Matthews JC, Baker SP. Human-relevant potency threshold (HRPT) for ER α agonism. *Arch Toxicol.* 2018;92:1685–702.
- Borgert CJ, Fuentes C, Burgoon LD. Principles of dose-setting in toxicology studies: the importance of kinetics for ensuring human safety. *Arch Toxicol.* 2021;95:3651–64.
- Browne P, Judson RS, Casey WM, Kleinstreuer NC, Thomas RS. Screening chemicals for estrogen receptor bioactivity using a computational model. *Environ Sci Technol.* 2015;49:8804–14.
- Burgoon LD, Fuentes C, Borgert CJ. A novel approach to calculating the kinetically derived maximum dose. *Arch Toxicol.* 2022;96:809-16.
- Burgoon LD, Borgert CJ, Fuentes C, Klaunig JE. Kinetically-derived maximal dose (KMD) indicates lack of human carcinogenicity of ethylbenzene. *Arch Toxicol.* 2023;98:327-34.
- ^[10] Cragg ST, Clarke EA, Daly IW, Miller RR, Terrill JB, Ouellette RE. Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. *Fund Appl Toxicol.* 1989;13:399-408.
- Delclos KB, Weis CC, Bucci TJ, Olson G, Mellick P, Sadovova N, et al. Overlapping but distinct effects of genistein and ethinyl estradiol (EE(2)) in female Sprague-Dawley rats in multigenerational reproductive and chronic toxicity studies. *Reprod Toxicol.* 2009;27: 117–32.
- EDSTAC, Endocrine Disruptor Screening and Testing Advisory Committee. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Final Report. Washington, D.C.: US Environmental Protection Agency, 1998.
- European Parliament. Council of the European Union. REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), Regulation (EC) No 1907/2006. *Off J Eur Union.* 2006;L396:1–849.
- European Parliament. Council of the European Union. BPR (Biocidal Products Regulation), Regulation (EU). 2012;528. *Off J Eur Union.* 2012;L167:1-123.

⁵ The exponents mark the thirteen studies that were used in the ToxRTool in the appendix.

⁶ Results were also published in: Hardin BD, Bond GP, Sikov MR, Andrew FD, Beliles RP, Niemeier RW. Testing of selected workplace chemicals for teratogenic potential. *Scand J Work Environ Health.* 1981;7(Suppl 4):66-75.

- [2] Faber WD, Roberts LS, Stump DG, Tardif R, Krishnan K, Tort M, et al. Two generation reproduction study of ethylbenzene by inhalation in crl-cd rats. *Birth Defects Res B Dev Reprod Toxicol.* 2006;77(1):10-21.
- [12] Faber, WD, Roberts LS, Stump, DG, Beck, M, Kirkpatrick D, Regan KS, et al. Inhalation developmental neurotoxicity study of ethylbenzene in Crl-CD rats. *Birth Defects Res B Dev Reprod Toxicol.* 2007;80:34-48.
- Gong X, Lin Y, Bell ML, Zhan FB. Associations between maternal residential proximity to air emissions from industrial facilities and low birth weight in Texas, USA. *Environ Int.* 2018;120:181-98.
- Gong X, Huang Y, Duong J, Leng S, Zhan FB, Guo Y, et al. Industrial air pollution and low birth weight in New Mexico, USA. *J Environ Manage.* 2023;348:119236.
- Gori GB. The EPA and the courts: inching toward a showdown. *Regul Toxicol Pharmacol.* 1999;30:167-8.
- Gori GB. The costly illusion of regulating unknowable risks. *Regul Toxicol Pharmacol.* 2001;34-3:205-12.
- Gori GB. Considerations on guidelines of epidemiologic practice. *Ann Epidemiol.* 2002;12:73-8.
- Gori GB. Conflict of interest and public policy. *Regul Toxicol Pharmacol.* 2009a;53:159-60.
- Gori GB. Scientific integrity. *Regul Toxicol Pharmacol.* 2009b;54:213.
- Gori GB. Regulating unknown risk. *Regulation.* 2010;33(1):16-21.
- Harrath AH, Alrezaki A, Jalouli M, Aldawood N, Aldahmash W, Mansour L, et al. Ethylbenzene exposure disrupts ovarian function in Wistar rats via altering folliculogenesis and steroidogenesis-related markers and activating autophagy and apoptosis. *Ecotoxicol Environ Saf.* 2022;229:113081.
- Kleinstreuer NC, Ceger P, Watt ED, Martin M, Houck K, Browne P, et al. Development and validation of a computational model for androgen receptor activity. *Chem Res Toxicol.* 2017;30:946-64.
- Klimisch HJ, Andreae M, Tillmann U. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regul Toxicol Pharmacol.* 1997;25:1-5.
- Kuiper GG, van den Bemd GJ, van Leeuwen JP. Estrogen receptor and the SERM concept. *J Endocrinol Invest.* 1999;22:594-603.
- Krimsky S. The weight of scientific evidence in policy and law. *Am J Public Health.* 2005;95(Suppl 1):S129-36.
- La Merrill MA, Vandenberg LN, Smith MT, Goodson W, Browne P, Patisaul, HB, et al. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nat Rev Endocrinol.* 2020;16:45-57.
- Lei T, Qian H, Yang J, Hu Y. The association analysis between exposure to volatile organic chemicals and obesity in the general USA population: A cross-sectional study from NHANES program. *Chemosphere.* 2023;315:137738.
- [7] Li AA, Maurissen JP, Barnett JF, Foss J, Freshwater L, Garman RH, et al. Oral gavage subchronic neurotoxicity and inhalation subchronic immunotoxicity studies of ethylbenzene in the rat. *Neurotoxicology.* 2010;31: 247-58.
- Marty MS, Borgert C, Coady K, Green R, Levine SL, Mihaich E, et al. Distinguishing between endocrine disruption and non-specific effects on endocrine systems. *Regul Toxicol Pharmacol.* 2018;99:142-58.
- Matthews JC. A mechanistic evaluation of the potential for octamethylcyclotetrasiloxane to produce effects via endocrine modes of action. *Crit Rev Toxicol.* 2021;51: 571-90.
- McCarty LS, Borgert CJ, Mihaich EM. Information quality in regulatory decision-making: peer review versus good laboratory practice. *Environ Health Perspect.* 2012;120:927-34.
- [3] Mellert W, Deckardt K, Kaufmann W, van Ravenzwaay B. Ethylbenzene: 4-and 13-week rat oral toxicity. *Arch Toxicol.* 2007;81:361-70.
- Mihaich EM, Borgert CJ. Hypothesis-driven weight-of-evidence analysis for the endocrine disruption potential of benzene. *Regul Toxicol Pharmacol.* 2018; 100:7-15.
- Mihaich E, Capdevielle M, Urbach-Ross D, Slezak B. Hypothesis-driven weight-of-evidence analysis of endocrine disruption potential: a case study with triclosan. *Crit Rev Toxicol.* 2017;47:263-85.
- Mills LJ, Gutjahr-Gobell RE, Horowitz DB, Denslow ND, Chow MC, Zaroogian GE. Relationship between reproductive success and male plasma vitellogenin concentrations in cunner, *Tautoglabrus adspersus*. *Environ Health Perspect.* 2003;111:93-100.
- Mills LJ, Chichester C. Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? *Sci Total Environ.* 2005; 343:1-34.

- Nakhjirgan P, Kashani H, Naddafi K, Nabizadeh R, Amini H, Yunesian M. Maternal exposure to air pollutants and birth weight in Tehran, Iran. *J Environ Health Sci Eng.* 2019;17:711-7.
- Neal BH, Bus J, Marty MS, Coady K, Williams A, Staveley J, et al. Weight-of-the-evidence evaluation of 2,4-D potential for interactions with the estrogen, androgen and thyroid pathways and steroidogenesis. *Crit Rev Toxicol.* 2017;47:1–57.
- ^[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies). NTP Tox. 10. NIH Publication No. 92-3129. PB93-149722. Research Triangle Park, NTP, U.S. Dept. of Health and Human Services, 1992.
- ^[1] NTP (National Toxicology Program). Toxicology and carcinogenesis studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (Inhalation Studies). NTP Tech Rep Ser. 1999; 466:1-231.
- NTP (National Toxicology Program). Multigenerational reproductive toxicology study of ethinyl estradiol (CAS No. 57-63-6) in Sprague-Dawley rats. NTP TR 547. Research Triangle Park, NC: National Institutes of Health, 2010.
- OECD (Organisation for Economic Co-operation and Development). Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption. ENV/JM/MONO(2012)22. Paris: OECD/IOMC, 2012.
- Rhomberg LR, Goodman JE, Bailey LA, Prueitt RL, Beck NB, Bevan C, et al. A survey of frameworks for best practices in weight-of-evidence analyses. *Crit Rev Toxicol.* 2013;43:753-84.
- Rouget F, Bihannic A, Cordier S, Multigner L, Meyer-Monath M, Mercier F, et al. Petroleum and chlorinated solvents in meconium and the risk of hypospadias: a pilot study. *Front Pediatr.* 2021;9:640064.
- Rotroff DM, Dix DJ, Houck KA, Knudsen TB, Martin MT, McLaurin KW, et al. Using in vitro high throughput screening assays to identify potential endocrine-disrupting chemicals. *Environ Health Perspect.* 2013; 121:7–14.
- ^[5] Saillenfait AM, Gallissot F, Morel G, Bonnet P. Developmental toxicities of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure. *Food Chem Toxicol.* 2003;41:415-29.
- ^[6] Saillenfait AM, Gallissot F, Sabaté JP, Bourges-Abella N, Cadot R, Morel G, et al. Developmental toxicity of combined ethylbenzene and methylethylketone administered by inhalation to rats. *Food Chem Toxicol.* 2006;44:1287-98.
- ^[4] Saillenfait AM, Gallissot F, Sabate JP, Bourges-Abella N, Muller S. Developmental toxic effects of ethylbenzene or toluene alone and in combination with butyl acetate in rats after inhalation exposure. *J Appl Toxicol.* 2007;27:32-42.
- Schapaugh AW, McFadden LG, Zorrilla LM, Geter DR, Stuchal LD, Sunger N, et al. Analysis of EPA's endocrine screening battery and recommendations for further review. *Regul Toxicol Pharmacol.* 2015;72: 552-61.
- Schneider K, Schwarz M, Burkholder I, Kopp-Schneider A, Edler L, Kinsner-Ovaskainen A, et al. "ToxRTool", a new tool to assess the reliability of toxicological data. *Toxicol Lett.* 2009;189:138–44.
- Schreider J, Barrow C, Birchfield N, Dearfield K, Devlin D, Henry S, et al.. Enhancing the credibility of decisions based on scientific conclusions: transparency is imperative. *Toxicol Sci.* 2010;116:5-7.
- Slikker W, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, et al. Dose-dependent transitions in mechanisms of toxicity. *Toxicol Appl Pharmacol.* 2004;201:203–25.
- ^[8] Stott WT, Johnson KA, Bahnemann R, Day SJ, McGuirk RJ. Evaluation of potential modes of action of inhaled ethylbenzene in rats and mice. *Toxicol Sci.* 2003;71:53-66.
- Subcommittee on Energy and Environment, US House of Representatives, Committee on Energy and Commerce. Endocrine-disrupting chemicals in drinking water: risks to human health and the environment. Hearing before the Subcommittee on Energy and Environment of the Committee on Energy and Commerce, House of Representatives, 111 Congress, 2nd session, February 25, 2010. Serial No. 111-99. <https://www.govinfo.gov/content/pkg/CHRG-111hhrg76011/pdf/CHRG-111hhrg76011.pdf>.
- Subcommittee on Health, US House of Representatives Committee on Energy and Commerce, Hearing report of 4/22/2010; Hearing Transcript at pages 79 & 80. 111 Congress, 2nd session, April 22, 2010. Serial No. 111-112. Washington, DC: GPO, 2012. <https://www.govinfo.gov/content/pkg/CHRG-111hhrg76567/pdf/CHRG-111hhrg76567.pdf>.

Trubiroha A, Kroupova H, Wuertz S, Frank SN, Sures B, Kloas W. Naturally-induced endocrine disruption by the parasite *Ligula intestinalis* (Cestoda) in roach (*Rutilus rutilus*). *Gen Comp Endocrinol*. 2010;166:234-40.

^[11] Ungváry G, Tátrai E. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits. *Arch Toxicol*. 1985(Suppl. 8):425-30.

US EPA, Environmental Protection Agency. Endocrine Disruptor Screening Program: Tier 1 Screening Order Issuing Announcement. 74 FR 54422, Doc. No. E9-25352, Oct. 21, 2009a. <https://www.federalregister.gov/documents/2009/10/21/E9-25352/endocrine-disruptor-screening-program-tier-1-screening-order-issuing-announcement>.

US EPA, Environmental Protection Agency. Final contaminant candidate list 3 chemicals: Screening to a PCCL. EPA 815-R-09-007, August 2009b. https://www.epa.gov/sites/default/files/2014-05/documents/ccl3chem_screening_to_pccl_08-31-09_508v2.pdf.

US EPA, Environmental Protection Agency. Weight-of-Evidence: Evaluating results of EDSP Tier 1 Screening to Identify the Need for Tier 2 Testing. EPA-HQ-OPPT-2013-0275-0004. Washington, DC: US EPA, Office of Chemical Safety and Pollution Prevention, 2011.

US EPA, Environmental Protection Agency. Endocrine Disruptor Screening Program (EDSP); Near-Term Strategies for Implementation; Notice of Availability and Request for Comment. EPA-HQ-OPP-2023-0474; FRL-11384-01-OCSPP. 2023.

US Public Law 114-182. Frank R. Lautenberg Chemical Safety for the 21st Century Act, 15 U.S.C. § 2601 et seq. 15 U.S.C. 2603, Sec. 4. Testing of Chemical substances and Mixtures. 2016. <https://www.congress.gov/114/plaws/publ182/PLAW-114publ182.pdf>.

Werder EJ, Engel LS, Blair A, Kwok RK, McGrath JA, Sandler DP. Blood BTEX levels and neurologic symptoms in Gulf states residents. *Environ Res*. 2019;175:100-7.

Werder EJ, Beier JI, Sandler DP, Falkner KC, Gripshover T, Wahlang B, et al. Blood BTEXS and heavy metal levels are associated with liver injury and systemic inflammation in Gulf states residents. *Food Chem Toxicol*. 2020;139:111242.

Wheeler JR, Gimeno S, Crane M, Lopez-Juez E, Morrill D. Vitellogenin: A review of analytical methods to detect (anti) estrogenic activity in fish. *Toxicol Mech Meth*. 2005;15:293-306.

WHO, World Health Organization; IPCS, International Program on Chemical Safety. Global assessment of the state-of-the-science of endocrine disruptors. WHO/PCS/EDC/02.2. Geneva, WHO, 2002.

WHO, World Health Organization; UNEP, United Nations Environment Program. State of the science of endocrine disrupting chemicals. Geneva: WHO Press, 2012.

Wu C, Yuan D, Liu B. Rapid determination of vitellogenin in fish plasma by anion exchange high performance liquid chromatography using postcolumn fluorescence derivatization with o-phthalaldehyde. *Anal Sci*. 2006;22:1593-6.

Appendix A: ToxRTool Summary

Study Number in Supplementary Tables 1-6 and Text	Study	Study Details	ToxRTool Score (Maximum Score) in vivo:(21) in vitro:(18)	Deficiency/Comment
[1]	NTP 1999	Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene (EB) by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 weeks (mice).	21 (21)	
[2]	Faber et al., 2006	Four groups of CrI:CD(SD)IGS BR rats (30/sex/group for F ₀ and 25/sex/group for F ₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F ₀ and F ₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F ₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F ₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F ₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F ₂ generation was not directly exposed.	21 (21)	

Study Number in Supplementary Tables 1-6 and Text	Study	Study Details	ToxRTool Score (Maximum Score) in vivo:(21) in vitro:(18)	Deficiency/Comment
[3]	Mellert et al., 2007	EB was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bodyweight/day, administered am/pm as half doses.	21 (21)	In the published manuscript, the study results are not transparent and complete for all endpoints investigated. The publication lists histological examination of the epididymides, mammary gland (female only), ovaries, prostate, seminal vesicle, testes, thyroid glands and uterus of the exposed and control animals, but the findings are not described, nor is there a general statement regarding these evaluations. The publication thus warrants a score of 20, but since the original study report contains this information and was made available, the overall score for [3] is 21 for purposes of this WoE evaluation.
[4]	Saillenfait et al., 2007	The combined effects of EB and <i>n</i> -butyl acetate were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or <i>n</i> -butyl acetate, separately or in combination, 6 h day ⁻¹ , on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm <i>n</i> -butyl acetate or mixtures of 250 ppm EB + 500 ppm <i>n</i> -butyl acetate, 250 ppm EB + 1500 ppm <i>n</i> -butyl acetate, 1000 ppm EB + 500 ppm <i>n</i> -butyl acetate, or 1000 ppm EB + 1500 ppm <i>n</i> -butyl acetate.	21 (21)	
[6]	Saillenfait et al., 2006	Pregnant Sprague–Dawley rats were exposed to EB (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.	21 (21)	

Study Number in Supplementary Tables 1-6 and Text	Study	Study Details	ToxRTool Score (Maximum Score) in vivo:(21) in vitro:(18)	Deficiency/Comment
[7]	Li et al., 2010	In the neurotoxicity study, EB was administered orally via gavage twice daily to Sprague-Dawley male and female rats at 0, 25, 125, or 250 mg/kg per dose (total daily dosages of 0, 50, 250, or 500 mg/kg bodyweight/day) for 13 weeks.	21 (21)	
[8]	Stott et al., 2003	Male and female Fischer 344 rats and B6C3F1 mice were exposed to 0 or 750 ppm EB vapor 6 h/day for one or four weeks. Livers from 6 (one-week study) or 8 (four-week study) mice/sex/dose were examined and weighed.	21 (21)	
[9]	NTP et al., 1992	Inhalation toxicity of EB was studied by exposing groups of seven-week-old F344/N rats and B6C3F1 mice of each sex to EB vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.	21 (21)	
[10]	Cragg et al., 1989	Mice, rats and rabbits (five/sex/group) were exposed by inhalation to EB vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.	21 (21)	

Study Number in Supplementary Tables 1-6 and Text	Study	Study Details	ToxRTool Score (Maximum Score) in vivo:(21) in vitro:(18)	Deficiency/Comment
[11]	Ungváry and Tátrai, 1985	Groups of pregnant CFY rats were exposed by inhalation of EB at 0, 138, 276 or 553 ppm for 24 h/day from day 7 to day 15 of gestation. Fetuses were evaluated on gestational day 21. CFLP mice were exposed to inhalation of EB at 0, 115 or 230 ppm for 24 h/day (no data provided for these groups) or for 3-4 hours/day intermittently from day 6 to 15 of pregnancy. The fetuses were evaluated on gestational days 18. NZ rabbits were exposed to 0, 115, or 230 ppm EB for 24 h/day from day 7 to day 20 gestation. Fetuses were examined on gestational day 30. The three rabbit does in the 230-ppm dose group aborted.	11 (21)	Information on the source/origin of the substance not given; Information on the nature and/or physico-chemical properties of the test item, deemed indispensable for judging data, were not provided; Age or body weight of the test organisms at the start of the study not given; Frequency and duration of exposure as well as time-points of observations not explained; Insufficient details of the administration scheme given to judge the study; Achieved concentrations not analytically verified or stability of the test substance not ensured; Study endpoints or their methods of determination not clearly described; Description of the study results for all endpoints investigated not transparent; Statistical methods applied for data analysis not applied in a transparent manner; Quantitative study results not reliable.

