Information Quality Guidelines Staff
U.S. Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC, 20460

Re: Request for Correction of Information under the Information Quality Act: The Toxic Substances Control Act (TSCA) Risk Evaluation for 1,3-Butadiene

Dear Sir or Madam:

The American Chemistry Council (ACC) 1,3-Butadiene TSCA Risk Evaluation Consortium (Consortium) submits this Request for Correction of information (RFC) on the final "*Risk Evaluation for 1,3-Butadiene CASRN: 106-99-0*" (Final 1,3-BD RE) issued by the U.S. Environmental Protection Agency's (EPA) Office of Pollution Prevention and Toxics (OPPT) on December 31, 2025.[1] This RFC is submitted under the Information Quality Act (IQA) and the implementing guidelines issued by the Office of Management and Budget (OMB) and EPA.[2] [3] [4]

Specifically, this RFC seeks correction of certain technical content and quantitative risk estimates in the final 1,3-BD RE, including: (a) cancer dose-response modeling and the resulting inhalation unit risk (IUR); (b) life-table risk calculations and parameterization; (c) selection and treatment of epidemiological datasets and endpoints; and (d) the noncancer hazard assessment and rejection of data-derived extrapolation factors (DDEFs).

This RFC seeks correction of the underlying methods and results identified below. These corrections are necessary to ensure the Agency's information satisfies IQA requirements for objectivity, utility, and integrity, is aligned with EPA's own scientific guidance and procedures, and complies with the Toxic

[1] EPA (2025) Final Risk Evaluation for 1,3 Butadiene CASRN: 106-99-0, EPA available at https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-evaluation-13-butadiene

[2] Pub. L. No. 106-554, Section 515.

[3] OMB (2002) *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies*, 67 Fed. Reg. 8452.

[4] EPA (2002) *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity, of Information Disseminated by the Environmental Protection Agency*, EPA/260R-02-008 (Oct. 2002), available at https://www.epa.gov/sites/default/files/2020-02/documents/epa-info-quality-guidelines_pdf_version.pdf.

Substances Control Act's (TSCA) requirements to incorporate best available science and make decisions based on the weight of the scientific evidence in risk evaluations.[5]

The Consortium identified a mathematical error in EPA's calculation of an IUR for leukemia, which is used to support all risk calculations in the risk evaluation as well as the Occupational Exposure Value (OEV) for 1,3-BD. Furthermore, the use of overly conservative assumptions in the cancer hazard assessment based on epidemiological studies has resulted in a final OEV that does not align with IQA requirements and the principles of Gold Standard Science referenced in Executive Order (EO) 14303.[6] The EO emphasizes that agency publications must rely on the best available science, clearly communicate error and uncertainty, and undergo unbiased peer review.

1. **Name and contact information for the individual or organization submitting a complaint; identification of an individual to serve as a contact.**

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2. **A description of the information the person believes does not comply with EPA or OMB guidelines, including specific citations to the information and to the EPA or OMB guidelines, if applicable.**

The June 22, 2016, amendments to TSCA required EPA to initiate risk evaluations on chemical substances. In December 2019, OPPT published a list of twenty high priority chemical substances, which included 1,3-BD.

OPPT released the draft risk evaluation for 1,3-BD with a request for comment on December 03, 2024. OPPT held a peer review meeting from April 1-4, 2025, with its Science Advisory Committee on Chemicals (SACC)[7] to review the draft risk evaluation. It should be noted that the SACC meeting did not reflect best peer-review practices and is significantly in variance with the

[5] 15 U.S.C. § 2625(h), (i).
[6] https://www.whitehouse.gov/presidential-actions/2025/05/restoring-gold-standard-science/
[7] Peer Review of 2024 Draft Risk Evaluation for 1,3-Butadiene | US EPA https://www.epa.gov/tsca-peer-review/peer-review-2024-draft-risk-evaluation-13-butadiene

Agency's own guidance on the conduct of peer review.[8] [9]

On December 31, 2025, EPA announced the availability of the Final Risk Evaluation for 1,3-BD. EPA noted in that announcement that it was required, for those conditions of use (COU) for which unreasonable risks were identified, to "initiate regulatory action to address those risks through risk management measures enumerated in 15 U.S.C. 2605(a) [TSCA Section 6(a)]."

We note that in developing the Final 1,3-BD RE, the Agency included a mathematical error in its calculation of an IUR, which is used to support all risk calculations in the risk evaluation. Furthermore, the agency relied on overly conservative assumptions that produced a cascading effect throughout the assessment, ultimately yielding an occupational exposure value (OEV) that does not comport with TSCA's best available science standard or Gold Standard Science. TSCA's scientific requirements are fully aligned with the purpose and intent of the IQA, which is to ensure and maximize the quality, objectivity, utility, and integrity of information disseminated by the Agency.

The IQA applies to the Final 1,3-BD RE because it constitutes information disseminated by EPA to the public. In addition, the Final 1,3-BD RE qualifies as "influential" scientific information, as OPPT must rely on it to propose and promulgate regulations under TSCA Section 6 to address the unreasonable risks identified therein—regulations that bear a "clear and substantial impact" on significant public policies and private sector decisions. Accordingly, it is imperative that EPA's risk management actions be grounded in the best available science and the weight of the scientific evidence. Moreover, TSCA Section 26 requires EPA to meet this standard.[10]

EPA interpreted TSCA Section 26(i) in its "Procedures for Chemical Risk Evaluation under the Amended Toxic Substances Control Act" issued in 2017 (the 2017 RE Rule) as:[11]

> *Weight of scientific evidence means a systematic review method*, applied in a manner suited to the nature of the evidence or decision, *that uses a pre-established protocol* to comprehensively, objectively, transparently, and consistently, identify and evaluate each stream of evidence, including strengths, limitations, and

[8] Appendix E-ACC1,3-Butadiene TSCA Risk Evaluation Consortium Letter dated April 30,2025. https://www.regulations.gov/document/EPA-HQ-OPPT-2024-0425-0125

[9] https://www.regulations.gov/document/EPA-HQ-OW-2019-0583-0125

[10] 15 U.S.C. § 2625(h), (i).

[11] 82 Fed. Reg. 33726 (July 20, 2017) (emphasis added). The 2017 RE Rule applies to the 1,3-BD risk evaluation because it was initiated in 2019, prior to revisions to the rule in 2024.

relevance of each study and *to integrate evidence* as necessary and appropriate based upon strengths, limitations, and relevance.

3. **An explanation of how the information does not comply with EPA or OMB guidelines and a recommendation of corrective action. EPA considers that the complainant has the burden of demonstrating that the information does not comply with EPA or OMB guidelines and that particular corrective action would be appropriate.**

Below, we outline how EPA's mathematical errors and use of overly conservative assumptions in the cancer hazard assessment based on epidemiological studies are inconsistent with the scientific standards under TSCA. These standards do not supersede the requirements under the IQA or EPA's requirements for complying with the IQA. The scientific standards under TSCA are, however, consistent with the intent of the IQA for "Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the [EPA]."[12]

The following outline is also supplemented with the supporting appendices which provide further details and are included as part of this RFC.

a.) Errors in the Inhalation Unit Risk (IUR) – A mathematical error was identified in EPA's calculation of an IUR for leukemia, which is used to support all risk calculations in the risk evaluation, as well as the OEV for 1,3-BD. Specifically, an error was made in calculating a 95th percentile value from the 97.5th percentile value of the Cox Proportional Hazards (CPH) slope term for leukemia, derived from Sathiakumar et al. (2021a). This value appears in the lifetable calculation spreadsheet "*28-1-3-butadiene-lifetable-analysis-of-leukemia-and-bladder-cancer-public-release-dec-2025.xlsx*." EPA's 95th percentile value, along with a corrected value, is summarized below.

EPA calculation:

- Maximum likelihood estimate of CPH slope term reported by Sathiakumar et al. (2021a): 9.94×10^{-4}
- 97.5th percentile of the CPH slope term reported by Sathiakumar et al. (2021a): 1.8×10^{-3}
- EPA's 95th percentile value: **1.79×10^{-3}** ← *value cannot be replicated*

Corrected calculation:

[12] EPA (2002), supra note 4, at 3-4.

- Standard error for CPH slope term (assuming normality): $(1.8 \times 10^{-3} - 9.94 \times 10^{-4}) / 1.96 = \mathbf{4.11 \times 10^{-4}}$
- Corrected 95th percentile for the CPH slope term: $9.94 \times 10^{-4} + 1.645 \times 4.11 \times 10^{-4} = \mathbf{1.67 \times 10^{-3}}$

This error results in an underestimation of the point of departure and worker IUR value, as shown in the table below.

Parameter	EPA Value	Corrected Value
CPH Slope Term (95th %)	1.79×10^{-3}	1.67×10^{-3}
LEC01 (Point of Departure)	2.046 ppm	2.2 ppm
Worker IUR	0.0049 per ppm	0.0045 per ppm

Additional spreadsheet formula errors were identified in the lifetable calculation spreadsheet for workers, but do not materially impact the IUR. However, these changes are important for correctness and future use and are summarized in the table below.

Cell	Current Formula	Corrected Formula
H27	=SUM(H4:H26)	=SUM(H9:H26)
O27	=SUM(O4:O26)	=SUM(O9:O26)
I9 onward	"X.495"	"X.5"

b.) EPA's Cancer Assessment Results in an IUR and OEV That Are Not Reflective of Best Available Science or Gold Standard Science – Epidemiology data-based dose-response assessments are less established (i.e., far fewer conducted compared to animal-based assessments) than animal data-based assessments, more *ad hoc* in nature since many of the decisions are not adequately covered by existing agency guidelines, and in practice result in the generation of worst-case toxicity values.

The following table (**Table 1**) includes rows for decisions made by EPA at each step of the cancer dose-response assessment. Due to its increased complexity, there are more decision points (rows in **Table 1**) in epidemiology-based assessments than for animal-based assessments. The *ad hoc* nature of epidemiology-based assessments, due to the lack of clear guidelines, results in overly conservative decisions made at all steps in the process. Due to cascading conservative decisions at each step in the process, epidemiology-based assessments, like the one conducted for 1,3-BD, result in a worst-case and unrealistic

estimate of cancer potency rather than a realistic upper bound estimate. These decisions appear in Section 4.2 of the Hazard Assessment document.[13]

Table 1. Breakdown of Sequential Conservative Decisions within EPA's Epidemiology-Based Cancer Assessment for 1,3-BD. Yellow shading indicates conservative decisions

Decision Point	Nature of Decision	Impact on IUR
1) Data set	Trimmed data used; excluding data for unexposed workers and highly exposed workers not justified	~4x increase over use of all data
2) Endpoint	Inclusion of bladder cancer without demonstration of a causal association	~30% increase over use of leukemia alone
3) Dose measure/ Covariate	High intensity tasks (HITs) excluded as covariate	~2x increase including HITs
4) Dose response model	Not assessed; only Cox proportional hazards model considered	
5) Lag assumption	Lag assumption had no meaningful impact on the assessment	
6) BMR	Benchmark response rate too high and results in point of departure at high end of range of observation	~20-30% increase over use of a lower BMR
7) POD Confidence Limit	Use of 95% lower confidence limit instead of maximum likelihood estimate	~60-80% increase over use of maximum likelihood
8a) Lifetable: Mortality vs incidence	Incidence used without consideration of appropriateness	~2x increase over use of mortality
b) Lifetable: Lifetime definition	85 years used in lifetable instead of 78 years	~20-30% increase over use of 78 years
8c) Lifetable: Work years definition	69 years used in lifetable instead of 40 years	~20-30% increase over use of 40 years
9) Low-dose extrapolation	Low-dose linearity assumed based on genotoxicity	NA

[13] EPA (2025) Final Risk Evaluation for 1,3 Butadiene.

10) ADAF application	Worker IUR: Not applicable; General population: Ignores evidence suggesting early life at lower risk	~70% increase over adult IUR for general population

c.) **EPA's Noncancer Assessment Results in Unrealistic Point of Departure and an OEV for 1,3-BD That is Not Consistent with the Best Available Science** – EPA's decision for interspecies extrapolation in the non-cancer dose-response assessment (Section 4.2.2 of the Hazard Assessment document), does not reflect best available science. Specifically, the assumption that mice and humans are equally sensitive to a given external concentration of 1,3-BD in air is contradicted by the scientific weight of evidence. Large species differences are well-documented across in vitro, in situ, and in vivo studies (as reviewed in Kirman et al., 2022), as outlined in **Appendix B, Figure B-1**. For a given exposure to 1,3-BD, mice, rats, and humans experience very different internal exposures to 1,3-BD's toxic metabolites, both in terms of magnitude (mice>rats>humans) and composition (DEB predominates in mice and rats; EBD predominates in humans). As such, the three species are internally exposed to different mixtures of 1,3-BD metabolites, and mice and humans are not equally sensitive (i.e., the size and composition of the pie chart depicted for mice is not the same as that depicted for humans).

d.) **The Cancer Assessment for 1,3-BD is Inconsistent with EPA and OMB IQA Guidelines** – Due to the mathematical error in the cancer assessment (see Section 2.b.i), it does not meet the IQA's quality and accuracy requirements.

e.) **The Cancer Assessment for 1,3-BD is Inconsistent with EPA Guidelines and EO 14303** – Adoption of best available science as a matter of risk assessment policy represents a commitment to add information to the dose-response assessment process to evolve away from protective policy default estimates towards science-based predictive estimates (USEPA, 1994). Within exposure assessment, clear policy has been established by EPA for defining a Reasonable Maximum Exposure (RME) scenario that reflects a balanced combination of upper bound (precautionary) and central tendency (accurate) parameter values to avoid unrealistic, worst-case exposure estimates while ensuring safety (USEPA, 1989). In the same way, use of animal data for cancer dose-response is a mature practice with clear guidelines from EPA (USEPA, 2005, 2012), and hundreds of assessments conducted across the agency. When all of the decisions that go into an animal-based dose-response assessment are unpacked, it is clear that not all are precautionary in nature. Standard practice using animal data results in IUR values (USEPA, 2005, 2012) that reflect a mixture of accurate and precautionary decisions at each step in the process. In general, decisions for the dose measure selection, dose-response model selection, benchmark response rate, and low-dose extrapolation method are intended to be accurate rather than precautionary (**Table 2**). By combining accurate and precautionary decisions, EPA's standard practice for animal-based assessments have evolved to produce values that are protective, realistic upper-bound estimate of potency rather than a worst-case

estimate. By comparison, the pattern of decisions (accurate vs. precautionary) listed in **Table 1** are different from those listed in **Table 2**. As a result of the cascade of precautionary decisions within the cancer dose response assessment, the resulting IUR and OEV represent worst-case values for 1,3-BD. As such, the cascade of decisions is inconsistent with EO 14303, which directs federal agencies to move away from using worst-case scenario projections in regulatory decision-making.

Table 2. Example of Balancing Accurate and Precautionary Decisions: Standard Practice for Animal Data-Based Dose-Response Assessments (USEPA, 2005; 2012). Yellow shading indicates precautionary decisions; Blue shading indicates accurate decisions.

Decision Point	Standard Practice/Policy
Data set	Rely on most sensitive data set relevant to human health
Endpoint	Rely on most sensitive endpoint relevant to human health
Dose measure	Rely on dose measure that is consistent with MOA (not the one generating the lowest human equivalent concentration)
DR Model	Rely on the best fitting model (not the one generating in the lowest POD)
BMR	Rely on a BMR that results in POD near the low end of the range of observation that serves as a good point for extrapolating to lower exposure (not the one generating the highest slope value)
POD Confidence Limit	Rely on 95% LCL
Low-dose extrapolation	Linear or nonlinear based upon consideration of MOA
Adjustments (ADAF)	Rely on an assumption of early life susceptibility, particularly for genotoxic carcinogens

As such, the cascading effect of precautionary decisions made by EPA in the cancer assessment (Table 1) are inconsistent with EPA's Guidelines for Carcinogen Risk Assessment (USEPA, 2005), which state:

> *"In constructing high end estimates of risk, the assessor should bear in mind that the high-end risk is a plausible estimate of the risk for those persons at the upper end of the risk distribution (U.S. EPA, 1992a). The intent of this approach is to convey an estimate of risk in the upper range of the distribution, but to avoid estimates that are beyond the true*

distribution. Overly conservative assumptions, when combined, can lead to unrealistic estimates of risk. This means that when constructing estimates from a series of factors (e.g., emissions, exposure, and unit risk estimates) not all factors should be set to values that maximize exposure, dose, or effect, since this will almost always lead to an estimate that is above the 99th-percentile confidence level and may be of limited use to decisionmakers. This is particularly problematic when using unbounded lognormal factor distributions. While it is an appropriate aim to assure protection of health and the environment in the face of scientific uncertainty, common sense, reasonable applications of assumptions and policy, and transparency are essential to avoid unrealistically high estimates."

In addition, several of the decisions listed in **Table 1** for the cancer assessment are inconsistent with EPA guidelines. These decisions are discussed in detail in **Appendix A**, and summarized below:

- The exclusion of subsets (unexposed and highly exposed workers) of the styrene-butadiene rubber worker cohort data for leukemia (Sathiakumar et al., 2021a), by EPA in the cancer dose-response assessment is inconsistent with agency guidelines (USEPA, 2005, 2012) and standard practice and is supported by rationale that is scientifically flawed (See **Appendix A, Section A.1**).
- The use of a benchmark response rate of 1% is inconsistent with agency guidelines (USEPA, 2012) and standard practice and supported by rationale that is scientifically flawed (See **Appendix A, Section A.4**).
- Assumptions made by EPA in the lifetable calculation spreadsheet and their impact on the IUR and OEV for 1,3-BD are not transparent and are mislabeled in the hazard assessment document (See **Appendix A, Section A.6**).
- The rationale and impact of other decisions made in the assessment (e.g., combining leukemia and bladder cancer risk, ignoring the role of High Intensity Tasks in the observed cancer response, use of background data for cancer incidence in the lifetable calculations) are not transparent (See **Appendix A, Sections A.2, A.3, A.5**).

f.) The Non-Cancer Assessment for 1,3-BD is Inconsistent with Decisions by Other Agencies, EPA Guidelines, and EO 14303, and EPA's Rationale for Interspecies Extrapolation is Scientifically Flawed – Based on the weight of evidence supporting large species differences (**Appendix B, Figure B-1**), risk assessors have taken different approaches for 1,3-BD hazard assessment:

- ATSDR 2012: "*due to the large species differences in the metabolism of 1,3-BD and the lack of chemical-specific data to adjust for these differences, which may result in the MRL overestimating the risk to humans.*"
- ECHA 2024: No 8h-TWA value for noncancer effects "*is proposed considering the marked uncertainties in the extrapolation of animal data to humans*"

- EPA TSCA RE: Species differences and uncertainties are recognized in the qualitative discussion, but are ignored in the quantitative assessment (i.e., humans are assumed to be equally sensitive as mice to a given concentration of 1,3-BD in air) citing uncertainties in the MOA and data that could be used to support data-derived extrapolation factor (DDEF) values.
    - This decision invokes a worst-case assumption that is not supported by the weight of evidence. As such, this decision is inconsistent with EO 14303, which directs federal agencies to move away from using worst case scenario projections in regulatory decision-making.
- Kirman et al. (2022, 2025, 2026): In a series of publications, the authors provide full quantification of species differences utilizing DDEF calculations based on hemoglobin adducts and MOA. To ensure consistency with best available science, independent advisory panels were engaged to guide key decisions in the calculation of DDEF values and their application to human health risk assessment. The MOA advisory panel included six former regulatory senior scientists (EPA, Health Canada), two of whom co-authored EPA's DDEF guidelines (USEPA, 2014) (**Appendix B, Table B.1**). A manuscript summarizing the panel's conclusions has been submitted to *Critical Reviews in Toxicology* (Kirman et al., 2026[14]). In contrast to EPA conclusions, this independent panel concluded:
    - Overall confidence in the noncancer MOA, which includes four key events, is medium.
    - Overall confidence in interspecies extrapolation based on DDEF values that consider all three epoxide metabolites is high.
    - Confidence in assuming equal sensitivity between mice and humans for a given concentration of BD is low.

Importantly, EPA's DDEF guidelines (USEPA, 2014) explicitly state: *"Information on MOA is important in DDEF derivation, even when a complete understanding of the mechanism is not available."* This statement allows for DDEF values to be used in cases when uncertainty in the MOA remains. As such, EPA's decision to rely upon a low confidence assumption (equal sensitivity) by requiring a higher degree of understanding of mechanism is inconsistent with the DDEF guidelines.

In addition, as detailed in **Appendix B**, EPA's rationale for supporting its default assumption and rejecting best available science using DDEF is scientifically flawed.

g.) **Challenges in Translating Risk Evaluation Conclusions to Risk Management in the Workplace** – **Appendix C** identifies several critical challenges in applying EPA's 1,3-BD risk evaluation conclusions to practical workplace risk management. First, most unreasonable-risk findings occur only under high-end sentinel exposure scenarios, which do not reflect how exposures actually occur or are controlled in facilities. EPA's use of Conditions of Use (COUs) and Occupational Exposure Scenarios (OES) does not align with standard industrial hygiene

[14] Appendix D-Kirman et al (2026). Evaluation of the Modes of Action for Key Noncancer Effects of 1,3-Butadiene: Input from an Independent Expert Panel to Support Derivation of Data-Derived Extrapolation Factors. Critical Reviews of Toxicology- Accepted for publication

practice, where exposure is evaluated by similar exposure groups (SEGs) and controlled at the task level rather than by full-shift assumptions. As a result, high-end sentinel scenarios overstate worker exposures and do not reflect real-world protective practices (e.g., respirator use during short-duration tasks), as detailed in **Appendix C**.

Second, EPA's application of task-level sampling data as if it represents full-shift exposures—particularly for loading/unloading and waste-handling tasks—mischaracterizes exposure potential. Although EPA acknowledges the short duration of these tasks, unreasonable-risk conclusions were still drawn for full-shift exposure, resulting in an overestimation of workplace risk. For risk-management purposes, **Appendix C** recommends using task-level data to identify targeted controls, while full-shift data should be compared to the final OEV.

Third, EPA's assumption that occupational non-users (ONUs) experience exposures equivalent to central-tendency worker exposures contradicts both EPA's own definition of ONUs and OSHA's regulated-area requirements under the 1,3-BD standard.[15] ONUs do not perform hands-on process tasks, have restricted access to higher-exposure zones, and experience transient, intermittent presence in process areas. Modeling shows ONU exposure would be substantially lower than worker exposures in regulated areas. EPA's ONU assumptions compound multiple conservative defaults—co-location with high-exposure tasks, no mobility, no temporal variability—resulting in unrealistic exposure estimates.

Fourth, unreasonable-risk findings for all workers in the "Processing as a Reactant" COU, including those in polymerization operations, do not reflect the variability of exposures across different job roles. **Appendix C** recommends that EPA evaluate exposures by SEGs to ensure risk conclusions and subsequent risk management requirements match actual workplace conditions.

h.) Conclusions and Recommended Corrective Actions – To be consistent with the IQA and requirements of TSCA, we urge EPA to consider our recommendations for improving the Hazard Assessment, Exposure Assessment and Risk Assessment for 1,3-BD to ensure consistency with available guidelines, as summarized below.

- ***Recommend EPA Correct Errors in the Cancer Dose-Response Assessment*** - All errors in the lifetable calculations should be corrected, and the methods for deriving 95th percentile values from 97.5th percentile values — including the normality assumption — should be documented transparently in the risk evaluation text. These corrections have a ripple effect that impacts all subsequent calculations for an IUR value based on the combined endpoints (leukemia and bladder cancer), cancer risk calculations for workers and general populations, and OEV calculations.

[15] https://www.osha.gov/butadiene/standards

- ***Recommend EPA Rely Upon a Balance of Accurate and Precautionary Decisions in the Cancer Dose-Response Assessment*** – Some, but not all, of these decision points in the epidemiology-based assessment should be accurate rather than precautionary (i.e., some yellow rows in the Table 1 should be blue). An example of a balance between accurate and precautionary decisions is provided in Table 3. By including a balance of accurate and precautionary decisions, an assessment results in a value that is protective but not worst-case, consistent with standard practices for exposure assessment and animal-based toxicity assessment.

Table 3. Balancing Accurate and Precautionary Decisions in the Cancer Dose-Response Assessment for 1,3-BD. Yellow shading indicates precautionary decisions; Blue shading indicates accurate decisions

Decision Point	***Recommended Change***	***Impact on IUR***
1) Data set	*Because the criteria for excluding portions of the data have not been met (i.e., lack of statistically acceptable fit), the entire data set should be used'*	
2) Endpoint	*Until a causal association between BD exposure and bladder cancer is proven, cancer potency should be based on leukemia, and combined risk considered in sensitivity analysis/discussion*	
3) Dose measure/ Covariate	*High Intensity Tasks (HITs) excluded as covariate*	*~2x increase over inclusion of HITs*
4) Dose response model	*NA*	
5) Lag assumption	*NA*	
6) BMR	*To remain consistent with standard practice when using animal studies and BMD guidelines, a BMR of 0.0001 should be selected since it falls at the low end of the range of observation*	
7) POD Confidence Limit	*Use of 95% LCL*	*~60-80% increase over the maximum likelihood*
8a) Lifetable: Mortality vs incidence	*Incidence used without consideration of appropriateness*	*~2x increase over use of mortality data*

8b) Lifetable: Lifetime definition	*A lifetime of 78 years should be used as a default value, with other values (e.g., 70, 85) used to support sensitivity analysis/discussion*	
8c) Lifetable: Work years definition	*A work years of 40 years should be used as a default value, with other values (e.g., 20, 62) used to support sensitivity analysis/discussion*	
Low-dose extrapolation	*Low-dose linearity assumed based on genotoxicity*	*NA*
ADAF	*Worker IUR: Not applicable; General population: Ignores evidence suggesting early life at lower risk*	*~70% increase for general population over adult only*

- ***Recommend EPA Rely on DDEF Values Based on Best Available Science to Quantify Important Species Differences in the Noncancer Dose-Response Assessment*** – EPA's default, low confidence assumption for interspecies extrapolation (mice and humans are equally sensitive to a given concentration of 1,3-BD in air), should be replaced with an approach that quantifies based upon available data used within EPA DDEF framework. The noncancer MOA and hemoglobin data described in Kirman et al. (2026; **Appendix D**) are of sufficient quality and certainty to move away from the worst-case approach in the risk assessment for 1,3-BD with reasonable confidence.