Study Number in Supplementary Tables 1-6 and Text	Study	Study Details	ToxRTool Score (Maximum Score) in vivo:(21) in vitro:(18)	Deficiency/Comment
[12]	Faber et al., 2007	Four groups of CrI:CD(SD)IGS BR rats (30/sex/group for F ₀ and 25/sex/group for F ₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure of the F ₀ and F ₁ females continued throughout mating, from pregnancy through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on post-natal day (PND) 21 and exposure of the F ₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F ₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F ₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F ₂ generation was not directly exposed.	21 (21)	
[13]	Andrew et al., 1981	Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm EB for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through gestational day 19 (rats) and 24 (rabbits).	21 (21)	

Supplementary information to:

Original article:

**HYPOTHESIS-DRIVEN WEIGHT OF EVIDENCE EVALUATION
INDICATES ETHYLBENZENE LACKS ENDOCRINE DISRUPTION
POTENTIAL BY EATS PATHWAYS**

Christopher J. Borgert , PhD

Applied Pharmacology and Toxicology Inc, Gainesville FL, 32605 and
University of Florida College of Veterinary Medicine, Dept. Physiological Sciences,
Gainesville FL, 32610. Tel.: +1 352-219-8551

<https://dx.doi.org/10.17179/excli2024-7822>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License
(<http://creativecommons.org/licenses/by/4.0/>).

- Supplemental material A: Ethylbenzene references reviewed
- Supplemental material B: OSRI evaluation for ethylbenzene – Summaries of studies
- Supplemental material C: Rationale for excluding studies
- Supplementary Table 1: Estrogen agonist hypothesis; guideline toxicity studies
- Supplementary Table 2: Estrogen antagonist hypothesis; guideline toxicity studies
- Supplementary Table 3: Androgen agonist hypothesis; guideline toxicity studies
- Supplementary Table 4: Androgen antagonist hypothesis; guideline toxicity studies
- Supplementary Table 5: Thyroid inhibition hypothesis; guideline toxicity studies
- Supplementary Table 6: Interaction with steroidogenesis enzymes hypothesis;
guideline toxicity studies
- Supplementary Table 7: Summary of endpoints from all tables

Ethylbenzene References Reviewed Supplemental Material A

- Acuna-Askar, K., Villarreal-Chiu, J.F., Gracia-Lozano, M.V., Garza-Gonzalez, M.T., Chavez-Gomez, B., Rodriguez-Sanchez, I.P., and Barrera-Saldana, H.A. (2004). BTE-OX biodegradation kinetics with MTBE through bioaugmentation. *Water Sci Technol* 50, 85-92
- Adebambo, T. H., Fox, D. T., & Otitolaju, A. A. (2020). Toxicological Study and Genetic Basis of BTEX Susceptibility in *Drosophila melanogaster*. *Front Genet*, 11, 594179.
<https://doi.org/10.3389/fgene.2020.594179>
- Aguilera, I., Garcia-Esteban, R., Iñiguez, C., Nieuwenhuijsen, M.J., Rodríguez, A., Paez, M., Ballester, F., and Sunyer, J. (2010). Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA Sabadell cohort. *Environ Health Perspect* 118, 705-711.
- Aguilera, I., Guxens, M., Garcia-Esteban, R., Corbella, T., Nieuwenhuijsen, M.J., Foradada, C.M., and Sunyer, J. (2009). Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA Sabadell Cohort. *Environ Health Perspect* 117, 1322-27.
- Ahmadi, Z., Moradabadi, A., Abdollahdokht, D., Mehrabani, M., & Nematollahi, M. H. (2019). Association of environmental exposure with hematological and oxidative stress alteration in gasoline station attendants. *Environ Sci Pollut Res Int*, 26(20), 20411-20417.
- Akdeniz, N., Jacobson, L.D., and Hetchler, B.P. (2013). Health risk assessment of occupational exposure to hazardous volatile organic compounds in swine gestation, farrowing and nursery barns. *Environ Sci Process Impacts* 15, 563-572.
- Alabdulhadi, A., Ramadan, A., Devey, P., Boggess, M., & Guest, M. (2019). Inhalation exposure to volatile organic compounds in the printing industry. *J Air Waste Manag Assoc*, 69(10), 1142-1169.
- Andrew, F. D; Buschbom, R.L.; Cannon, W.C.; Miller, R.A.; Montgomery, L.F.; Phelp, D.W.; Sikov, M.R. 1981: Teratologic Assessment of Ethylbenzene and 2-Ethoxyethanol (publication), NTIS Report No. PB83-208074.
- ATSDR 2010. Toxicological Profile for Ethylbenzene. November 2010. U.S. department of health and human services Public Health Service Agency for Toxic Substances and Disease Registry.
- Baines, C.J., McKeown-Eyssen, G.E., Riley, N., Cole, D.E., Marshall, L., Loescher, B., and Jazmaji, V. (2004). Case-control study of multiple chemical sensitivity, comparing haematology, biochemistry, vitamins and serum volatile organic compound measures. *Occup Med (Lond)* 54, 408-418.
- Berthet, A., de Batz, A., Tardif, R., Charest-Tardif, G., Truchon, G., Vernez, D., and Droz, P.O. (2010). Impact of biological and environmental variabilities on biological monitoring--an approach using toxicokinetic models. *J Occup Environ Hyg* 7, 177-184.
- Bolden AL, Kwiatkowski CF, Colborn T. 2015. New Look at BTEX: Are Ambient Levels a Problem. *Environ Sci Technol* 49: 5261–76.
- Bolden AL, Schultz K, Pelch KE, Kwiatkowski CF. 2018. Exploring the endocrine activity of air pollutants associated with unconventional oil and gas extraction. *Environ Health* 17: 26.
- Boyle EB, Viet SM, Wright DJ, Merrill LS, Alwis KU, Blount BC, Mortensen ME, Moye JJ, Dellarco M. 2016. Assessment of Exposure to VOCs among Pregnant Women in the National Children's Study. *Int J Environ Res Public Health* 13: 376.
- Brajenovic N, Karaconji IB, Bulog A. 2015. Evaluation of Urinary Btex, Nicotine & Cotinine as Biomarkers of Airborne Pollutants in Nonsmokers & Smokers. *J Toxicol Environ Health A* 78:1133-6.

Ethylbenzene References Reviewed Supplemental Material A

Brennan, R.J., Kandikonda, S., Khrimian, A.P., DeMilo, A.B., Liquido, N.J., and Schiestl, R.H. (1996). Saturated and monofluoro analogs of the oriental fruit fly attractant methyl eugenol show reduced genotoxic activities in yeast. *Mutat Res* 369, 175-181.

Brown DR, Lewis C, Weinberger BI. 2015. Human exposure to unconventional natural gas development: A public health demonstration of periodic high exposure to chemical mixtures in ambient air. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 50: 460–72.

Cao, Y. M., Gao, W. M., & Liu, J. (2018). [Study on the health effects of occupational exposure to low concentrations of benzene]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*, 36(6), 435-438.

Capella, K. M., Roland, K., Geldner, N., Rey deCastro, B., De Jesús, V. R., van Bommel, D., & Blount, B. C. (2019). Ethylbenzene and styrene exposure in the United States based on urinary mandelic acid and phenylglyoxylic acid: NHANES 2005-2006 and 2011-2012. *Environ Res*, 171, 101-110.

Cardozo, T.R., Rosa, D.P., Feiden, I.R., Rocha, J.A., de Oliveira, N.C., da Silva Pereira, T., Pastoriza, T.F., da Motta Marques, D., de Lemos, C.T., et al. (2006). Genotoxicity and toxicity assessment in urban hydrographic basins. *Mutat Res* 603, 83-96.

Caron-Beaudoin, É., Whitworth, K. W., Bosson-Rieutort, D., Wendling, G., Liu, S., & Verner, M. A. (2021). Density and proximity to hydraulic fracturing wells and birth outcomes in Northeastern British Columbia, Canada. *J Expo Sci Environ Epidemiol*, 31(1), 53-61.

Caron-Beaudoin, É., Whyte, K. P., Bouchard, M. F., Chevrier, J., Haddad, S., Copes, R., Frohlich, K. L., Dokkie, D., Juul, S., Bouchard, M., & Verner, M. A. (2022). Volatile organic compounds (VOCs) in indoor air and tap water samples in residences of pregnant women living in an area of unconventional natural gas operations: Findings from the EXPERIVA study. *Sci Total Environ*, 805, 150242.

Casey, J. A., Goin, D. E., Rudolph, K. E., Schwartz, B. S., Mercer, D., Elser, H., Eisen, E. A., & Morello-Frosch, R. (2019). Unconventional natural gas development and adverse birth outcomes in Pennsylvania: The potential mediating role of antenatal anxiety and depression. *Environ Res*, 177, 108598.

Cassidy-Bushrow, A. E., Burmeister, C., Birbeck, J., Chen, Y., Lamerato, L., Lemke, L. D., Li, J., Mor, G., O’Leary, B. F., Peters, R. M., Reiners, J. J. J., Sperone, F. G., Westrick, J., Wiewiora, E., & Straughen, J. K. (2021). Ambient BTEX exposure and mid-pregnancy inflammatory biomarkers in pregnant African American women. *J Reprod Immunol*, 145, 103305.

Cassidy-Bushrow, A. E., Burmeister, C., Lamerato, L., Lemke, L. D., Mathieu, M., O’Leary, B. F., Sperone, F. G., Straughen, J. K., & Reiners, J. J. J. (2020). Prenatal airshed pollutants and preterm birth in an observational birth cohort study in Detroit, Michigan, USA. *Environ Res*, 189, 109845.

Chambers, D.M., McElprang, D.O., Waterhouse, M.G., and Blount, B.C. (2006). An improved approach for accurate quantitation of benzene, toluene, ethylbenzene, xylene, and styrene in blood. *Anal Chem* 78, 5375-383.

Chan, P.C., Hasemani, J.K., Mahleri, J., and Aranyi, C. (1998). Tumor induction in F344/N rats and B6C3F1 mice following inhalation exposure to ethylbenzene. *Toxicol Lett* 99, 23-32.

Ethylbenzene References Reviewed Supplemental Material A

- Chang M, Lee D, Park H, Ha M, Hong YC, Kim Y, Kim BN, Kim Y, Lim YH, Ha EH. 2018. Prenatal TVOCs exposure negatively influences postnatal neurobehavioral development. *Sci Total Environ* 618: 977–81.
- Chang M, Park H, Ha M, Hong YC, Lim YH, Kim Y, Kim YJ, Lee D, Ha EH. 2017. The effect of prenatal TVOC exposure on birth and infantile weight: the Mothers and Children’s Environmental Health study. *Pediatr Res* 82: 423–8.
- Chen X, Feng L, Luo H, Cheng H. 2016. Health risk equations and risk assessment of airborne benzene homologues exposure to drivers and passengers in taxi cabins. *Environ Sci Pollut Res Int* 23: 4797–811.
- Chen, C.S., Hseu, Y.C., Liang, S.H., Kuo, J.Y., and Chen, S.C. (2008). Assessment of genotoxicity of methyl-tert-butyl ether, benzene, toluene, ethylbenzene, and xylene to human lymphocytes using comet assay. *J Hazard Mater* 153, 351-56.
- Cragg, S.T., Clarke, E.A., Daly, I.W., Miller, R.R., Terrill, J.B., and Ouellette, R.E. (1989). Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. *Fundam Appl Toxicol* 13, 399-408.
- Cruz SL, Gauthereau-Torres MY, Rivera-Garcia MT. 2016. Structure-activity relationship for the anticonvulsant effects of organic solvents. *Neurotoxicology* 57: 121–7.
- Cushing, L. J., Vavra-Musser, K., Chau, K., Franklin, M., & Johnston, J. E. (2020). Flaring from Unconventional Oil and Gas Development and Birth Outcomes in the Eagle Ford Shale in South Texas. *Environ Health Perspect*, 128(7), 77003.
- da Silva, M.L., and Alvarez, P.J. (2010). Indole-based assay to assess the effect of ethanol on *Pseudomonas putida* F1 dioxygenase activity. *Biodegradation* 21, 425-430.
- Dai H, Jing S, Wang H, Ma Y, Li L, Song W, Kan H. 2017. VOC characteristics and inhalation health risks in newly renovated residences in Shanghai, China. *Sci Total Environ* 577: 73–83.
- Davidson, C. J., Hannigan, J. H., & Bowen, S. E. (2021). Effects of inhaled combined Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX): Toward an environmental exposure model. *Environ Toxicol Pharmacol*, 81, 103518.
- De Celis, R., Feria-Velasco, A., Gonzalez-Unzaga, M., Torres-Calleja, J., and Pedron-Nuevo, N. (2000). Semen quality of workers occupationally exposed to hydrocarbons. *Fertil Steril* 73, 221-28.
- Dehghani F, Omid F, Heravizadeh O, Barati Chamgordani S, Gharibi V, Sotoudeh Manesh A. 2018. Occupational health risk assessment of volatile organic compounds emitted from the coke production unit of a steel plant. *Int J Occup Saf Ergon* 1–6.
- Dehghani M, Fazlzadeh M, Sorooshian A, Tabatabaee HR, Miri M, Baghani AN, Delikhoon M, Mahvi AH, Rashidi M. 2018. Characteristics and health effects of BTEX in a hot spot for urban pollution. *Ecotoxicol Environ Saf* 155: 133–43.
- Dellefratte, K., Stingone, J. A., & Claudio, L. (2019). Combined association of BTEX and material hardship on ADHD-suggestive behaviours among a nationally representative sample of US children. *Paediatr Perinat Epidemiol*, 33(6), 482-489.
- Ding, N., Batterman, S., & Park, S. K. (2020). Exposure to Volatile Organic Compounds and Use of Feminine Hygiene Products Among Reproductive-Aged Women in the United States. *J Womens Health (Larchmt)*, 29(1), 65-73.

Ethylbenzene References Reviewed Supplemental Material A

- Doherty BT, Kwok RK, Curry MD, Ekenge C, Chambers D, Sandler DP, Engel LS. 2017. Associations between blood BTEX concentrations and hematologic parameters among adult residents of the U.S. Gulf States. *Environ Res* 156: 579–87.
- Du Z, Mo J, Zhang Y. 2014. Risk assessment of population inhalation exposure to volatile organic compounds and carbonyls in urban China. *Environ Int* 73: 33–45.
- El-Metwally D, Chain K, Stefanak MP, Alwis U, Blount BC, LaKind JS, Bearer CF. 2018. Urinary metabolites of volatile organic compounds of infants in the neonatal intensive care unit. *Pediatr Res*
- Elliott EG, Ettinger AS, Leaderer BP, Bracken MB, Deziel NC. 2017. A systematic evaluation of chemicals in hydraulic-fracturing fluids and wastewater for reproductive and developmental toxicity. *J Expo Sci Environ Epidemiol* 27: 90–9.
- Elliott EG, Trinh P, Ma X, Leaderer BP, Ward MH, Deziel NC. 2017. Unconventional oil and gas development and risk of childhood leukemia: Assessing the evidence. *Sci Total Environ* 576: 138–47.
- Engelhardt, G. (2006). In vivo micronucleus test in mice with 1-phenylethanol. *Arch Toxicol* 80, 868-872.
- Ethylbenzene. IARC Monogr Eval Carcinog Risks Hum. 2000, 77, 227-266.
- Faber, W.D., Roberts, L.S., Stump, D.G., Beck, M., Kirkpatrick, D., Regan, K.S., Tort, M., Moran, E., and Banton, M. (2007). Inhalation developmental neurotoxicity study of ethylbenzene in Crl-CD rats. *Birth Defects Res B Dev Reprod Toxicol* 80, 34-48.
- Faber, W.D., Roberts, L.S., Stump, D.G., Tardif, R., Krishnan, K., Tort, M., Dimond, S., Dutton, D., Moran, E., and Lawrence, W. (2006). Two generation reproduction study of ethylbenzene by inhalation in Crl-CD rats. *Birth Defects Res B Dev Reprod Toxicol* 77, 10-21.
- Fabian E, Bordag N, Herold M, Kamp H, Krennrich G, Looser R, Ma-Hock L, Mellert W, Montoya G, Peter E, Prokudin A, Spitzer M, Strauss V, Walk T, Zbranek R, van Ravenzwaay B. 2016. Metabolite profiles of rats in repeated dose toxicological studies after oral and inhalative exposure. *Toxicol Lett* 255: 11–23.
- Francioni, E., Fillmann, G., Hamacher, C., Wagener, A.d.e. .L., Depledge, M.H., Readman, J.W., and Meniconi, M.d.e. .F. (2003). Evaluation of a commercially available ELISA kit as a tool to determine BTEX in groundwater. *Environ Technol* 24, 665-670.
- Franck U, Weller A, Roder SW, Herberth G, Junge KM, Kohajda T, von Bergen M, Rolle-Kampczyk U, Diez U, Borte M, Lehmann I. 2014. Prenatal VOC exposure and redecoration are related to wheezing in early infancy. *Environ Int* 73: 393–401.
- Fustinoni, S., Giampiccolo, R., Pulvirenti, S., Buratti, M., and Colombi, A. (1999). Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine. *J Chromatogr B Biomed Sci Appl* 723, 105-115.
- Gherardi, M., Gordiani, A., and Gatto, M. (2010). Development and validation of method for analysis of some ototoxic solvents in saliva matrix by headspace gas chromatography/mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 878, 2391-96.
- Ghosh JK, Wilhelm M, Su J, Goldberg D, Cockburn M, Jerrett M, Ritz B. 2012. Assessing the influence of traffic-related air pollution on risk of term low birth weight on the basis of land-use-based regression models and measures of air toxics. *Am J Epidemiol* 175: 1262–74.