- **Recommend EPA Align the Occupational Exposure Scenarios (OES) with standard industrial hygiene practices** – EPA's unreasonable risk conclusions for 1,3-BD are driven largely by high-end sentinel exposure assumptions that do not reflect how exposures are actually managed in workplaces, where controls are implemented at the task level and exposure groups vary substantially. These assumptions also extend to ONUs, whose true exposure potential is far lower due to restricted access and regulated -area requirements, resulting in overly conservative and unrealistic exposure estimates.

We urge EPA to make these corrections and address these substantial issues to ensure the risk evaluation for 1,3-BD is consistent with requirements of the IQA and TSCA.

Appendix A: Detailed Comments for 1,3-Butadiene Cancer Assessment

A.1 EPA's Data Set Selection is Inconsistent with EPA Guidelines and Standard Practice and Adds to Compounded Conservative Decisions in the IUR

EPA relied upon the sensitivity analysis results of Sathiakumar et al. (2021a) that were based on a "trimmed" data set for the Styrene-Butadiene Rubber (SBR) worker cohort that excluded: (1) unexposed worker data; and (2) highly exposed worker data, with exposures above the 95%ile. In its response to comments, EPA stated "since the purpose of the 1,3-butadiene IUR derivation is for 1,3-butadiene exposure and cancer, the models including unexposed people were not considered in EPA's evaluation" (USEPA 2025).

This statement implies that the purpose of the IUR is to characterize risk in the range of observation (i.e., where we have exposed worker data). In the absence of guidelines for when it is appropriate to exclude subsets of epidemiology data, this practice should instead be considered against what is standard practice for the treatment for use of animal data for dose-response assessment. EPA's rationale is ill-advised since it suggests that control animal groups should be dropped from animal-based IUR derivations, a practice that is rarely done. Typically, when control animal data are questionable, consideration of historical control data is included to determine if there is a treatment-related effect. Control animal data are never excluded from dose-response modeling efforts.

Since the IUR serves as an estimate of a chemical's cancer potency below the range of observation (i.e., dose-response slope at low exposures), an understanding of the low dose slope for cancer risk is anchored at the low end by the response in unexposed subjects (animal or human). Furthermore, because the CPH model is modeling rate ratios (i.e., rate in exposed/baseline rate), the unexposed worker data improve the robustness of the denominator for all data modeled. As such, the exclusion of unexposed workers is inappropriate.

Further, in its hazard assessment, EPA states "Exposure-response curves tend to dimmish at higher exposure levels. IUR represents a lower exposure range (Stayner et al., 2003) so the concern about high-exposure workers is not as relevant to IUR derivation." (USEPA, 2025),

EPA appears to be making inferences about the shape of the dose-response relationship based upon the appearance of the epidemiology data. This runs contrary to EPA cancer risk assessment guidelines (USEPA, 2005), which state:

> For epidemiologic studies, including those with grouped data, analysis by linear models in the range of observation is generally appropriate unless the fit is poor. The relatively small exposure range observed in many epidemiologic studies, for example, makes it difficult to discern the shape of the exposure-or dose-response curve. Exposure misclassification and errors in exposure estimation also obscure the shape of the dose-response curve. When these errors are unsystematic or

> random, the result is frequently to bias the risk estimates toward zero. When a linear model fits poorly, more flexible models that allow for low-dose linearity, for example, a linear-quadratic model or a Hill model (Murrell et al., 1998), are often considered next. (USEPA, 2005)

Because the standard CPH model is linear at low exposures, and provides an acceptable, and slightly better fit, to the whole data set than to the trimmed subset used by EPA, the criteria for excluding data or assessing more complex models have not been met in this case. As such, EPA's decision to rely upon a subset of the data is inconsistent with its guidelines and standard practice and contributes to the compounded conservatism in the IUR value.

Recommendations for resolving this issue are summarized below:

- To remain consistent with standard practice using animal data, EPA should use the entire cohort data set from Sathiakumar et al. (2021a) to support the IUR. In the same way that the trimmed data were part of a sensitivity analysis by Sathiakumar et al., use of the trimmed data set should be included in the uncertainty discussion of the assessment.

A.2 EPA's Combining Cancer Endpoints is Not Appropriate for the IUR and Adds to Compounded Conservative Decisions

In a change from the draft risk assessment, which relied upon leukemia risk alone to derive an IUR, EPA relies upon risks combined for leukemia and bladder cancer for the final IUR. This approach assumes that these endpoints are independent and IURs are normally distributed, neither of which may be valid. The assumptions are not stated in the hazard assessment. Although Sathiakumar et al. (2021b) reported a statistical association between BD exposure and bladder cancer mortality in workers, a causal association has not been established, and therefore its inclusion in the quantitative assessment for the IUR is premature. The role of smoking confounding needs to be addressed before causation can be concluded. This decision contributes to the compounded conservatism in the IUR value for BD.

Recommendations to resolve this issue in the assessment are summarized below:

- The hazard assessment should rely upon leukemia risk for the IUR. Consideration of bladder cancer should be included in the discussion of uncertainty.
- In Table 5-1 for evidence integration, the human evidence for bladder cancer should be downgraded from "Moderate" to "Slight" since a causal relation has not been established.

A.3 EPA's Ignoring High Intensity Tasks (HITs) as an Important Covariate is Not Appropriate and Adds to Compounded Conservative Decisions in the IUR

In its response to comments, EPA states "Including HIT as a covariate can result in an over-adjustment of the association between 1,3-butadiene exposure and cancer."

One of the limitations of cumulative dose measures like ppm-years is that a "40-year exposure to 1 ppm" and a "1-year exposure to 40 ppm" are treated equivalently from a mathematical perspective, whereas from a biological perspective these two exposure scenarios may be very different in their contributions to a cancer response. Consideration of HITs as a covariate provides an opportunity to improve upon this limitation. HITs is the number of peak exposures (>100 ppm) that serves measure of the degree of exposure intensity, but not a direct measure of cumulative exposure itself (i.e., ppm-years associated with peaks are not ignored by its inclusion). Conceptually, adjusting for HITs would be like adjusting for other commonly used covariates in epidemiology studies (e.g., employment duration, job titles/task, period of hire). Those are a more indirect approximation of degree of exposure compared to HITs but would also be positively correlated with the estimated cumulative exposure. Adjusting for HITs allows all data to be included (even on the high end of exposure) while controlling for individuals more likely to be assigned frequent high exposure tasks. This is an important factor to consider since occupational cohorts, particularly in the past, experience peak exposures, whereas the general population does not. CPH model slopes should be adjusted for peak exposures (i.e., HITs) to improve extrapolation to the general population. Exclusion of HITs has contributed to the compounded conservatism in the IUR value calculated for BD.

Recommendations for resolving this issue are summarized below:

- We recommend that HITs be considered for the primary assessment, and at a minimum should be discussed in the uncertainty/sensitivity discussion.

A.4 EPA's Benchmark Response Rate is Inconsistent with EPA Guidelines and Adds to Compounded Conservative Decisions in the IUR

In defense of its selection of a benchmark response rate (BMR) of 1%, EPA states in the response to comments document that "According to the EPA Benchmark Dose Technical Guidance (U.S. EPA, 2012b), 1 percent extra risk is often used as a BMR for epidemiological data, unless the health outcome is a rare cancer."

This justification is inadequate. EPA's benchmark dose guidelines (USEPA, 2012) do not provide specific recommendations on a default BMR for epidemiology data sets. Instead, it includes an example for when BMR values less than the default of 10% may be considered,

> a BMR of 1% has typically been used for quantal human data from epidemiology studies. (USEPA, 2012).

However, the BMD guidelines also more definitively state:

> Typically, a BMR near the low end of the observable range is selected as the basis for obtaining BMDs and BMDLs to serve as potential PODs for deriving quantitative estimates… Because different study designs have different dose selections and different sensitivities (i.e., statistical power) to observe adverse effects at various

> doses, the low end of the observations can correspond to disparate response levels across studies. (USEPA, 2012)

For this reason, the BMR decision is study-specific based upon consideration of the domain (range of observation) defined by the data being used to support the assessment. As such, for BMR selection, one size (e.g., 1%) does not fit all epidemiology studies, and is specifically contra-indicated for the epidemiology data used for BD (i.e., Sathiakumar et al., 2021a,b).

In addition, in the response to comments, EPA states:

> The Surveillance, Epidemiology, and End Results Program (SEER) of the National Cancer Institute indicated that the lifetime risk of bladder cancer is 2.1 percent and leukemia is 1.5 percent. Compared to a much lower level of BMR, such as 0.1 percent or 0.01 percent, a BMR of 1 percent is more appropriate because 1 percent is closer to the lifetime risks of bladder cancer (2.1%) and leukemia (1.5%).

EPA's rationale for citing background cancer rates in the US is unclear and confusing. Since leukemia and bladder risk are assessed in terms of a relative risk measure (i.e., rate ratio) that is converted to an extra risk measure, both of which account for background rates regardless of their magnitude, the magnitude of the background rates is irrelevant to the BMR selection. If EPA is attempting to indicate that the BMR and background rates are of a similar magnitude, implying that it represents a detectable change in risk, this justification is inconsistent with EPA's BMD guidelines, which clearly state that "It is important to recognize that the BMR need not correspond to a response that the study could detect as statistically significantly different from the control response." (USEPA, 2012).

There are several reasons why EPA's use of a 1% BMR is problematic in this case for the epidemiology data set (i.e., SBR cohort of Sathiakumar et al., 2021a,b):

- First, for the BD epidemiology data set, the CPH model is linear at low exposures (less than 30 ppm-years), near-linear for exposures between approximately 30-300 ppm-years, and nonlinear (curving upwards) at high exposures (greater than approximately 300 ppm-years). EPA's use of a BMR of 1% results in POD values that fall in the nonlinear region of the CPH curve (see **Figure A.1**). This results in the BMR decision having a quantitative impact on the IUR (overestimation by approximately 20-30%).
- Second, for the SBR cohort, a BMR of 1% is clearly NOT near the low end of the range of observation and instead results in all POD values near the high end (see **Figure A.1**). As such, this definition is inconsistent with EPA guidelines (USEPA, 2012). This inconsistency is not immediately obvious since: (1) the dose-response assessment is performed in terms of units (ppm-years) that differ from the units used for the final POD values (ppm), making direct comparison of POD values to the range of observation difficult; (2) EPA has not prepared plots that compare the range of observation for the cohort data set and possible POD values (e.g., **Figure A.1**) in the draft or final RE.

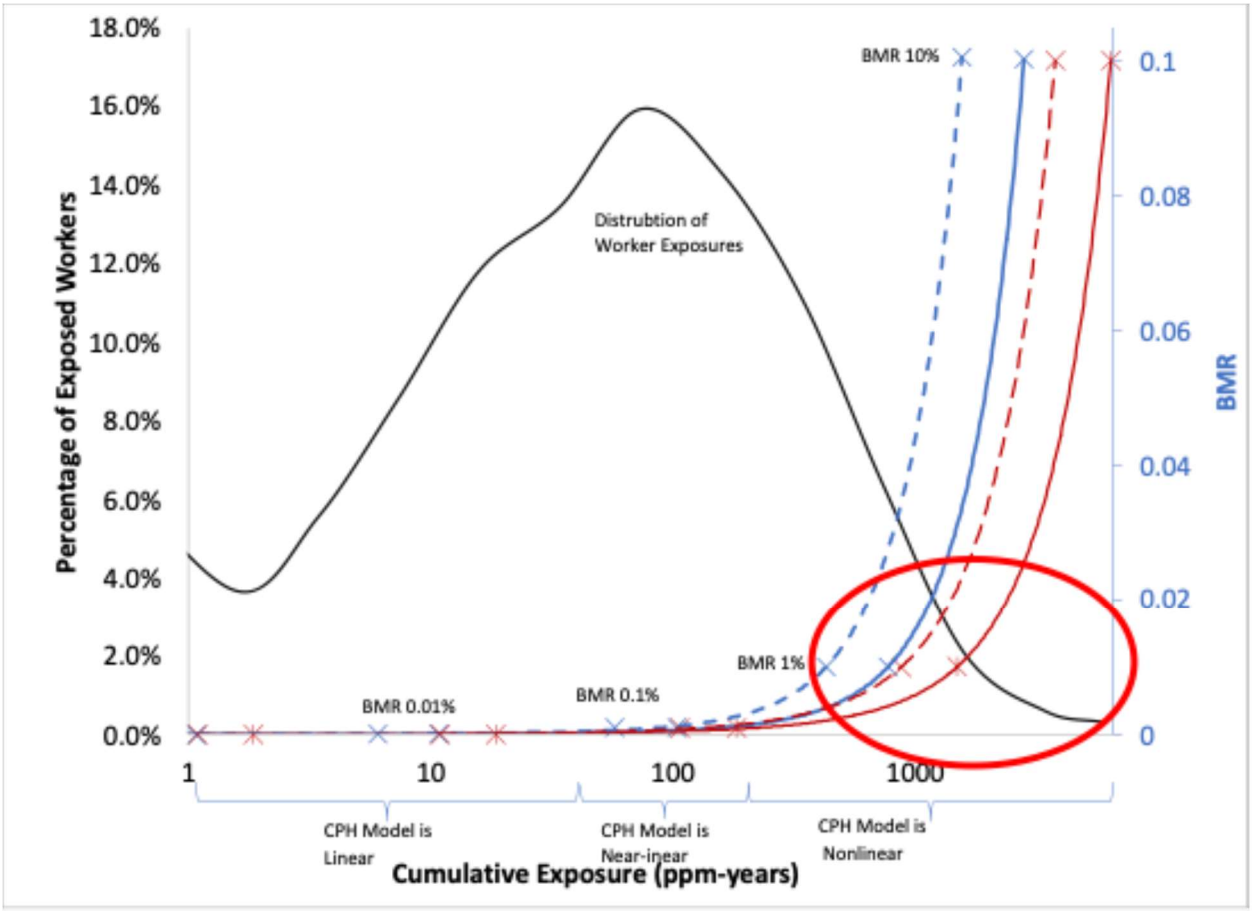
- Third, the problem with a 1% BMR is exacerbated by EPA's decisions to rely upon the "trimmed" data set for the SBR cohort (Sathiakumar et al., 2021a) (see **Section A.1** above). Specifically, because the trimmed data set excludes worker exposures above the 95%ile, the range of observation for leukemia is artificially truncated at the high end. Because EPA's BMD guidelines indicate that "residuals in the most relevant region of the data range… models that result in low scaled residuals for dose groups near the BMD are preferred" (USEPA, 2012), the ability to assess model fit near the POD is severely compromised by excluding data for the top 5% of the worker population (red oval in **Figure A.1**). As such, EPA's use of a 1% response rate is incompatible with their decision to rely upon the "trimmed" data set.

As a result, EPA has effectively selected a point of departure (POD) from the high end of the range of observation for the epidemiology data set, where CPH is nonlinear, then extrapolating across most of this range of observation where the data and CPH are linear (see **Figure A.1**). This practice: (1) contributes to the compounded conservatism in the IUR, resulting in an IUR value that is approximately 20-30% higher than if an appropriate BMR used (e.g., 0.01%); and (2) is inconsistent with EPA guidelines (USEPA, 2012).

Recommendations for resolving this issue are summarized below:

- For the BD assessment, BMR of 0.01% (i.e., LEC0001) is considered an appropriate point of departure since: (1) it falls within the 1st quartile of exposed workers exposures; and (2) it falls in the linear range of the CPH model behavior. BMR selection impacts Sections 5.4.3.2 and 5.4.3.6 but also impacts subsequent sections and risk calculations.
- To improve transparency, the hazard assessment should include consideration of multiple BMR values (e.g., 0.001%, 0.01%, 0.1%, 1%) so that the impact of the BMR decision is clear to the reader.

Figure A.1. Considering the Range of Observation for the Epidemiology Data to Establish an Appropriate BMR. Red lines indicate CPH model predictions for bladder cancer; Blue lines indicate CPH model predictions for leukemia (solid = MLE; dashed = LCL); note that log-linear x-axis used to show lower BMR values and range of observation for exposed workers exaggerates nonlinear appearance of CPH model.



A.5 EPA's Extrapolation of Cox Proportional Hazards Slopes from Cancer Mortality Analyses to Prediction Cancer Incidence is Not Appropriate and Adds to Compounded Conservative Decisions in the IUR

Within the lifetable calculations, EPA has applied Cox Proportional Hazards (CPH) model slopes derived from analyses of cancer mortality data for leukemia and bladder cancer (Sathiakumar et al., 2021) and applied them to the prediction of cancer incidence. This practice is an extrapolation outside the range of observation of the SBR data set that is not transparent to the reader. This decision contributes to the compounded conservatism in the IUR, and results in approximately a 2-fold increase in the value compared to that obtained from use of background rates for cancer mortality. The appropriateness of this extrapolation, from biological and statistical perspectives, is not discussed in the assessment. For cancer types that are not highly lethal, incidence and mortality can behave very differently, and CPH slopes may not be directly transferable. In addition, this decision should also include consideration of mode of action. Specifically, potential role of chemical exposures in late events in the MOA relating to disease progression and severity should also be considered.

Recommendations for resolving this

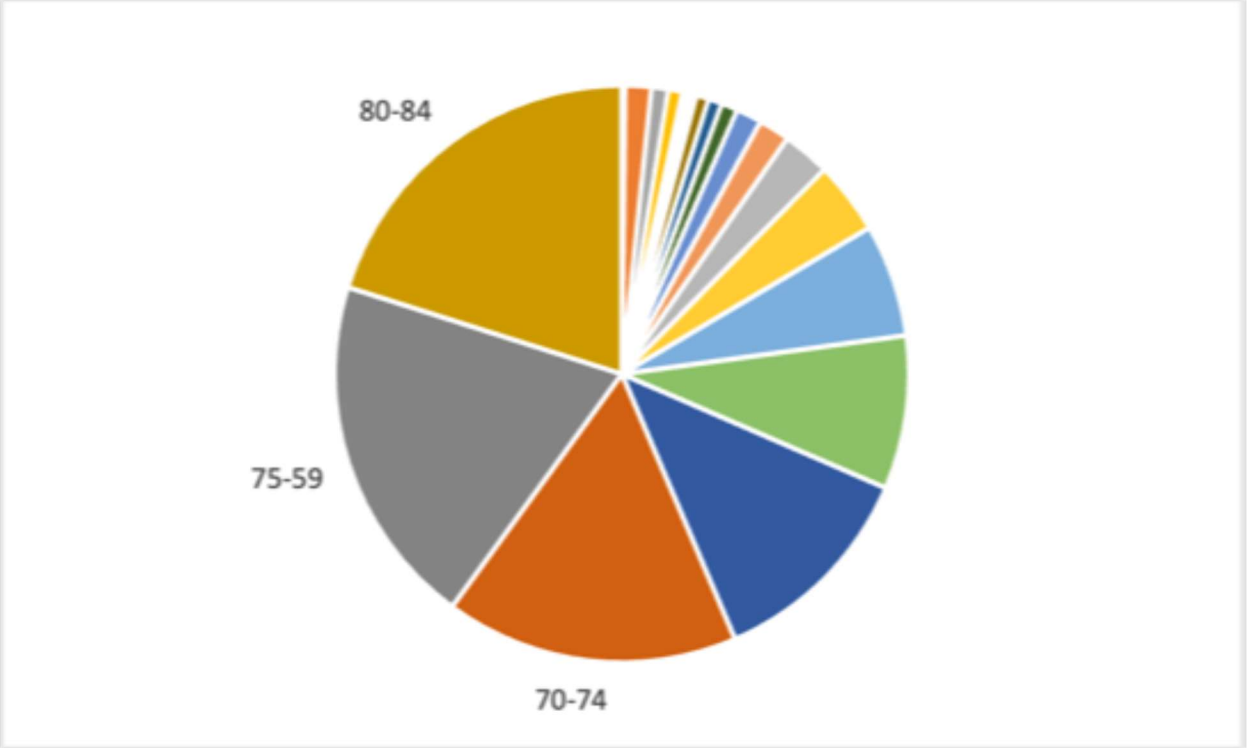
- The appropriateness of extrapolating slope for mortality to incidence rates need to be considered within the RE. If not appropriate or too uncertain, IUR lifetable calculations should rely on background mortality rates.
- Lifetable calculations should be performed using both background mortality and incidence rates so that the impact on the IUR is transparent.

mA.6 EPA's Lifetable Value Parameter Values Create Internal Inconsistencies and Adds to Compounded Conservative Decisions in the IUR

EPA's lifetable calculations for BD include parameter value definitions that are inconsistent with the values used in the exposure assessment. Specifically, EPA has adopted overly conservative values for Lifetime (85 years) and Worker Years (69 years) that do not match the values used in the exposure assessment (78 years and 40 years, respectively). These values are inconsistent with recommended values in EPA's Exposure Factors Handbook (USEPA, 2011). Furthermore, this practice creates internal inconsistency in the risk assessment and contributes to the compounded conservative decisions made by EPA in the IUR in a way that is not transparent.

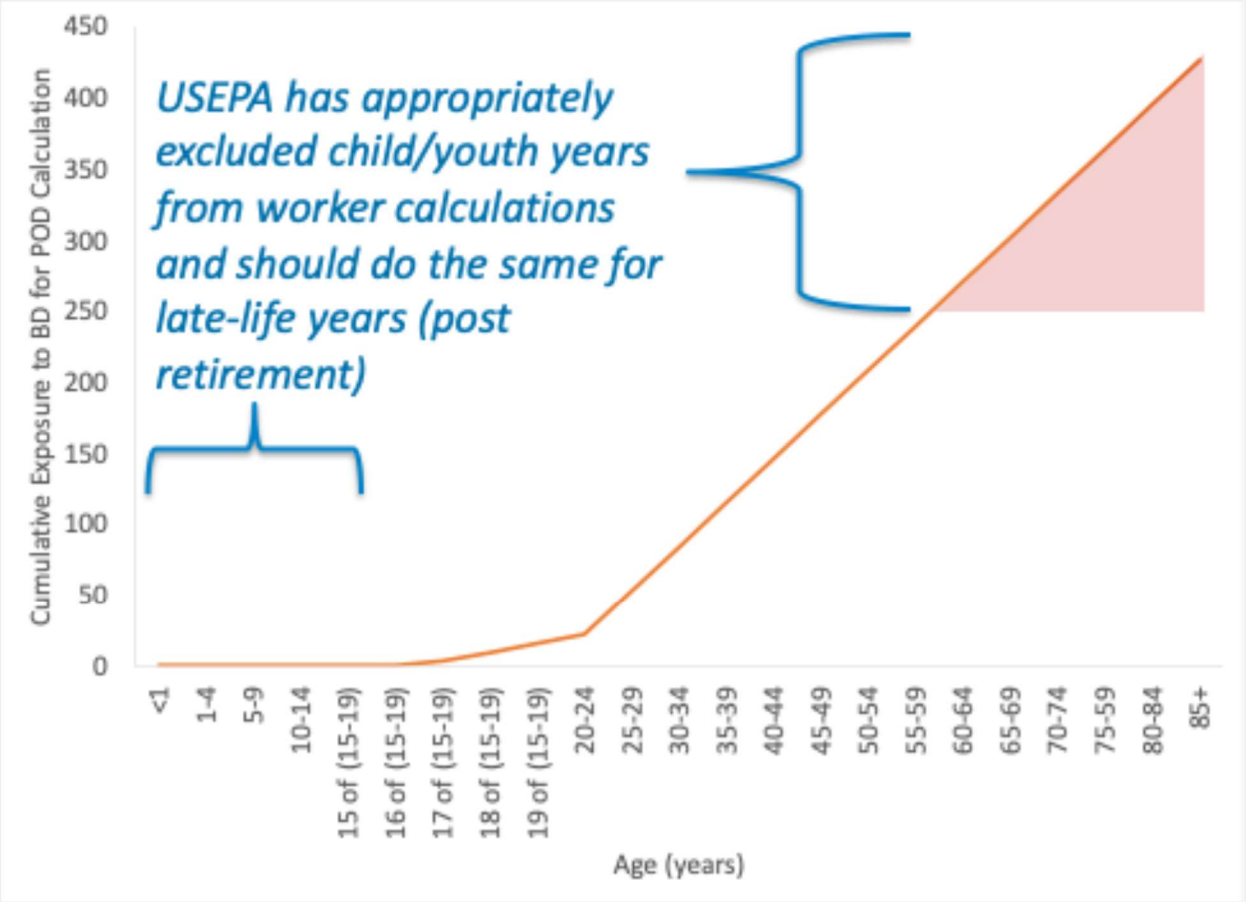
The background rates for leukemia and bladder cancer for the U.S. population during ages 79-85 years have no biological bearing on the prediction of cancer risks over a 78-year lifetime, yet EPA's calculations result in their driving the IUR value approximately 20-30% higher than is appropriate for the risk evaluation for BD (**Figure A.2**). This practice effectively squeezes 85 years of risk into a 78-year lifetime. Transparency in the risk assessment suffers since readers of the risk assessment are unaware of the inconsistencies between the exposure assessment and lifetable calculations. This problem is exacerbated in the hazard assessment and risk evaluations documents by EPA referring to the IURs as "78 year" and "62 year" values (e.g., Tables 5-9, 5-13 of the Hazard Assessment). Because of the 85-year lifetime assumption, these labels are misleading and incorrect, since the values calculated by EPA are actually "85 year" and "69 year" values.

Figure A.2. Age Group-Specific Contributions to Leukemia Potency Estimates Based On EPA's Lifetable Calculations



A similar problem of internal inconsistency and compounded conservative decisions arises from EPA's decision for defining Work Years. Specifically, EPA defined work years as 40 years in the exposure assessment, but defined work years as 69 years in the lifetable calculations. In the lifetable spreadsheet, the equations for cumulative exposure continue to increase after 40 years (i.e., for ages 57-85 in cells J20-25). For workers who do work for 40 years, cumulative exposures should remain constant during these years (i.e., exposure post-retirement should be excluded (see red shaded region in **Figure A.3**).

Figure A.3. Worker Exposures Are Inappropriately Assumed to Continue Post-Retirement in EPA's Lifetable Calculations



Specific recommendations to resolve the compound conservatism and internal inconsistency issues are summarized below:

- A value of 78 years for lifetime should consistently be used across the exposure assessment and hazard assessment (lifetable). This impacts Sections 5.4.3.2 and 5.4.3.6 but also impacts subsequent sections and risk calculations.
- A value of 40 years for work years should consistently be used across the exposure assessment and hazard assessment (lifetable). EPA has appropriately excluded early-life period (i.e., no working as a child/youth) and should do the same with the late-life period. This impacts Sections 5.4.3.2 and 5.4.3.6 but also impacts subsequent sections and all risk calculations.
- The lifetable spreadsheets would benefit from additional documentation/instruction to benefit the reader. This documentation should include quantitative evaluations of alternative values (e.g., 70, 78, and 85 year values for lifetime; 20, 40, and 69 years for work years) to improve transparency on the impact of these decisions. Consideration of alternative values for lifetime is appropriate and useful for sensitivity analyses, as well as for future risk managers who opt to treat lifetime parameter probabilistically.

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Appendix B: Detailed Comments for 1,3-Butadiene Noncancer Assessment

B.1 A Noncancer Mode of Action Has Been Sufficiently Defined to Support Data-Derived Extrapolation Factors (DDEFs) for BD

In its response to comment document, EPA states:

> EPA has reviewed the proposed mode of action (MOA) based on "general toxicity." In the revised Section 4.2.2.1 EPA determines that a well-defined MOA and robust mechanistic data are lacking for any non-cancer effect other than ovarian toxicity, and it is unknown whether a particular metabolite or set of metabolites are responsible for the observed critical outcomes. The cited document does not provide strong evidence to support species-specific toxicokinetic adjustments from the mouse data, and it even proposes "a plausible role for other BD metabolites, (including EB and EBD, the predominant epoxide metabolite BD estimated in humans). (USEPA, 2025)

The noncancer MOA has undergone additional review by an independent expert panel, and has been submitted for publication (Kirman et al., 2026). This independent panel included multiple former senior EPA scientists), including two who served as co-authors of EPA's DDEF guidelines (USEPA, 2014).

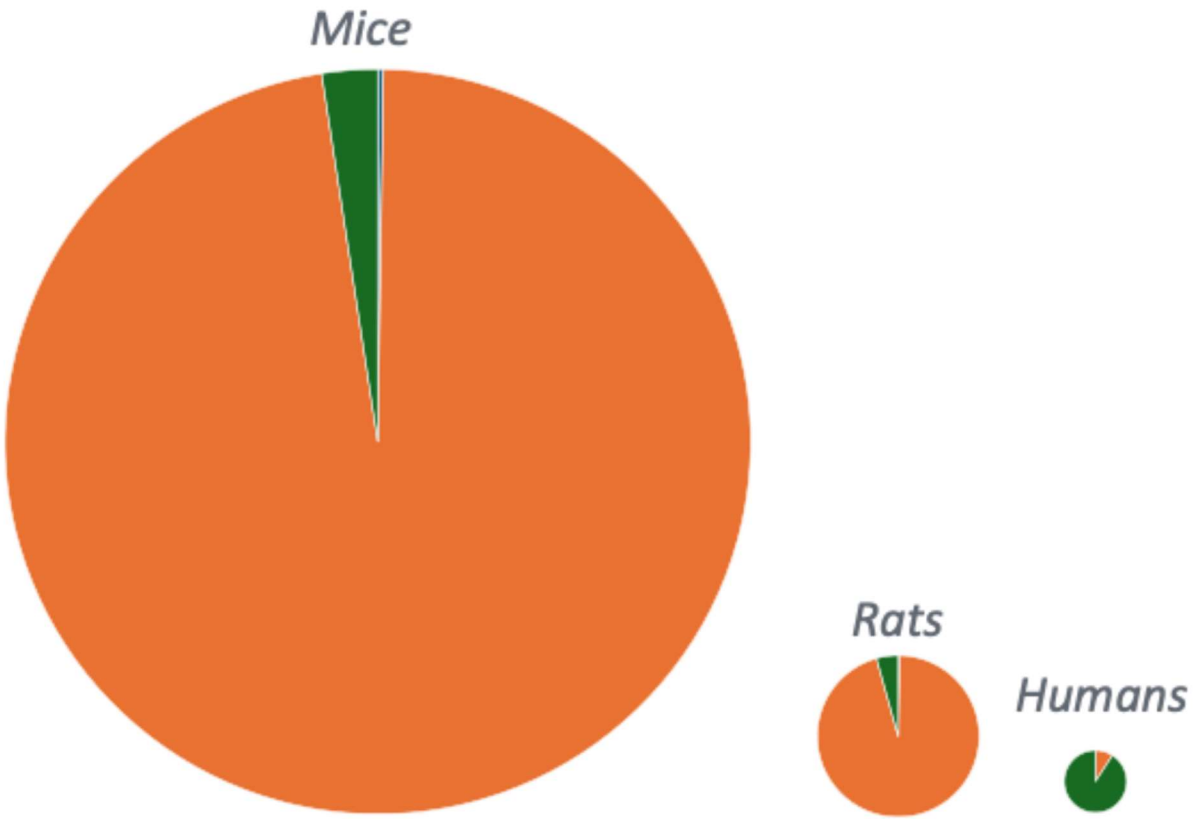
The limitations of the MOA for fetal body weight changes are recognized; however, "robust mechanistic data" should be considered as preferred but not a requirement for use. As stated in EPA's DDEF guidelines (USEPA, 2014), "Information on MOA is important in DDEF derivation, even when a complete understanding of the mechanism is not available."

For BD, there is moderate evidence available, particularly for early events related to toxicokinetics, that can be used to support dosimetry decisions. The independent advisory panel expressed medium confidence in the MOA for fetal body weight changes (Kirman et al., 2026; Appendix D). Under the best available science approach, moderate evidence in the MOA with medium confidence is recommended over a default policy assumption with low confidence.

The species differences in BD metabolism to toxic metabolites are large and are consistently supported by the weight of evidence provided by in vitro, in situ, and in vivo studies for BD (as summarized in Kirman et al. 2026; **Appendix D**) (**Figure B.1**). As such, the default assumption that mice and humans are equally sensitive to a given concentration of BD in air is directly refuted by this evidence. There is sufficient evidence to support the involvement of BD diepoxide metabolite (DEB) in causing weight changes: (1) DEB is consistently identified as the most potent metabolite of BD across studies and

endpoints assessed for metabolites individually (as summarized in Kirman et al., 2022); (2) DEB has been shown to produce weight changes in mice (response to BD) and rats following direct exposure to the metabolite (Doerr et al., 1995); and (3) DEB contributes greater than 95% of circulating toxicity equivalents in mouse blood (Kirman et al., 2022). Because EBD is the primary circulating epoxide metabolite of BD in humans, a conservative assumption was made that it also can contribute to fetal body weight changes via an MOA involving decreased glutathione (Kirman et al., 2026; **Appendix D**).

Figure B.1. Hemoglobin Adduct Data Support Interspecies Comparison of BD's Epoxide Metabolites. Surface area of pies are proportionate to magnitude of internal doses. Colors indicate contribution of each metabolite to toxicity: Orange=diepoxide, DEB; Blue=monoepoxide, EB; Green=epoxide diol, EBD.



These data strongly refute the default assumption that mice and humans are equally sensitive to a given air concentration of BD, an assumption that was rejected by ATSDR in its assessment over concerns of overestimating risk to human populations (ATSDR, 2012).

With respect to interspecies extrapolation for BD toxicokinetics, the independent advisory panel considered two possible MOAs: (1) fetal body weight changes can be attributed to

the diepoxide metabolite DEB alone; and (2) fetal body weight changes can be attributed to the combined action of BD's three epoxide metabolites (EB, DEB, EBD). The independent panel concluded:

- The panel expressed *high confidence* in using hemoglobin adduct data to account for species differences in all three epoxide metabolites;
- The panel expressed *medium confidence* in using hemoglobin adduct data to account for species differences in the diepoxide metabolite alone; and
- The panel expressed *low confidence* when species differences are ignored (i.e., assuming equivalent sensitivity to mice and humans for a given concentration of BD in air, as assumed by USEPA in the risk assessment).

B.2 Human Hemoglobin Adduct Data Are Robust and Can Support DDEF Calculations for BD

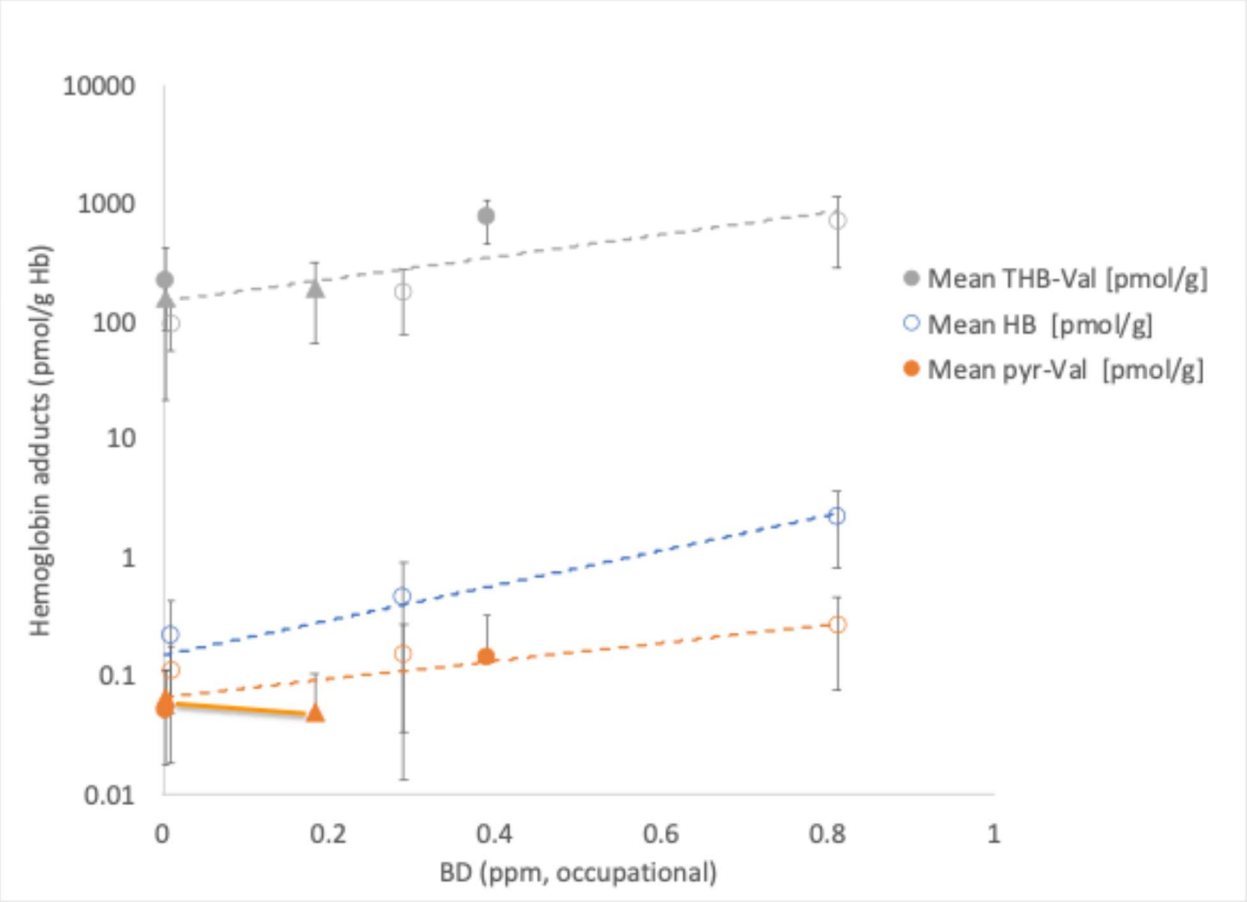
In its response to comment document (USEPA, 2025), EPA states:

> Additionally, high inter and intra individual variability, weak or inconsistent exposure response relationships, detectable background levels in unexposed populations, limited cohort diversity, and sex-dependent differences reduce confidence in species specific toxicokinetic adjustments based on available human Hb adduct data.

EPA's rationale for not adopting a DDEF value is flawed for several reasons.

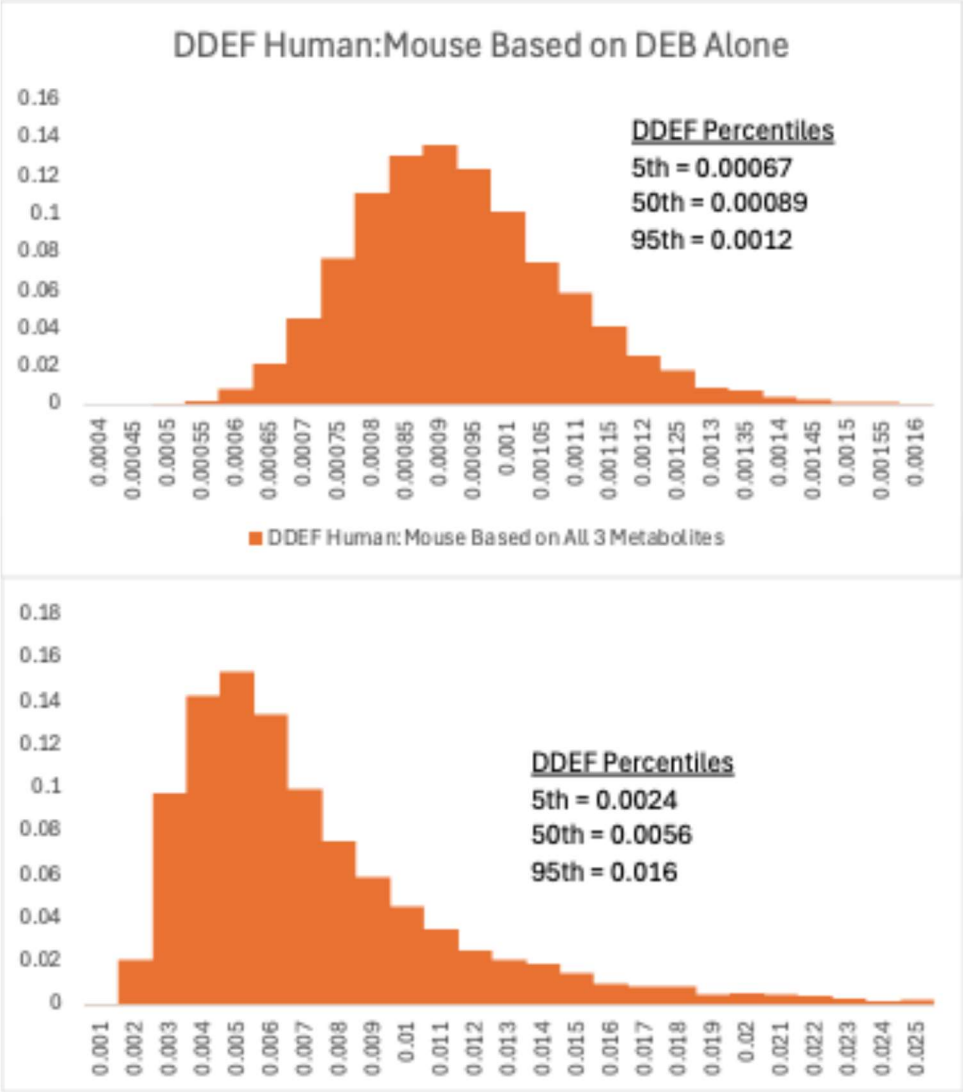
- First, hemoglobin adduct available for BD's epoxide metabolites are robust and reflect best available science. Paired hemoglobin adduction measurements for all three epoxide metabolites and personal air measurements over previous months have been collected in male and female workers (n=177) collected across two studies (Figure B.2). Companion data sets are also available for mice and rats, which indicate much higher levels, particularly for the most toxic metabolite DEB (Figure B.1).

Figure B.2. Mean Hemoglobin Adduct Burdens for BD Metabolites in Exposed Workers (Georgieva et al., 2025). HB adduct corresponds to EB; pyr-Val adduct corresponds to DEB; THB-Val adduct corresponds to EBD; Symbols = Arithmetic mean; Error bars = SD; Solid circles = Male workers from Study 1 (Albertini et al. 2003); Hollow circles = Male workers from Study 2 (Vacek et al. 2010); Solid triangles = Female workers from Study 2 (Vacek et al. 2010).From Kirman et al. 2026 (Attachment 1).



- Second, "inter and intra individual variability" are not factors that should be considered in the decision to use a DDEF value for interspecies adjustment. These are factors that are considered in the decision to use a DDEF value for intraspecies variation. Hemoglobin adduct data can also be used to support DDEF values for intra-human variability, which has been done (Kirman et al., 2025). Uncertainty and variability in the underlying data used to calculate DDEF values has been characterized using Monte Carlo methods and are relatively small (**Figure B.3**) and manageable for risk assessment purposes.

Figure B.3 Monte Carlo Evaluation of DDEF Values Based on Hemoglobin Adducts for Interspecies Extrapolation (Kirman et al., 2025)



- Third, inconsistent results for unexposed and exposed workers for one of the three adducts (DEB) do not detract from their use in risk assessment since: (1) these data remain consistent with the overall trend noted for the data set as a whole (**Figure B.2**); and (2) the key point for interspecies extrapolation is that the orange dashed line for pyr-val adducts in humans is orders of magnitude lower than the corresponding dashed line in mice, which remains the same whether or not female worker data are included or excluded. Kirman et al., 2026 Appendix D
- provides multiple options for including/excluding the female worker data in the DDEF calculations to provide flexibility to risk assessors.
- Fourth, background exposures do not preclude the use of worker hemoglobin adduct data for calculating DDEF values. Background contributions are subtracted out to calculate the unit AUC values per ppm exposure of workers to BD, as per the methods of Motwani and Tornqvist (2014).

- Lastly, sex-specific differences are readily accounted for using sex-specific data that are available for all three metabolites in mice, rats, and humans. Kirman et al., 2026; Appendix D we provides multiple options for calculating sex-specific DDEF values as well as calculating values for sexes combined so that risk assessors have flexibility in their application.

B.3 Requirements Defined by EPA for DDEF Application Have Been Met for BD

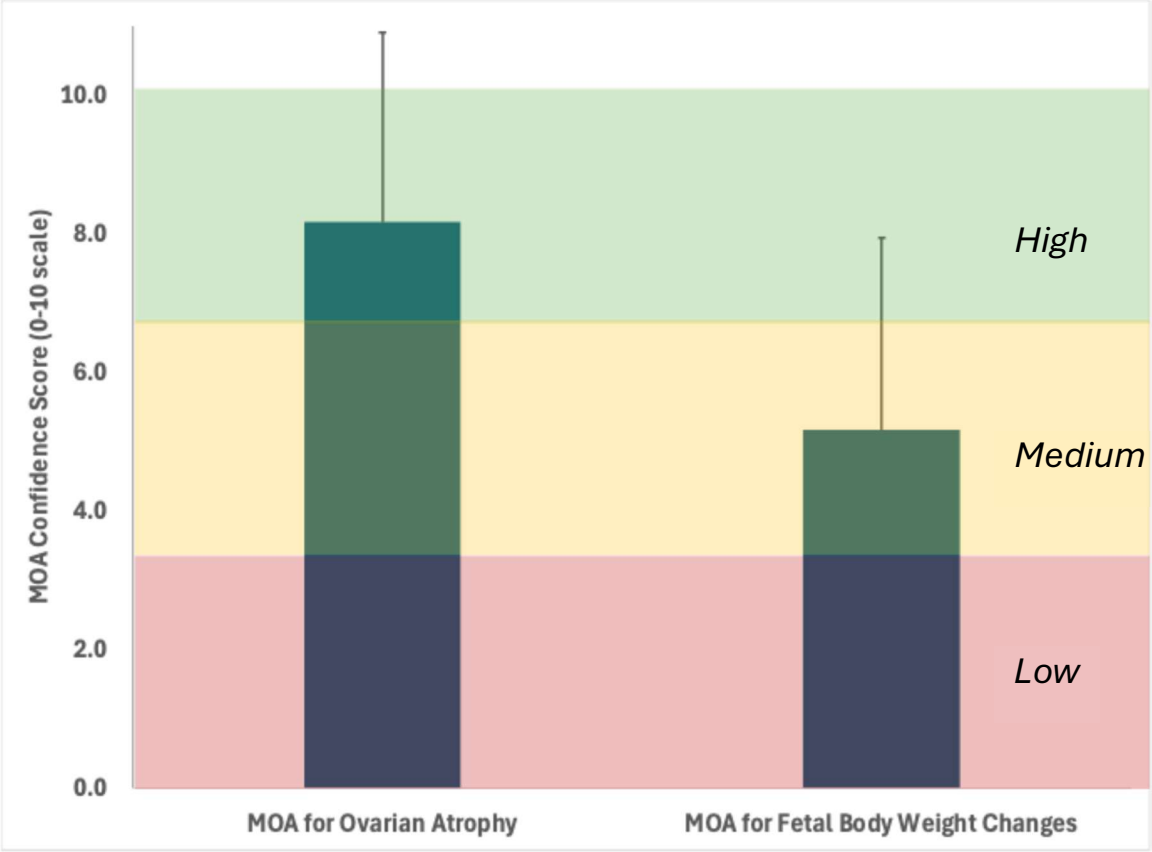
In its response to comment document (USEPA, 2025), EPA defines requirements for DDEF adoptions as follows:

> The commenter appears to be recommending the use of a data derived extrapolation factor (DDEF) to adjust human equivalent concentrations/doses. According to EPA guidance for application of DDEFs (U.S. EPA, 2014), a DDEF requires:
>
> 1) sufficient information on an endpoint-specific mode of action,
>
> 2) data specific to the affected tissue, and
>
> 3) identification of the most appropriate dose metric
>
> Because there is insufficient data across all these factors, the agency has determined not to use Hb adduct data for deriving a data-derived extrapolation factor and instead relied on default dosimetric adjustments and uncertainty factors to account for interspecies differences and variability.

These three requirements have been met for the BD noncancer assessment.

- With respect to requirement 1, an independent panel has reviewed the available evidence supporting an MOA for the fetal body weight changes observed in mice and assigned medium confidence to the MOA (**Figure B.4**) (Kirman et al., 2026; Attachment D). Medium confidence is considered sufficient for DDEF application, particularly when weighed against the low confidence assigned to an assumption of equal sensitivity between mice and humans (as assumed by EPA in the BD noncancer hazard assessment; USEPA, 2025). As such, DDEF application reflects best science for the BD noncancer hazard assessment.

Figure B.4. Panel Confidence in the Noncancer MOAs for BD. MOA for Premature Ovarian Failure: mean=8.2±0.8 out of 10 ; MOA for General Toxicity (e.g., reduced fetal body weight gain): mean=5.2±1.7 out of 10 (Kirman et al., 2026; Appendix D



- With respect to requirement 2, estimates of maternal blood dose serve as an appropriate dose measure for assessing fetal body weight changes. This dose measure has been used in the past by EPA in lieu of fetal tissue data, most recently in the TSCA risk assessment for N-methylpyrollidone (USEPA, 2020), which states,

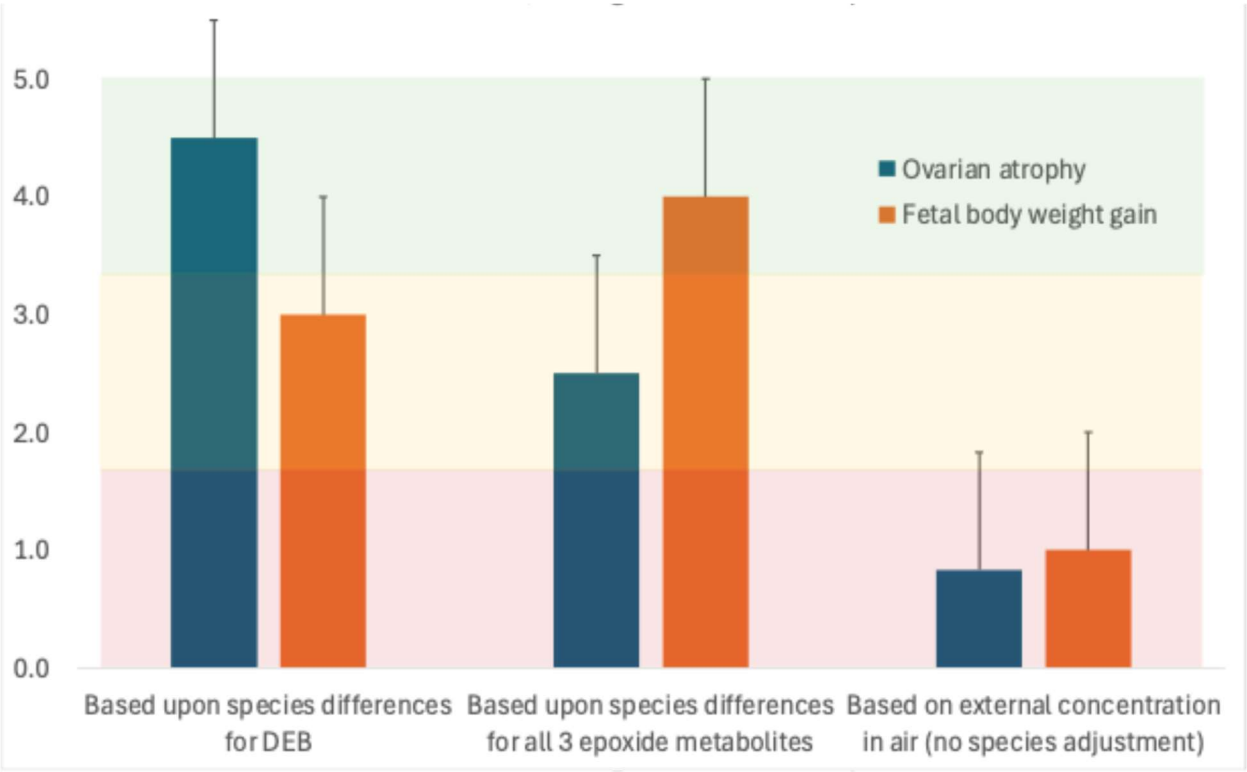
  > ...decreased fetal body weight is expected to be better represented by the AUC of average maternal blood concentration during the vulnerable period of fetal development.