Ethylbenzene References Reviewed Supplemental Material A

Gill, R., Hatchett, S.E., Osselton, M.D., Wilson, H.K., and Ramsey, J.D. (1988). Sample handling and storage for the quantitative analysis of volatile compounds in blood: the determination of toluene by headspace gas chromatography. *J Anal Toxicol* 12, 141-46.

Gong, X., Lin, Y., Bell, M. L., & Zhan, F. B. (2018). Associations between maternal residential proximity to air emissions from industrial facilities and low birth weight in Texas, USA. *Environ Int*, 120, 181-198.

Gong, X., Huang, Y., Duong, J., Leng, S., Zhan, F. B., Guo, Y., Lin, Y., & Luo, L. (2023). Industrial air pollution and low birth weight in New Mexico, USA. *J Environ Manage*, 348, 119236. <https://doi.org/10.1016/j.jenvman.2023.119236>

Gonzalez JL, Pell A, Lopez-Mesas M, Valiente M. 2017. Simultaneous determination of BTEX and their metabolites using solid-phase microextraction followed by HPLC or GC/MS: An application in teeth as environmental biomarkers. *Sci Total Environ* 603-604: 109–17.

Groth C, Banerjee S, Ramachandran G, Stenzel MR, Sandler DP, Blair A, Engel LS, Kwok RK, Stewart PA. 2017. Bivariate Left-Censored Bayesian Model for Predicting Exposure: Preliminary Analysis of Worker Exposure during the Deepwater Horizon Oil Spill. *Ann Work Expo Health* 61: 76–86.

Gunsch, C.K., Kinney, K.A., Szaniszlo, P.J., and Whitman, C.P. (2006). Quantification of homogentisate-1,2-dioxygenase expression in a fungus degrading ethylbenzene. *J Microbiol Methods* 67, 257-265.

Haigler, B.E., and Spain, J.C. (1989). Degradation of p-chlorotoluene by a mutant of *Pseudomonas* sp. strain JS6. *Appl Environ Microbiol* 55, 372-79.

Hardin BD, Bond GP, Sikov MR, Andrew FD, Beliles RP, Niemeier RW. 1981. Testing of selected workplace chemicals for teratogenic potential. *Scand J Work Environ Health* 7 Suppl 4: 66–75.

Harrath, A. H., Alrezaki, A., Jalouli, M., Aldawood, N., Aldahmash, W., Mansour, L., & Alwasel, S. (2022). Ethylbenzene exposure disrupts ovarian function in Wistar rats via altering folliculogenesis and steroidogenesis-related markers and activating autophagy and apoptosis. *Ecotoxicol Environ Saf*, 229, 113081.

HEI-Energy Research Committee. Potential human health effects associated with unconventional Oil and gas development: a systematic review of the epidemiology literature. Special Report 1.

<https://www.heienergy.org/system/files/hei-energy-epi-lit-review.pdf>

Heibati B, Godri Pollitt KJ, Charati JY, Ducatman A, Shokrzadeh M, Karimi A, Mohammadyan M. 2018. Biomonitoring-based exposure assessment of benzene, toluene, ethylbenzene and xylene among workers at petroleum distribution facilities. *Ecotoxicol Environ Saf* 149: 19–25.

Heibati B, Pollitt KJG, Karimi A, Yazdani Charati J, Ducatman A, Shokrzadeh M, Mohammadyan M. 2017. BTEX exposure assessment and quantitative risk assessment among petroleum product distributors. *Ecotoxicol Environ Saf* 144: 445–9.

Henderson, L., Brusick, D., Ratpan, F., and Veenstra, G. (2007). A review of the genotoxicity of ethylbenzene. *Mutat Res* 635, 81-89.

Hill, M., Stabile, C., Steffen, L.K., and Hill, A. (2002). Toxic effects of endocrine disrupters on freshwater sponges: common developmental abnormalities. *Environ Pollut* 117, 295-300.

Ethylbenzene References Reviewed Supplemental Material A

Hong JY, Yu SY, Kim GW, Ahn JJ, Kim Y, Lim S, Son SW, Hwang SY. 2016. Identification of time-dependent biomarkers and effects of exposure to volatile organic compounds using high-throughput analysis. *Environ Toxicol* 31: 1563–70.

Hongyan, L., Zexiong, Z., Shiwei, X., He, X., Yinian, Z., Haiyun, L., & Zhongsheng, Y. (2019). Study on transformation and degradation of bisphenol A by *Trametes versicolor* laccase and simulation of molecular docking. *Chemosphere*, 224, 743-750.

Huff, J. (2002). Chemicals studied and evaluated in long-term carcinogenesis bioassays by both the Ramazzini Foundation and the National Toxicology Program: in tribute to Cesare Maltoni and David Rall. *Ann N Y Acad Sci* 982, 208-230.

Inayat-Hussain SH, Fukumura M, Muiz Aziz A, Jin CM, Jin LW, Garcia-Milian R, Vasiliou V, Deziel NC. 2018. Prioritization of reproductive toxicants in unconventional oil and gas operations using a multi-country regulatory data-driven hazard assessment. *Environ Int* 117: 348–58.

Jain RB. 2015. Levels of selected urinary metabolites of volatile organic compounds among children aged 6-11 years. *Environ Res* 142: 461–70.

Janitz, A. E., Dao, H. D., Campbell, J. E., Stoner, J. A., & Peck, J. D. (2019). The association between natural gas well activity and specific congenital anomalies in Oklahoma, 1997-2009. *Environ Int*, 122, 381-388.

Jephcote, C., Brown, D., Verbeek, T., & Mah, A. (2020). A systematic review and meta-analysis of haematological malignancies in residents living near petrochemical facilities. *Environ Health*, 19(1), 53.

Kasemy, Z. A., Kamel, G. M., Abdel-Rasoul, G. M., & Ismail, A. A. (2019). Environmental and Health Effects of Benzene Exposure among Egyptian Taxi Drivers. *J Environ Public Health*, 2019, 7078024.

Kassotis CD, Klemp KC, Vu DC, Lin CH, Meng CX, Besch-Williford CL, Pinatti L, Zoeller RT, Drobnis EZ, Balise VD, Isiguzo CJ, Williams MA, Tillitt DE, Nagel SC. 2015. Endocrine-Disrupting Activity of Hydraulic Fracturing Chemicals and Adverse Health Outcomes After Prenatal Exposure in Male Mice. *Endocrinology* 156: 4458–73.

Kassotis CD, Tillitt DE, Lin CH, McElroy JA, Nagel SC. 2016. Endocrine-Disrupting Chemicals and Oil and Natural Gas Operations: Potential Environmental Contamination and Recommendations to Assess Complex Environmental Mixtures. *Environ Health Perspect* 124: 256–64.

Kassotis CD, Vu DC, Vo PH, Lin CH, Cornelius-Green JN, Patton S, Nagel SC. 2018. Endocrine-Disrupting Activities and Organic Contaminants Associated with Oil and Gas Operations in Wyoming Groundwater. *Arch Environ Contam Toxicol*

Kim, B.M., Park, E.k., LeeAn, S.Y., Ha, M., Kim, E.J., Kwon, H., Hong, Y.C., Jeong, W.C., Hur, J., et al. (2009). [BTEX exposure and its health effects in pregnant women following the Hebei Spirit oil spill]. *J Prev Med Public Health* 42, 96-103.

Kim, J.H., Moon, J.Y., Park, E.-Y., Lee, K.-H., and Hong, Y.-C. (2011). Changes in Oxidative Stress Biomarker and Gene Expression Levels in Workers Exposed to Volatile Organic Compounds. *Ind Health* 49, 8-14.

Ethylbenzene References Reviewed Supplemental Material A

- Kim, M.N., Park, H.H., Lim, W.K., and Shin, H.J. (2005). Construction and comparison of *Escherichia coli* whole-cell biosensors capable of detecting aromatic compounds. *J Microbiol Methods* 60, 235-245.
- Kljaković-Gašpić, Z., Herceg Romanić, S., Bituh, T., Kašuba, V., Brčić Karačonji, I., Brajenović, N., Franulović, I., Jurasović, J., Klinčić, D., Kopjar, N., Marović, G., Milić, M., Orct, T., Sekovanić, A., & Želježić, D. (2018). Assessment of multiple anthropogenic contaminants and their potential genotoxicity in the aquatic environment of Plitvice Lakes National Park, Croatia. *Environ Monit Assess*, 190(11), 694.
- Kuster, M., Díaz-Cruz, S., Rosell, M., López de Alda, M., and Barceló, D. (2010). Fate of selected pesticides, estrogens, progestogens and volatile organic compounds during artificial aquifer recharge using surface waters. *Chemosphere* 79, 880-86.
- Lee, E.G., Slaven, J., Bowen, R.B., and Harper, M. (2011). Evaluation of the COSHH Essentials model with a mixture of organic chemicals at a medium-sized paint producer. *Ann Occup Hyg* 55, 16-29.
- Lei, T., Qian, H., Yang, J., & Hu, Y. (2023). The association analysis between exposure to volatile organic chemicals and obesity in the general USA population: A cross-sectional study from NHANES program. *Chemosphere*, 315, 137738. <https://doi.org/10.1016/j.chemosphere.2023.137738>
- Li J, Lu S, Liu G, Zhou Y, Lv Y, She J, Fan R. 2015. Co-exposure to polycyclic aromatic hydrocarbons, benzene and toluene and their dose-effects on oxidative stress damage in kindergarten-aged children in Guangzhou, China. *Sci Total Environ* 524-525: 74–80.
- Li, A.A., Maurissen, J.P., Barnett, J.F., Foss, J., Freshwater, L., Garman, R.H., Peachee, V.L., Hong, S.J., Stump, D.G., and Bus, J.S. (2010). Oral gavage subchronic neurotoxicity and inhalation subchronic immunotoxicity studies of ethylbenzene in the rat. *Neurotoxicology* 31, 247-258.
- Lim SK, Shin HS, Yoon KS, Kwack SJ, Um YM, Hyeon JH, Kwak HM, Kim JY, Kim TY, Kim YJ, Roh TH, Lim DS, Shin MK, Choi SM, Kim HS, Lee BM. 2014. Risk assessment of volatile organic compounds benzene, toluene, ethylbenzene, and xylene (BTEX) in consumer products. *J Toxicol Environ Health A* 77: 1502–21.
- Liu B, Jia C. 2015. Effects of exposure to mixed volatile organic compounds on the neurobehavioral test performance in a cross-sectional study of US adults. *Int J Environ Health Res* 25: 349–63.
- Liu FF, Escher BI, Were S, Duffy L, Ng JC. 2014. Mixture effects of benzene, toluene, ethylbenzene, and xylenes (BTEX) on lung carcinoma cells via a hanging drop air exposure system. *Chem Res Toxicol* 27: 952–9.
- Liu FF, Peng C, Ng JC. 2015. BTEX in vitro exposure tool using human lung cells: trips and gains. *Chemosphere* 128: 321–6.
- Liu Y, Li H, Fu X, Guo H, Meng R, Lu W, Zhao M, Wang H. 2016. Health risk impacts analysis of fugitive aromatic compounds emissions from the working face of a municipal solid waste landfill in China. *Environ Int* 97: 15–27.
- Liu, K. P., Su, Y. W., Zhang, J. W., Wang, Z., Ma, Y. Y., Liu, Y. M., & Xiao, Y. M. (2021). [The effects of ethylbenzene on HEI-OC1 cells proliferation and oxidative stress level]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*, 39(1), 44-47.
- Liu, X.T., Yang, D.Y., Wang, Y.R., Wang, Q., Kuang, D., Zhang, M., Qiao, L.J., Li, J.G., Yang, X.Y., and Zhao, S.L. (2013). [Influence of ethylbenzene on oxidative damage and apoptosis in rat renal epithelial cells NRK-52e]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 31, 133-36.

Ethylbenzene References Reviewed Supplemental Material A

- Liu, Y., Zhou, Q., Xie, X., Lin, D., and Dong, L. (2010). Oxidative stress and DNA damage in the earthworm *Eisenia fetida* induced by toluene, ethylbenzene and xylene. *Ecotoxicology* 19, 1551-59.
- López-Vargas, R., Méndez-Serrano, A., Albores-Medina, A., Oropeza-Hernández, F., Hernández-Cadena, L., Mercado-Calderón, F., Alvarado-Toledo, E., Herrera-Morales, S., Arellano-Aguilar, O., García-Vargas, G., & Montero-Montoya, R. (2018). Oxidative stress index is increased in children exposed to industrial discharges and is inversely correlated with metabolite excretion of voc. *Environ Mol Mutagen*, 59(7), 639-652.
- Lu, F., Li, S., Shen, B., Zhang, J., Liu, L., Shen, X., & Zhao, R. (2020). The emission characteristic of VOCs and the toxicity of BTEX from different mosquito-repellent incenses. *J Hazard Mater*, 384, 121428.
- Madaniyazi, L., Jung, C. R., Fook Sheng Ng, C., Seposo, X., Hashizume, M., & Nakayama, S. F. (2022). Early life exposure to indoor air pollutants and the risk of neurodevelopmental delays: The Japan Environment and Children's Study. *Environ Int*, 158, 107004.
- Maiolini, E., Knopp, D., Niessner, R., Eremin, S., Bolelli, L., Ferri, E.N., and Girotti, S. (2010). Chemiluminescent ELISA for the BTEX determination in water and soil. *Anal Sci* 26, 773-77.
- Mannisto T, Mendola P, Laughon Grantz K, Leishear K, Sundaram R, Sherman S, Ying Q, Liu D. 2015. Acute and recent air pollution exposure and cardiovascular events at labour and delivery. *Heart* 101: 1491-8.
- Marchand A, Aranda-Rodriguez R, Tardif R, Nong A, Haddad S. 2015. Human inhalation exposures to toluene, ethylbenzene, and m-xylene and physiologically based pharmacokinetic modeling of exposure biomarkers in exhaled air, blood, and urine. *Toxicol Sci* 144: 414-24.
- Marchand A, Aranda-Rodriguez R, Tardif R, Nong A, Haddad S. 2016. Evaluation and modeling of the impact of coexposures to VOC mixtures on urinary biomarkers. *Inhal Toxicol* 28: 260-73.
- Martínez, C., Ramírez, N., Gómez, V., Pocurull, E., and Borrull, F. (2013). Simultaneous determination of 76 micropollutants in water samples by headspace solid phase microextraction and gas chromatography-mass spectrometry. *Talanta* 116, 937-945.
- Martins EM, Borba PF, Dos Santos NE, Dos Reis PT, Silveira RS, Correa SM. 2016. The relationship between solvent use and BTEX concentrations in occupational environments. *Environ Monit Assess* 188: 608.
- Mazzeo, D.E., Matsumoto, S.T., Levy, C.E., de Angelis, D.d.e. .F., and Marin-Morales, M.A. (2013). Application of micronucleus test and comet assay to evaluate BTEX biodegradation. *Chemosphere* 90, 1030-36.
- Mellert, W., Deckardt, K., Kaufmann, W., & van Ravenzwaay, B. (2007). Ethylbenzene: 4-and 13-week rat oral toxicity. *Archives of Toxicology*, 81(5), 361-370.
- Mendola P, Wallace M, Liu D, Robledo C, Mnnist T, Grantz KL. 2016. Air pollution exposure and preeclampsia among US women with and without asthma. *Environ Res* 148: 248-55.
- Meyer-Monath M, Beaumont J, Morel I, Rouget F, Tack K, Lestremau F. 2014. Analysis of BTEX and chlorinated solvents in meconium by headspace-solid-phase microextraction gas chromatography coupled with mass spectrometry. *Anal Bioanal Chem* 406: 4481-90.

Ethylbenzene References Reviewed Supplemental Material A

Meyer-Monath M, Chatellier C, Rouget F, Morel I, Lestremau F. 2014. Development of a multi-residue method in a fetal matrix: analysis of meconium. *Anal Bioanal Chem* 406: 7785–97.

Miri M, Rostami Aghdam Shendi M, Ghaffari HR, Ebrahimi Aval H, Ahmadi E, Taban E, Gholizadeh A, Yazdani Aval M, Mohammadi A, Azari A. 2016. Investigation of outdoor BTEX: Concentration, variations, sources, spatial distribution, and risk assessment. *Chemosphere* 163: 601–9.

Montero-Montoya, R., López-Vargas, R., & Arellano-Aguilar, O. (2018). Volatile Organic Compounds in Air: Sources, Distribution, Exposure and Associated Illnesses in Children. *Ann Glob Health*, 84(2), 225-238.

Moolla R, Curtis CJ, Knight J. 2015. Occupational exposure of diesel station workers to BTEX compounds at a bus depot. *Int J Environ Res Public Health* 12: 4101–15.

Mueller, S., Dennison, G., & Liu, S. (2021). An Assessment on Ethanol-Blended Gasoline/Diesel Fuels on Cancer Risk and Mortality. *Int J Environ Res Public Health*, 18(13).

Mullen, K. R., Rivera, B. N., Tidwell, L. G., Ivanek, R., Anderson, K. A., & Ainsworth, D. M. (2020). Environmental surveillance and adverse neonatal health outcomes in foals born near unconventional natural gas development activity. *Sci Total Environ*, 731, 138497.

Nakhjirgan, P., Kashani, H., Naddafi, K., Nabizadeh, R., Amini, H., & Yunesian, M. (2019). Maternal exposure to air pollutants and birth weight in Tehran, Iran. *J Environ Health Sci Eng*, 17(2), 711-717.

National Toxicology Program (1999). NTP Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). *Natl Toxicol Program Tech Rep Ser* 466, 1-231.

Neghab, M., Nourozi, M. A., Shahtaheri, S. J., Mansoori, Y., Bazzaz, J. T., & Nedjat, S. (2018). Effects of Genetic Polymorphism on Susceptibility to Nephrotoxic Properties of BTEXs Compounds. *J Occup Environ Med*, 60(8), e377-e382.

Nicole, W. (2020). On Wells and Wellness: Oil and Gas Flaring as a Potential Risk Factor for Preterm Birth. *Environ Health Perspect*, 128(11), 114004. <https://doi.org/10.1289/EHP7952>

Niederlehner, B.R., Cairns, J., and Smith, E.P. (1998). Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to *Ceriodaphnia dubia*. *Ecotoxicol Environ Saf* 39, 136-146.

North MA, Rodriguez-Estival J, Smits JEG. 2017. Biomarker Sensitivity to Vehicle Exhaust in Experimentally Exposed European Starlings. *Environ Sci Technol* 51: 13427–35.

OECD SIDS Ethylbenzene - SIDS Initial Assessment Report for SIAM 14. March 2002.

Okamoto, Y., Hayashi, T., Matsunami, S., Ueda, K., Toda, C., Kawanishi, S., and Kojima, N. (2006). Formation of DNA damaging product from light-irradiated nonylphenol. *JOURNAL OF HEALTH SCIENCE* 52, 91-95.

Onink, F., Meindersma, W., Burghoff, B., Weggemans, W., Aerts, G., and de Haan, A. (2014). Ion Chromatography as a Novel Method to Quantify the Solubility of Pyridinium Ionic Liquids in Organic Solvents. *J Chromatogr Sci*

Paciência, I., Cavaleiro Rufo, J., Silva, D., Martins, C., Mendes, F., Farraia, M., Delgado, L., de Oliveira Fernandes, E., Padrão, P., Moreira, P., Severo, M., Barros, H., & Moreira, A. (2019).

Ethylbenzene References Reviewed Supplemental Material A

Exposure to indoor endocrine-disrupting chemicals and childhood asthma and obesity. *Allergy*, 74(7), 1277-1291.

Paez-Martinez, N., Lopez-Rubalcava, C., and Cruz, S.L. (2003). Basic research advances on the in vivo effects of abused solvents. *SALUD MENTAL* 26, 8-16.

Pankiewicz-Sperka M, Stanczyk K, Plaza GA, Kwasniewska J, Nalecz-Jawecki G. 2014. Assessment of the chemical, microbiological and toxicological aspects of post-processing water from underground coal gasification. *Ecotoxicol Environ Saf* 108: 294–301.

Peng C, Lee JW, Sichani HT, Ng JC. 2015. Toxic effects of individual and combined effects of BTEX on *Euglena gracilis*. *J Hazard Mater* 284: 10–8.

Philibert DA, Philibert CP, Lewis C, Tierney KB. 2016. Comparison of Diluted Bitumen (Dilbit) and Conventional Crude Oil Toxicity to Developing Zebrafish. *Environ Sci Technol* 50: 6091–8.

Phoenix, P., Keane, A., Patel, A., Bergeron, H., Ghoshal, S., and Lau, P.C. (2003). Characterization of a new solvent-responsive gene locus in *Pseudomonas putida* F1 and its functionalization as a versatile biosensor. *Environ Microbiol* 5, 1309-327.

Price, K., and Krishnan, K. (2011). An integrated QSAR-PBPK modelling approach for predicting the inhalation toxicokinetics of mixtures of volatile organic chemicals in the rat. *SAR QSAR Environ Res* 22, 107-128.

Quiros-Alcala L, Wilson S, Witherspoon N, Murray R, Perodin J, Trousdale K, Raspanti G, Sapkota A. 2016. Volatile organic compounds and particulate matter in child care facilities in the District of Columbia: Results from a pilot study. *Environ Res* 146: 116–24.

Rafiee, A., Delgado-Saborit, J. M., Sly, P. D., Amiri, H., & Hoseini, M. (2022). Exploring urinary biomarkers to assess oxidative DNA damage resulting from BTEX exposure in street children. *Environ Res*, 203, 111725.

Rajendran, R., Ragavan, R. P., Al-Sehemi, A. G., Uddin, M. S., Aleya, L., & Mathew, B. (2022). Current understandings and perspectives of petroleum hydrocarbons in Alzheimer's disease and Parkinson's disease: a global concern. *Environ Sci Pollut Res Int*, 29(8), 10928-10949.

Ramakrishnan, A., Lupo, P.J., Agopian, A.J., Linder, S.H., Stock, T.H., Langlois, P.H., and Craft, E. (2013). Evaluating the effects of maternal exposure to benzene, toluene, ethyl benzene, and xylene on oral clefts among offspring in Texas: 1999-2008. *Birth Defects Res A Clin Mol Teratol* 97, 532-37.

Ran J, Qiu H, Sun S, Tian L. 2018. Short-term effects of ambient benzene and TEX (toluene, ethylbenzene, and xylene combined) on cardiorespiratory mortality in Hong Kong. *Environ Int* 117: 91–8.

Reutman, S.R., LeMasters, G.K., Knecht, E.A., Shukla, R., Lockey, J.E., Burroughs, G.E., and Kesner, J.S. (2002). Evidence of reproductive endocrine effects in women with occupational fuel and solvent exposures. *Environ Health Perspect* 110, 805-811.

Riedel TP, DeMarini DM, Zavala J, Warren SH, Corse EW, Offenbergh JH, Kleindienst TE, Lewandowski M. 2018. Mutagenic atmospheres resulting from the photooxidation of aromatic hydrocarbon and NOx mixtures. *Atmos Environ* (1994) 178: 164–72.

Robrock, K.R., Mohn, W.W., Eltis, L.D., and Alvarez-Cohen, L. (2011). Biphenyl and ethylbenzene dioxygenases of *Rhodococcus jostii* RHA1 transform PBDEs. *Biotechnol Bioeng* 108, 313-321.

Ethylbenzene References Reviewed Supplemental Material A

- Rouget, F., Bihannic, A., Cordier, S., Multigner, L., Meyer-Monath, M., Mercier, F., Pladys, P., & Garlantezec, R. (2021). Petroleum and Chlorinated Solvents in Meconium and the Risk of Hypospadias: A Pilot Study. *Front Pediatr*, 9, 640064.
- Ruiz, P., Emond, C., McLanahan, E. D., Joshi-Barr, S., & Mumtaz, M. (2020). Exploring Mechanistic Toxicity of Mixtures Using PBPK Modeling and Computational Systems Biology. *Toxicol Sci*, 174(1), 38-50.
- Saalberg Y, Wolff M. 2016. VOC breath biomarkers in lung cancer. *Clin Chim Acta* 459: 5–9.
- Saillenfait, A.-M., Gallissot, F., Sabate, J.-P., Bourges-Abella, N., and Muller, S. (2007). Developmental toxic effects of ethylbenzene or toluene alone and in combination with butyl acetate in rats after inhalation exposure. *J Appl Toxicol* 27, 32-42.
- Saillenfait, A.M., Gallissot, F., Morel, G., and Bonnet, P. (2003). Developmental toxicities of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure. *Food Chem Toxicol* 41, 415-429.
- Saillenfait, A.M., Gallissot, F., Sabaté, J.P., Bourges-Abella, N., Cadot, R., Morel, G., and Lambert, A.M. (2006). Developmental toxicity of combined ethylbenzene and methylethylketone administered by inhalation to rats. *Food Chem Toxicol* 44, 1287-298.
- Sammarco PW, Kolian SR, Warby RA, Bouldin JL, Subra WA, Porter SA. 2016. Concentrations in human blood of petroleum hydrocarbons associated with the BP/Deepwater Horizon oil spill, Gulf of Mexico. *Arch Toxicol* 90: 829–37.
- Sapouckey SA, Kassotis CD, Nagel SC, Vandenberg LN. 2018. Prenatal Exposure to Unconventional Oil and Gas Operation Chemical Mixtures Altered Mammary Gland Development in Adult Fetonic Mice. *Endocrinology* 159: 1277–89.
- Serrano-Lomelin, J., Nielsen, C. C., Jabbar, M. S. M., Wine, O., Bellinger, C., Villeneuve, P. J., Stieb, D., Aelicks, N., Aziz, K., Buka, I., Chandra, S., Crawford, S., Demers, P., Erickson, A. C., Hystad, P., Kumar, M., Phipps, E., Shah, P. S., Yuan, Y., . . . Osornio-Vargas, A. R. (2019). Interdisciplinary-driven hypotheses on spatial associations of mixtures of industrial air pollutants with adverse birth outcomes. *Environ Int*, 131, 104972.
- Silvestre, R. T., Bravo, M., Santiago, F., Delmonico, L., Scherrer, L., Otero, U. B., Liehr, T., Alves, G., Chantre-Justino, M., & Ornellas, M. H. (2020). Hypermethylation in Gene Promoters Are Induced by Chronic Exposure to Benzene, Toluene, Ethylbenzene and Xylenes. *Pak J Biol Sci*, 23(4), 518-525.
- Sirotkin AV, Harrath AH. 2017. Influence of oil-related environmental pollutants on female reproduction. *Reprod Toxicol* 71: 142–5.
- Sisto, R., Cavallo, D., Ursini, C. L., Fresegna, A. M., Ciervo, A., Maiello, R., Paci, E., Pignini, D., Gherardi, M., Gordiani, A., L'Episcopo, N., Tranfo, G., Capone, P., Carbonari, D., Balzani, B., & Chiarella, P. (2020). Direct and Oxidative DNA Damage in a Group of Painters Exposed to VOCs: Dose - Response Relationship. *Front Public Health*, 8, 445.
- Skurský, L., Khan, A.N., Saleem, M.N., and al-Tamer, Y.Y. (1992). A new potent inhibitor of horse liver alcohol dehydrogenase: p-methylbenzyl hydroperoxide. *Biochem Int* 26, 899-904.

Ethylbenzene References Reviewed Supplemental Material A

- Song MK, Ryu JC. 2015. Blood miRNAs as sensitive and specific biological indicators of environmental and occupational exposure to volatile organic compound (VOC). *Int J Hyg Environ Health* 218: 590–602.
- Spinder, N., Prins, J. R., Bergman, J. E. H., Smidt, N., Kromhout, H., Boezen, H. M., & de Walle, H. E. K. (2019). Congenital anomalies in the offspring of occupationally exposed mothers: a systematic review and meta-analysis of studies using expert assessment for occupational exposures. *Hum Reprod*, 34(5), 903-919.
- Sreng L, Temime-Roussel B, Wortham H, Mourre C. 2017. Chemical Identification of “Maternal Signature Odors” in Rat. *Chem Senses* 42: 211–22.
- Staudt, A. M., Whitworth, K. W., Chien, L. C., Whitehead, L. W., & Gimeno Ruiz de Porras, D. (2019). Association of organic solvents and occupational noise on hearing loss and tinnitus among adults in the U.S., 1999-2004. *Int Arch Occup Environ Health*, 92(3), 403-413.
- Stephens, S.M., Alkindi, A.Y.A., Waring, C.P., and Brown, J.A. (1997). Corticosteroid and thyroid responses of larval and juvenile turbot exposed to the water-soluble fraction of crude oil. *JOURNAL OF FISH BIOLOGY* 50, 953-964.
- Stingone JA, McVeigh KH, Claudio L. 2017. Early-life exposure to air pollution and greater use of academic support services in childhood: a population-based cohort study of urban children. *Environ Health* 16: 2.
- Stott WT, Day SJ, McGuirk RJ, Johnson KA. 2001. Ethylbenzene: Four-Week Mechanism of Tumorigenicity Study in Fischer 344 Rats and B6C3F1 Mice. Report of Toxicology & Environmental Research and Consulting The Dow Chemical Company, Midland, Michigan .
- Stott WT, Johnson KA, Bahnemann R, Day SJ, McGuirk RJ. 2003. Evaluation of potential modes of action of inhaled ethylbenzene in rats and mice. *Toxicol Sci* 71: 53–66.
- Stott WT, Johnson KA, Day SJ, McGuirk RJ. 1999. Ethylbenzene: Mechanism of tumorigenicity in Fischer 344 rats and B6C3F1 mice. Report of Toxicology & Environmental Research and Consulting The Dow Chemical Company, Midland, Michigan
- Sweeney LM, Kester JE, Kirman CR, Gentry PR, Banton MI, Bus JS, Gargas ML. 2015. Risk assessments for chronic exposure of children and prospective parents to ethylbenzene (CAS No. 100-41-4). *Crit Rev Toxicol* 45: 662–726.
- Szaleniec, M., Witko, M., Tadeusiewicz, R., and Goclon, J. (2006). Application of artificial neural networks and DFT-based parameters for prediction of reaction kinetics of ethylbenzene dehydrogenase. *J Comput Aided Mol Des* 20, 145-157.
- Takagi, S., Sato, Y., Kokubun, A., Inomata, E., & Agatsuma, Y. (2020). Odor-active compounds from the gonads of *Mesocentrotus nudus* sea urchins fed *Saccharina japonica* kelp. *PLoS One*, 15(4), e0231673.
- Take, M., Takeda, T., Ishikawa, H., Matsumoto, M., Nagano, K., & Fukushima, S. (2020). Area under the blood concentration-time curve (AUC) of ethylbenzene concentration in rats: relationship to inhalation and oral administration route-dose. *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 55(14), 1596-1603.

Ethylbenzene References Reviewed Supplemental Material A

- Takeda, H., Yamada, A., Miyauchi, K., Masai, E., and Fukuda, M. (2004). Characterization of transcriptional regulatory genes for biphenyl degradation in *Rhodococcus* sp. strain RHA1. *J Bacteriol* 186, 2134-146.
- Tallandier, V., Chalansonnet, M., Merlen, L., Boucard, S., Thomas, A., Campo, P., & Pouyatos, B. (2021). An in vitro model to assess the peripheral vestibulotoxicity of aromatic solvents. *Neurotoxicology*, 84, 105-113.
- The Committee for Recommendation of Occupational Exposure Limits, Japan Society for Occupational Health. (2020). Occupational exposure limits for ethyl benzene, dimethyl terephthalate and hydrogen fluoride, and carcinogenicity and reproductive toxicant classifications. *J Occup Health*, 62(1), e12151.
- The Committee for Recommendation of Occupational Exposure Limits, Japan Society for Occupational Health. (2021). Occupational exposure limits for acetaldehyde, 2-bromopropane, glyphosate, manganese and inorganic manganese compounds, and zinc oxide nanoparticle, and the biological exposure indices for cadmium and cadmium compounds and ethylbenzene, and carcinogenicity, occupational sensitizer, and reproductive toxicant classifications. *J Occup Health*, 63(1), e12294.
- Tizzard, A.C., and Lloyd-Jones, G. (2007). Bacterial oxygenases: in vivo enzyme biosensors for organic pollutants. *Biosens Bioelectron* 22, 2400-07.
- Toda, C., Uchida, T., Midorikawa, K., Murata, M., Hiraku, Y., Okamoto, Y., Ueda, K., Kojima, N., and Kawanishi, S. (2003). DNA damage by ethylbenzenehydroperoxide formed from carcinogenic ethylbenzene by sunlight irradiation. *Biochem Biophys Res Commun* 304, 638-642.
- Tran, K. V., Casey, J. A., Cushing, L. J., & Morello-Frosch, R. (2020). Residential Proximity to Oil and Gas Development and Birth Outcomes in California: A Retrospective Cohort Study of 2006-2015 Births. *Environ Health Perspect*, 128(6), 67001.
- Tsangari X, Andrianou XD, Agapiou A, Mochalski P, Makris KC. 2017. Spatial characteristics of urinary BTEX concentrations in the general population. *Chemosphere* 173: 261–6.
- Ueda, K. (2009). Effect of Environmental Chemicals on Genes and the Expression. *Yakugaku Zasshi* 129, 1501-06.
- Ungváry, G., and Tátrai, E. (1985). On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits. *Arch Toxicol Suppl* 8, 425-430.
- Valcke M, Haddad S. 2015. Assessing human variability in kinetics for exposures to multiple environmental chemicals: a physiologically based pharmacokinetic modeling case study with dichloromethane, benzene, toluene, ethylbenzene, and m-xylene. *J Toxicol Environ Health A* 78: 409–31.
- Vigano, L. (1993). Reproductive strategy of *Daphnia magna* and toxicity of organic compounds. *Water Res* 27, 903-09.
- Wang T, Bo P, Bing T, Zhaoyun Z, Liyu D, Yonglong L. 2014. Benzene homologues in environmental matrixes from a pesticide chemical region in China: Occurrence, health risk and management. *Ecotoxicol Environ Saf* 104: 357–64.
- Wathier L, Venet T, Thomas A, Nunge H, Bonfanti E, Cosnier F, Parietti-Winkler C, Campo P, Tsan P, Bouguet-Bonnet S, Gansmuller A. 2016. Membrane fluidity does not explain how solvents act on the middle-ear reflex. *Neurotoxicology* 57: 13–21.

Ethylbenzene References Reviewed Supplemental Material A

Webb E, Bushkin-Bedient S, Cheng A, Kassotis CD, Balise V, Nagel SC. 2014. Developmental and reproductive effects of chemicals associated with unconventional oil and natural gas operations. *Rev Environ Health* 29: 307–18.

Webb E, Hays J, Dyrszka L, Rodriguez B, Cox C, Huffling K, Bushkin-Bedient S. 2016. Potential hazards of air pollutant emissions from unconventional oil and natural gas operations on the respiratory health of children and infants. *Rev Environ Health* 31: 225–43.

Webb E, Moon J, Dyrszka L, Rodriguez B, Cox C, Patisaul H, Bushkin S, London E. 2018. Neurodevelopmental and neurological effects of chemicals associated with unconventional oil and natural gas operations and their potential effects on infants and children. *Rev Environ Health* 33: 3–29.

Weinstein JR, Asteria-Penaloza R, Diaz-Artiga A, Davila G, Hammond SK, Ryde IT, Meyer JN, Benowitz N, Thompson LM. 2017. Exposure to polycyclic aromatic hydrocarbons and volatile organic compounds among recently pregnant rural Guatemalan women cooking and heating with solid fuels. *Int J Hyg Environ Health* 220: 726–35.

Werder, E. J., Beier, J. I., Sandler, D. P., Falkner, K. C., Gripshover, T., Wahlang, B., Engel, L. S., & Cave, M. C. (2020). Blood BTEXS and heavy metal levels are associated with liver injury and systemic inflammation in Gulf states residents. *Food Chem Toxicol*, 139, 111242.

Werder, E. J., Engel, L. S., Blair, A., Kwok, R. K., McGrath, J. A., & Sandler, D. P. (2019). Blood BTEX levels and neurologic symptoms in Gulf states residents. *Environ Res*, 175, 100-107.