  The panel expressed high confidence in using hemoglobin adducts to account for species differences in lieu of a physiologically based pharmacokinetic model, and medium confidence in relying upon hemoglobin adduct data from nonpregnant humans and animals in lieu of similar data in pregnant humans and animals (Kirman et al., 2026;Appendix D). Extrapolation from nonpregnant humans and animals to pregnant humans and animals is a small one with comparatively low uncertainty,

particularly when contrasted to the large extrapolation made by EPA from mice to humans that has comparatively high uncertainty. As such, estimates of maternal internal dose based on hemoglobin adducts reflects best science for the BD noncancer hazard assessment.

- With respect to requirement 3, an independent advisory panel evaluated three different dose measures for assessing fetal body weight changes. The panel assigned high confidence for using hemoglobin adducts to quantify internal doses for all 3 epoxide metabolites; medium confidence for using hemoglobin adducts to quantify internal dose of the diepoxide metabolite DEB alone; and low confidence in relying upon a dose measure based on external air concentration BD with an assumption of equivalent sensitivity between mice and humans (as assumed by EPA in the noncancer hazard assessment (**Figure B.5**) (Kirman et al., 2026; **Appendix D**). As such, the use of hemoglobin adducts to quantify internal doses for all 3 epoxide metabolites reflects best science for the BD noncancer hazard assessment.

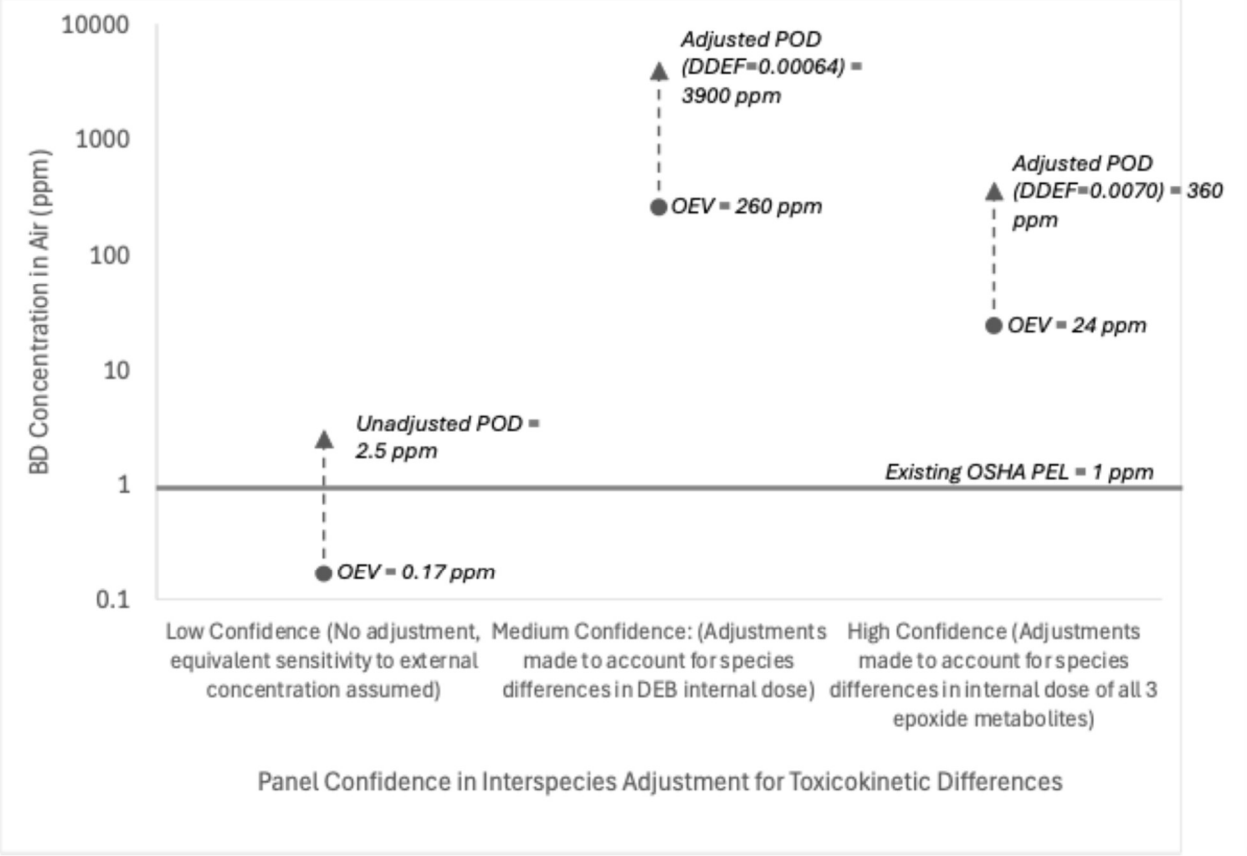
Figure B.5. Panel Confidence in Dosimetry Decisions to Account for Species Differences BD Toxicokinetics



The dosimetry decision for interspecies extrapolation in the noncancer hazard assessment for BD is of critical importance to establishing exposure levels that are protective of worker

populations. The default policy approach adopted by EPA for this decision (assumption of equal sensitivity for mice and humans exposed to a given concentrations) was rejected by other agencies due to concerns that it would overestimate risks to human health and was too uncertain (ATSDR, 2012; ECHA, 2025). EPA's default assumption for BD results in a conclusion that the existing OSHA PEL of 1 ppm is not protective of worker populations (**Figure B.6**). In contrast, best available science for interspecies adjustments results in OEV values that are substantially larger than calculated by EPA with greater confidence, and suggest that the existing OSHA PEL of 1 ppm has a sufficient margin of safety to protect workers from the noncancer effects of BD.

Figure B.6. Calculation of Occupational Exposure Values for BD Based Upon Lower Fetal Body Weight in Mice Using Different Approaches for Interspecies Extrapolation (Triangles = human equivalent concentration for the point of departure; dashed lines = application of an uncertainty factor of 30; Circles = OEV values). Kirman et al. 2026 in press



B.4 EPA's Rationales in the Hazard Assessment for Excluding DDEF Values in the Noncancer Hazard Assessment are Flawed

In its hazard assessment, EPA states:

> EPA did not apply a DDEF for any of the critical hazard outcomes. EPA guidance (U.S. EPA, 2014) on the use of DDEFs requires a strong understanding of the MOA for the endpoint of interest, supported by relevant quantitative data that inform specific key events and characterize the associated toxicokinetic exposure response relationship. Supporting data must be specific to the affected tissue and identification of the most appropriate dose metric. (USEPA, 2025)

Please see Sections B.2 and B.3 for rebuttal of this statement.

EPA also states:

> In the case of fetal body weight reduction, which is identified as the most sensitive and reliable endpoint, substantial uncertainties remain regarding its mode of action. It is unknown if any particular metabolite is responsible for the observed developmental toxicity, and emerging research has identified additional bifunctional metabolites across species (Section 3.3). (USEPA, 2025)

Please see Section B.2 and B.3 for rebuttal of portions of this statement. In addition, emerging research on additional bifunctional metabolites (Elfarra and Zhang, 2012; Wang et al., 2018; Wu et al., 2019; Nakamura et al., 2021) is currently at hypothesis stage. The proposed metabolites have not been measured in animals or humans exposed to BD, the toxicity of these metabolites relative to other BD metabolite (e.g., EB, DEB, EBD) has not been evaluated in any test system, and their potential role in any effects (including fetal body weight changes) has not been evaluated. In the same way that proposal of novel dioxin-like chemicals or novel polycyclic aromatic hydrocarbons does not invalidate a relative potency approach applied to established mixture components, the proposal of new metabolites for BD does not invalidate the application of a relative potency approach to established BD metabolite mixtures. Instead, as more data are collected for new metabolites, the relative potency approach adopted for BD's three epoxide metabolites can readily be expanded to accommodate additional metabolites as needed.

Further, EPA states:

> While Kirman et al. (2022) proposed a DDEF based on a cytotoxicity or general toxicity MOA, these hypotheses lack experimental validation of key events directly linking 1,3-butadiene exposure to fetal body weight reduction. Critical data gaps include limited characterization of 1,3-butadiene metabolism during pregnancy and within fetal tissues, as well as uncertainty

> regarding the primary metabolites responsible for the reduced fetal body weight. (USEPA, 2025)

Please see Section B.2 and B.3 for rebuttal of portions of this statement. In addition, information on the ontogenesis of the enzymes (e.g., cytochrome P450) suggests that fetal metabolism of BD under conditions of Hackett et al. (1987a) is negligible. Specifically, expression of most cytochrome P450 isozymes, including CYP2E1 which is important for BD metabolism, is absent in fetal tissues approximately 2 days prior to birth in mice, with expression starting and then increasing shortly thereafter (Hart et al., 2009; Cui et al., 2012). Because the exposure period used by Hackett et al. (1987a,b) (GD5-15) occurs well before CYP expression becomes important in developing mice, fetal metabolism of BD is expected to be negligible during the exposure period. Instead, delivery of the toxic metabolites of BD is expected to be driven by maternal metabolism and partitioning and therefore is expected to be proportionate to the internal dose of metabolites in maternal blood.

Finally, EPA states:

> Furthermore, the mechanistic data underlying the Kirman et al. DDEF was derived from in vitro cytotoxicity assays conducted across diverse cell lines (e.g., human bone marrow, TK6 cells, rodent fibroblasts, chicken lymphoid cells) display considerable variability and have not been demonstrated to directly predict fetal body weight outcomes in vivo. EPA guidance explicitly states that MOAs from one tissue or outcome cannot be extrapolated to support DDEF for another (U.S. EPA, 2014). (USEPA, 2025)

EPA's statement is misleading for several reasons:

- First, all DDEF values calculated in Kirman et al. are primarily based upon robust *in vivo* data for hemoglobin adducts to estimate internal doses for all three epoxide metabolites in mice, rats, and humans (e.g., **Figure B.2**; Kirman et al., 2026; **Appendix D**). The DDEF values, calculated under an assumption that toxicity is attributed to the diepoxide metabolite (DEB) alone, are based solely on the *in vivo* data. For DDEF values calculated under an assumption that effects are attributed to the combined action of BD's epoxide metabolite, *in vitro* data have been used to estimate relative potency of the metabolites in combination with *in vivo* data to estimate internal dose. The practice of relying upon *in vitro* studies from other tissues and test systems (including bacterial) is well established and has been used by EPA to determine relative potencies for chemical mixtures (e.g., polycyclic aromatic hydrocarbons USEPA, 1993; dioxin-like chemicals, USEPA, 2009). The

application of *in vitro* studies in Kirman et al. within a relative potency framework for mixtures of BD metabolites is consistent with EPA's past practices.

- Second, variability in the DDEF calculations, including variation in underlying data for internal dose estimates and underlying data used to estimate in relative potency, has been assessed quantitative using Monte Carlo methods (Kirman et al., 2022; 2025) (**Figure B.3**). Based upon this evaluation, variation in the underlying data is manageable (less 3-fold variation in resulting DDEF value) and therefore this variation is not a rationale for rejecting the use of DDEF values.
- Lastly, application of DDEF values has been shown to improve dose-response concordance across mice and rats (Kirman et al., 2022), thereby improving confidence in extrapolation to humans, and therefore, contrary to EPA's statement, the DDEF values have been used "to directly predict fetal body weight outcomes."

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Appendix C.

Challenges in Translating Risk Evaluation Conclusions to Risk Management in the Workplace

C.1 Unreasonable risk is concluded for 9 of the Conditions of Use (COU) only under the high-end exposure assumptions

Unreasonable risk to workers was concluded for nine of the COUs for full-shift exposures but only under the high-end, sentinel exposure scenarios. While the use of high-end sentinel exposure scenarios may be appropriate for risk screening, the conclusions do not translate to risk management in actual workplaces. The high-end sentinel scenarios do not represent the distribution of exposures at individual workplaces and miss critical insights into the distribution of the frequency of activities and the specific conditions that may drive risk.

EPA's use of COU and Occupational Exposure Scenarios (OES) are TSCA organizing concepts that reflect how chemicals move through commerce, whereas industrial hygienists in workplaces organize occupational risk assessment using similar exposure groups (SEG) and task level exposure assessment. As such there is a fundamental disconnect between the EPA risk evaluation and how risks are evaluated and managed in actual workplaces. Table C1 shows the contrast in objectives and approaches.

Table C1. Contrast between EPA TSCA Risk Evaluation and Standard Workplace Occupational Risk Assessment

TSCA Risk Evaluation	Workplace Occupational Risk Assessment
Problem formulation – Intended Use of Data	
Characterize human health risk for conditions of use at national level	Assess exposure potential for compliance with OEL and/or risk management at individual workplace
Approach to data collection and analysis	
Data aggregation to characterize exposure profile (random sampling of the entire COU / OES, including ONUs).	Tiered assessment based on exposure potential (targeted sampling for workers with highest exposure potential). Define and use similar exposure groups (SEG) to represent individual worker exposures.
Analytical tools/Data interpretation	
Average and high-end percentile of distribution across entire COU. Metric based on the nature of the toxicity	Metrics depend on type of data (full shift/ short term), type of hazard (chronic /acute), and decision (compliance, control, etc.).
Alternatives to quantitative assessments	

Sentinel scenarios by COU / OES	Tiered approaches include models and data.

An important concept missing in the use of the high-end sentinel exposure scenarios is that elevated air concentrations of butadiene may occur during certain task activities and therefore the full-shift air sample concentrations for workers performing those tasks will include the higher air concentrations that occur during short duration tasks. However, because worker exposure is controlled at the task level (i.e., respirators are worn), the worker is not actually exposed to the air concentration reflected in the high-end exposure scenarios. Although EPA has considered the impact of respirator use in Table 5-5 of the Final Risk Evaluation, the agency's assumption implies that the PPE would be worn for the full-shift. In order to inform risk management however, EPA should acknowledge that in actual workplaces, worker exposures are controlled at the task level to ensure full-shift exposures below the OSHA PEL. This standard work practice is shown in Figure C.1.

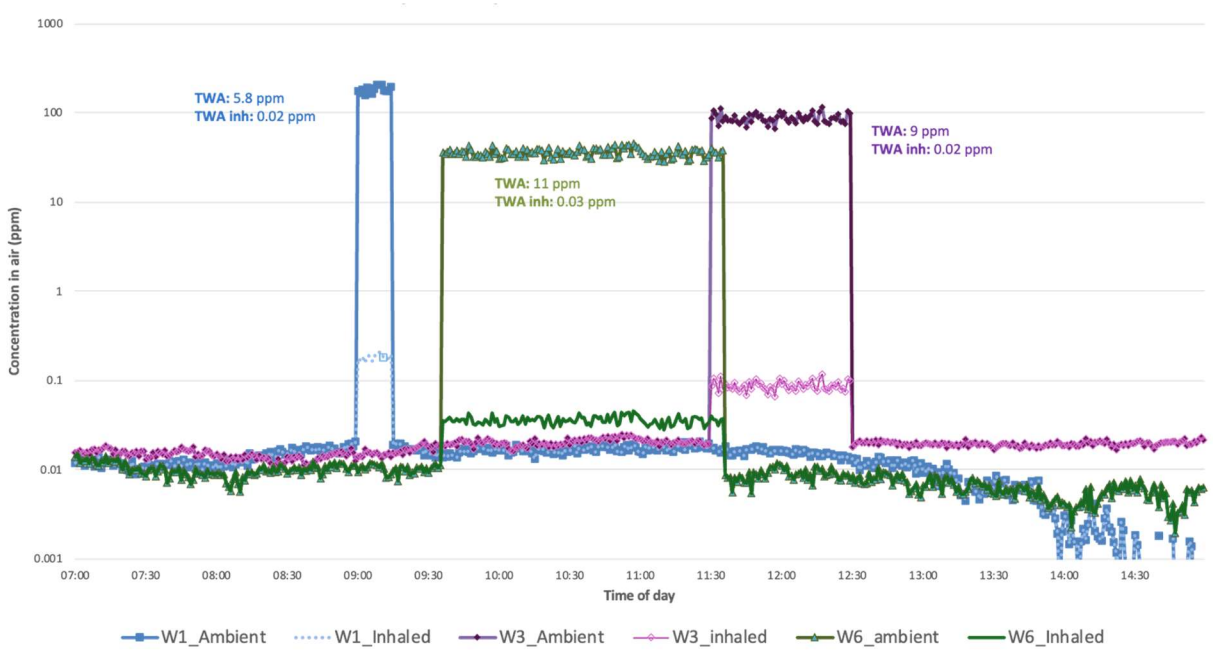


Figure C.1 – Demonstration of potential for higher exposures during certain job tasks, that when controlled at the task level, result in acceptable full-shift exposures.

C.2. Unreasonable risk has been concluded for four COUs based on task level data provided by the Consortium but assumed by EPA to be representative of both task exposures and full shift exposures.

EPA has used the task level air sample data for loading/unloading provided by the Consortium and applied it as analogous to the COU/OES of repackaging and the Consortium task level air sampling data for waste handling and applied it as analogous to

the Recycling and Disposal COUs. For each of the task level datasets, the Consortium indicated the sample time – loading/unloading (16-218 minutes) and waste handling (6 – 135 minutes), which is also reflective of the range of task durations. Although the Agency acknowledges this in the risk evaluation, the unreasonable risk conclusion has still been drawn for the full-shift assumption This has resulted in some workplaces being designated as unreasonable risk at both the Central Tendency and High End even though their work activities do not comport with the assumptions.

In order to inform risk management in the Manufacturing and Processing as a Reactant COU, it is recommended that the task level data be used to target exposure controls where warranted. The full-shift data should be used to compare to the final OEV.

C3. Unreasonable risk was concluded for ONUs associated with repackaging, recycling and disposal because their exposure was assumed to be equivalent to worker central tendency exposures

The risk evaluation concluded unreasonable risk for ONUs associated with repackaging, recycling and disposal because their exposure was assumed to be equivalent to worker central tendency exposures The Agency's assumption that the ONU exposures are equivalent to worker exposures is inconsistent with EPA's definition of an ONU:

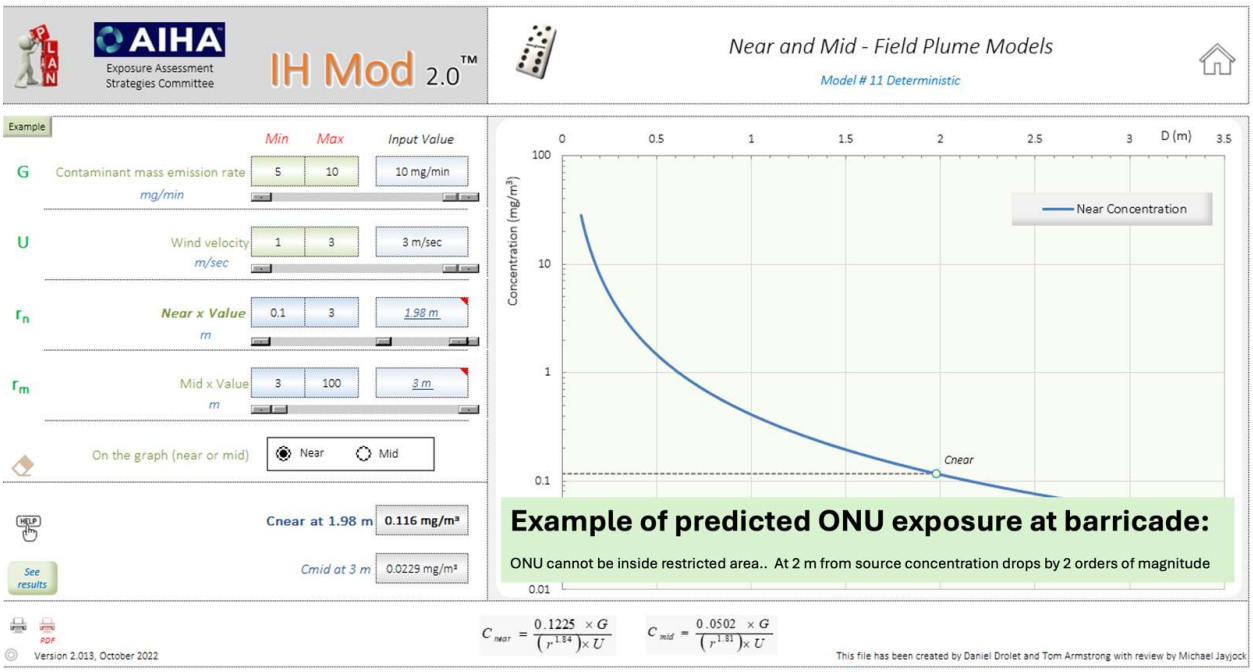
> In addition, EPA plans to evaluate exposure to occupational non-users (ONUs) (i.e., workers who do not directly handle the chemical but perform work in the area whet the chemical is present. (Final Scope of the Risk Evaluation for 1,3-butadiene, p. 35)[1]

Although the term ONU is not typically used by industrial hygienists in the workplace, it is well recognized that workers who are not specifically trained in the hazards of 1,3-butadiene and required to wear PPE for their work are limited in terms of access to areas of a facility where there is a potential for over exposure. This concept is regulated in the OSHA 1,3-butadiene standard (29 CFR 1910.1051), where employers are required to establish regulated areas. The regulated areas are designated, demarcated zones that are established to control and limit access to only those employees who are authorized to work in the area.

Although it is understood that EPA did not have air monitoring for the ONUs in repackaging, recycling and disposal COUs, it is possible to estimate the ONU exposure using simple

[1] Final Scope of the Risk Evaluation for 1,3-Butadiene, CASRN 106-99-0. EPA 740-R-20-011. August 2020. Office of Chemical Safety and Pollution Prevention.

industrial hygiene models (e.g., IH-Mod 2.0™)[2] based on the knowledge that ONUs are prohibited from working in a regulated area. An example of this approach is shown in Figure C2. This model demonstrates that the ONU exposure will not be equal to the worker exposure.



In addition to recognizing the use of regulated areas in accordance with the OSHA standard, EPA's risk management approach should consider the real differences between ONUs and workers. First, ONUs are typically not stationed in process areas, rather they generally perform administrative, support, logistics, or intermittent oversight duties, not prolonged hands-on process work. In addition to regulated areas, work patterns inherently limit time in process areas. For example, even short "walkthrough" exposures are transient and episodic, not continuous. Second, monitoring data from other worker categories are not automatically representative of ONU exposures. Specifically, data collected for production workers or operators represent the highest feasible exposure category, not ONUs, and cannot be directly applied without upward bias. As indicated in Table C.1, industrial hygiene sampling strategy is often risk-based, targeting areas more likely to show higher exposure. Additionally, assumption of steady, unmitigated exposure does not reflect real practice for ONUs given the nature of their work. The agency's risk evaluation has included conservative assumptions that compound unreasonably, notably assuming:

[2] Armstrong, T. W., Drolet, D., and Jayjock, M. (downloaded 2025). IH Mod 2.0™: Deterministic and Probabilistic Exposure Models for Industrial Hygiene and Consumer Product Applications (Version 2.0xx). Fairfax, VA: American Industrial Hygiene Association (AIHA)

- 100% co-location with high-exposure tasks
- no mobility
- no variability across days or seasons
- full working lifetime exposure at this level

Individually, these assumptions might be justified as cautious; together, they yield an implausible worst-case scenario that far exceeds credible ONU exposures.

C.4. Unreasonable risk was concluded for all workers in the Processing as a Reactant COU and the occupational exposure scenario for the polymerization process.

Similar to the Manufacturing and Processing as a Reactant COUs, exposures to polymerization workers will vary by job title and function. As such exposure estimates used in the risk evaluation are unlikely to reflect exposures to all workers in this COU/OES. In order to inform risk management, the Agency should reconsider the exposure literature used to characterize workers and ONU exposure in this COU and assess risk by similar exposure group.

Evaluation of the Modes of Action for Key Noncancer Effects of 1,3-Butadiene: Input from an Independent Expert Panel to Support Derivation of Data-Derived Extrapolation Factors

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Abstract: 1,3-Butadiene (BD) is an important industrial chemical used in the production of plastics, rubbers, and polymers. This evaluation synthesizes mode of action (MOA) information to support interspecies extrapolations for key noncancer effects of BD (ovarian atrophy, lower fetal weight). Data-derived extrapolation factors (DDEFs) were calculated to account for substantial toxicokinetic differences between mice, rats, and humans. MOAs for the key noncancer effects were developed using the IPCS framework and modified Bradford-Hill considerations. An independent panel of six topic experts reviewed the weight of evidence, assessed human relevance, and provided recommendations on dosimetry approaches. DDEF values were calculated using newly published hemoglobin adduct data, including

measurements from female workers, to quantify species differences in internal doses of BD's reactive epoxide metabolites. For ovarian atrophy, the MOA focuses on BD's metabolism to the diepoxide metabolite). The expert panel expressed high confidence (mean score: 8.2/10) in this MOA. For fetal weight effects, the proposed MOA involves metabolism to multiple epoxide metabolites, glutathione depletion, and subsequent toxicity. The panel expressed medium confidence (5.2/10) in this MOA, noting data gaps regarding mechanisms in pregnant animals. Based on hemoglobin adduct data from exposed workers and laboratory animals, preferred DDEFs for mouse-to-human extrapolation were 0.00064 for ovarian atrophy (based on BD's diepoxide metabolite) and 0.0070 for fetal body weight effects (based on all three epoxide metabolites of BD). Application of MOA-informed DDEFs has substantial impact on BD risk assessment. Using EPA's proposed occupational exposure value calculation as an example, incorporating species differences in BD metabolism and pharmacokinetics yields protective values of 24-260 ppm (compared to 0.17 ppm without quantitative pharmacokinetic adjustment), all well above the current OSHA permissible exposure limit of 1 ppm. These findings demonstrate that existing occupational standards are protective for BD's noncancer endpoints when best available science on species differences in toxicokinetics is appropriately considered. This work illustrates the critical importance of incorporating MOA evidence and quantitative dosimetry data to ensure risk assessments reflect scientific understanding and avoid potentially overestimating human health risks.

Key Words: 1,3-butadiene, mode of action, ovarian atrophy, fetal weight, interspecies extrapolation, data-derived extrapolation factors, hemoglobin adducts, risk assessment.

Abbreviations:

AEGL = Acute Exposure Guideline Level; AIHA = American Industrial Hygiene Association; ATSDR = Agency for Toxic Substances and Disease Registry; AUC = Area Under the Curve; BD = 1,3-Butadiene; BMDSD = Benchmark Dose, Standard Deviation; CI = Cytotoxicity Index; CYP = Cytochrome P450 (enzyme); CYP2E1 = Cytochrome P450 2E1; DDEF = Data-Derived Extrapolation Factor; DEB = 1,2,3,4-Diepoxybutane; DNA = Deoxyribonucleic Acid; EB = 2,3-Epoxy-1-butene; EBD = 3,4-Epoxybutane-1,2-diol; ED50 = Effective Dose 50%; EF_AK = Extrapolation Factor for interspecies differences in toxicokinetics; EH = Epoxide Hydrolase; ERPG = Emergency Response Planning Guideline; GD = Gestational Day; GSH = Glutathione; GST = Glutathione-S-Transferase; Hb = Hemoglobin; HBVal = N-(2-hydroxy-3-butenyl)valine; HEC = Human Equivalent Concentration; IPCS = International Programme on Chemical Safety; KE = Key Event; Km = Michaelis-Menten constant; LD50 = Lethal Dose 50%; LOAEL = Lowest Observed Adverse Effect Level; LOD = Limit of Detection; MOA = Mode of Action; MRL = Minimal Risk Level; NAS = National Academy of Sciences; NOAEL = No Observed Adverse Effect Level; NPSH = Non-Protein Sulfhydryl; NTP = National Toxicology Program; OEV = Occupational Exposure Value; OEHHA = Office of Environmental Health Hazard Assessment; OSHA = Occupational Safety and Health Administration; PBPK = Physiologically-Based Pharmacokinetic (model); PEL = Permissible Exposure Limit; POD = Point of Departure; ppm = Parts per million; pyr-Val = N,N-(2,3-dihydroxy-1,4-butadiyl)-valine; ROS = Reactive Oxygen Species; RP = Relative Potency; SD = Standard Deviation; TCEQ = Texas Commission on Environmental Quality; THBVal = N,N,N-Tris-(2-hydroxy-3-butenyl)valine (hemoglobin adduct from EBD); USEPA = United States

67 Environmental Protection Agency; VCD = 4-Vinylcyclohexene Diepoxide; VCH = 4-

68 Vinylcyclohexene; VCM = Vinylcyclohexene Monoepoxide; Vmax = Maximum velocity; WOE =

69 Weight of Evidence

Table of Contents

1. Introduction

1,3-Butadiene (BD) is a chemical that serves as the building block to produce plastics, rubbers, and polymers. In addition to its known carcinogenic effects, exposures to BD are associated with a variety of noncancer effects in experimental laboratory animals (as reviewed by ATSDR, 2012 and USEPA, 2024). The most sensitive noncancer endpoints for BD risk assessment include ovarian atrophy and lower fetal body weight in mice. These endpoints have been used by many regulatory agencies to support noncancer risk assessment of BD over the past few decades. For example, ovarian atrophy in mice has served as the basis for the Health Canada's chronic noncancer assessment (Health Canada, 2000), USEPA's chronic reference concentration (USEPA, 2002), OEHHA's chronic reference exposure level (OEHHA, 2013), and TCEQ's chronic reference value (TCEQ, 2015). Similarly, lower fetal body weight in mice has served as the basis for USEPA's subchronic reference concentration (USEPA, 2002), OEHHA's acute reference exposure level (OEHHA, 2013), and TCEQ's acute reference value (TCEQ, 2015). In contrast, ATSDR decided not to rely on either endpoint to derive its minimal risk levels (MRLs) for BD "*due to the large species differences in the metabolism of 1,3-butadiene and the lack of chemical-specific data to adjust for these differences, which may result in the MRL overestimating the risk to humans*" (ATSDR, 2012). Since then, additional data and methods have become available for assessing BD.

The goal of this evaluation is to consider mode of action (MOA) information to support interspecies extrapolations in the assessment of noncancer endpoints for BD using best

118 available science. Specifically, interspecies extrapolations can be performed through the
119 application of data-derived extrapolation factors (DDEFs) (USEPA, 2014) to account for
120 substantial toxicokinetic differences between mice, rats, and humans based upon best available
121 methods and recent toxicokinetic data for BD. As noted by USEPA's DDEF guidelines,
122 "*Information on MOA is important in DDEF derivation, even when a complete understanding of*
123 *the mechanism is not available*" (USEPA, 2014). An independent expert panel was engaged to
124 do the following: (1) consider the weight of evidence supporting the proposed MOAs and
125 associated confidence, as well as to identify data gaps and limitations; (2) consider the
126 relevance of the noncancer endpoints of toxicity to human health; and (3) provide input on the
127 most appropriate dose metric for human health risk assessment for BD.
128
129

130 2. Background

131
132 Metabolism is an important determinant of all adverse health effects (toxicity and
133 carcinogenicity) of BD. BD itself is biologically inert, in that it does not bind to cellular
134 macromolecules or to receptors. The metabolism of BD to reactive epoxide metabolites,
135 including 2,3-epoxy-1-butene (EB), 1,2,3,4-diepoxybutane (DEB), and 3,4epoxybutane-1,2-diol
136 (EBD), has been well studied in mice, rats, and humans (as reviewed in Himmelstein et al., 1997;
137 Albertini et al., 2003; Kirman et al., 2010; Filser et al., 2010). These reactive metabolites readily
138 bind to cellular molecules, with differing potencies. The metabolic pathways for BD are

qualitatively similar across species, but they exhibit large quantitative differences. Internal doses of these metabolites reflect pathways for their formation (e.g., oxidation) as well as their clearance (e.g., hydrolysis, conjugation) (**Figure 1**).

Large species differences in the metabolism of BD are consistently reported *in vitro, in situ*, and *in vivo* studies. *In vitro* studies on Michaelis-Menten constants (Vmax and Km values) for activation and detoxication pathways of BD in microsomes indicate that mice have a significantly higher ratio of EB activation-to-detoxification than either rats or humans (Csanady et al., 1992; Schmidt and Loeser, 1985; Krause and Elfarra, 1997; Bond et al., 1993; Kreuzer et al., 1991; Seaton et al., 1995; Motwani and Tornqvist, 2014). In the effluent of mouse livers perfused with BD, all three epoxides (EB, DEB, and EBD) and BD-diol were observed, while in effluents from rat livers perfused with BD, only EB and BD-diol were detected. When the mouse and rat livers were perfused with EB, Filser et al., (2001, 2010) found that BD-diol, EBD, and DEB were formed, with BD-diol predominating in both species. DEB formation was greater in mice than in rat livers (Filser et al., 2010). Following *in vivo* exposures of rats and mice to BD via inhalation, differences in circulating DEB levels have been reported to be over 100-fold greater in mice than in rats (Filser et al., 2007; Thornton-Manning et al., 1995a,b).

Quantitative differences in the *in vivo* production of BD metabolites are also reflected in the accumulations of metabolite-specific hemoglobin adducts. A DEB-specific hemoglobin adduct, N,N-(2,3-dihydroxy-1,4-butadiyl)-valine (pyr-Val), has been identified and measured, providing insights into species differences in BD metabolism (Boysen et al., 2012). The formation of pyr-

Val hemoglobin adducts has been studied in male and female mice and rats exposed to 1.0 ppm by inhalation for 6 hours/day for four weeks (Swenberg et al., 2007), in which adduct burdens (i.e., concentrations in blood due to cumulative exposure) in rats were more than 30-fold lower than the corresponding values in mice. The formation of pyr-Val adducts in rats and mice of both sexes was assessed following 4-week inhalation exposures to either 1, 6.25, or 62.5 ppm BD for 6 hours/day (Georgieva et al., 2010). The difference in adduct levels between species was large (mice>rat by approximately an order of magnitude) and dose-dependent, with larger differences observed at higher concentration compared to low concentrations. Swenberg et al. (2007) compared results in occupationally-exposed workers in the Czech Republic to results in BD-exposed mice and rats for pyr-Val. Pyr-Val adducts were not detected (LOD of 0.3 pmol/g Hb) in occupationally exposed men and women with the mean exposures ranging from 0.18-0.8 ppm (Albertini et al., 2003, 2007). Using analytical methods with improved sensitivity, Swenberg et al. (2011)/Boysen et al. (2012) detected pyr-Val in humans. For a given exposure to BD, DEB blood levels in humans (estimated from measured pyr-Val adducts) were approximately 16-fold lower than the DEB blood levels in rats, which in turn are approximately 45-fold lower than the DEB blood levels in mice.

Motwani and Tornqvist (2014) estimated internal dose (i.e., blood AUCs per unit exposure) for BD metabolites in mice, rats, and humans using two approaches: (1) estimating blood dose from hemoglobin adduct data using second-order rate constants for adduct formation and erythrocyte half-lives; and (2) scaling up metabolite clearance rates from *in vitro* studies. For DEB, both approaches yielded consistent results in which substantial differences are estimated

across species (mice>rats>humans). Of primary importance to human health risk assessment, relative species differences in DEB AUC between mice and humans are exceptionally large (approximately 2 to 3 orders of magnitude) (Motwani and Tornqvist, 2014). Based on hemoglobin adduct biomarkers (Motwani and Tornqvist, 2014) and urinary biomarker data (Kotapati et al., 2015), there is clear evidence that mice, rats, and humans are exposed internally to mixtures of BD metabolites that are qualitatively similar, but they have important quantitative differences.

Because of its importance, the definition of MOA has been extended here to specifically include toxicokinetic events in addition to toxicodynamic events. Understanding of BD's metabolism helps explain important species differences in binding to cellular macromolecules, glutathione, and adverse health effects:

- ***Adduct Formation*** – The formation of adducts with cellular macromolecules following BD exposures occurs to a greater extent in mice than in rats. This conclusion applies to adducts formed with hemoglobin, for which metabolite-specific adducts have been measured (EB forms HBVal; DEB forms pyr-Val; EBD forms THBVal (Motwani and Tornqvist, 2014; Boysen et al., 2012, 2022; Georgieva et al., 2010, 2025) and with DNA (as summarized in Albertini et al., 2010).
- ***Glutathione Depletion/ Acute Toxicity*** – Acute exposures to 2,000 ppm produces a nearly complete depletion (by 80% after 7 hours, by 96% after 15 hours) of hepatic non-protein sulfhydryls (NPSH) in mice (Kreiling et al., 1988), to which all three epoxide

metabolites can bind. NPSH depletion was accompanied by signs of acute toxicity in exposed mice. In contrast, similarly exposed rats experienced only moderate NPSH depletion (by 20-35%) with no signs of acute toxicity. BD itself does not react with NPSH, and these results are best explained by the large species differences in the formation of reactive metabolites that conjugate with NPSH. For comparison, acute exposures to 2,000-8,000 ppm in humans, who form much less epoxides than mice, were tolerated with only mild subjective complaints (difficulty focusing, eye irritation) [Carpenter et al., 1944; this study serves as the basis for the NAS (2009) Acute Exposure Guideline Level-1 (AEGL-1) and the American Industrial Hygiene Association (AIHA, 2015) Emergency Response Guideline-2 (ERPG-2) for BD]. GSH depletion is more pronounced and appears at lower BD concentrations mice compared to similarly exposed rats (Himmelstein et al., 1995). *In vitro* studies indicate that all three epoxide metabolites of BD can deplete cellular GSH levels and that cytotoxicity is correlated with the potency by which these metabolites deplete GSH levels (Nieusma et al., 1997, 1998).

- ***Repeat Dose Toxicity*** – Information on the species differences in the reproductive and developmental toxicity of BD in mice and rats is summarized below (**Tables 1-2**). In general, adverse effects are noted in mice, the species with the highest internal dose of BD metabolites, and are generally absent in rats, who have much lower internal doses of BD metabolites. Rats are, however, responsive to direct exposures to BD metabolites, particularly DEB.
- ***Carcinogenicity*** – BD is a multisite carcinogen in male and female rats and mice. Inhalation unit risk values for cancer based on mouse studies (the species with the

highest internal dose of BD metabolites) are more than two orders of magnitude higher than corresponding values based on rat studies (Kirman and Hays, 2022). Adjusting for species differences in the internal dose of BD metabolites resulted in improved concordance of inhalation unit risk values across species.

The weight of evidence for BD from all endpoints supports a general conclusion that species differences in the formation of reactive metabolites underlie species differences in the manifestation of noncancer and cancer effects. The text below summarizes proposed MOAs for the effects of BD on mouse ovary (**Section 2.1**) and mouse fetal body weight (**Section 2.2**), followed by a discussion of recent hemoglobin adduct data and potential DDEF values that reflect species differences in the metabolic activation of BD (**Section 2.3**).

2.1 Proposed MOA for Ovarian Atrophy

The IPCS framework for MOA and human relevance analysis generalized for noncancer endpoints (Boobis et al., 2008), is adopted here. The section below provides a brief description of the Key Events (KEs) in the proposed MOA for ovarian atrophy in rodents, the weight of evidence (WOE) supporting the MOA in rodents within the context of the modified Bradford-Hill considerations (Boobis et al., 2008), an assessment of human relevance, and the DDEF value used to support the noncancer risk assessment. The KEs for the proposed MOA for the ovarian

effects are depicted in **Figure 2**. The WOE supporting the proposed MOA is summarized in **Tables 1, S1**, and are discussed below.

2.1.1 Key Events

Evidence supporting KEs for the ovarian atrophy effects of BD are available from studies of BD itself, its epoxide metabolites (EB and DEB), a structural analog 4-vinylcyclohexene (VCH, a dimer of BD via Diels-Alder reaction) and its epoxide metabolites (monoepoxide VCM and diepoxide VCD) (**Tables 1, S1**).

- ***KE1: Metabolism of BD to DEB*** – As described above BD is initially oxidized to EB, a reaction mediated primarily by P450 isozyme CYP2E1 although other isozymes such as CYP2A6 have also been shown to be involved. Further oxidation of EB by P450 produces the DEB that has been shown to be the causative agent for ovarian toxicity by BD (Doerr et al., 1995, 1996). Similarly, VCD has been shown to be the causative agent for VCH. Metabolism of BD occurs predominantly in the liver and lungs, resulting in systemic dose of BD metabolites. DEB has been detected in animal tissues *in vivo, in situ* (Filser et al., 2001, 2010), and *in vitro* (Seaton et al., 1995; Motwani and Tornqvist, 2014). pyr-Val adducts, a specific biomarker that forms because of a reaction between DEB and hemoglobin, has been detected in rats and mice (Swenberg et al., 2007; Georgieva et al., 2010). Large species differences (mice>rat>human) have been quantified for the internal doses of DEB (based on measured pyr-Val adducts) following exposures to BD

(Motwani and Tornqvist, 2014). Local tissue metabolism of BD in rodent ovary is not expected based upon data collected for a structurally similar chemical (4-vinylcyclohexene or VCH, which is a dimer of BD) that produces the same effects on mouse ovary due to diepoxide metabolite formation (Doerr et al., 1995, 1996). Specifically, rat and mouse ovaries did not have detectable capacity to metabolize either oxidation step for VCH to its diepoxide (VCD) (Keller et al., 1997). However, mRNA for CYP isoenzymes responsible for BD and VCH metabolism can be detected in mouse ovary, and enzyme activity can be induced (Cannady et al., 2003). Based upon *in vitro* study of mouse ovaries showing reduced follicle counts exposed to the monoepoxide metabolite, VCM (125-1000 μM), mouse ovary does have some capacity to oxidize the VCM to VCD (Rajapaksa et al., 2007). However, since these effects were not observed in mice following *in vivo* exposures to VCM (2.72 mmol/kg-day) or VCH (7.4 mmol/kg-day), the authors concluded that hepatic metabolism dominates the contribution made by the ovary in bioactivation of VCM to VCD (Rajapaksa et al., 2007). VCD has been detected in blood following exposure to single doses of VCH (7.5 mmol/kg; 800 mg/kg), with much higher concentrations detected in mice compared to rats (Smith et al., 1990b; Doerr et al., 1995).

- ***KE2: Distribution of DEB to Ovary*** – Wide distribution of DEB has been reported based on direct measurements in multiple tissues, including ovary, in rats and mice (Thornton-Manning et al., 1995, 1997, 1998; Himmelstein et al. 1995). Studies identifying VCD concentrations in systemic tissues following VCH exposure were not identified.

- ***KE3: Molecular Crosslinks (or similar molecular lesion requiring a bifunctional alkylating agent)*** - The precise molecular event responsible for ovarian toxicity by DEB is not known. However, the results of Doerr et al. (1995, 1996) strongly support a conclusion that diepoxide formation is required for ovarian effects to occur. Bifunctional alkylating agents such as DEB cause crosslinks (DNA-DNA, DNA-protein, and/or other macromolecules) (Goggin et al. 2009, 2011; Sangaraju et al., 2012; Jelitto et al., 1989; Vangala et al., 1993; Park and Tretyakova, 2004; Michaelson-Richie et al., 2010), which likely differentiates DEB and VCD ovotoxic potency from their respective mono-alkylating epoxide metabolites (EB, EBD; VCM). This may help explain: (1) observations of ovarian toxicity being limited to agents that either are or are metabolized to diepoxides (BD epoxides, VCH and its epoxides, isoprene); and (2) the lack of ovarian effects following exposure to structurally similar mono-epoxides that are unable to form diepoxides (ethylcyclohexene oxide, vinylcyclohexane oxide, cyclohexene oxide, epoxybutane) (Doerr et al., 1995, 1996). The potential for VCD to produce crosslinks has not been studied: however, based upon its metabolism and reactivity Chiappe et al. (2003) noted that the ovotoxicity of VCH is expected to be due to the biological reactivity of VCD via crosslinking.
- ***KE4: Increase in Cell Death via Apoptosis/Autophagy*** – The precise effects of DEB within ovarian follicles have not been assessed. However, because VCD is used as a research tool to induce early onset menopause in laboratory animals (Van Kempen et al. 2011; Kappeler and Hoyer, 2012), its effects on cellular processes in ovarian follicles have been well-studied. There are multiple and complex responses that occur in ovary

follicles because of exposure to VCD (and by analogy DEB). Thus, KE4 to be an "umbrella" event, which includes mechanistic processes such as the induction of cell death via apoptosis and autophagy, and may include other mechanistic processes. In human granulosa cells exposed to VCD there were concentration dependent increases in apoptosis, which was accompanied by decreases in cell proliferation, increases in markers for oxidative stress, and altered gene expression (Song et al. 2023a). Rats exposed to VCD for 5 days exhibited increased apoptosis in oocyte and granulosa cells (Sen Halicioglu et al., 2021). Mice exposed to VCD for 15 days had increased autophagy of ovarian granulosa cells (Niu et al., 2021). In rats exposed to VCD for 15 days, expression of autophagy proteins was increased (Zhou et al., 2023) as were apoptotic cells (Liu et al., 2023). These changes in mice and rats exposed to VCD were accompanied by a variety of other changes including the induction of oxidative stress, inhibition of IGF1R/AKT/mTOR pathways, inhibition of c-kit signaling, reduced expression of miR-144, and altered expression of caspase and many other genes (Song et al., 2023a; Sen Halicioglu et al., 2021; Zhou et al., 2023; Li et al., 2023, 2024; Abolaji et al., 2016; Niu et al., 2025). As additional research is conducted, additional mechanistic processes and events could be refined and further split into separate KEs.

- ***KE5: Decreased Ovarian Follicle Counts/Ovarian Weight*** – Ovarian toxicity in laboratory animals is indicated by reduced ovary weights and follicle counts. Ovary weights are reduced in a dose-dependent manner in mice by the BD metabolites EB and DEB (Doerr et al., 1996). Follicle counts were also reduced in a clear dose-dependent manner in mice exposed to EB and DEB. For both endpoints, rats were generally not responsive to

EB but did show dose-dependent changes with DEB (Doerr et al., 1996). ED50 values for follicle loss calculated for VCH and its metabolites were highest (least potent) for the parent chemical, intermediate for the monoepoxide (VCM), and lowest (most potent) for the diepoxide (VCD) (Smith et al., 1990a). In rats exposed to VCD, reduced follicle counts were accompanied by increased hemorrhage and congestion, follicular cell degeneration, vacuolization, and increased collagen fibers (Sen Halicioglu et al., 2021).

- ***KE6/AO: Premature Ovarian Failure*** – Premature ovarian failure (i.e., complete loss of follicles, ovarian atrophy; early onset menopause) has been observed in mice exposed to BD for subchronic and chronic durations (NTP, 1984, 1993; Bevan et al., 1996), but not in rats exposed to much higher concentrations (Owen et al., 1987; Bevan et al., 1996). The pattern of effects is similar in animals exposed to VCH and VCD (Bevan et al., 1996; NTP, 1989).

2.1.2 MOA Weight of Evidence Using Modified Bradford-Hill Considerations

Dose-Response Concordance

Dose-response information for the KEs in the MOA for ovarian atrophy are summarized in **Tables 1** and **S1**. Although the adverse outcome has been observed only in exposed mice, because rats experience lower internal doses of DEB than do mice for a given exposure to BD (by a factor of approximately 20), the rat data supporting KEs in this MOA are included to provide additional dose-response context. Integration of the data for VCH/VCD into **Table S1** is complicated by differences in route of exposure (ip, oral, and dermal exposures to VCH/VCD;

356 inhalation exposures to BD) and so was not attempted. Instead, the table includes comparison
357 of the epoxide metabolites of both chemicals assessed within the same test system (reduced
358 follicle counts in rodents following ip exposures; Smith et al., 1990a; Doerr et al., 1996).
359 Unfortunately, Doerr et al. (1996) did not assess BD in this test system. Based on information
360 for KEs 5 and 6 from other studies, the dose-response curve for BD in mice would be less potent
361 than EB, and that the dose-response curve for BD in rats would be unchanged. Characterization
362 of dose-response concordance is also complicated by the fact that data for some events reflect
363 characterization of dose-incidence (AO, premature ovarian failure) whereas data for other
364 events reflect characterization of dose-severity. For this reason, a complete assessment of the
365 dose-response concordance for this MOA is not possible. Instead, a semi-quantitative
366 consideration of dose concordance of the KEs is summarized below.

367 • ***KE1*** – The formation of epoxide metabolites following BD exposure has been quantified
368 across a broad range of exposures in mice and rats (0.1-625 ppm; Georgieva et al.,
369 2010), an exposure range that extends lower than the range producing premature
370 ovarian failure in mice (6.25-625 ppm; NTP, 1993). For VCH, the formation of epoxide
371 metabolites is less well-studied, but VCD has been detected in blood following exposure
372 to single doses of VCH (7.5 mmol/kg; 800 mg/kg), with much higher concentrations
373 detected in mice compared to rats (Smith et al., 1990b; Doerr et al., 1995).
374 • ***KE2*** – The distribution of DEB to a variety of systemic tissues (including ovary) has been
375 measured in mice and rats across a moderate range of exposures (62-8000 ppm), a
376 range that overlaps the range produce premature ovarian failure in mice (6.25-625

ppm). Because KE3 has been measured at even lower concentrations (e.g., 0.5 ppm), the occurrence of KE2 at lower exposures can be inferred.

- ***KE3*** – Macromolecular crosslinks (e.g., DNA-DNA, DNA-protein) have been reported in mice and rats in systemic tissues, but not for ovary specifically, across a wide range of exposures (0.5-2000 ppm; Goggin et al., 2009, 2011; Sangaraju et al., 2012; Jelitto et al., 1989; Vangala et al., 1993). This range extends lower than the range producing premature ovarian failure in mice (6.25-625 ppm; NTP, 1993).
- ***KE4*** – Dose-response data for KE4 following BD exposures are not available. Instead, data for induction of apoptosis are available for VCD (a structural analog of DEB) across a relatively narrow dose range (100-500 mg/kg-day), which generally covers the dose range that VCD is typically used as a research tool to induce early onset menopause in laboratory rodents (Sen Halicioglu et al., 2021; Zhou et al., 2023; Liu et al., 2023; Niu et al., 2025; Abolaji et al., 2016).
- ***KE5*** - Dose-response data for KE5 following BD exposures are not available. However, excellent dose-response data are available for decreased follicle counts and ovarian weight in mice and rats exposed directly (via ip injection) to a broad range of doses of EB (0.005-1.4 mmol/kg-day or ~0.35-100 mg/kg-day) and DEB (0.002-0.29 mmol/kg-day or ~0.17-25 mg/kg-day) (Doerr et al., 1995, 1996). Additional dose-response data are also available in these papers for VCH and its metabolites. For comparison purposes, assuming standard body weights and breathing rates in mice and 100% absorption, the exposure range for BD in producing premature ovarian failure in mice (6.25-625 ppm; NTP, 1993) corresponds to a dose range of approximate 23-2300 mg/kg-day.