Wickliffe, J. K., Stock, T. H., Howard, J. L., Frahm, E., Simon-Friedt, B. R., Montgomery, K., Wilson, M. J., Lichtveld, M. Y., & Harville, E. (2020). Increased long-term health risks attributable to select volatile organic compounds in residential indoor air in southeast Louisiana. *Sci Rep*, 10(1), 21649.

Xiong F, Li Q, Zhou B, Huang J, Liang G, Zhang L, Ma S, Qing L, Liang L, Su J, Peng X, Li Q, Zou Y. 2016. Oxidative Stress and Genotoxicity of Long-Term Occupational Exposure to Low Levels of BTEX in Gas Station Workers. *Int J Environ Res Public Health* 13:

Xu, J., Zheng, L., Yan, Z., Huang, Y., Feng, C., Li, L., & Ling, J. (2020). Effective extrapolation models for ecotoxicity of benzene, toluene, ethylbenzene, and xylene (BTEX). *Chemosphere*, 240, 124906.

Xu, Z., Mulchandani, A., and Chen, W. (2003). Detection of benzene, toluene, ethyl benzene, and xylenes (BTEX) using toluene dioxygenase-peroxidase coupling reactions. *Biotechnol Prog* 19, 1812-15.

Yamada, A., Kishi, H., Sugiyama, K., Hatta, T., Nakamura, K., Masai, E., and Fukuda, M. (1998). Two nearly identical aromatic compound hydrolase genes in a strong polychlorinated biphenyl degrader, *Rhodococcus* sp. strain RHA1. *Appl Environ Microbiol* 64, 2006-012.

Yang, R. (1993). NTP technical report on the toxicity studies of a Chemical Mixture of 25 Groundwater Contaminants Administered in Drinking Water to F344/N Rats and B6C3F(1) Mice. *Toxic Rep Ser* 35, 1-112.

Yousefian, F., Mahvi, A. H., Yunesian, M., Hassanvand, M. S., Kashani, H., & Amini, H. (2018). Long-term exposure to ambient air pollution and autism spectrum disorder in children: A case-control study in Tehran, Iran. *Sci Total Environ*, 643, 1216-1222.

Ethylbenzene References Reviewed Supplemental Material A

- Yu, S. Y., Koh, E. J., Kim, S. H., Lee, S. Y., Lee, J. S., Son, S. W., & Hwang, S. Y. (2021). Integrated analysis of multi-omics data on epigenetic changes caused by combined exposure to environmental hazards. *Environ Toxicol*, 36(6), 1001-1010.
- Zapór, L., Skowroń, J., and Gołofit-Szymczak, M. (2002). The cytotoxicity of some organic solvents on isolated hepatocytes in monolayer culture. *Int J Occup Saf Ergon* 8, 121-29.
- Zhang M, Wang Y, Wang X, Liu J, Zhang J, Gu Q. 2016. Roles of oxidative stress, apoptosis, and heme oxygenase-1 in ethylbenzene-induced renal toxicity in NRK-52E cells. *Toxicol Ind Health* 32: 1952–60.
- Zhang M, Wang Y, Yang D, Zhang J, Gu Q. 2015. Roles of oxidative damage and mitochondria-mediated apoptosis in ethylbenzene-induced hepatotoxic effects in rat. *Inhal Toxicol* 27: 64–73.
- Zhang, L., Zhang, C., Cheng, Z., Yao, Y., and Chen, J. (2013). Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by the bacterium *Mycobacterium cosmeticum* byf-4. *Chemosphere* 90, 1340-47.
- Zhang, M., Wang, Y., Wang, Q., Yang, J., Yang, D., Liu, J., and Li, J. (2010). Involvement of mitochondria-mediated apoptosis in ethylbenzene-induced renal toxicity in rat. *Toxicol Sci* 115, 295-303.
- Zhang, S., Cawley, G.F., Eyer, C.S., and Backes, W.L. (2002). Altered ethylbenzene-mediated hepatic CYP2E1 expression in growth hormone-deficient dwarf rats. *Toxicol Appl Pharmacol* 179, 74-82.
- Zheng S, Zhou Q. 2017. Intoxication and biochemical responses of freshwater snail *Bellamya aeruginosa* to ethylbenzene. *Environ Sci Pollut Res Int* 24: 189–98.
- Zheng, S., Wang, Y., Zhou, Q., and Chen, C. (2013). Responses of oxidative stress biomarkers and DNA damage on a freshwater snail (*Bellamya aeruginosa*) stressed by ethylbenzene. *Arch Environ Contam Toxicol* 65, 251-59.

Table of Contents

Supplemental Material B	5
OSRI Evaluation for Ethylbenzene	5
Summaries of Studies	5
1. Estrogen Agonist Hypothesis	5
1.1 Rank 2: Repeat Dose Toxicity – Epididymis histopathology	5
1.2 Rank 2: Repeat Dose Toxicity – Epididymis weight	6
1.3 Rank 2: Repeat Dose Toxicity – Ovary histopathology	6
1.4 Rank 2: Repeat Dose Toxicity – Testis histopathology (atrophy)	7
1.5 Rank 2: Repeat Dose Toxicity – Testis weight	8
1.6 Rank 2: Repeat Dose Toxicity – Uterus histopathology	8
1.7 Rank 2: Repeat Dose Toxicity – Vaginal histopathology	9
1.8 Rank 2: Developmental Toxicity – Corpora lutea	9
1.9 Rank 2: Developmental Toxicity – Post-implantation loss	10
1.10 Rank 2: Developmental Toxicity – Pre-implantation loss	11
1.11 Rank 2: Reproductive Toxicity – Estrous cyclicity	12
1.12 Rank 2: Reproductive Toxicity – Fertility	12
1.13 Rank 2: Developmental Toxicity – Gestational length	13
1.14 Rank 2: Reproductive Toxicity – Implantations	13
1.15 Rank 2: Reproductive Toxicity – Litter size	14
1.16 Rank 2: Reproductive Toxicity – Mating index	14
1.17 Rank 2: Reproductive Toxicity – Ovarian follicle count in offspring	14
1.18 Rank 2: Reproductive Toxicity – Sperm count	15
1.19 Rank 2: Reproductive Toxicity – Time to mating	15
1.20 Rank 2: Reproductive Toxicity – Time to vaginal patency	16
1.21 Rank 3: Repeat Dose Toxicity – Gross pathology	16
2. Estrogen Antagonist Hypothesis	17
2.1 Rank 2: Repeat Dose Toxicity – Epididymis histopathology	17
2.2 Rank 2: Repeat Dose Toxicity – Ovary histopathology	18
2.3 Rank 2: Repeat Dose Toxicity – Prostate histopathology	19
2.4 Rank 2: Repeat Dose Toxicity – Seminal vesicle histopathology	19
2.5 Rank 2: Repeat dose toxicity – Testis histopathology (atrophy)	20

2.6 Rank 2: Repeat Dose Toxicity – Testis weight	21
2.7 Rank 2: Developmental Toxicity – Corpora lutea.....	22
2.8 Rank 2: Reproductive Toxicity – Estrous cyclicity.....	22
2.9 Rank 2: Reproductive Toxicity – Fertility	23
2.10 Rank 2: Reproductive Toxicity – Litter size	23
2.11 Rank 2: Reproductive Toxicity – Sperm count	23
2.12 Rank 2: Reproductive Toxicity – Time to mating.....	24
2.13 Rank 2: Reproductive Toxicity – Time to vaginal patency in offspring	24
2.14 Rank 3: Repeat Dose Toxicity – Gross Pathology.....	25
3. Androgen Agonist Hypothesis.....	25
3.1 Rank 2: Repeat Dose Toxicity – Ovary histopathology	25
3.2 Rank 2: Repeat Dose Toxicity – Sperm count.....	26
3.3 Rank 2: Repeat dose toxicity – Testis histopathology (atrophy).....	26
3.4 Rank 2: Repeat Dose Toxicity – Testis weight	27
3.5 Rank 2: Developmental Toxicity – Implantations.....	28
3.6 Rank 2: Developmental Toxicity – Litter size	29
3.7 Rank 2: Developmental Toxicity – Sex ratio	30
3.8 Rank 2: Reproductive Toxicity – Estrous cyclicity.....	30
3.9 Rank 2: Reproductive Toxicity – Fertility.....	31
3.10 Rank 2: Reproductive Toxicity – Implantations	31
3.11 Rank 2: Reproductive Toxicity – Litter size	32
3.12 Rank 2: Reproductive Toxicity – Mating index	32
3.13 Rank 2: Reproductive Toxicity – Prostate weight	33
3.14 Rank 2: Reproductive Toxicity – Sex ratio.....	33
3.15 Rank 2: Reproductive Toxicity – Sperm count	33
3.16 Rank 2: Reproductive Toxicity – Time to balano-preputial separation	34
3.17 Rank 2: Reproductive Toxicity – Time to mating.....	34
3.18 Rank 2: Reproductive Toxicity – Time to vaginal patency.....	35
3.19 Rank 3: Repeat Dose Toxicity – Gross pathology	35
4. Androgen Antagonist Hypothesis.....	36
4.1 Rank 2: Repeat Dose Toxicity – Epididymal weight	36
4.2 Rank 2: Repeat Dose Toxicity – Epididymis histopathology	36
4.3 Rank 2: Repeat Dose Toxicity – Ovary histopathology	37
4.4 Rank 2: Repeat Dose Toxicity – Prostate histopathology.....	38

4.5 Rank 2: Repeat Dose Toxicity – Seminal vesicle histopathology	39
4.6 Rank 2: Repeat Dose – Testis histopathology (atrophy)	40
4.7 Rank 2: Repeat Dose Toxicity – Testis weight	41
4.8 Rank 2: Repeat Dose Toxicity – Uterus histopathology.....	41
4.9 Rank 2: Reproductive Toxicity – Estrous cyclicity	42
4.10 Rank 2: Reproductive Toxicity – Fertility	42
4.11 Rank 2: Reproductive Toxicity – Gross pathology	43
4.12 Rank 2: Reproductive Toxicity – Litter size	43
4.13 Rank 2: Reproductive Toxicity – Prostate weight	44
4.14 Rank 2: Reproductive Toxicity – Sperm count	44
4.15 Rank 2: Reproductive Toxicity – Sperm motility	45
4.16 Rank 2: Reproductive Toxicity – Time to balano-preputial separation	45
4.17 Rank 2: Reproductive Toxicity – Time to mating.....	46
4.18 Rank 3: Repeat Dose Toxicity – Gross pathology	46
5. <i>Thyroid Inhibition Hypothesis</i>	46
5.1 Rank 2: Repeat Dose Toxicity – Thyroid follicular cell histopathology	46
5.2 Rank 2: Developmental Toxicity – Fetal survival	47
5.3 Rank 2: Developmental Toxicity – Fetal weight.....	49
5.4 Rank 2: Reproductive Toxicity – Pup growth.....	50
5.5 Rank 2: Reproductive Toxicity – Pup survival.....	51
5.6 Rank 2: Reproductive Toxicity – Thyroid weight.....	51
5.7 Rank 3: Repeat Dose Toxicity – Liver weight.....	52
5.8 Rank 3: Reproductive Toxicity – Liver weight.....	53
5.9 Rank 3: Developmental Neurotoxicity – Auditory startle.....	54
5.10 Rank 3: Developmental Neurotoxicity – Brain morphometry	55
5.11 Rank 3: Developmental Neurotoxicity – Learning and memory	55
5.12 Rank 3: Developmental Neurotoxicity – Motor activity	56
6. <i>Interaction with Steroidogenesis Enzymes Hypothesis</i>.....	56
6.1 Rank 2: Repeat Dose Toxicity – Ovary histopathology	56
6.2 Rank 2: Repeat Dose toxicity – Testis histopathology.....	57
6.3 Rank 2: Repeat Dose Toxicity – Uterus histopathology.....	58
6.4 Rank 2: Developmental Toxicity – Sex ratio	59
6.5 Rank 2: Reproductive Toxicity – Estrous cyclicity	60
6.6 Rank 2: Reproductive Toxicity – Fertility	60

6.7 Rank 2: Reproductive Toxicity – Live births	61
6.8 Rank 2: Reproductive Toxicity – Mating index.....	61
6.9 Rank 2: Reproductive Toxicity – Sex ratio.....	62
6.10 Rank 2: Reproductive Toxicity –Sperm count.....	62
6.11 Rank 3: Repeat Dose Toxicity – Gross Pathology.....	63

Supplemental Material B

OSRI Evaluation for Ethylbenzene

Summaries of Studies

1. Estrogen Agonist Hypothesis

1.1 Rank 2: Repeat Dose Toxicity – Epididymis histopathology

[1] **NTP (National Toxicology Program). (1999)** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] **NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histologic changes in the epididymides of ethylbenzene-exposed mice or rats.

[10] **Cragg and colleagues (1989)** Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

1.2 Rank 2: Repeat Dose Toxicity – Epididymis weight

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: The tabular data indicate there was a significant decrease in the epididymal weight in mice exposed to ethylbenzene in the 1000-ppm group. The authors note that this was not considered biologically significant since spermatid counts, sperm motility, and caudal weight were normal. The narrative portion of the report states that this significant difference was found in the epididymal weight of rats, not mice – a likely error (p. 17). The tabular data show that there was no difference in the epididymal weight of rats at any ethylbenzene exposure level.

1.3 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

1.4 Rank 2: Repeat Dose Toxicity – Testis histopathology (atrophy)

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no effects observed on sperm or testicular morphology in rats exposed to ethylbenzene.

1.5 Rank 2: Repeat Dose Toxicity – Testis weight

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

1.6 Rank 2: Repeat Dose Toxicity – Uterus histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to

ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the uterus of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The uterus of high-exposure and controls animals of all species was subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in this organ.

1.7 Rank 2: Repeat Dose Toxicity – Vaginal histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results used for WOE: The histological examination of vaginal tissue did not reveal significant differences between the chamber controls and any of the exposure groups in rats or mice.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

1.8 Rank 2: Developmental Toxicity – Corpora lutea

[5] Saillenfait and colleagues (2003) The developmental toxicity of ethylbenzene was studied in

Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of corpora lutea per dam did not differ between dams in any of the treatment groups and control dams.

[13] Andrew et al., (1981); Hardin et al. (1981) Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of Corpora Lutea was unchanged by exposure to 100 and 1,000 ppm ethylbenzene relative to controls.

1.9 Rank 2: Developmental Toxicity – Post-implantation loss

[4] Saillenfait and colleagues (2007) The combined effects of EB and butyl acetate (BA) were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day⁻¹, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] Saillenfait and colleagues (2003) The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm. Maternal weight was significantly reduced on GD 21 at 1000 ppm and on GD 13 and 21 at 2000 ppm. Dams exposed to 1000 or 2000 ppm showed significant decreases in maternal weight gain and food consumption throughout exposure, and in corrected weight gain

Results included in WOE: The number of implantations was comparable among groups. Although the difference was not statistically significant, the incidence of non-live implants and resorptions was higher at 2000 ppm than in the control group. This was likely due to the 100% postimplantation loss seen in three of the 21 pregnant females exposed to 2000 ppm (0 in other

groups).

[6] **Saillefaït and colleagues (2006)** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: EB and MEK, alone or in combination, have no effect on the average number of implantations and live fetuses, and in the incidence of non-live implants and resorptions.

[11] **Ungváry, G. & Tátrai, E. (1985)** Groups of CFY rats were exposed to inhalation of ethylbenzene at 0, 138, 276 or 553 ppm for 24 h/day from day 7 to day 15 of pregnancy. Fetuses were evaluated on pregnancy day 21. CFLP mice were exposed to inhalation of ethylbenzene at 0, 115 or 230 ppm for 24 h/day (no data provided for these groups) or for 3-4 hours/day intermittently from day 6 to 15 of pregnancy. The fetuses were evaluated on pregnancy days 18. NZ rabbits were exposed to 0, 115, or 230 ppm ethylbenzene for 24 h/day from day 7 to day 20 gestation. Fetuses were examined on pregnancy day 30. The three rabbit does in the 230-ppm dose group aborted.

Limitations: The data for mice was only provided for the animals in the 115-ppm exposure group and maternal toxicity information was lacking. The authors mention that the maternal toxic effects of ethylbenzene in rats were “moderate and dose-dependent” but fail to describe or quantify these effects. The contribution of general toxicity effects to all study findings should be considered.

Results included in WOE: The percentage of dead or resorbed fetuses was significantly increased in all ethylbenzene-exposed groups in rats (138, 276 and 553 ppm). There was no significant difference in the percentage of dead or resorbed fetuses in ethylbenzene exposed mice or rabbits compared with controls.

[13] **Andrew et al., (1981); Hardin et al. (1981)** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of implantations was comparable among groups. Post-implantation loss was inferred from the number of live fetuses, which was slightly reduced in rabbits, but not in rats, exposed to ethylbenzene at 1,000 ppm, a concentration that produced some indications of maternal systemic effects. This finding is therefore unlikely to have been produced by and endocrine mode of action.

1.10 Rank 2: Developmental Toxicity – Pre-implantation loss

[13] **Andrew et al., (1981); Hardin et al. (1981)** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for

3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Pre-implantation loss was inferred from the number of implantations per corpora lutea, which was comparable between control groups and groups exposed to 100 and 1,000 ppm ethylbenzene in both rats and rabbits.

1.11 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3 days) was significantly reduced for the F₀, 500 ppm group when compared to the F₀ control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4- to 5-day estrous cycles. Mean estrous cycle length did not differ between control and experimental F₁ offspring.

1.12 Rank 2: Reproductive Toxicity – Fertility

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ animals.

1.13 Rank 2: Developmental Toxicity – Gestational length

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No effects from ethylbenzene exposure of F₀ or F₁ animals were observed on reproductive performance parameters (mating and fertility indices, gestation lengths, former implantation sites and unaccounted sites).

1.14 Rank 2: Reproductive Toxicity – Implantations

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No effects from ethylbenzene exposure of F₀ or F₁ rats were observed on reproductive performance parameters (mating and fertility indices, gestation lengths, former implantation sites and unaccounted sites).

1.15 Rank 2: Reproductive Toxicity – Litter size

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F₁ and F₂ pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

1.16 Rank 2: Reproductive Toxicity – Mating index

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The male and female mating indices (%) were not different between any of the treatment animals and controls.