Temporal Concordance

Temporal information of the KEs for the MOA for ovarian effects of BD are summarized in **Table 1**. Temporal concordance of the KEs is well supported by available evidence.

- ***KE1*** – Metabolism of BD to DEB has been measured i*n vitro* (Motwani and Tornqvist, 2014), *in situ* (Filser et al., 2001, 2010), and *in vivo* following short-term exposures to BD (Georgieva et al., 2010), time points that occur before the first appearance of premature ovarian failure in BD-exposed mice at 13 weeks (Bevan et al., 1996).
- ***KE2*** – The widespread distribution of DEB to systemic tissues has been measured following acute exposures to BD (Thornton-Manning et al., 1995, 1997, 1998; Himmelstein et al. 1995), time points that occur before the first appearance of premature ovarian failure in mice at 13 weeks (Bevan et al., 1996).
- ***KE3*** – Macromolecular crosslinks have been measured *in vitro* in human cervical carcinoma cells (Michaelson-Richie et al., 2010) and in multiple systemic tissues (but not ovary specifically) following acute and short-term exposures to BD (Jelitto et al., 1989; Vangala et al. 1993; Goggin et al., 2009, 2011), time points that occur before the first appearance of premature ovarian failure in BD-exposed mice at 13 weeks (Bevan et al., 1996).
- ***KE4*** – Increase in cell death via apoptosis/autophagy has not been specifically measured following exposures to BD. Instead, this KE has been measured i*n vitro* for VCD (as summarized in Kappeler and Hoyer, 2012) and following short-term exposures to VCD (Sen Halicioglu et al., 2021; Niu et al., 2025; Zhou et al., 2023; Liu et al. 2023; Abolaji et

al., 2016). By analogy, this KE occurs at time points before the first appearance of premature ovarian failure in BD-exposed mice at 13 weeks (Bevan et al., 1996).

- **KE5** – There are no data on follicle counts following exposures to BD. Direct exposure of mice and rats to BD metabolites EB and DEB following short-term exposures (Doerr et al., 1995, 1996) decreases follicle counts at time points before the first appearance of premature ovarian failure in BD-exposed mice at 13 weeks (Bevan et al., 1996).

Strength, Consistency, and Specificity

Ovarian toxicity is consistently observed in mice exposed to BD (Doerr et al., 1996; NTP, 1984, 1993; Bevan et al., 1996), and is consistently absent in rats exposed to BD (Doerr et al., 1996; Owen et al., 1987; Bevan et al., 1996), but observed when rats are exposed directly to BD metabolites (Doerr et al., 1996). The proposed MOA is consistent with observed the metabolic activation of BD to a diepoxide intermediate (mouse>rat; Filser et al., 2001, 2007, 2010; Thornton-Manning et al., 1995a,b; Motwani and Tornqvist, 2014) and sensitivity to ovarian effects (mouse>rat; Doerr et al., 1996; NTP, 1984, 1993; Bevan et al., 1996; Owen et al., 1987).

There are marked species differences in effects observed between rats, with no BD-induced ovarian atrophy observed following chronic exposures as high as 8,000 ppm (Owen et al., 1987), and for mice, which BD-induced ovarian atrophy results from chronic exposures as low as 6.25 ppm BD (NTP, 1993). Furthermore, *in vivo* exposure to the mono-epoxide metabolite of BD, EB, has been shown to be toxic to mouse ovary but not to rat ovary, reflecting greater hepatic/pulmonary conversion of EB to DEB in mice. Direct exposure to DEB was toxic to the

443 ovary of both species, albeit with a lower efficacy in rats than in mice (Doerr et al., 1996).
444 Toxicodynamic differences between rats and mice exposed to DEB (rat<mouse) are supported
445 by the data of Doerr et al. (1996), which were estimated to be approximately 11-fold in Kirman
446 et al. (2022), and may be larger to explain the absence of effects in rats chronically exposed to
447 8,000 ppm (Owen et al., 1987)
448
449 Species differences in premature ovarian failure (mouse>rat) also correlate well with species
450 differences noted for KE1, KE2, KE3, and KE5 (**Table 1**). Species differences (mouse>rat) in the
451 formation of DEB and DEB-specific hemoglobin adducts (pyr-Val) are consistently reported across
452 available studies (see Section 2 summary of metabolism above).
453 The overall pattern for ovarian toxicity (diepoxide>monoepoxide) and species differences
454 (mice>rats) observed for BD are the same as those observed for VCH (Doerr et al., 1995, 1996).
455
456 **Biological Plausibility and Coherence**
457 There is convincing evidence that ovarian atrophy is mediated by the formation of diepoxides,
458 such as the BD diepoxide metabolite DEB (Doerr et al., 1995; 1996) and the diepoxide
459 metabolite of VCH (VCD). Ovarian toxicity was observed following exposure to diepoxides (DEB,
460 VCD) and diepoxide precursors (EB, BD dimer, or VCH, VCM, isoprene), but was absent
461 following exposure to structural analogues that do not form diepoxides (ethyl cyclohexene
462 oxide, vinyl cyclohexane oxide, cyclohexene oxide) (Doerr et al. 1995, 1996). Although the
463 molecular mechanism(s) are not fully understood, diepoxides appear to selectively destroy the
464 primordial and primary follicles via increased cell death by apoptosis and autophagy, thereby

accelerating the normal process of atresia (Springer et al., 1996; Hoyer and Sipes, 2007; Sen Halicioglu et al., 2021; Zhou et al., 2023; Liu et al., 2023; Niu et al., 2025). Accelerated oocyte depletion leads eventually to premature ovarian failure (i.e., complete loss of follicles) and cessation of the estrous cycle.

Other MOAs

No other potential MOAs are envisaged for the effects of BD on ovarian atrophy. The parent chemical is inert and is considered "inactive" for the estrogen receptor (agonist, antagonist, or binding) (Mansouri et al., 2016). Based upon studies in VCD, increased cell death in ovarian follicles via necrosis/toxicity is not supported (Kappeler and Hoyer, 2012). In addition, GSH depletion does not appear to be involved in the ovotoxicity of VCD (Devine et al., 2001). The other epoxide metabolites of BD (EB and EBD) are not diepoxides, and therefore are not expected to directly contribute to the ovarian effects of BD. This information does not detract from the weight of evidence supporting the proposed MOA for ovarian described above.

Uncertainties, Inconsistencies, Data Gaps

Uncertainties, inconsistencies, and data gaps on some aspects of the MOA are discussed below.

- ***Data Gaps for KEs*** – As noted above, the precise molecular event(s) (KE3) responsible for ovarian effects are unknown. Additional studies to identify the specific crosslink(s) responsible for this KE would further extend our understanding of this MOA and its application to risk assessment. For KE4, information is lacking for BD. However, due to its use as a research tool to induce premature ovarian failure in laboratory animals, there is

a robust database of studies available for VCD and the mechanistic processes it affects, which are believed to be directly applicable to DEB. For this reason, uncertainty in extrapolating information collected for VCD to DEB for KE4 is expected to be low. However, additional studies designed to characterize KE4 (reliance on studies for VCD for data on increased cell death via apoptosis/autophagy) and KE5 (reliance on studies for BD metabolites, EB and DEB, for data on decreased follicle counts) following exposure to BD at multiple exposure levels would serve to increase confidence in the MOA.

- ***Uncertainty Associated with Recently Proposed Metabolites*** - Researchers have recently proposed the potential formation of additional bifunctional metabolites for BD, including the formation of a chlorinated metabolite via myeloperoxidase and hypochlorous acid (Elfarra and Zhang, 2012; Wang et al., 2018; Wu et al., 2019) and ketone/aldehyde metabolites of EBD via alcohol dehydrogenase in isogenic chicken cells *in vitro* (Nakamura et al., 2021). The formation of these metabolites *in vivo* following exposure to BD, as well as the ability of these hypothesized bifunctional metabolites to cause effects (noncancer or cancer) has not been demonstrated (i.e., a role for these potential metabolites in the cancer effects BD was proposed by the study authors). If future research shows these metabolites to be important to both internal dose and to contribute to ovarian atrophy, a relative potency approach could be extended and applied to include contributions from additional metabolites for ovarian atrophy.
- ***Essentiality of KEs Has Not Been Assessed*** – Although the data of Doerr et al. (1995, 1996) strongly support the requirement for diepoxides in the ovarian effects of BD and VCD, studies assessing the essentiality of the KEs for this MOA are lacking. Additional

studies in P450 knockout mice (to assess the essentiality of KE1) and similarly designed studies for other KEs would serve to increase confidence in the overall MOA.

- ***Toxicodynamic Differences –*** As noted above, toxicodynamic differences between mice and rats are suggested by the data of Doerr et al. (1996). However, toxicodynamic difference have not been investigated to level to support quantifying these differences (e.g., DDEF values to toxicodynamics). Additional studies that quantify these differences, as well as studies that assess potential toxicodynamic differences in humans would serve to increase confidence in the overall MOA.

2.1.3 Human Relevance of MOA

Key questions identified for considering the human relevance of the MOA (Boobis et al., 2008) are summarized below, and considered by an independent expert panel (see **Section 4**):

- ***Is the weight of evidence sufficient to establish a mode of action in animals?***

  The MOA for ovarian toxicity in experimental animals exposed to BD, through the formation of a diepoxide metabolite (DEB), appears to be well supported by available literature as summarized in **Tables 1, S1** and described above, with weaknesses noted for KE4 (hence reliance on VCD data).

- ***Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?***

Ovarian toxicity is observed when rats are exposed directly to DEB (Doerr et al. 1995, 1996), indicating that this endpoint is not specific to mice. Data from a structural analog VCD lend additional support to this conclusion. Like DEB, the structural analog VCD also produces ovarian toxicity in rats following direct administration. Additionally, ovarian toxicity was observed in nonhuman primates exposed to VCD via intramuscular injection or surgical implantation of a degradable fiber (Appt et al., 2006, 2010). Lastly, *in vitro* studies show that VCD produces increased intracellular ROS, DNA damage, and alters the expression of genes related to apoptosis and oxidative stress, resulting in increased apoptosis in human ovarian (granulosa) cells (Song et al., 2023a). All the KEs are expected to occur in humans exposed to BD, albeit with clear quantitative species differences. Together, the weight of evidence appears to support the conclusion that qualitatively the endpoint of rodent ovarian toxicity is relevant to human health.

- ***Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?***

Point of departure (POD) values for premature ovarian failure in mice and rats vary widely depending upon the approach used to quantify differences in toxicokinetics: (1)

no adjustment (mouse BMDSD= ~1.5 ppm; rat NOAEL = ~180 ppm); (2) accounting for differences in DEB internal dose (mouse BMDSD= ~1700 ppm; rat NOAEL = ~11,000 ppm); and (3) accounting for differences in the internal doses of all three epoxide metabolites (mouse BMDSD= ~260 ppm; rat NOAEL = ~1,400 ppm) (Kirman et al., 2022). These interspecies adjustments are based on hemoglobin adduct data for male workers (as described in Kirman et al., 2022), and do not reflect the recently published hemoglobin adduct data in female workers (described in **Section 2.3** and applied in **Section 5**). For comparison purposes, average concentrations of BD in U.S. ambient air are 0.000058 ppm (Kirman et al., 2025), whereas the average concentration of BD in workplace air for BD workers range from 0.012-0.16 ppm (Panko et al., 2023).

There are profound quantitative differences among mice, rats, and humans with respect to circulating levels of DEB following exposure to BD, which need to be considered in risk assessment. Studies of hemoglobin biomarkers (Swenberg et al., 2011; Boysen et al., 2012; Motwani and Tornqvist, 2014) demonstrate that for a given exposure to BD, estimated DEB blood levels in humans are several orders of magnitude lower than corresponding DEB blood levels in mice (see Table 3 of Motwani and Tornqvist, 2014). Recently published data (Georgieva et al., 2025; discussed further in **Section 2.3**), which includes findings in female workers, which suggests that internal doses of DEB in women may be lower than those measured in men following exposures to BD.

2.2 Proposed MOA for the General Toxicity of BD (e.g., Reduced Body Weight and Weight Gain)

The section below provides a brief description of the KEs in the proposed MOA for the general toxicity of BD. General toxicity is defined here as an "umbrella" endpoint that includes signs of toxicity as indicated by reduced body weight gain and weight gain in maternal, fetal, and nonpregnant animals following *in vivo* exposures to BD, and cytotoxicity as measured in cells following *in vitro* exposures to BD. The weight of evidence supporting this proposed MOA is presented below within the context of the modified Bradford-Hill considerations, along with an assessment of human relevance (Boobis et al., 2008). The KEs for the proposed MOA for general toxicity (e.g., reduced body weight and weight gain) are depicted in **Figure 3**. The WOE supporting the proposed MOA is summarized in **Tables 2 and S2** and is discussed below.

2.2.1 Key Events

Information on the MOA for the general toxicity of BD is limited. KEs for BD's proposed MOA are summarized below. As noted above, because metabolism is an important determinant of BD's toxicity, and because of the large species differences (mouse>rat>human) in the metabolic activation of BD to reactive metabolites, the definition of MOA has been extended to specifically include toxicokinetic events in addition to toxicodynamic events.

- ***KE1: Metabolism of BD to Reactive Epoxide metabolites*** – As summarized in **Section 2**, BD is metabolized to multiple epoxide metabolites (EB, DEB, EBD). Hemoglobin adducts that reflect circulating blood levels of all three epoxide metabolites of BD have been characterized in mice, rats, and humans (EB producing HBVal, DEB producing pyr-Val, and EBD producing THBVal; Swenberg et al., 2007; Georgieva et al., 2010; Boysen et al., 2012). These adducts have been used to quantify internal doses (AUC in blood) for all three metabolites (Motwani and Tornqvist, 2014). These data indicate that there are significant differences between species for the internal doses of BD epoxides (mouse>rat>human). More recently, additional data have been published for these hemoglobin adducts in exposed workers (including female workers) that can further support quantification of internal doses (Georgieva et al., 2025; see discussion in **Section 2.3**).
- ***KE2: Distribution of Epoxide Metabolites to Systemic Tissues (e.g., maternal and fetal)*** – Although studies on the distribution of BD metabolites to maternal and fetal tissues during pregnancy are not available, wide distribution of BD's metabolites has been reported based on direct measurements in multiple systemic tissues, including uterus, in rats and mice (Thornton-Manning et al., 1995, 1997, 1998; Himmelstein et al. 1995). Consistent with tissue partitioning, systemic tissue concentrations of these epoxides are generally within a factor of 2-3 of the concentrations measured in blood. Tissue concentrations of epoxide metabolites are higher in mouse tissues compared to rat tissues. Based upon their wide systemic distribution, the distribution of BD's epoxide metabolites to all maternal and fetal following BD exposure is inferred.

- ***KE3: GSH Depletion Due to Epoxide Conjugation*** - All three epoxide metabolites of BD are conjugated with GSH via glutathione-S-transferases. As a result of this conjugation, GSH levels can become depleted, as measured *in vitro* in rat hepatocytes (Nieusma et al., 1997, 1998). Acute exposures of rats and mice to a broad range of concentrations of BD (10-8000 ppm) indicate that systemic tissue concentrations of GSH are reduced in a concentration-dependent manner, with much larger reductions in GSH levels measured in mouse tissues compared to rats (Deutschman and Laib, 1989; Himmelstein et al., 1995; Kreiling et al., 1988). Based upon the measurement of GSH depletion in systemic tissues, depletion of all maternal and fetal following BD exposure is inferred.
- ***KE4/AO: General Toxicity in Dams/Fetus*** – Depletions of GSH levels is associated with increased signs of general toxicity. KE4 is considered a "umbrella" event since it includes different measures of general toxicity (cytotoxicity *in vitro*; reduced body weight and weight gain *in vivo*), and since there may be additional events that occur after GSH depletion but before signs of toxicity (see discussion of uncertainty below). As additional information is collected for BD, this KE could be split into separate KEs.

In vitro studies in rat hepatocytes indicate that the cytotoxic potency of BD's epoxide metabolite enantiomers mirrored their ability to deplete cellular GSH levels (i.e., the most cytotoxic epoxide enantiomers also exhibited the greatest depletion of cellular GSH). *In vitro* studies indicate that DEB is highly embryotoxic, with an LD50 value of 5 uM for growth (Clerici et al., 1995). Signs of acute toxicity were reported in mice following acute exposures to BD that resulted in GSH depletion (Kreiling et al., 1988).

638 Similarly exposed rats experienced only a moderate decrease in GSH and did not exhibit
639 signs of acute toxicity.
640
641 Signs of general toxicity, as indicated by reduced maternal weight gain and fetal body
642 weight, were reported in pregnant mice and rats exposed to BD (Hackett et al., 1987a,b;
643 Hazleton, 1981). In rats, one study reported no treatment-related effects of maternal
644 weight gain or fetal body weight following exposures to up to 1,000 ppm on GD 6-15
645 (Hackett et al., 1987b). A second study reported decreases in maternal body weight gain
646 at doses lower than those associated with decreases in fetal body weight (Hazleton,
647 1981). Significant reductions in maternal body weight gain were noted on GD 6-9
648 following exposures to 1,000 or 8,000 ppm BD. Small but significant reductions in fetal
649 weight were measured in pregnant rats exposed to the highest concentration (8,000
650 ppm). Exposure to BD during gestation (GD 5-15) in mice resulted in concentration-
651 dependent decreased maternal weight gain (particularly on GD11-16 when fetal growth
652 would be highest in the study) as well as decreases in fetal body weight (Hackett et al.,
653 1987a). The study authors identified the lowest concentration (40 ppm) as statistically
654 significant for fetal body weight and the middle concentration (200 ppm) as statistically
655 significant for maternal body weight gain. However, Christian (1996) noted that the
656 authors' statistical analysis was inappropriate, and Green (2003) identified errors in their
657 analysis, concluding that the lowest concentration was instead a NOAEL for fetal body
658 weight effects. Rather than focus on NOAEL and LOAEL comparisons to determine which
659 endpoint is more sensitive, inspection of a plot of maternal weight gain *vs*. fetal weight

expressed as a percentage of control values reveals that the impact on both endpoints as a function of BD concentration are remarkably similar (**Figure 4**).

In mature (non-growing), nonpregnant mice, BD exposures of 40-1000 ppm had no effect on body weights (i.e., body weights remained unchanged at approximately 30 grams for all concentrations and time points; Hackett et al., 1987a). However, studies in young (growing) nonpregnant mice indicate that BD's epoxide metabolites (EB, DEB) can reduce body weight and weight gain. Direct exposure of mice to either EB or DEB produced dose-dependent decreases in weight gain over 5-30 days (Doerr et al., 1996). In nonpregnant rats, EB did not result in any notable change in weight gain; however, DEB produced a significant decrease in weight gain that was more pronounced than that measured in mice and was accompanied by increased mortality at the highest dose (Doerr et al., 1996). These data lend support to the role of metabolites, particularly DEB, in the effects of BD on weight in growing animals.

No developmental toxicity studies were identified for the structural analog VCH. However, a single developmental toxicity study was identified for VCD in mice, which reported reduced fetal body weight (by approximately 20%) at the highest dose (240 mg/kg-day VCD) (Song et al., 2023b). Maternal body weight measurements were not reported in this study.

NAS (2009) reviewed the data for maternal and fetal body weight effects in animals exposed to BD (Hackett et al., 1987a) and ultimately concluded that "*These effects were only observed in the presence of maternal toxicity (growth reduction). Such a pattern of effects can be typically considered to represent non-specific growth retardation due to the maternal condition.*"

2.2.2 MOA Weight of Evidence Using Modified Bradford-Hill Considerations

Dose-Response Relationships

Dose-response data for KEs in the MOA for decreased body weight and weight gain are summarized in **Tables 2** and **S2**.

- ***KE1*** – The formation of epoxide metabolites following BD exposure has been quantified across a broad range of exposures in mice and rats (0.1-625 ppm; Georgieva et al., 2010; Boysen et al., 2007), an exposure range that includes and extends below the range producing decreases in body weight effects in mice (40-1000 ppm; Hackett et al., 1987a). Measurements for KE1 were lower in rats compared to mice for a given exposure to BD.
- ***KE2*** – The distribution of BD's epoxide metabolites to a variety of systemic tissues (including ovary) has been measured in mice and rats across a moderate range of exposures (62-8000 ppm; Himmelstein et al., 1995; Thornton-Manning et al., 1995, 1997, 1998), a range that overlaps that producing decreases in body weight and weight

gain in mice (40-1000 ppm; Hackett et al., 1987a). Again, measurements for KE2 were lower in rats compared to mice for a given exposure to BD.

- ***KE3*** – GSH depletion has been measured in mice and rats across a broad range of concentrations of BD (10-2000 ppm; Kreiling et al., 1988; Deutschman and Laib, 1989; Himmelstein et al., 1995), a range that includes and extends below the range producing decreases in body weight and weight gain in mice (40-1000 ppm; Hackett et al., 1987a). Response measurements for KE3 were lower in rats compared to mice for a given exposure to BD.

Additionally, dose-response data are available for BD metabolites supporting their role in producing signs of general toxicity (e.g., decreased body weight and weight gain) in growing nonpregnant animals:

- In growing mice receiving EB via daily ip injections for 30 days, a 10% decrease in body weight gain was noted at the highest tested dose (1.43 mmol/kg-day; Doerr et al., 1996). In contrast, no notable change in body weight gain was noted in similarly exposed rats. These results are consistent with mice producing more DEB from EB than is produced in rats.
- In growing mice receiving DEB via daily ip injections for 30 days, a 15% decrease in body weight gain was noted at the highest dose evaluated (0.29 mmol/kg-day; Doerr et al., 1996). In rats, a 15% decrease in body weight gain was caused by a lower dose of DEB (0.14 mmol/kg-day; Doerr et al., 1996). Rats were more sensitive to the highest dose of

DEB (0.29 mmol/kg-day) than mice, exhibiting a 50% decrease in body weight gain by day 25, with only 4/10 animals surviving until day 30. These data support a role for the systemic dose of DEB as an important determinant of reduced weight gains.

Together the available data provide good dose-response concordance. Measurements for rats for KE1-KE3 are consistently lower than measured in mice, providing additional dose-response context for KE4, for which BD-exposed rats were either nonresponsive (Hackett et al., 1987b) or required higher exposures (Hazleton, 1981).

Temporal Association

Temporal association data for KEs in the MOA for effects on body weight and weight gain are summarized in **Table S2**.

- ***KE1*** – The formation of epoxide metabolites following BD exposure has been measured following short-term exposures to BD in mice and rats (1-10 days; Georgieva et al., 2010; Boysen et al., 2007), a range of durations that includes and is shorter than the duration producing decreases in body weight and weight gain in mice (10 days; Hackett et al., 1987a).
- ***KE2*** – The distribution of BD's epoxide metabolites to a variety of systemic tissues (including ovary) has been measured in mice and rats following acute exposures to BD (single day; Himmelstein et al., 1995; Thornton-Manning et al., 1995, 1997, 1998), a duration that is shorter than that producing decreases in body weight and weight gain in mice (10 days; Hackett et al., 1987a).

- ***KE3*** – GSH depletion has been measured in mice and rats following acute exposures to BD (single day; Kreiling et al., 1988; Deutschman and Laib, 1989; Himmelstein et al., 1995), a duration that shorter than that producing decreases in body weight and weight gain in mice (10 days; Hackett et al., 1987a).

Changes in body weight gain in growing, nonpregnant mice were evident as soon as 5 days of direct exposure to BD metabolites EB or DEB (Doerr et al., 1996), which is temporally consistent with the response of Hackett et al. (1987a) after 10 days of exposure to BD. *In vitro* exposure of mouse pre-implantation embryos to DEB (widely considered to be the most potently toxic metabolite of BD) for 24 hours was sufficient time to result in signs of toxicity (Clerici et al., 1995), and as such is temporally consistent with observations of reduced weight gain during gestation.

Strength, Consistency, and Specificity

The weight of evidence supporting this MOA has some limitations, primarily its reliance on studies conducted in nonpregnant animals to characterize KEs in pregnant animals. The WOE does, however, provide a consistent depiction of the KEs in both mice and rats, with the rats being consistently less sensitive than mice for all events (**Tables 2, S2**).

The data from Doerr et al. (1996) provide strong support for the role of BD metabolites, particularly DEB, in causing body weight changes in growing non-pregnant mice. In addition, there is some evidence supporting the role for DEB in the fetotoxic endpoints of BD:

- DEB is specifically considered to be "*highly embryotoxic in preimplantation mouse embryos in vitro at micromolar concentrations*" (Clerici et al., 1995).
- When administered directly, DEB also produces fetotoxicity, including reduced growth and viability, in the nonresponsive species rats (Chi et al., 2002), suggesting that species differences in metabolite formation underly species differences to responsiveness for this endpoint. This conclusion is consistent with that reached in a review of BD's reproductive and developmental effects (Christian, 1996). Reports of reduced fetal body weight in rats exposed to higher concentrations (8,000 ppm; Hazleton, 1981), indicate that this effect is not specific to the mouse.
- Potential toxicity of BD's other epoxide metabolites (i.e., other than DEB) is supported by their ability to deplete cellular GSH in a manner that correlates with their cytotoxicity (Nieusma et al., 1997, 1998). Empirical support for this inference from improved dose-response concordance across species was reported in Kirman et al. (2022; see Figure 5C, D) when adjustments were made to account for species differences in internal dose for BD metabolites.

Biological Plausibility and Coherence

Decreases in maternal and fetal weight both serve as signs of general toxicity. The relationship between these two endpoints is less clear. In rats, decreases in maternal weight gain occur at lower concentrations of BD than those producing decreases in fetal weight (200 vs 8,000 ppm; Hazleton, 1981). However, in mice these two endpoints occur at the same concentration level

(**Figure 4**; Hackett et al., 1987a). Therefore, the relationship between these endpoints is uncertain. Fetal effects secondary to maternal toxicity is a common finding in developmental toxicity studies (Chernoff et al., 2008), particularly when a maximum tolerated dose is utilized as part of the study design. The general findings of their review of 125 developmental toxicity studies included the following: (1) lowest observable adverse effect levels (LOAELs) were determined by reduced maternal gestational weight gain or fetal weight at term; (2) Maternal weight reductions are associated with reduced food intake for a variety of dissimilar test agents; (3) Lower fetal weights were associated with reduced maternal weight gains late in gestation; (4) The degree of fetal weight reduction is correlated with the extent of maternal weight loss. This finding is consistent with NAS (2009) conclusions based upon review of the data from Hackett et al. (1987a).

A potential role for DEB in causing the effects in dams/fetus is supported. DEB is consistently identified as the most toxic metabolite of BD utilizing multiple test systems (*in vitro* and in *vivo*). However, a role for other metabolites contributing to toxicity, particularly when present at high concentrations in the body, is supported by their ability to deplete GSH and corresponding cytotoxicity (Nieusma et al., 1997, 1998).

Since BD is biologically inert, its toxicity mainly results from the formation of reactive metabolites such as EB, DEB, or EBD. In a review of the reproductive and developmental toxicity of BD, Christian (1996) stated that, "*Regardless of the strain used, mice were always affected by BD at lower doses than rats, an expected observation, based on well recognized differences in*

pharmacokinetic (PK) parameters in these two species." Specifically, mice have been shown to produce higher internal doses of the reactive epoxide metabolites of BD than corresponding internal doses in other species (e.g., rats, humans), as quantified in Motwani and Tornqvist (2014).

By analogy, maternal weight gain and fetal body weights were reduced in mice exposed to a structurally similar chemical (isoprene), whose toxicity is also attributed to the formation of reactive epoxide metabolites (limited details from an unpublished report summarized in Anderson, 2001). Like the effects of BD exposure, these effects of isoprene were observed only in mice, whereas similarly exposed rats were unaffected by treatment. Although no developmental toxicity studies were identified for VCH, a developmental toxicity study conducted in mice for VCD (80, 160, 240 mg/kg-day) reported a decrease in fetal body weight at the highest dose (Song et al., 2023b). Maternal body weights were not reported in this study.

Other MOAs

Unfortunately, feed intake data were not available to assess the potential role of reduced caloric intake in the reduced maternal weight gains and fetal body weight in the key study for KE5 (Hackett et al., 1987a). Reduced feed intake, rather than the redirection of maternal nutrition in response to the toxic action of BD metabolites, is a plausible alternative MOA by which the effects on weight could have occurred.

Spencer et al. (2001) and Chi et al. (2002) proposed a specific mechanism for DEB in rats involving reduced serum progesterone, placental pituitary adenylate cyclase-activating polypeptide expression, matrix metalloproteinase activity. The authors noted that the ovotoxicity of DEB may indirectly affect decidual proliferation by reducing progesterone, the preeminent endocrine regulator of deciduoma development. Therefore, it is possible that the effects of BD on body weight and weight gain are secondary to its effects on the ovary (via DEB as described in **Section 2.1**). A proposed MOA involving a pituitary-placental axis would not be able to explain the reduced body weight effects of BD metabolites (EB and DEB) in growing nonpregnant animals (Doerr et al., 1996), which would therefore require separate MOAs to explain these effects.

Uncertainties, Inconsistencies, Data Gaps

Sources of uncertainty, inconsistency, and data gaps include consideration of the following.

- ***Lack of Data in Pregnant Animals*** - Studies supporting the KEs for the proposed MOA were largely collected in nonpregnant animals, which is an important source of uncertainty. The evidence from nonpregnant supporting KEs 1-3 is qualitatively relevant to pregnant animals, but it is recognized that there are changes during pregnancy (e.g., increases in volume of distribution, changes in P450 and GST activity) that can have quantitative impacts on these KEs. Additional studies in pregnant animals exposed to BD for measurements related to KEs 1-3 would help increase confidence in the proposed MOA.

- ***Role of Metabolic Activation in Fetal Tissues*** - With respect to KEs 1 and 2, there is some uncertainty associated with potential metabolic activation of BD via fetal tissues. There are no direct data regarding the metabolism of BD in fetal tissues that might impact internal doses to the fetus. However, information on the ontogenesis of the enzymes (e.g., cytochrome P450) suggest that fetal metabolism of BD under conditions of Hackett et al. (1987a) is negligible. Specifically, expression of most cytochrome P450 isozymes, including CYP2E1 which is important for BD metabolism, is negligible in fetal tissues until a few days prior to birth in mice, with expression as determined by mRNA levels becoming detectable and then increasing shortly thereafter (Hart et al., 2009; Cui et al., 2012; Golden et al., 2025). Because the exposure period used by Hackett et al. (1987a,b) (GD6-15) concludes prior to the initiation of CYP expression in developing mice, metabolism of BD in the fetal compartment is expected to be negligible. . Instead, delivery of the toxic metabolites of BD is expected to be driven by maternal metabolism and partitioning and therefore is expected to be proportionate to the internal dose of metabolites in maternal blood. This expectation is consistent with the lower adduct levels reported for the epoxide metabolites of other chemicals (acrylamide and its epoxide metabolite, glycidamide, also mediated via CYP2E1) in human cord blood compared to maternal blood levels (von Stedingk et al., 2011). Consistent with maternal delivery of epoxide metabolites (rather than epoxide formation in fetal tissues), mean cord blood adduct levels were ~2.4-fold lower than corresponding mean maternal levels in nonsmokers, and ~3.6- to 7-fold lower than corresponding mean maternal levels in

smokers. More importantly, the ratio of glycidamide:acrylamide hemoglobin adducts in cord blood was slightly lower than the ratio in maternal blood (0.7 vs 0.8), indicating that fetal tissues are not a significant source of the epoxide metabolite. For this reason, the potential contribution of fetal tissues to the metabolic activation of BD is considered low.

- ***Relationship Between Maternal Weight Gain and Fetal Body Weight*** - With respect to KE4, the potential relationship between decreases in maternal body weight gains and fetal body weight in animals exposed to BD is uncertain. In rats, maternal and fetal body weight effects were either unaffected by BD exposure (Hackett et al., 1987b) or maternal effects on weight gain were observed at lower doses compared to fetal effects (200 vs 8000 ppm; Hazleton, 1981). In mice, weight gains in the dam and body weights in the fetus are decreased by approximately the same magnitude at each exposure BD level (**Figure 4**; Hackett et al., 1987a). Due to the potential uncertainty in the relationship between these two endpoints (i.e., dependent, or independent), both have been lumped together in KE4 as signs of general toxicity from BD exposure.

- ***Additional Detail/KEs*** - As an umbrella KE, there is additional uncertainty in the KE4. Specifically, there are likely additional events between GSH depletion (KE3) and toxicity (KE4), but data are generally lacking. For example, GSH depletion can do the following: (1) leave cells more susceptible to oxidative stress; (2) decrease the metabolic clearance of BD's epoxide metabolites result in greater binding of BD epoxide metabolites to cellular macromolecules; and/or (3) alter energy metabolism (e.g., GSH depletion via

898 buthionine sulfoximine exposure results reduced body weight gain in mice fed a high fat
899 diet; Elshorbagy et al., 2016). Additional studies that explore late events that occur after
900 GSH depletion (e.g, oxidative stress measures, binding to macromolecules,
901 transcriptomics) in pregnant animals across a range of BD exposure levels (below and up
902 to those producing changes to fetal body weight) permit separating them out from the
903 umbrella definition of KE4 and would serve to reduce uncertainty in the MOA.
904
905 • ***Essentiality of KEs*** - Lastly, studies assessing the essentiality of the KEs for this MOA are
906 lacking. Additional studies in P450 knockout mice (to assess the essentiality of KE1) and
907 similarly designed studies for other KEs would serve to increase confidence in the overall
908 MOA.
909

910 ### 2.2.3 Human Relevance

911
912 Based upon this evaluation, the key questions identified for evaluating the human relevance of
913 the MOA (Boobis et al., 2008) are discussed as follows.
914
915 • ***Is the weight of evidence sufficient to establish a mode of action in animals?***
916
917 Although limited, there is evidence to support the importance of BD metabolism in MOA
918 for general toxicity (e.g., reduced body weight and weight gain), with evidence supporting

metabolism as a key determinant of BD's toxicity. The evidence supports the role of epoxide-mediated GSH depletion (Nieusma et al., 1997, 1998; Kreiling et al., 1988). Species differences in the KEs (mouse>rat; **Table 2**) help explain the clear species differences noted for sensitivity to the adverse outcome (mouse>rat; Hackett et al., 1987a,b; Hazleton, 1981).

- ***Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?***

KEs 1-3 in the proposed MOA are all expected to occur in humans but with important quantitative differences. Humans form less epoxide metabolites than mice or rats (see quantitation in **Section 2.3**). In addition, humans may be at lower risk of GSH depletion due to greater reliance on epoxide hydrolase over GSH conjugation when compared to mice and rats (Albertini, 2004). The effects of BD on body weight and weight gain (dams and fetus) were measured in rats at higher BD exposures (Hazleton, 1981) than measured in mice (Hackett et al., 1987a). Therefore, this endpoint does not appear to be unique to mice exposed to BD, and decreases in body weight and weight gain are qualitatively assumed to be relevant to all mammalian species, including humans.

- ***Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?***

To provide some context, average concentrations of BD in U.S. ambient air are 0.000058 ppm (Kirman et al., 2025), whereas the average concentration of BD in workplace air for BD workers range from 0.012-0.16 ppm (Panko et al., 2023). In contrast, point of departure (POD) values for decreases in fetal body weight in mice and rats vary widely depending upon the approach used to quantify differences in toxicokinetics: (1) no adjustment (mouse BMDSD= ~15 ppm; rat NOAEL = ~250 ppm); (2) accounting for differences in DEB internal dose (mouse BMDSD= ~17,000 ppm; rat NOAEL = ~16,000 ppm); and (3) accounting for differences in the internal doses of all three epoxide metabolites (mouse BMDSD= ~2,600 ppm; rat NOAEL = ~2,000 ppm) (Kirman et al., 2022). These interspecies adjustments are based on hemoglobin adduct data for male workers (as described in Kirman et al., 2022), and do not reflect the recently published hemoglobin adduct data in female workers (described in **Section 2.3** and applied in **Section 5**).

There are clear quantitative differences among mice, rats, and humans with respect to circulating levels of epoxide metabolites following BD exposure, which need to be considered in BD risk assessment. Swenberg et al. (2011), Boysen et al. (2012), and Motwani and Tornqvist (2014) showed that for a given exposure to BD, BD metabolite levels in humans are lower than the levels in rats, which in turn are lower than levels in mice. Newly published data (Georgieva et al., 2025; discussed further in **Section 2.3**), which includes female workers, suggests that women may experience lower internal doses of DEB and EBD than men following exposures to BD. Humans are also much less

963 sensitive to GSH depletion than rats or mice due to species differences the metabolic
964 clearance of epoxides (conjugation via GST vs. hydrolysis via EH). Based on an analyses of
965 urinary metabolite ratios, Albertini (2004) estimated the relative percentages of each
966 epoxide clearance pathway (%hydrolysis, % conjugation) in mice (~24%, ~76%), rats
967 (~51%, ~49%) and humans (~99%, ~1%), indicating there is a large difference between
968 mouse and human reliance on GSH conjugation for epoxide clearance (76% vs 1%). These
969 profound species differences in metabolic activation and sensitivity to GSH depletion may
970 help explain why BD concentrations associated with acute toxicity in mice (2000 ppm;
971 Kreiling et al., 1988) appear to be well tolerated by human volunteers (2,000-8,000 ppm;
972 Carpenter, 1944).
973

974 2.3 Use of Hemoglobin Adduct Data to Quantify Species Differences in
975 the Internal Doses of BD Metabolites

976

977 To support noncancer dose-response assessments (e.g., reference concentration derivation) for
978 BD, Kirman et al. (2022) relied upon internal dose estimates for BD metabolites (blood AUC
979 values per ppm BD exposure) as estimated by Motwani and Tornqvist (2014). The internal dose
980 estimates of Motwani and Tornqvist (2014) were based upon hemoglobin adduct data collected
981 in mice and rats (Boysen et al., 2007; Georgieva et al., 2010) and in exposed workers (Czech
982 Republic workers Study 1; Albertini et al., 2003; Boysen et al., 2012). More recently, Georgieva
983 et al. (2025) published a more complete set of hemoglobin adduct data that includes the data

from Study 1, but also includes hemoglobin adduct data from a second study in Czech Republic workers (Study 2; Vacek et al., 2010), including pyr-Val data that have not been published previously. Study 2 is considered important since it also includes data collected from female workers (i.e., Study 1 assessed only male workers), which may be considered more relevant for the key noncancer endpoints of BD (i.e., ovarian atrophy and fetal body weight changes). The mean adduct burdens as a function of mean BD exposures from both studies are depicted in **Figure 5**.

Two limitations have been identified for the adduct data from Study 2:

- *Adduct Data for HBVal Not Available* – Study 2 included measurements of hemoglobin adducts THBVal (which reflects circulating EBD levels) and pyr-Val (which reflects circulating DEB levels), but not for adduct HBVal (which reflects circulating EB levels). This is not considered to be a substantial limitation since EB does not contribute directly to the ovarian atrophy endpoint (requires a diepoxide), and when the metabolites are considered together (i.e., cytotoxicity index of Kirman et al., 2022; see their Fig. 4) the contribution of EB was negligible when compared to DEB and EBD.
- *Lack of Trend for Female Worker pyr-Val data* – In Figure 2 of Georgieva et al. (2025), a general negative trend was noted for the measurement of pyr-Val for control workers exposed to low-level BD concentrations, and exposed workers exposed to higher BD concentrations. This behavior can also be seen in **Figure 5** above (2nd orange triangle from the left is slightly lower than the 1st triangle). Based upon the observed differences between male and female workers, the study authors postulated that "*human males*

may be at higher risk for BD-induced toxicity." This behavior may be explained by an increased prevalence of unreported smoking (or other non-occupational exposures to BD) in the female control group compared to the exposed group, sex-specific inhibition of metabolic enzymes, sex-specific exposure-related toxicity to erythrocytes (resulting in higher cell turnover and loss of adducts), or stochasticity associated with the sample population and/or analytical methods (i.e., both data points are within 1SD of the overall trend line in **Figure 5**, and by chance the control group falls approximately on the overall trend line and the exposed group falling slightly below). Motwani and Tornqvist (2014) did not report any enzyme inhibition in *in vitro* metabolism studies utilizing concentrations of up to 2 mM for each metabolite. Although some sex differences in metabolism have been reported in rodents and humans, these differences have been relatively modest and would not result in a decrease in pyr-Val as a function of exposure. Interpretation of the female worker data will be an important determinant of their potential application to DDEF calculations to account for species differences in internal dose (depicted in **Figure 6** for pyr-Val adducts in mice, rats, and humans).

Internal doses of BD metabolites (AUC in blood) were estimated from the hemoglobin adduct data from both worker studies using the methods described in Motwani and Torqvist (2014) (**Table 3**). Values for rats and mice in the top half of the table are as reported by Motwani and Tornqvist (2014). All human values in the bottom half of the table are calculated here from the raw adduct data (Georgieva et al., 2025). The resulting AUC for workers from Study 1 are slightly different from those reported by Motwani and Tornqvist (2014) since the calculations

1028 are based on raw data rather than relying upon summary statistics. For the female worker data,
1029 two sets of AUC values were calculated: (1) a negative value of -0.011 nM-hr/ppm-hr. BD based
1030 upon an arithmetic mean pyr-Val in exposed female workers that is lower than the arithmetic
1031 mean for control female workers; and (2) a positive value of 0.13 nM-hr/ppm-hr. BD based on a
1032 conservative difference between the upper 90% CI value for the arithmetic mean pyr-Val in
1033 exposed workers and the lower 90% CI value for the arithmetic mean pyr-Val in control
1034 workers.
1035
1036 The limitations noted for the female worker data contribute uncertainty to their application to
1037 DDEF value calculation. For this reason, a conservative upper bound estimate value for these
1038 data is proposed, and thus the incremental mean increase in internal dose may be lower than
1039 depicted in the DDEF calculations. Additional studies that provide a better characterization of
1040 BD exposures outside of the workplace, gender differences in BD metabolism to epoxides, and
1041 their variation in humans may help resolve the limitations noted for the female worker data for
1042 pyr-val adducts of Georgieva et al. (2025).
1043
1044 A robust and current physiologically based pharmacokinetic (PBPK) model, which is often
1045 considered the gold standard for supporting interspecies extrapolations, is not available for BD.
1046 Instead, our DDEF calculations rely upon a simpler and acceptable (USEPA, 2014) approach
1047 using hemoglobin biomarkers to quantify species differences in the internal doses of BD
1048 metabolites experienced in mice, rats, and humans. The use of hemoglobin adducts for BD here
1049 is consistent with USEPA's practice in the assessment of other chemicals (e.g., USEPA's IRIS

assessment for acrylamide; USEPA, 2010). For this reason, the lack of a complete PBPK model for BD was not considered to be a limitation that contributed uncertainty to the calculations. If a complete PBPK model were developed, it is expected that the predictions and calculations would maintain consistency with the hemoglobin adduct data, as was noted for internal dose estimates for BD metabolites estimated from in vitro enzyme toxicokinetics data (Motwani and Tornqvist, 2014).

It is also recognized that all hemoglobin adduct data for BD's metabolites were collected from nonpregnant animals and humans. For this reason, for the application of these DDEF values to extrapolate data for BD's noncancer effects from mice to humans, the following assumption is made:

$$\frac{[Human\ Internal\ Dose]_{nonpregnant}}{[Rodent\ Internal\ Dose]_{nonpregnant}} \sim \frac{[Human\ Internal\ Dose]_{pregnant}}{[Rodent\ Internal\ Dose]_{pregnant}}$$

This assumption serves as a continuation of the assumption that laboratory rodents serve as appropriate models for human pregnancy and development, and as such any changes during pregnancy that may impact internal dose (e.g., volume of distribution, metabolic activation) will be similar in both rodents and humans. A PBPK model developed for acetaminophen and its conversion to N-acetyl-p-benzoquinone imine (primarily via CYP2E1) in women indicated that molar fraction of parent chemical metabolized via this pathway did change over the course of pregnancy (trimester 1>trimester 2>trimester 3), but these changes were relatively small (i.e., all within a factor of 2 of the conversion determined for nonpregnant women) (Mian et al.,

2020). Nevertheless, the proportionality assumption for pregnant and nonpregnant females introduces some uncertainty into DDEF values calculated for BD. Additional studies comparing the dosimetry of BD metabolites (e.g., hemoglobin adduct burdens) in pregnant and nonpregnant animals would help evaluate the validity of this assumption.

3. Methods

3.1 Independent Expert Panel

An independent expert panel was engaged to do the following: (1) consider the weight of evidence supporting the MOAs and determine the degree of confidence in them, as well as identify data gaps and limitations; (2) provide conclusions on relevance of the noncancer endpoints to human health; and (3) provide input on how dosimetry decisions should be made in human health risk assessment for BD that reflect best available science. An independent panel of experts was recruited, selected, and engaged utilizing the methods described in Kirman et al. (2019). Coverage of this panel included the following expertise areas: reproductive/developmental effects, mode of action evaluation, and/or dose metric decisions for risk assessment. The review manager (SciPinion) defined review material to include summaries of information needed to support the assessment (provided in **Section 2**), along with publications and reports (**Appendix A**). Panelists were also given the opportunity to

request access to additional publications/reports as needed to support their engagement. Multiple design elements were included in this review to minimize potential sources of bias and groupthink, and to improve transparency of the review, including the following: (1) a triple-blinded process was used (the review sponsor was blinded to panelists identities; the panelists were blinded to the sponsor identity during recruitment and engagement; and the panelists were blinded to one another during engagement (e.g., identified only as Expert 1, Expert 2, etc. during all online deliberations). (2) A multi-round, modified Delphi format was adopted to collect both independent and deliberative input from the topic experts to minimize potential groupthink. (3) Individual responses and comments from the panelists were recorded and are provided in their entirety (**Appendix A**) to ensure transparency and minimize potential reporting bias. Finally (4) although individual responses are provided in this appendix, they are attributed to panelist's anonymous display names (e.g., to Expert 1, Expert 2, etc.). This was done to ensure candid sharing of scientific opinion(s) on areas of controversy with minimal concern of potential adverse within-panel or external professional perceptions. Demographic, expertise metrics, and qualification statements from the panelists are provided in **Table 4**.

3.2 Quantifying Species Differences in the Toxicokinetics of BD

To support DDEF calculations, internal doses of BD epoxides were estimated using the methods described in Motwani and Tornqvist (2014) (**Table 3**). DDEF values to quantify species differences in the toxicokinetics of BD (EF_{AK}) were calculated from these internal dose estimates

using the methods described in Kirman et al. (2022). For endpoints such as ovarian atrophy whose MOA can be attributed to a single metabolite (DEB; see MOA summary above), the contributions from other reactive BD metabolites are assumed to be zero. Species differences in the toxicokinetics of BD were accounted for using a DDEF (USEPA, 2014; Equation 3) calculated based on DEB internal doses using the following equations:

$$DDEF\ for\ Ovarian\ Atrophy = C_A / C_H = Unit\ AUC_{DEB\_H} / Unit\ AUC_{DEB\_A}$$

$$DDEF\ for\ Fetal\ Weight = CI_H / CI_A$$

Where,

- EF_{AK} = Data-derived extrapolation factor for interspecies extrapolation due to toxicokinetic differences (unitless).
- C_A = Air concentration in animals producing an internal dose of DEB at or near the point of departure, AUC_A (ppm).
- C_H = Air concentration in humans producing an internal dose of DEB at or near the point of departure, AUC_A (ppm).
- *Unit* AUC_{DEB_H} = Unit internal dose of DEB per external exposure in humans (AUC per ppm; **Table 3**);
- *Unit* AUC_{DEB_A} = Unit internal dose of DEB per external exposure in animals (mice or rats; AUC per ppm; **Table 3**); and

- *CI* = Cytotoxicity index weight was calculated separately for humans (H) and animals (A; mouse or rat) using the equation below (Kirman et al., 2022):

$$CI_S = \sum(Unit\ AUC_{EB} \times RP_{EB} + Unit\ AUC_{DEB} \times RP_{DEB} + Unit\ AUC_{EBD} \times RP_{EBD})$$

- CI_S = Cytotoxicity index, calculated separately for mice, rats, and humans (AUC per cumulative BD exposure or nM*hr. per ppm*hr BD);
- *Unit* AUC_{EB} = Species-specific unit AUCs for EB (nM*hr. per ppm*hr. BD; **Table 3**);
- *Unit* AUC_{EBD} = Species-specific unit AUCs for EBD (nM*hr. per ppm*hr. BD; **Table 3**); and
- *RP* = Relative potency of each metabolite (EB, DEB, EBD), as compared to EB, for producing cytotoxicity in cell systems *in vitro* (unitless; Kirman et al., 2022).

Consistent with USEPA guidelines (USEPA, 2014), DDEF values below a value of 1 were permitted when indicated by underlying data.

4. Results

4.1 Panel Recommendations/Conclusions on the MOA for Premature Ovarian Failure by BD

The panelists reviewed summary material (provided in **Section 2**) on the MOA for premature ovarian failure and reached several conclusions. A majority of the panel (5/6 responding "Yes", 1/6 responding "Somewhat") considered the weight of evidence for establishing a MOA for the ovarian effects in animals to be sufficient (**Figure 7A**), since it was supported by multiple studies with various exposure durations and dose levels. There was agreement within the panel for the role of BD's epoxide metabolites (particularly DEB) in causing ovarian toxicity, with species differences in sensitivity between mice and rats explained by underlying differences in metabolism. The panel was unanimous (6/6) in concluding that the relevance of the ovarian effects to human health cannot be excluded due to qualitative differences (**Figure 7B**), noting that the metabolism pathways for BD are qualitatively similar across species, ovarian toxicity was observed in non-human primates exposed to VCD, and *in vitro* studies have shown VCD produces increased intracellular ROS, DNA damage, and altered gene expression in human ovarian cells (see **Section 2**). In contrast, there was some support (2/6 responding "Yes", 3/6 responding "Somewhat", 1/6 responding "No") for concluding that the ovarian effects of BD are not relevant to human health due to quantitative differences (**Figure 7C**), noting substantial quantitative differences in metabolism between species regarding DEB production (mice > rats > humans), which as noted by at least one panelist was complicated by a lack of guidance or precedence on the magnitude of species difference (e.g., x-fold difference to serve as a cutoff) required to exclude human relevance. Overall, the panel expressed high confidence in the MOA for premature ovarian failure by BD (mean confidence score of 8.2 out of 10; **Figure 8**).