1.17 Rank 2: Reproductive Toxicity – Ovarian follicle count in offspring

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70

consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: In the F₁ females, the mean number of primordial follicles in the 500-ppm dose group was no significantly different from controls.

1.18 Rank 2: Reproductive Toxicity – Sperm count

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F₀ and F₁ males were not different between any treatment group and controls.

1.19 Rank 2: Reproductive Toxicity – Time to mating

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22.

Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ animals.

1.20 Rank 2: Reproductive Toxicity – Time to vaginal patency

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age of acquisition of vaginal patency for all exposed groups (25, 100 and 500 ppm ethylbenzene) was statistically significantly lower than the mean for the concurrent control group value in F₁ female offspring; similar differences were not observed in the F₂ female pups. The authors felt these differences were not biologically important because the mean values were comparable to the historical control mean value.

1.21 Rank 3: Repeat Dose Toxicity – Gross pathology

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5$ /dose/sex) and 13 weeks ($n = 10$ /dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

2. Estrogen Antagonist Hypothesis

2.1 Rank 2: Repeat Dose Toxicity – Epididymis histopathology

[1] **NTP (National Toxicology Program). (1999)** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] **NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the epididymides of mice or rats compared with controls.

[10] **Cragg and colleagues (1989)** Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

2.2 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

2.3 Rank 2: Repeat Dose Toxicity – Prostate histopathology

[1] **NTP (National Toxicology Program). (1999)** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] **NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the prostates of mice or rats compared with controls.

2.4 Rank 2: Repeat Dose Toxicity – Seminal vesicle histopathology

[1] **NTP (National Toxicology Program). (1999)** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the seminal vesicles of mice or rats compared with controls.

2.5 Rank 2: Repeat dose toxicity – Testis histopathology (atrophy)

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

2.6 Rank 2: Repeat Dose Toxicity – Testis weight

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

2.7 Rank 2: Developmental Toxicity – Corpora lutea

[5] **Saillenfait and colleagues (2003)** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of corpora lutea per dam did not differ between dams in any of the treatment groups and control dams.

[13] **Andrew et al., (1981); Hardin et al. (1981)** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of Corpora Lutea was unchanged by exposure to 100 and 1,000 ppm ethylbenzene relative to controls.

2.8 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3 days) was significantly reduced for the F₀, 500ppm group when compared to the F₀ control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4-5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F₁ offspring.

2.9 Rank 2: Reproductive Toxicity – Fertility

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ animals.

2.10 Rank 2: Reproductive Toxicity – Litter size

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F₁ and F₂ pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

2.11 Rank 2: Reproductive Toxicity – Sperm count

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of

the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F₀ and F₁ males were not different between any treatment group and controls.

2.12 Rank 2: Reproductive Toxicity – Time to mating

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ animals.

2.13 Rank 2: Reproductive Toxicity – Time to vaginal patency in offspring

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age of acquisition of vaginal patency for all exposed groups (25, 100 and 500 ppm ethylbenzene) was statistically significantly lower than the mean for the concurrent control group value in F₁ female offspring; similar differences were not observed in the F₂ female pups. The authors felt these differences were not biologically important because the mean values were comparable to the historical control mean value.

2.14 Rank 3: Repeat Dose Toxicity – Gross Pathology

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

3. Androgen Agonist Hypothesis

3.1 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] **NTP (National Toxicology Program). (1999)** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

3.2 Rank 2: Repeat Dose Toxicity – Sperm count

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Sperm counts (sperm count/gram testis) were not significantly different between control and ethylbenzene-exposed mice or rats.

3.3 Rank 2: Repeat dose toxicity – Testis histopathology (atrophy)

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

3.4 Rank 2: Repeat Dose Toxicity – Testis weight

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action.

Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

3.5 Rank 2: Developmental Toxicity – Implantations

[4] Saillenfait and colleagues (2007) The combined effects of EB and BA were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day⁻¹, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] Saillenfait and colleagues (2003) The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of implantation sites per litter did not differ between any of the treatment groups and controls.

[6] Saillenfait and colleagues (2006) Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: EB and MEK, alone or in combination, have no effect on the average number of implantations and live fetuses, and in the incidence of non-live implants and resorptions.

[13] Andrew et al., (1981); Hardin et al. (1981) Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: In both rats and rabbits, the number of implantations per doe and per corpora lutea was unaffected by exposure to 100 or to 1,000 ppm ethylbenzene relative to unexposed controls.

3.6 Rank 2: Developmental Toxicity – Litter size

[4] Saillenfait and colleagues (2007) The combined effects of EB and BA were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day⁻¹, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] Saillenfait and colleagues (2003) The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm. Maternal weight was significantly reduced on GD 21 at 1000 ppm and on GD 13 and 21 at 2000 ppm. Dams exposed to 1000 or 2000 ppm showed significant decreases in maternal weight gain and food consumption throughout exposure, and in corrected weight gain

Results included in WOE: The number of implantations was comparable among groups. Although the difference was not statistically significant, the incidence of non-live implants and resorptions was higher at 2000 ppm than in the control group. This was likely due to the 100% postimplantation loss seen in three of the 21 pregnant females exposed to 2000 ppm (0 in other groups).

[6] Saillenfait and colleagues (2006) Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: EB and MEK, alone or in combination, have no effect on the average number of implantations and live fetuses, and in the incidence of non-live implants and resorptions.

[13] Andrew et al., (1981); Hardin et al. (1981) Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Litter size, inferred from the number of live fetuses per litter, was slightly reduced in rabbits, but not in rats, exposed to ethylbenzene at 1,000 ppm, a concentration that produced some indications of maternal systemic effects. This finding is therefore unlikely to have been produced by an endocrine mode of action.

3.7 Rank 2: Developmental Toxicity – Sex ratio

[5] **Saillenfait and colleagues (2003)** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percentage of males per litter did not differ between any of the treatments groups and controls.

[13] **Andrew et al., (1981); Hardin et al. (1981)** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Sex ratio in rats and rabbits was unaffected by exposure to 100 or to 1,000 ppm ethylbenzene relative to unexposed controls.

3.8 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] **Faber and colleagues (2006)** Four groups of CrI:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart).

Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3 days) was significantly reduced for the F₀ 500ppm group when compared to the F₀ control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4-5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F₁ offspring.

3.9 Rank 2: Reproductive Toxicity – Fertility

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ animals.

3.10 Rank 2: Reproductive Toxicity – Implantations

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No effects from ethylbenzene exposure of F₀ or F₁ rats were observed on reproductive performance parameters (mating and fertility indices, gestation lengths, former implantation sites and unaccounted sites).

3.11 Rank 2: Reproductive Toxicity – Litter size

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F₁ and F₂ pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

3.12 Rank 2: Reproductive Toxicity – Mating index

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The male and female mating indices (%) were not different between the any of the treatment animals and controls.

3.13 Rank 2: Reproductive Toxicity – Prostate weight

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were statistically significant decreases in absolute prostate weights in the F₀ male 500 ppm group but not when these organ weights were expressed as relative to body weight. There was no significant difference in absolute or relative prostate weights in F₁ males.

3.14 Rank 2: Reproductive Toxicity – Sex ratio

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The sex distribution, measured by the % males/litter, was not different in either F₁ or F₂ litters compared with control litters.

3.15 Rank 2: Reproductive Toxicity – Sperm count

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout

mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F₀ and F₁ males were not different between any treatment group and controls.

3.16 Rank 2: Reproductive Toxicity – Time to balano-preputial separation

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age at acquisition of balanopreputial separation was significantly greater in the F₁ offspring in the 500-ppm treatment group compared with controls (PND 44.7 ± 2.0 vs. PND 43.5 ± 2.2). The mean value for the 500 ppm F₁ male group (PND 44.7) was similar to the value obtained in the F₂ generation control group (PND 45.3) and essentially equivalent to the mean historical control value (44.8 days) for the laboratory and as such, the authors stated that the significant finding was not considered biologically important. F₂ data for this measure were not published. However, the data is available in the full study report.

3.17 Rank 2: Reproductive Toxicity – Time to mating

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND)

21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ offspring.

3.18 Rank 2: Reproductive Toxicity – Time to vaginal patency

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age of acquisition of vaginal patency for all exposed groups (25, 100 and 500ppm ethylbenzene) was statistically significantly lower than the mean for the concurrent control group value in F₁ female offspring; similar differences were not observed in the F₂ female pups. The authors felt these differences were not biologically important because the mean values were comparable to the historical control mean value.

3.19 Rank 3: Repeat Dose Toxicity – Gross pathology

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bodyweight/day (mg/kg bw/day), administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

4. Androgen Antagonist Hypothesis

4.1 Rank 2: Repeat Dose Toxicity – Epididymal weight

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: The tabular data indicate there was a significant decrease in the epididymal weight in mice exposed to ethylbenzene in the 1000-ppm group. The authors note that this was not considered biologically significant since spermatid counts, sperm motility, and caudal weight were normal. The narrative portion of the report states that this significant difference was found in the epididymal weight of rats, not mice – a likely error (p. 17). The tabular data show that there was no difference in the epididymal weight of rats at any ethylbenzene exposure level.

4.2 Rank 2: Repeat Dose Toxicity – Epididymis histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5$ / dose/sex) and 13 weeks ($n = 10$ /dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was

studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the epididymides of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

4.3 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5$ / dose/sex) and 13 weeks ($n = 10$ /dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was

studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

4.4 Rank 2: Repeat Dose Toxicity – Prostate histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the prostates of mice or rats compared with controls.

4.5 Rank 2: Repeat Dose Toxicity – Seminal vesicle histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the seminal vesicle of mice or rats compared with controls.

4.6 Rank 2: Repeat Dose – Testis histopathology (atrophy)

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/$ dose/sex) and 13 weeks ($n = 10/$ dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

4.7 Rank 2: Repeat Dose Toxicity – Testis weight

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

4.8 Rank 2: Repeat Dose Toxicity – Uterus histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5$ /dose/sex) and 13 weeks ($n = 10$ /dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the uteri of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The uterus of high-exposure and controls animals of all species was subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in this organ.

4.9 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] Faber and colleagues (2006) Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3 days) was significantly reduced for the F₀, 500ppm group when compared to the F₀ control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4- to 5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F₁ offspring.

4.10 Rank 2: Reproductive Toxicity – Fertility

[2] Faber and colleagues (2006) Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three

equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ animals.

4.11 Rank 2: Reproductive Toxicity – Gross pathology

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No adverse exposure-related macroscopic pathology was noted at any level.

4.12 Rank 2: Reproductive Toxicity – Litter size

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F₁ and F₂ pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

4.13 Rank 2: Reproductive Toxicity – Prostate weight

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were statistically significant decreases in absolute prostate weights in the F₀ male 500ppm group but not when these organ weights were expressed relative to body weight. There was no significant difference in absolute or relative prostate weights in F₁ males.

4.14 Rank 2: Reproductive Toxicity – Sperm count

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F₀ and F₁ males were not significantly different between any treatment group and controls.

4.15 Rank 2: Reproductive Toxicity – Sperm motility

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean percentage of motile sperm did not differ significantly between any of the treatment group animals compared with controls.

4.16 Rank 2: Reproductive Toxicity – Time to balano-preputial separation

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 hr/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 hr apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 hr after a 6-hr inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age at acquisition of balanopreputial separation was significantly greater in the F₁ offspring in the 500ppm treatment group compared with controls (PND 44.7± 2.0 vs. PND 43.5 ± 2.2). The mean value for the 500ppm F₁ male group (PND 44.7) was similar to the value obtained in the F₂ generation control group (PND 45.3) and essentially equivalent to the mean historical control value (44.8 days) for the laboratory and as such, the authors stated that the significant finding was not considered biologically important. F₂ data for this measure were not published. However, the data is available in the full study report.

4.17 Rank 2: Reproductive Toxicity – Time to mating

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ animals.

4.18 Rank 3: Repeat Dose Toxicity – Gross pathology

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

5. Thyroid Inhibition Hypothesis

5.1 Rank 2: Repeat Dose Toxicity – Thyroid follicular cell histopathology

[1] **NTP (National Toxicology Program). (1999)** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Positive trends in the incidences of thyroid follicular cell hyperplasia occurred in mice in both males (control: 21:50; 75 ppm: 21:50; 250 ppm: 29:50; 750 ppm: 32:50) and females (18:50, 23:50, 25:50, 35:50) with significant increases in incidences relative to chamber

controls in 750 ppm males and females. There were no significant differences between control and exposed rat thyroids upon histopathological examination.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic changes seen in the thyroid glands of mice or rats.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

5.2 Rank 2: Developmental Toxicity – Fetal survival

[4] Saillenfait and colleagues (2007) The combined effects of EB and BA were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day⁻¹, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] Saillenfait and colleagues (2003) The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percent dead fetuses per litter was not significantly different between ethylbenzene treated and control dams.

[6] Saillenfait and colleagues (2006) Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no increase in embryoletality for fetuses whose mothers were exposed to ethylbenzene alone or in combination with methylethylketone.

[11] Ungváry, G. & Tátrai, E. (1985) Groups of CFY rats were exposed to inhalation of ethylbenzene at 0, 138, 276 or 553 ppm for 24 h/day from day 7 to day 15 of pregnancy. Fetuses were evaluated on pregnancy day 21. CFLP mice were exposed to inhalation of ethylbenzene at 0, 115 or 230 ppm for 24 h/day (no data provided for these groups) or for 3-4 h/day intermittently from day 6 to 15 of pregnancy. The fetuses were evaluated on pregnancy days 18. NZ rabbits were exposed to 0, 115, or 230 ppm ethylbenzene for 24 h/day from day 7 to day 20 gestation. Fetuses were examined on pregnancy day 30. The three rabbit does in the 230-ppm dose group aborted.

Limitations: The data for mice was only provided for the animals in the 115-ppm exposure group and maternal toxicity information was lacking. The authors mention that the maternal toxic effects of ethylbenzene in rats were “moderate and dose-dependent” but fail to describe or quantify these effects. The contribution of general toxicity effects to all study findings should be considered.

Results included in WOE: All rabbit dams (3/3) in the 230 ppm dose group aborted resulting in the loss of all fetuses.

[13] Andrew et al., (1981); Hardin et al. (1981) Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of implantations was comparable among groups. The number of live fetuses was slightly reduced in rabbits, but not in rats, exposed to ethylbenzene at 1,000 ppm, a concentration that produced some indications of maternal systemic effects. This finding is therefore unlikely to have been produced by an endocrine mode of action.

5.3 Rank 2: Developmental Toxicity – Fetal weight

[4] Saillenfait and colleagues (2007) The combined effects of EB and BA were investigated. Groups of 18 bred rats (15–18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day⁻¹, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: Fetal body weight was significantly decreased after exposure to 1000 ppm EB alone.

[5] Saillenfait and colleagues (2003) The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm. Maternal weight was significantly reduced on GD 21 at 1000 ppm and on GD 13 and 21 at 2000 ppm. Dams exposed to 1000 or 2000 ppm showed significant decreases in maternal weight gain and food consumption throughout exposure, and in corrected weight gain

Results included in WOE: Ethylbenzene produced a concentration-related reduction in fetal weights that achieved statistical significance at 1000 ppm. These decreases amounted to 7 and 18% of the control values at 1000 and 2000 ppm, respectively.

[6] Saillenfait and colleagues (2006) Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: The body weight of the fetuses (all, males, females) was significantly lower than control after exposure to the high concentration of EB, 1000 ppm.

[11] **Ungváry, G. & Tátrai, E. (1985)** Groups of CFY rats were exposed to inhalation of ethylbenzene at 0, 138, 276 or 553 ppm for 24 h/day from day 7 to day 15 of pregnancy. Fetuses were evaluated on pregnancy day 21. CFLP mice were exposed to inhalation of ethylbenzene at 0, 115 or 230 ppm for 24 h/day (no data provided for these groups) or for 3-4 h/day intermittently from day 6 to 15 of pregnancy. The fetuses were evaluated on pregnancy days 18. NZ rabbits were exposed to 0, 115, or 230 ppm ethylbenzene for 24 h/day from day 7 to day 20 gestation. Fetuses were examined on pregnancy day 30. The three rabbit does in the 230-ppm dose group aborted.

Limitations: The data for mice was only provided for the animals in the 115-ppm exposure group and maternal toxicity information was lacking. The authors mention that the maternal toxic effects of ethylbenzene in rats were “moderate and dose-dependent” but fail to describe or quantify these effects. The contribution of general toxicity effects to all study findings should be considered.

Results included in WOE: The percentage of weight-retarded fetuses was significantly greater in the group of rats exposed to ethylbenzene at a concentration of 553 ppm and in female rabbit fetuses at 115 ppm compared with controls. There was not a significant difference in mean fetal weights in mice exposed to ethylbenzene 3-4 hours/day intermittently at 115 ppm.

[13] **Andrew et al., (1981); Hardin et al. (1981)** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Fetal weights were unchanged relative to controls in rats or rabbit exposed to 100 or to 1,000 ppm ethylbenzene.

5.4 Rank 2: Reproductive Toxicity – Pup growth

[2] **Faber and colleagues (2006)** Four groups of CrI:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean body weight gain of males and females in the F₁ and F₂ offspring, between postnatal days 1-4, did not differ significantly from the control animals.

5.5 Rank 2: Reproductive Toxicity – Pup survival

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percentage of F₁ and F₂ pups surviving from birth to PND 4 and from PND 4-21 did not differ between treatment animals and controls.

5.6 Rank 2: Reproductive Toxicity – Thyroid weight

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. The authors note that because this finding was only found in the F₁ males and not in the F₁ females or the F₂ treatment animals that the finding was considered to be the result of normal biological variation and not related to ethylbenzene exposure. However, we could not ascertain that a histopathologic examination of the thyroid tissue was carried out to rule out pathologic changes.

Results included in WOE: Increases (approximately 18–20% and statistically significant) in absolute and relative thyroid weights in the F₀ males in the 100 and 500 ppm groups were not replicated in the F₁ male group nor were they observed in the female groups exposed to these concentrations of ethylbenzene.

5.7 Rank 3: Repeat Dose Toxicity – Liver weight

[3] **Mellert and colleagues (2007)** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. However, the histological examination of liver tissue demonstrated only centrilobular hypertrophy of hepatocytes suggesting an adaptive response.

Results included in WOE: Liver weight was increased in a dose-related fashion in both male and female rats in the mid and high dose exposure groups.

[6] **Saillenfait and colleagues (2006)** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. High doses were used, and it is unclear if the endpoint responses are secondary to general toxicity. Additionally, histological evaluation of the liver revealed no pathological effects attributable to solvent exposures therefore we agree with the authors that the positive liver weight changes are likely an adaptive response.

Results included in WOE: Compared with control, both absolute and relative liver weight were significantly elevated in animals treated with 250 and 1000 ppm ethylbenzene.

[7] **Li and colleagues (2010)** In the neurotoxicity study, ethylbenzene was administered orally via gavage twice daily to Sprague-Dawley male and female rats at 0, 25, 125, or 250 mg/kg per dose (total daily dosages of 0, 50, 250, or 500 mg/kg bw/day) for 13 weeks and the functional observational battery (FOB), automated tests for motor activity and neuropathological examination were conducted.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. In addition, there were no treatment-related microscopic lesions observed in the liver suggesting the increased relative liver weight finding represents an adaptive response rather than a pathological change.

Results included in WOE: The weights of the liver relative to terminal body weights were significantly increased ($p \leq 0.05$) in male rats at 250 and 500 mg/kg bw/day and in female rats at 500 mg/kg bw/day.

[8] **Stott and colleagues (1999)** Male and female Fischer 344 rats and B6C3F1 mice were exposed to 0 or 750 ppm ethylbenzene vapor 6 h/day for one or four weeks. Livers from 6 (one-week study) or 8 (four-week study) mice/sex/dose were examined and weighed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis,

a positive result could be due to several mechanisms. In addition, increased liver weights were not accompanied by histological changes suggesting an adaptive rather than a pathologic response.

Results included in WOE: The relative liver weight of male and female mice exposed to 750 ppm EB for one week and female mice exposed to 750 ppm for four weeks were significantly higher than those of control animals. There were no significant differences in liver weight between male mice and male controls in the four-week study.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity. There were no treatment-related microscopic lesions associated with the increased liver weights, therefore this change is considered to be an adaptive response rather than a pathological finding.

Results included in WOE: Significant increases in liver weights were seen in male rats in the 250-, 500-, 750- and 1000 ppm exposure groups and in female rats in the 500-, 750- and 1000 ppm exposure groups; significant increases in liver weights were seen in male and female mice in the 750- and 1000-ppm exposure groups.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were statistically significant increases in the liver/body weight ratios of male rats in the 782 ppm dose group and female rats in the 382 ppm and 782 ppm dose groups. Absolute liver weight was increased for female rats at 782 ppm and for male rats at 382 and 782 ppm. The absolute liver weight for female mice was also significantly increased at 782 ppm. We agree with the authors note that the absence of accompanying liver histopathology or abnormal clinical chemistry indicates that the increases were due to an adaptive induction of hepatic function rather than toxicity. Liver weights were unchanged in rabbits exposed to EB at any concentrations up to 1610 ppm.

5.8 Rank 3: Reproductive Toxicity – Liver weight

[2] Faber and colleagues (2006) Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of

the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, histological evaluation of the liver revealed no pathological effects attributable to solvent exposures therefore we agree with the authors that the positive liver weight changes are likely an adaptive response.

Results included in WOE: Absolute and relative liver weights were slightly increased (3–7%) in the 500ppm groups compared to the control group. The increases in relative liver weight were statistically significant in the F₀ and F₁ females. These increases in the liver weights were considered related to ethylbenzene exposure but not a pathological finding.

5.9 Rank 3: Developmental Neurotoxicity – Auditory startle

[12] **Faber and colleagues (2007)** Four groups of CrI:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed. Neurobehavioral development of one F₂-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no statistically significant differences in parameter of the acoustic startle test for the F₂ treatment derived offspring of either gender. On PND 20, the maximum startle amplitudes in the control group were much lower than the mean historical control values for this assessment age. Also on PND 60, a statistically significant main effect of treatment was obtained for maximum startle amplitude in the F₂ males, however there was an outlier response of three control males which inflated the mean. When these responses were removed, the within group distributions were not markedly different and closely matched historical control values. Therefore, the differences noted in males at this age were attributed to unusual control mean values and were not considered to be related to parental ethylbenzene exposure.

5.10 Rank 3: Developmental Neurotoxicity – Brain morphometry

[12] **Faber and colleagues (2007)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed. Neurobehavioral development of one F₂-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No brain morphometric changes were noted at either age (PNDs 21, 72) in the measurements taken in the height of the hemisphere and vertical thickness of the cortex, the radial thickness of the cortex, vertical heights between hippocampal pyramidal neuron layers, vertical height of the dentate hilus, the length of the ventral limb of the dentate hilus or the vertical thickness of the brainstem and base of cerebellar lobule 9, in animals of either gender.

5.11 Rank 3: Developmental Neurotoxicity – Learning and memory

[12] **Faber and colleagues (2007)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed. Neurobehavioral development of one F₂-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Assessments of straight-alley escape times and aspects of learning and memory in the Biel water maze task were initiated on PND 26 and PND 62. There were no biologically meaningful differences noted in animals of either gender at either testing age.

5.12 Rank 3: Developmental Neurotoxicity – Motor activity

[12] Faber and colleagues (2007) Four groups of CrI:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed. Neurobehavioral development of one F₂-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no statistically significant differences among the groups in cumulative session activity counts during the pre-weaning period (PND13, 17, 21). There was a significant main effect of treatment found in the repeated measure of analysis of variance for total activity counts for females on PND 61, but due to the relatively slight change in this behavior and the lack of any suggested dose-response relationship in either gender, this difference was not considered to be related to parental ethylbenzene exposure.

6. Interaction with Steroidogenesis Enzymes Hypothesis

6.1 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5$ /dose/sex) and 13 weeks ($n = 10$ /dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

6.2 Rank 2: Repeat Dose toxicity – Testis histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in

females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

6.3 Rank 2: Repeat Dose Toxicity – Uterus histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber

control animals and treatment group animals, in either species, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5/\text{dose/sex}$) and 13 weeks ($n = 10/\text{dose/sex}$) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992) Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the uteruses of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The uterus of high-exposure and controls animals of all species was subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in this organ.

6.4 Rank 2: Developmental Toxicity – Sex ratio

[5] Saillenfait and colleagues (2003) The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percentage of males per litter did not differ between any of the treatments groups and controls.

[13] **Andrew et al., (1981); Hardin et al. (1981)** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Sex ratio in rats and rabbits was unaffected by exposure to 100 or to 1,000 ppm ethylbenzene relative to unexposed controls.

6.5 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3 days) was significantly reduced for the F₀, 500ppm group when compared to the F₀ control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4-5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F₁ offspring.

6.6 Rank 2: Reproductive Toxicity – Fertility

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last

gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F₀ or F₁ offspring.

6.7 Rank 2: Reproductive Toxicity – Live births

[2] Faber and colleagues (2006) Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F₁ and F₂ pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

6.8 Rank 2: Reproductive Toxicity – Mating index

[2] Faber and colleagues (2006) Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The male and female mating indices (%) were not different between the any of the treatment animals and controls.

6.9 Rank 2: Reproductive Toxicity – Sex ratio

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The sex distribution, measured by the % males/litter, was not different in either F₁ or F₂ litters compared with control litters.

6.10 Rank 2: Reproductive Toxicity –Sperm count

[2] **Faber and colleagues (2006)** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F₀ and 25/sex/group for F₁) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F₀ and F₁ females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F₁ generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F₁ dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F₁ dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F₂ generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F₀ and F₁ males were not different between any treatment group and controls.

6.11 Rank 3: Repeat Dose Toxicity – Gross Pathology

[3] *Mellert and colleagues (2007)* Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 ($n = 5$ /dose/sex) and 13 weeks ($n = 10$ /dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/ kg bodyweight/day (mg/kg bw/day), administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

Supplemental Material C

Rationale for Excluding Studies

Gong et al., 2023.

Gong, X., Huang, Y., Duong, J., Leng, S., Zhan, F.B., Guo, Y., Lin, Y., Luo, L. 2023. Industrial air pollution and low birth weight in New Mexico, USA. *Journal of Environmental Management*, 348, part. no. 119236, DOI: 10.1016/j.jenvman.2023.119236.

This study evaluated the relationship between exposure to air pollution and Low Birth Weight (LBW) among 22,375 LBW cases and 233,340 controls in New Mexico, where the incidence of LBW has exceeded the national average in recent decades. Exposure focused on 14 common chemicals listed in the Toxic Release Inventory (TRI) and monitoring datasets, which have abundant monitoring samples. The Emission Weighted Proximity Model (EWPM) was used to calculate maternal air pollution exposure intensity. Adjusted odds ratios (adjORs) were calculated using binary logistic regressions to examine the association between maternal residential air pollution exposure and LBW, while controlling for potential confounders, such as the maternal age, race/ethnicity, gestational age, prenatal care, education level, consumption of alcohol during pregnancy, public health regions, child's sex, and the year of birth. Multiple comparison correction was applied using the False Discovery Rate approach. Maternal residential exposure to 1,2,4-trimethylbenzene, benzene, chlorine, ethylbenzene, and styrene was associated with LBW in offspring, with adjusted odds ratios ranging from 1.10 to 1.13. These five chemicals remained as significant risk factors after dividing the estimated exposure intensities into four categories. In addition, significant linear trends were found between LBW and maternal exposure to each of the five identified chemicals. Furthermore, 1,2,4-trimethylbenzene was identified as a risk factor to LBW for the first time.

Although statistically significant, the adjusted odds ratios reported in this study are of such low magnitude that their biological relevance is uncertain. Despite reporting significant linear trends for all five chemicals individually, the adjusted odds ratios are not quantifiable for ethylbenzene exposure alone, as the exposure was to air pollution generally rather than to specific chemicals. Exposures were estimated based on residential locations, excluded potential workplace exposures, and did not consider several potentially-important confounders for LBW, such as genetic factors, exposure to tobacco smoke, and exposure to other air pollutants, including those on the Criteria Air Pollutants (CAPs) list. As such, the results are not interpretable for use in the WoE evaluation.

Gong et al., 2018.

Gong, X., Lin, Y., Bell, M. L., & Zhan, F. B. (2018). Associations between maternal residential proximity to air emissions from industrial facilities and low birth weight in Texas, USA. *Environ Int*, 120, 181–198. <https://doi.org/10.1016/j.envint.2018.07.045>

Gong et al., (2018) is a forerunner to Gong et al., 2023 that investigated associations between maternal residential exposure to industrial air pollutants during pregnancy and low birth weight (LBW) in offspring using a case-control design that included 94,106 term LBW cases and 376,424 controls. The analysis covered 78 air pollutants common to both the Toxic Release Inventory and ground air quality monitoring databases in Texas during 1996 – 2008. The authors report an adjusted odds ratios for ethylbenzene of 1.05 (95% CI: 1.03 – 1.06). Although statistically significant, the biological significance of such a low odds ratio is questionable. Confounding exposures and inadequate control of other factors that could influence birth weight render the results uninterpretable for a WoE analysis of potential endocrine activity for ethylbenzene.

Supplemental Material C

Rationale for Excluding Studies

Harrath et al., 2022.

Harrath AH, Alrezaki A, Jalouli M, Aldawood N, Aldahmash W, Mansour L, & Alwasel S. (2022). Ethylbenzene exposure disrupts ovarian function in Wistar rats via altering folliculogenesis and steroidogenesis-related markers and activating autophagy and apoptosis. *Ecotoxicol Environ Saf*, 229, 113081. doi:10.1016/j.ecoenv.2021.113081.

In this repeat dose toxicity study, Harrath et al., (2022) exposed rats to EB for 30 minutes per day for 30 consecutive days to 2,000 ppm, 4,000 ppm, and 8,000 ppm EB. Ovary weight was slightly reduced at 2,000 ppm, but not at 4,000 or 8,000 ppm. Abnormal follicles were observed at 2,000 and 4,000 ppm but the effect was barely significant at 8,000 ppm. Circulating estradiol levels were increased at 4,000 but not at 2,000 or 8,000 ppm. Circulating testosterone was increased at 2,000 and 8,000 ppm, but not at 4,000 ppm and estrogen receptor numbers were also altered.

The lack of clear dose-response relationships for these various effects makes the results difficult to interpret, but this is the least of the problems with this study. All exposure levels produced significant apoptosis in the affected organs, which confounds the interpretation of an endocrine MoA as each endpoint was likely affected secondary to induction of apoptosis. The authors assert that apoptosis is the primary mechanism underlying various other effects observed in the study. Moreover, the reported levels of exposure strain credibility. The reported exposure concentrations are equivalent to 2.5X, 5X, and 10X the IDLH value* for human occupational exposures, and near within 1/4, 1/2, and 1X the explosive limit of the chemical. The lowest exposure level used in this study equals or exceeds the highest level used in other studies, and is tenfold above the KMD for EB (Burgoon et al., 2023). Even though the exposure durations were short (30 minutes), the degree of kinetic overload these would produce and the obvious confounding by apoptosis and other unknown mechanisms renders the effects reported by Harrath et al. (2022) unreliable and uninterpretable for the purposes of an endocrine WoE analysis.

Lei, T., Qian, H., Yang, J., Hu, Y.

Lei, T., Qian, H., Yang, J., & Hu, Y. (2023). The association analysis between exposure to volatile organic chemicals and obesity in the general USA population: A cross-sectional study from NHANES program. *Chemosphere*, 315, 137738. <https://doi.org/10.1016/j.chemosphere.2023.137738>

This study attempted to evaluate whether recent reports of an association between exposure to volatile organic chemical (VOC) pollutants, measured as urinary metabolites, and obesity are general, or associated specifically with abdominal obesity. Data from the 6 survey cycles (2005–2006, 2011–2018, 2017–2020) of the NHANES program were analyzed by 4 separate models in a cross-sectional study among a total of 17,524 participants (4965 obesity, 7317 abdominal obesity). Participants in the obesity or abdominal obesity groups showed higher VOCs in urine than were present in the control group. OR for obesity in the Q2 to Q4 of model 3 was 1.169 (Q2, $p < 0.05$), 1.306 (Q3, $p < 0.001$) and 1.217 (Q4, $p < 0.01$) respectively. The OR for abdominal obesity in the Q2 to Q4 of model 3 was 1.222 (Q2, $p < 0.01$), 1.448 (Q3, $p < 0.001$) and 1.208 (Q4, $p < 0.05$) respectively. A significantly positive association between urine levels of VOCs (Acrolein, Acrylamide, Acrylonitrile, 1,3-Butadiene, Crotonaldehyde, Cyanide, N,N-Dimethylformamide, Ethylbenzene, Styrene, Propylene oxide, Toluene and Xylene) and BMI and waist circumference was reported.

None of the analyses were specific to ethylbenzene, and the models employed were incapable of determining whether the direction of the associations, i.e., whether exposure begat obesity or obesity enhanced absorption of VOCs. The endpoints measured were unusable in the WoE evaluation due to confounding by multiple chemical exposures.

Supplemental Material C

Rationale for Excluding Studies

Nakhjirgan et al. 2019.

Nakhjirgan P, Kashani H, Naddafi K, Nabizadeh R, Amini H, & Yunesian M. (2019). Maternal exposure to air pollutants and birth weight in Tehran, Iran. *J Environ Health Sci Eng*, 17(2), 711-717. doi:10.1007/s40201-019-00386-7

Although the authors mention EB in the context of air pollutants in urban Tehran, Iran, the study evaluated potential associations between air pollutants broadly and health outcomes in pregnant women. Since exposures to EB were unspecified and uncertain and the results indicative only of potential associations with urban air generally, the results of the study are not informative regarding potential endocrine MoAs for EB.

Rouget et al., 2021.

Rouget F, Bihannic A, Cordier S, Multigner L, Meyer-Monath M, Mercier F, Pladys P, Garlantezec R. (2021). Petroleum and Chlorinated Solvents in Meconium and the Risk of Hypospadias: A Pilot Study. *Front Pediatr*, 9, 640064. doi:10.3389/fped.2021.640064

Rouget et al. (2021) conducted a pilot case-control study in the maternity unit of the University Hospital in Rennes, France to evaluate possible associations between the occurrence of hypospadias and fetal exposure to petroleum and chlorinated solvents measured in meconium. Since exposures to EB were unspecified and uncertain and the results indicative only of potential associations with petroleum and chlorinated solvents generally, the results of the study are not useful for an endocrine WoE evaluation.

Werder et al. 2019.

Werder EJ, Engel LS, Blair A, Kwok RK, McGrath JA, & Sandler DP. (2019). Blood BTEX levels and neurologic symptoms in Gulf states residents. *Environ Res*, 175, 100-107. doi:10.1016/j.envres.2019.05.004

Werder et al. (2019) evaluated potential associations between blood levels of BTEX chemicals (benzene, toluene, EB, and xylene) in Gulf coast residents of the United States who were transiently exposed to BTEX during the Deepwater Horizon oil spill and/or the response to it. Although the publication mentions endocrine disruptive effects of BTEX, the authors generated no data relevant to specific endocrine effects of EB.

Werder et al. 2020.

Werder EJ, Beier JI, Sandler DP, Falkner KC, Gripshover T, Wahlang B, . . . Cave MC. (2020). Blood BTEXS and heavy metal levels are associated with liver injury and systemic inflammation in Gulf states residents. *Food Chem Toxicol*, 139, 111242. doi:10.1016/j.fct.2020.111242.

Werder et al., 2020 conducted a clinical cross-sectional analysis to evaluate possible associations of biomarkers with serum liver injury and adipocytokine biomarkers in a sample of 214 men. No data relevant to specific endocrine effects of EB were reported. However, with respect to endocrine disruptive effects as speculated by the authors, their results suggest that rather than an endocrine mechanism, liver toxicity would be the likely mechanism that secondarily affects endocrine parameters.

Supplementary Table 1. Estrogen Agonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	FSTRA	Vitellogenin	↑♂		
	Uterotrophic	Uterus weight (blotted or wet)	↑		
2	Uterotrophic	Conversion to estrus	↑		
	ERTA	Reporter gene activation	↑		
	FSTRA	Behavioral (sexual, mating)	Δ♂		
		Gonad histopathology	Δ♂		
		Tubercle score	↓♂		
	Female Pubertal	Age and body weight at vaginal opening	↓		
		Age at first estrus	↓		
		Ovary histopathology	Δ		
		Ovary weight	↓		
	Male Pubertal	Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
	Repeat Dose Toxicity	Epididymis histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Epididymis weight	↓	[9s,m]	[9s,r]
		Mammary histopathology	Δ		
		Ovary histopathology	Δ		[1c,m,r][3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary weight	↓		
		Prostate weight	↓		
		Seminal vesicle weight	↓		
		Testis histopathology (atrophy)	↑		[1c,m,r] [9s,m,r]
		Testis weight	↓		[9s,m,r]
Uterus histopathology		Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]	
Uterus weight		↑			
Vaginal histopathology		Δ		[1c,m,r] [3s,r]	
Developmental Toxicity		Corpora lutea	↓		[5r][13r,rb]
	Post-implantation loss	↑	[11r] [13rb]	[4r] [5r] [6r] [11m,rb] [13r]	

Supplementary Table 1. Estrogen Agonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene	
2	Developmental Toxicity	Pre-implantation loss	↑		[13r,rb]	
		Time to vaginal patency	↓			
	Reproductive Toxicity	Anogenital distance	Δ ♂, ♀			
		Corpora lutea	↓			
		Epididymis histopathology (atrophy)	Δ			
		Epididymis weight	↓			
		Estrous cyclicity	Δ	[2r,F ₀]	[2r,F ₁]	
		Fertility	↓ ♂, ♀		[2r,F ₀ ,F ₁]	
		Gestational length	↓ ♀		[2r,F ₀ ,F ₁]	
		Implantations	↓		[2r,F ₀ ,F ₁]	
		Litter size	↓		[2r,F ₀ ,F ₁]	
		Mammary histopathology	Δ ♀			
		Mating index	↓ ♂, ♀		[2r,F ₀ ,F ₁]	
		Ovarian follicle count in offspring	Δ		[2r,F ₁]	
		Ovary histopathology	Δ			
		Ovary weight in offspring	↓			
		Prostate histopathology (atrophy)	Δ			
		Prostate weight	↓			
		Seminal vesicle weight	↓			
		Sperm count	↓		[2r,F ₀ ,F ₁]	
		Testis histopathology (atrophy)	Δ			
		Testis weight (absolute)	↓			
		Time to mating	↑ ♂, ♀		[2r,F ₀ ,F ₁]	
		Time to preputial separation	↑ ♂			
		Time to vaginal patency	↓	[2r,F ₁]	[2r,F ₂]	
		Uterus histopathology	Δ			
	Uterus weight in offspring	↑				
Vaginal histopathology	Δ					
3	ERBA	Displacement of Estradiol	↑			
	FSTRA	Behavior	Δ			
		Estradiol level	↓ ♀			
		Fecundity	↓			

Supplementary Table 1. Estrogen Agonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
3	FSTRA	Fertilization success	↓ ♀		
		Follicular atresia	↑		
		Gonad somatic index	↓ ♂, ↑ ♀		
		Testosterone level	↓ ♂		
	Female Pubertal	Estrous cyclicity	↑		
		Growth	↑		
	Male Pubertal	Epididymis histopathology	Δ		
		Growth	Δ		
		Ventral prostate weight	Δ		
	Steroidogenesis	Estradiol level	↑		
	Repeat Dose Toxicity	Gross pathology	Δ ♂, ♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F₀ = F₀ generation; F₁ = F₁ generation; F₂ = F₂ generation.

Supplementary Table 2. Estrogen Antagonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Uterotrophic	Uterus weight increase w E2	↓		
2	ERBA	Displacement of estradiol	↑		
	FSTRA	Gonad histopathology	Δ ♀		
		Vitellogenin	↓ ♀		
	Female Pubertal	Age and body weight at vaginal opening	↑		
		Age at first estrus	↑		
	Repeat Dose Toxicity	Epididymis histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary histopathology	Δ		[1c,mr] [3s,r] [9s,m,r] [10s,m,r,rb]
		Prostate histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
		Seminal vesicle histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
		Testis histopathology (atrophy)	↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Testis weight	↓		[9s,m,r]
	Developmental Toxicity	Corpora lutea	↓		[5r] [13r,rb]
		Time to vaginal patency	↓		
	Reproductive Toxicity	Corpora lutea	↓		
		Epididymis histopathology (atrophy)	Δ		
		Estrous cyclicity	Δ	[2r,F ₀]	[2r,F ₁]
		Fertility	↓ ♂, ♀		[2r,F ₀ ,F ₁]
		Litter size	↓		[2r,F ₀ ,F ₁]
		Ovary histopathology	Δ		
		Prostate histopathology (atrophy)	Δ		
Seminal vesicle histopathology		Δ			

Supplementary Table 2. Estrogen Antagonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
2	Reproductive Toxicity	Sperm count	↓		[2r,F ₀ ,F ₁]
		Testis histopathology (atrophy)	Δ		
		Testis weight (absolute)	↓		
		Time to mating	↑ ♂, ♀		[2r,F ₀ ,F ₁]
		Time to vaginal patency	↑	[2r,F ₁]	[2r,F ₂]
3	Aromatase Inhibition	Aromatase inhibition	↓		
	FSTRA	Behavior	Δ ♀		
		Estradiol level	↓ ♀		
		Fecundity	↓		
		Fertilization success	↓ ♀		
		Gonad somatic index	↓ ♀, ♂		
		Testosterone level	↓ ♂		
	Female Pubertal	Estrous cyclicity	Δ		
		Ovary histopathology (atrophy)	Δ		
		Ovary weight (with atrophy)	↓		
	Steroidogenesis	Estradiol level	↓		
	Repeat Dose Toxicity	Gross pathology	Δ ♂, ♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F₀ = F₀ generation; F₁ = F₁ generation; F₂ = F₂ generation.

Supplementary Table 3. Androgen Agonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Hershberger	Concordance of 5 endpoints	↑		
	FSTRA	Secondary sexual characteristics: tubercles in females	↑		
2	ARBA	Displacement of testosterone	↑		
	FSTRA	Gonad histopathology	Δ		
		Vitellogenin	↓♀		
	Male Pubertal	Age & weight at preputial Separation: if accelerated	↓		
		Dorsolateral prostate weight	↑		
		Epididymis histopathology	Δ		
		Epididymis weight	↑		
		LABC weight	↑		
		Seminal vesicle + coagulating gland weight	↑		
		Testis histopathology (atrophy)	Δ		
		Testis weight	↑		
		Ventral prostate weight	↑		
		Hershberger	Concordance of 2 to 4 endpoints	↑	
	Repeat Dose Toxicity	Ovary histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary weight	↑		
		Prostate weight	↓		
		Seminal vesicle weight	↓		
		Sperm count	↓		[9s,m,r]
		Testis histopathology (atrophy)	↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Testis weight	↓		[9s,m,r]
		Uterus weight	↑		
	Developmental Toxicity	Implantations	↓		[4r] [5r] [6r] [13r,rb]
		Litter size	↓	[13rb]	[4r] [5r] [6r] [13r]
		Masculinization of female offspring	↑		
		Sex ratio	Δ ♂, ♀		[5r] [13r,rb]
		Time to balano-preputial separation	↓♂		
		Time to vaginal patency	↑		

Supplementary Table 3. Androgen Agonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
2	Reproductive Toxicity	Anogenital distance	↑ ♀		
		Estrous cyclicity	↓	[2r,F ₀]	[2r,F ₁]
		Fertility	↓ ♂, ♀		[2r,F ₀ ,F ₁]
		Implantations	↓		[2r,F ₀ ,F ₁]
		Litter size	↓		[2r,F ₀ ,F ₁]
		Masculinization of female offspring	↑ ♀		
		Mating index	↓ ♂, ♀		[2r,F ₀ ,F ₁]
		Nipple retention	↑ ♂		
		Ovarian follicle count	↓		
		Ovary histopathology	Δ		
		Ovary weight in offspring	↑		
		Prostate weight	↓		[2r,F ₀ ,F ₁]
		Seminal vesicle weight	↓		
		Sex ratio	Δ ♂, ♀		[2r,F ₁ ,F ₂]
		Sperm count	↓		[2r,F ₀ ,F ₁]
		Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
		Time to balano-preputial separation	↓ ♂	[2r,F ₁]	[2r,F ₂]
		Time to mating	↑ ♀		[2r,F ₀ ,F ₁]
		Time to vaginal patency	↑	[2r,F ₁]	[2r,F ₂]
3	Aromatase	Aromatase activity	↑		
	FSTRA	Behavior	Δ		
		Estradiol level	Δ		
		Fecundity	Δ		
		Fertilization success	Δ		
		Gonad somatic index	Δ		
		Testosterone level	Δ		
	Female Pubertal	Adrenals weight	↓		
		Age & weight at vaginal opening	↑		
		Growth	↑		
		Ovary histopathology	Δ		
		Ovary weight	↓		
		Uterus histopathology	Δ		
	Male Pubertal	Growth	↑		
		Testosterone level	↓		
	Steroidogenesis	Testosterone level	Δ		
	Hershberger	Concordance of 1 endpoint	↑		
Repeat Dose Toxicity	Gross pathology	Δ ♂, ♀		[3s,r]	

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F₀ = F₀ generation; F₁ = F₁ generation; F₂ = F₂ generation.

Supplementary Table 4. Androgen Antagonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Hershberger	Concordance of 5 endpoints	↓		
2	ARBA	Displacement of testosterone	↑		
	FSTRA	Gonad histopathology	Δ ♂		
		Secondary sexual characteristics	↓ ♂		
		Vitellogenin	↑ ♀		
	Male Pubertal	Age & weight at preputial separation: if delayed	↑		
		Dorsolateral prostate weight	↓		
		Epididymis histopathology	Δ		
		Epididymis weight	↓		
		LABC weights	↓		
		Seminal vesicle + coagulating gland weight	↓		
		Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
		Ventral prostate weight	↓		
	Hershberger	Concordance of 2 to 4 endpoints	↓		
	Repeat Dose Toxicity	Epididymal weight	↓	[9s,m]	[9s,r]
		Epididymis histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Prostate histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
		Prostate weight	↓		
		Seminal vesicle histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
Seminal vesicle weight		↓			
Testis histopathology (atrophy)		↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]	
Testis weight		Δ		[9s,m,r]	
Uterus histopathology		Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]	

Supplementary Table 4. Androgen Antagonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
2	Developmental Toxicity	Time to balano-preputial separation	↑ ♂		
		Anogenital distance	↓ ♂		
	Reproductive Toxicity	Epididymis weight	↓		
		Epididymis histopathology	Δ		
		Estrous cyclicity	↓	[2r,F ₀]	[2r,F ₁]
		Fertility	↓ ♂,♀		[2r,F ₀ ,F ₁]
		Gross pathology	Δ ♂,♀		[2r,F ₀ ,F ₁]
		Litter size	↓		[2r,F ₀ ,F ₁]
		Nipple retention	↑ ♂		
		Ovary histopathology	Δ		
		Prostate histopathology	Δ		
		Prostate weight	↓		[2r,F ₀ ,F ₁]
		Seminal vesicle histopathology	Δ		
		Seminal vesicle weight	↓		
		Sperm count	↓		[2r,F ₀ ,F ₁]
		Sperm motility	↓		[2r,F ₀ ,F ₁]
		Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
		Time to balano-preputial separation	↑ ♂	[2r,F ₁]	[2r,F ₂]
		Time to mating	↑ ♀		[2r,F ₀ ,F ₁]
Uterus histopathology	Δ				
3	FSTRA	Behavior	Δ		
		Estradiol level	Δ		
		Fecundity	↓		
		Fertilization success	↓		
		Gonad-somatic index	↓		
		Testosterone level	↑ ♂		
	Male Pubertal	Testosterone level	↑		
	Steroidogenesis	Testosterone level	Δ		
	Hershberger	Concordance of 1 endpoint	↓		
	Repeat Dose Toxicity	Gross pathology	Δ ♂,♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F₀ = F₀ generation; F₁ = F₁ generation; F₂ = F₂ generation.

Supplementary Table 5. Thyroid Inhibition Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Male Pubertal	Thyroid histopathology	Δ		
		Thyroid weight	↑		
	Female Pubertal	Thyroid (colloid area & follicular cell height)	↑		
		Thyroid weight	↑		
	AMA	Asynchronous development	↑		
Thyroid histopathology		Δ			
2	Female Pubertal	Age & weight at vaginal opening	↑		
		T4 level	↑		
		TSH level	↑		
	Male Pubertal	Liver weight	Δ		
		T4 level	↑		
		TSH level	↑		
	Repeat Dose Toxicity	Thyroid follicular cell histopathology	Δ ♂, ♀	[1c,m]	[1c,r][3s,r] [9s,m,r] [10s,m,r,rb]
		Thyroid hormones	Δ ♂, ♀		
		Thyroid weight	↑ ♂, ♀		
	Developmental Toxicity	Fetal survival	↓ ♂, ♀	[11rb] [13rb]	[4r] [5r] [6r] [11r] [13r]
		Fetal weight	↓ ♂, ♀	[4r] [5r] [6r] [11r,rb]	[11m] [13r,rb]
		Thyroid follicular cell histopathology	Δ ♂, ♀		
		Thyroid hormones	Δ ♂, ♀		
		Thyroid weight	↑ ♂, ♀		
	Reproductive Toxicity	Fetal weight	↓ ♂, ♀		
		Pup growth	↓ ♂, ♀		[2r,F ₁ ,F ₂]
		Pup survival	↓ ♂, ♀		[2r,F ₁ ,F ₂]
Pup weight		↓ ♂, ♀			
Thyroid follicular cell histopathology		Δ ♂, ♀			
Thyroid hormones		Δ ♂, ♀			
Thyroid weight		↑ ♂, ♀	[2r,F ₀ ♂]	[2r,F ₀ ♀,F ₁]	
3	Male Pubertal	Age & weight at preputial separation	↑		
		Growth	↓		
		Pituitary weight	↓		

Supplementary Table 5. Thyroid Inhibition Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
3	Female Pubertal	Estrous cyclicity (diestrus)	Δ		
		Ovary histopathology	Δ		
		Ovary weight	↓		
	AMA	Delayed development	↑		
		Hind limb length	↓		
		Snout-vent length	Δ		
		Wet weight	↑		
	Repeat Dose Toxicity	Liver weight	↑ ♂, ♀	[3s,r] [6r] [7s,r] [8s,m] [9s,m,r] [10s,m,r]	[10s,rb]
	Reproductive Toxicity	Liver weight	↑ ♂, ♀	[2r,F ₀ ,F ₁]	
	Developmental Neurotoxicity	Auditory Startle	↓ ♂, ♀		[12,F ₂]
		Behavioral Ontogeny	↓ ♂, ♀		
		Brain Morphometry	Δ ♂, ♀		[12F ₂]
		Learning and Memory	↓ ♂, ♀		[12F ₂]
		Liver weight	↑ ♂, ♀		
Motor Activity		Δ ♂, ♀		[12F ₂]	
Myelination		Δ ♂, ♀			
Pup growth		↓ ♂, ♀			
Pup survival	↓ ♂, ♀				

♂ = males; ♀ = females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F₀ = F₀ generation; F₁ = F₁ generation; F₂ = F₂ generation.

**Supplementary Table 6. Interaction with Steroidogenesis Enzymes Hypothesis;
Guideline Toxicity Studies**

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene	
1	Female Pubertal	Uterus weight	↓			
2	Female Pubertal	Ovary weight	↓			
	FSTRA	Gonad histopathology: males	Δ			
		Vitellogenin	↓ ♀			
	Steroidogenesis	Estradiol level	↓			
		Testosterone level	↓			
	Repeat Dose Toxicity	Ovary histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]	
		Ovary weight	↓			
		Testis histopathology (atrophy)	↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]	
		Uterus histopathology	Δ		[1,c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]	
		Uterus weight	↓			
	Developmental Toxicity	Sex ratio	Δ ♂, ♀		[5r] [13r,rb]	
	Reproductive Toxicity	Estrous cyclicity	Δ		[2r,F ₀]	[2r,F ₁]
		Fertility	↓ ♂, ♀			[2r,F ₀ ,F ₁]
		Live births	↓ ♀			[2rF ₀ ,F ₁]
		Mating index	↓ ♂			[2r,F ₀ ,F ₁]
		Ovary histopathology	Δ			
		Parturition	↓			
		Post-implantation loss	↑			
		Resorptions	↑			
		Sex ratio	Δ ♂, ♀			[2r,F ₀ ,F ₁]
Sexual behavior		Δ ♂				
Sperm count		↓			[2r,F ₀ ,F ₁]	
Testicular histopathology (atrophy)		Δ				
Uterus histopathology		Δ				
Uterus weight	↓					

**Supplementary Table 6. Interaction with Steroidogenesis Enzymes Hypothesis;
Guideline Toxicity Studies**

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
3	Aromatase	Aromatase activity	↓		
	Female Pubertal	Age & weight at vaginal opening	↑		
		Age at first estrus	↑		
	Male Pubertal	Testosterone level	Δ		
	FSTRA	Behavior	Δ		
		Estradiol level	Δ		
		Fecundity	Δ		
		Fertilization success	Δ		
		Gonad-somatic index	Δ		
	Testosterone level	Δ			
Repeat Dose Toxicity	Gross pathology	Δ ♂, ♀		[3s,r]	

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F₀ = F₀ generation; F₁ = F₁ generation; F₂ = F₂ generation.

Supplementary Table 7. Summary of Endpoints from all Tables

MoA	Fraction of Rank 1 Endpoints Tested	# of Rank 1 Endpoints Responding to Ethylbenzene	# of Rank 1 Endpoints Showing No Response to Ethylbenzene	Fraction of Rank 2 Endpoints Tested	# of Rank 2 Endpoints Responding to Ethylbenzene	# of Rank 2 Endpoints Showing No Response to Ethylbenzene	Fraction of Rank 3 Endpoints Tested	# of Rank 3 Endpoints Responding to Ethylbenzene	# of Rank 3 Endpoints Showing No Response to Ethylbenzene
Estrogen Agonist - Table 1	0 (2)	0	0	20 (53)	4	20	1 (15)	0	1
Estrogen Antagonist - Table 2	0 (1)	0	0	13 (26)	2	13	1 (12)	0	1
Androgen Agonist - Table 3	0 (2)	0	0	18 (47)	4	18	1 (19)	0	1
Androgen Antagonist - Table 4	0 (1)	0	0	17 (45)	3	17	1 (10)	0	1
Thyroid Inhibition - Table 5	0 (6)	0	0	6 (21)	4	6	6 (21)	2	5
Steroidogenesis - Table 6	0 (1)	0	0	10 (25)	1	10	1 (11)	0	1
Hershberger endpoints counted as one concordance response									