In addition to the uncertainties discussed in **Section 2.1**, sources of uncertainty considered by the panel included the following items. The MOA for premature ovarian failure relies upon information for a structural analog (VCH, VCD), particularly for KE4. Most of the panelists (5/6 responding "Yes", 1/6 responding "Somewhat") considered the reliance on VCH/VCD for BD's MOA to be appropriate noting structural similarities, comparable metabolic pathways leading to diepoxide formation, and similar species-specific ovarian toxicity patterns (mice > rats). However, additional studies for BD that address the dose-temporal concordance for KE4 could serve to reduce reliance on VCD for this KE and increase overall confidence (i.e., increasing the mean confidence score of 8.2 closer to 10). Panelists also noted that studies addressing the essentiality of the KEs would be useful for increasing confidence in this MOA. Uncertainty associated with the possibility of excluding human relevance due to quantitative species differences at the hazard identification stage was also discussed by the panel, with several panel members indicating that addressing quantitative differences warranted full consideration in a quantitative risk assessment (e.g., consideration of the magnitude of human exposures).

4.2 Panel Recommendations/Conclusions on the MOA for the Effects of BD on General Toxicity (Reduced Maternal Weight Gain and Lower Fetal Weight)

The independent panel reviewed summary material (see **Section 2**) on the MOA for general toxicity (e.g., reduced maternal weight gain and fetal weight) and reached several conclusions.

A majority of the panel considered there to be at least some evidence for establishing a MOA for reduced maternal weight gain/fetal weight in animals (1/6 responding "Yes", 4/6 responding "Somewhat", 1/6 responding "No") (**Figure 7D**), noting that there is a lack of specificity for some KEs, there may be additional KEs between KE3 and KE4, some uncertainties associated with the dose-response relationships, and the potential relationship between maternal weight gain and fetal weight. The panel was unanimous (6/6) in concluding that the relevance of body weight effects to human health cannot be excluded due to qualitative differences (**Figure 7E**), noting that the reactive epoxide metabolites of BD likely contribute to toxic responses through similar mechanisms across species (as measured in two species), and lack of data suggesting the MOA would not be operative in humans. In contrast, there was mixed support (2/6 responding "Yes", 1/6 responding "Somewhat", 3/6 responding "No" for concluding that the effects of BD on maternal and fetal weight are not relevant to human health due to quantitative differences (**Figure 7F**). The panel noted that substantial quantitative differences in metabolism between species are operative (mice > rats > humans), but that the possibility that effects in highly exposed humans (e.g., levels above the point of departure for effects) cannot be ruled out. Overall, the panel expressed medium confidence in the MOA for general toxicity by BD (mean confidence score of 5.2 out of 10 (**Figure 8**), with stronger evidence noted for early KEs pertaining to dosimetry, than for late KEs pertaining to the adverse outcome.

In addition to the uncertainties discussed in **Section 2.2**, sources of uncertainty considered by the panel included that evidence supporting the MOA for general toxicity (e.g., effects on maternal and fetal weight) is based primarily upon studies conducted in non-pregnant animals.

The panel's opinions were mixed on the appropriateness of relying upon data in non-pregnant (2/6 considered it appropriate, 2/6 considered it somewhat appropriate, 2/6 considered in not appropriate), noting that data from non-pregnant are helpful, but that there are important differences between pregnant and nonpregnant animals, and therefore more information is needed. Additional studies that investigate KEs in pregnant animals exposed to BD would help improve confidence in the MOA (i.e., increasing the mean confidence score of 5.2 closer to 10), as well as characterize the role of maternal weight gain in fetal weight changes following BD exposure. Some panelists noted in their explanations and comments that studies addressing the essentiality of the KEs would be useful for increasing confidence in this MOA.

4.3 Panel Recommendations/Conclusions on Dosimetry and Calculation of DDEF Values

With respect to the newly reported data for metabolite-specific hemoglobin adducts in female workers (Georgieva et al., 2025; see **Section 2.3**), the panel was generally supportive of the conservative approach described for using these data; that is relying upon the difference upper and lower confidence values to avoid calculation of negative incremental values. On this point 4/6 panelists responded "Yes", and 2/6 responded "Somewhat"). The panel expressed a preference for using the female worker data combined with male worker data for quantifying species differences (selected by 3/6 panelists; with 1 panelist indicating the female worker should serve as the primary basis, and 2 panelists indicating they would prefer to see a range of

options for using the worker adduct data). Based upon this input, DDEF values were calculated from internal dose estimates derived from hemoglobin adduct data (**Table 3**) and are provided in **Table 5.** This table includes preferred values that reflect panel recommendations, as well as possible alternate values.

Based upon the evidence supporting the MOA for premature ovarian failure (**Section 2.1**), interspecies extrapolation to account for species difference in the internal dose of DEB had the most support from the panel (selected by 3/6, with the remaining 3/6 recognizing the importance of DEB but interested in providing a more comprehensive approach that compares different dose adjustments and along with their degree of confidence). To include such an approach, the panelists were asked about their degree of confidence in possible dose adjustments for ovarian effects of BD, and expressed *high confidence* (mean confidence score of 4.5±0.5 out of 5) in adjustments based on DEB, *medium confidence* (mean confidence score of 2.5±1.7 out of 5) in adjustments based on all three epoxide metabolites, and *low confidence* (mean confidence score of 0.8±1.3 out of 5) in reliance on external air concentration/no species adjustment (**Figure 9**).

Based upon the evidence supporting the MOA for general toxicity (e.g., reduced body weight and weight gain; **Section 2.2**), interspecies extrapolation to account for species difference in the internal doses of all three epoxide metabolites had the most support (selected by 4/6 panelists, with the remaining interested in providing a comprehensive approach that compares different dose adjustments and their degree of confidence). To include such an approach, the panelists

were asked about their degree of confidence in possible dose adjustments for the general toxic effects of BD (e.g., reduced body weight and weight gain), and expressed *high confidence* (mean confidence score of 4.0±0.9 out of 5) in adjustments based on all three epoxide metabolites, *medium confidence* (mean confidence score of 3.0±0.9 out of 5) in adjustments based on DEB alone, and *low confidence* (mean confidence score of 1.0±1.1 out of 5) in reliance on external air concentration/no species adjustment (**Figure 9**).

In addition to the uncertainties discussed in **Section 2.3**, sources of uncertainty considered by the panel include the following items. Although physiologically-based pharmacokinetic (PBPK) models generally serve as the preferred basis for extrapolating toxicokinetic differences across species, available PBPK models for BD are outdated and they do not include all of the most recent toxicokinetic data available for BD. Panelists expressed high confidence (mean of 4.5±0.5 out of 5; **Appendix A, Question 4.4**) in relying upon hemoglobin adduct data as a measure of BD's epoxide metabolites in mice, rats and humans. The panel noted that hemoglobin adducts provide quantitative measures of internal reactive epoxide dose, that these empirical data are from actual animals and humans using reliable analytic methods, and that these data appropriately capture species differences in the internal doses of BD metabolites.

5. Discussion/Conclusions

Evidence on MOA is an important consideration for many decisions made within a human health risk assessment, including those related to dosimetry and interspecies extrapolation. The text above provides a summary of the evidence supporting the MOAs for key noncancer effects of BD (premature ovarian failure, reduced body weight, and weight gain) and data to support interspecies extrapolations in human health risk assessment. The use of hemoglobin adduct data collected from mice, rats, and humans exposed to BD represents the best available sciences for quantifying species differences in the internal doses of BD's epoxide metabolites. This information was reviewed by an independent expert panel, which concluded high confidence in the MOA for premature failure, and medium confidence in the MOA for general toxicity (e.g., reduced body weight and weight gain). The panel also provided input on how MOA evidence and hemoglobin adduct data should be used to support DDEF calculations for interspecies extrapolation (**Table 5**). Since the key effects for BD have been reported in mouse studies (Hackett et al., 1987a; NTP, 1993), the preferred DDEF to account for species differences in toxicokinetics (EF_{AK}) when relying on these studies for human health risk assessment are 0.00064 for ovarian atrophy and 0.0070 for fetal body weight. Sources of uncertainty and data that could serve to improve confidence in the MOAs were identified. Because the species differences in the internal doses of BD's metabolites are large (up to three orders of magnitude), decisions on dosimetry and interspecies extrapolation have substantial impacts on human health risk assessment.

To illustrate the quantitative impact of dosimetry decisions made for BD, USEPA's proposed Occupational Exposure Value (OEV) (USEPA, 2024) is considered. In this assessment, USEPA

derived an OEV value of 0.17 ppm for BD based upon the application of an uncertainty factor of 30 (3 for interspecies variation; 10 for intraspecies variation) to a point of departure value of 2.5 ppm (adjusted to 5 ppm accounting for exposure time, exposure frequency, and inhalation rate differences) for lower fetal body weight in mice (Hackett et al., 1987a). For this calculation, no adjustments were made to account for species differences in BD toxicokinetics (i.e., it was assumed that for a given exposure to BD in air mice and humans are equally sensitive). This approach corresponds to the one rejected by ATSDR in their assessment of BD's noncancer effects out of concerns for potentially "*overestimating the risk to humans*" (ATSDR, 2012) and it is the approach to which the independent expert panel attributed low confidence (**Figure 9**). This OEV calculation is compared to alternative interspecies adjustments using the DDEF values (0.00064 and 0.0070; **Table 5**) recommended with higher confidence by the panel (**Figure 10**). USEPA's previous calculation, which did not include an adjustment for species differences in BD's metabolism (approach assigned low confidence by the panel), resulted in an OEV value that is lower than the Occupational Safety and Health Administration's (OSHA) Permissible Exposure Limit (PEL) for BD of 1 ppm by a factor of more than five, suggesting that the existing PEL may not be sufficiently protective of worker health for BD's noncancer endpoints. In contrast, adjusting for species differences in DEB internal dose (assigned medium confidence by the panel) or in differences in the internal doses for all three epoxide metabolites (assigned high confidence by the panel) results in OEV values that are well above the existing PEL (approximately 260 and 24 ppm, respectively), suggesting that the PEL is already protective of worker health for noncancer endpoints. This comparison illustrates the importance of fully considering evidence for MOA to support decision-making, "*even when a complete*

understanding of the mechanism is not available" (USEPA, 2014), to ensure that human health risk assessment and subsequent risk management decisions reflect the best available science.

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Declaration of Interest

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Table 1. Dose Concordance for Key Events in the MOA for the Ovarian Effects of BD in Mice and Rats[1]

KE	KE1: Metabolism of BD to DEB		KE2: Distribution of DEB to Ovary		KE3: Macromolecular Crosslink (or similar molecular lesion specific to bifunctional alkylating agents)		KE4: Induction of Cell Death via Apoptosis/ Autophagy	KE5: Decreased Ovarian Follicle Counts/Ovary Weight	KE6/AO: Premature Ovarian Failure	
Key References	**Measured following short-term exposures to BD (Georgieva et al., 2010)**		**Measured following acute exposures to BD (Himmelstein et al. 1995; Thornton-Manning et al. 1995, 1997, 1998)**		**Measured following acute to short-term exposures to BD (Goggin et al. 2009, 2011; Sangaraju et al. 2012; Jelitto et al. 1989; Vangala et al. 1993)**		**Measured following short-term exposures to VCD (Sen Halicioglu et al. 2021; Zhou et al. 2023; Liu et al. 2023; Niu et al., 2025; Abolaji et al. 2016)**	**Measured following short-term exposures to EB, DEB, and VCH (Doerr et al. 1995, 1996; Smith et al. 1990a)**	**Measured following subchronic to chronic exposures to BD (NTP 1993; Owen et al. 1987)**	
ppm	**Mouse**	**Rat**	**Mouse**	**Rat**	**Mouse**	**Rat**	Data are lacking for this KE following BD exposures. Instead, this KE is supported by studies of the diepoxide metabolite for a structural analog (VCD; see **Table S1**)	Data are lacking for this KE following BD exposures. Instead, this KE is supported by dose-response data measured for a broad range of doses for BD metabolites (0.005, 0.02, 0.09, 0.36, 1.43 mmol/kg-day for EB; 0.002, 0.009, 0.036, 0.14, 0.29 mmol/kg-day for DEB), and for a structural analog (VCH) and its metabolites (VCM, VCD) (see **Table S1**)	**Mouse**	**Rat**
0.1	+	-								
0.5	++	+			+					
1	++	+			++					
1.5	++	+			++					
6.25	++	+			+++	-			++	
20									+++	
62.5	+++	++	++	+	+++	+			++++	
100					+*	-				
200	+++	+++			++++	+			++++	
250					+*	-				
500					+*	-				

625	++++	+++	+++	++	++++	+			++++	
1000					+*	-				-
1250			+++	++						
2000					+*	-				
8000				+++						-

[1]Although mice are the primary species of interest for this endpoint, data for rats are also summarized since they experience internal doses of BD metabolites that are lower than mice by approximately an order of magnitude, and therefore provide additional dose concordance information.

[2]Analytical methods indicate crosslinks are present but not quantified

Table 2. Dose Concordance for Key Events in the MOA for General Toxicity of BD (e.g., Reduced Weight Gain)[1]

KE	KE1: Metabolism of BD to Reactive Epoxide metabolites		KE2: Distribution of Epoxide Metabolites to Maternal and Fetal Tissues		KE3: GSH Depletion Due to Epoxide Conjugation with GSH		KE4/AO: General Toxicity (Reduced weight gain/growth)			
Key Reference	Measured following short-term exposures to BD (Georgieva et al. 2010; Boysen et al. 2007)		Measured following acute exposures to BD (Himmelstein et al. 1995; Thornton-Manning et al. 1995, 1997, 1998)		Measured following acute exposures to BD (Kreiling et al. 1988; Deutschman and Laib 1989; Himmelstein et al. 1995)		Measured following short-term exposure to BD (Hackett et al. 1987a,b; Hazleton 1981)			
ppm	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse: Decreased Maternal Weight Gain	Mouse: Decreased Fetal Weight Gain	Rat: Decreased Maternal Weight Gain	Rat: Decreased Fetal Weight Gain
0.1	+	-								
0.5	++	+								
1	++	+								
1.5	++	+								
6.25	++	+								
10					+	-				
40							+	+	-	-
50					+	-				
62.5	+++	++	++	+	+	-				
100					+	-				

200	+++	+++					++	++	-/+[2]	-
250					++	+				
500					++	+				
625	++++	+++	+++	++	++	-				
1000					+++	+	+++	+++	-/+[2]	-
1250	++++	+++	+++	++	++	++				
2000					+++	++				
8000				+++					++	+

[1]Although mice are the primary species of interest for this endpoint, data for rats are also summarized since they experience internal doses of BD metabolites that are lower than mice by approximately an order of magnitude, and therefore provide additional dose concordance information.

[2]Mixed results observed across studies; negative for Hackett et al. (1987b)/positive for Hazleton (1981)

Table 3. Internal Dose Estimates for BD Metabolites Based Upon Hemoglobin Adduct Data (Motwani and Tornqvist, 2014; Georgieva et al., 2025)

				AUC (nM-hr/ppm-hr BD)		
Species	**Sex**	**n**	**BD (ppm)**	**EB**	**DEB**	**EBD**
Mouse	F	4-5 per group	0.1-1.5	13±2	27±7	266±71
	M	3-5 per group	0.1-1.5	15±2	38±8	210±30
	Mouse Mean (MF combined)	7-10 per group	0.1-1.5	14±2	33±8	240±50
Rat	F	3-6 per group	0.5-1.5	0.77±0.1	1.45±0.2	19±2
	M	2-6 per group	0.5-1.5	0.72±0.1	1.37±0.3	19±0.9
	Rat Mean (MF combined)	5-12 per group	0.5-1.5	0.75±0.1	1.41±0.3	19±1.5
Human[1]	M (Study 1 monomer workers)	24	0.29	0.050±0.05	0.018±0.014	28±16
	M (Study 1 polymer workers)	34	0.81	0.15±0.09	0.023±0.017	73±44
	M (Study 2 workers)	27	0.39	--	0.029±0.035	130±55
	F (Study 2 workers)	22	0.18	--	0.013±0.012* (-0.011±0.012)*	19±13
	Human Mean 1 (male workers only)			0.15±0.09	0.023±0.006	77±51
	Human Mean 2 (male and female workers combined; female worker mean pyr-Val represented using a conservative value of 0.013)				0.021±0.007	63±51
	Human Mean 3 (male and female workers combined; female worker mean pyr-Val represented as a conservative value of 0.00)				0.018±0.013	
	Human Mean 4 (male and female workers combined female worker mean pyr-Val calculated as a negative value, -0.011)				0.015±0.014	

[1]Calculated from hemoglobin adduct levels using the methods described by Motwani and Tornqvist (2014) using an erythrocyte lifespan of 126 days, and hemoglobin binding constants (using the arithmetic mean of mouse and rat values) of 0.000345, 0.00005, 0.000021 L/g*hr for EB, DEB, and EBD, respectively.

[2]Two sets of AUC values were calculated: (1) a negative value of -0.011 nM-hr/ppm-hr BD based upon an arithmetic mean pyr-Val in exposed female workers that is lower than the arithmetic mean for control female workers; and (2) a positive value of 0.13 nM-hr/ppm-hr BD based on a conservative

difference between the upper 90% CI value for the arithmetic mean pyr-Val in exposed workers and the lower 90% CI value for the arithmetic mean pyr-Val in control workers.

Table 4. Demographics and Expertise Summary for Panel Members

Name	Employment Sector	Years Post-Degree	Advanced Degree	Country	Publications	Qualifications
Dr. John Lipscomb	Consulting; Former EPA	33	PhD	US	89	- Toxicologist and Toxicokinetics Expert - Former Toxicologist for EPA ORD - Former FDA Biologist, Division of Reproductive and Developmental Toxicology - Co-author of EPA guidelines for DDEFs - Co-author of multiple MOA papers - Responsible for decisions among dose metrics to use in EPA risk assessments
Dr. Bette Meek	Academia; Former Health Canada	43	PhD	Canada	250	- Toxicologist and Risk Assessment Expert - Former Manager Health Canada Bureau of Chemical Hazards - Internationally recognized expert in MOA & AOP evaluation - Author of multiple MOA framework papers including those for non-cancer endpoints
Dr. John Rogers	Consulting; Former EPA	42	PhD	US	147	- Developmental Toxicologist - Former EPA Chief of the Perinatal Toxicology Branch and Director of the Toxicity Assessment - Past President of the Teratology Society - Past President of SOT Reproductive and Developmental Toxicity Specialty Section - Editor in Chief of Birth Defects Reseach
Dr. Rita Schoeny	Consulting; Former EPA	47	PhD	US	97	- Risk Assessment Expert - Former Senior Science Advisor EPA OSP & Acting Director EPA RAF - Co-author of EPA guidelines for DDEFs, weight of evidence in MOA, & approaches to mixtures risk assessment - Co-author of multiple MOA & AOP papers

Dr. Jennifer Seed	Consulting; Former EPA	37	PhD	US	44	- Developmental Biologist/Toxicologist - Former Senior Science Advisor, Deputy Division Director, Branch Chief EPA OPPTS - Co-author of EPA test guidelines for developmental toxicity & reproductive toxicity - Co-author of multiple EPA guidelines on MOA and risk assessment - Involved in multiple international working groups on MOA for noncancer effects - Co-author of multiple MOA papers - Co-authors of multiple developmental toxicity papers
Dr. Babasaheb (Bob) Sonawane	Academia; Former EPA	53	PhD	US	137	- Developmental Toxicologist - Former EPA NCEA Supervisory Interdisciplinary Toxicologist & Acting Group Chief Quantitative Risk Management Group - Co-author of EPA guidelines for reproductive and developmental toxicity risk assessment

Table 5. DDEF Values for Interspecies Adjustment for Toxicokinetic Differences to Support Noncancer Assessment for BD

Dose Measure Basis (Noncancer Endpoint)	Species Extrapolation	Panel Recommendation	Data Sets Used to Calculate Internal Dose Ratio[1]	DDEF Value (unitless)
DEB Alone (Dose measure best supported by the panel for assessing premature ovarian failure by BD)	Mouse to Human	Alternate value	Female human[2] : Female mouse	0.00048
			Combined human : Female mouse	0.00077
			Male human : Female mouse	0.00086
			Male human : Male mouse	0.00061
		Preferred value	***Combined human : Combined mouse***	***0.00064***
	Rat to Human	Alternate value	Female human : Female rat	0.0090
			Combined human : Female rat	0.014
			Male human : Female rat	0.016
			Male human : Male rat	0.017
		Preferred value	***Combined human : Combined rat***	***0.015***
All the epoxide metabolites[3] (Dose measure best supported by the panel for assessing general toxicity/reduced weight gain by BD)	Mouse to Human	Alternate value	Female human : Female mouse	0.0028
			Combined human : Female mouse	0.0083
			Male human : Female mouse	0.010
			Male human : Male mouse	0.0073
		Preferred value	***Combined human : Combined mouse***	***0.0070***
	Rat to Human	Alternate value	Female human : Female rat	0.051
			Combined human : Female rat	0.15
			Male human : Female rat	0.19
			Male human : Male rat	0.19
		Preferred value	***Combined human : Combined rat***	***0.16***

[1]Due to limitations in the female worker data (see text), alternative DDEF values were calculated using male and female worker data combined (i.e., if female worker data are determined to be too limited to use alone) and using male worker data alone (i.e., if female worker data are determined to not be useful for quantifying species differences).

[2]All calculations using female human data reflect a conservative upper bound internal dose estimate of 0.013 nM-hr/ppm-hr BD from **Table 3**. For example, the value of 0.00064 for combined human and combined mice is calculated as 0.021 / 33.

[3]Relies upon the cytotoxicity index approach described in Kirman et al. (2022), with relative cytotoxic potencies of 1, 171, and 0.578 for EB, DEB, and EBD, respectively. For example, the value of 0.0070 for combined human and combined mice is calculated as (0.15*1 + 0.021*171 + 63*0.578) / (14*1 + 33*171 + 240*0.578).

Table S1. Detailed Support for Key Events in the MOA for the Ovarian Effects of BD

Duration	Test Agent	KE1: Metabolism of BD to DEB (primarily in the liver and lung)	KE2: DEB Distribution to Ovary	KE3: Macromolecular Crosslink (or similar molecular lesion specific to bifunctional alkylating agents)	KE4: Induction of Cell Death via Apoptosis/Autophagy	KE5: Decreased Ovarian Follicle Counts/Ovary Weight	KE6/AO: Premature Ovarian Failure (early onset menopause)
In vitro/In situ (1x)	BD and metabolites	Perfusion studies: 400 nmol/mL BD (Filser et al., 2001), 8.2 uM BD, 4.1 uM EB (Filser et al., 2010); In vitro studies: 20 uM-2 mM EB (Motwani and Tornqvist, 2014) parameters; 5-1000 uM BD (Seaton et al., 1995) to characterize epoxide formation and clearance Mice form more DEB than do rats		DNA-protein crosslinks reported in human cervical carcinoma cells at DEB concentrations of 5-100 mM in vitro (Michaelson-Richie et al., 2010)			

	VCH and metabolites	VCH is oxidized to VCM and VCD by mouse and rat microsomes (Fontaine et al., 2001). Bioactivation of VCH in rats was significantly less compared with that measured in mice.			Kappeler and Hoyer (2012) reviewed a series of in vitro studies on the mechanism of VCD and concluded that it causes cell death by apoptosis and not necrosis. Human granulosa cells were exposed to 30 uM-3 mM VCD showed a concentration dependent increases in apoptosis that were accompanied by decreases in proliferation and increases in markers for oxidative stress, and altered gene expression (Song et al. 2023)		

1x	BD	DEB detected in blood following 6 hours of exposure in mice to 62.5-1250 ppm in air (Thornton-Manning et al., 1995, 1997, 1998; Himmelstein et al. 1995); in mice exposed to 62 ppm for 6 hours, DEB level in blood was 345 pmol/g; in rats exposed to 8000 ppm for 6 hours, DEB level in blood was 14 pmol/g	Wide distribution of DEB in mice and rats in multiple tissues, including ovary following exposures to 62.5-8,000 ppm in air (Thornton-Manning et al., 1995, 1997, 1998; Himmelstein et al. 1995); in mice exposed to 62 ppm for 6 hours, DEB level in ovary was 169 pmol/g; in rats exposed to 8000 ppm for 6 hours, DEB level in ovary was 7 pmol/g	DNA-protein and DNA-DNA crosslinks reported in livers from mice exposed to 100-2000 ppm for 7 hours (Jelitto et al., 1989; Vangala et al. 1993); DNA- Crosslinks were not detected in similarly exposed rats			

10-20 days	BD	pyr-Val hemoglobin adducts from DEB detected in animals exposed to 0.1-625 ppm BD in air (Georgieva et al., 2010); pyr-Val levels are higher in mice (2.1-1980 pmol/g) than in rats (ND-125 pmol/g)		DNA crosslinks detected in multiple tissues (liver, lung, kidney, brain, thymus; ovary not specifically assessed) in mice exposed to 6.25-1250 ppm for 2weeks (Goggin et al., 2009, 2011). DNA crosslinks were also detected in mouse liver following exposures to 0.5-1.5 ppm BD for 2 weeks (Sangaraju et al., 2012) DNA crosslink concentrations in similarly exposed rats were lower than mice.			

30 days	EB					Doerr et al. (1995) mmol/kg (follical count % control) 1.4 (2%) Doerr et al. (1996) 0.005 (79%) 0.02 (91%) 0.09 (79%) 0.36 (27%) 1.4 (1.1%) mmol/kg rel ovarian wt (% control) 0.005 (119%) 0.02 (119%) 0.09 (108%) 0.36 (94%) 1.4 (58%) Rats are not responsive to EB	

30 days	DEB					Doerr et al. (1995) mmol/kg (follicle count % control) 0.14 (15%) Doerr et al. (1996) 0.002 (90%) 0.009 (80%) 0.036 (81%) 0.14 (13%) 0.29 (0.3%) mmol/kg ovarian wt (% control) 0.002 (106%) 0.009 (95%) 0.036 (98%) 0.14 (59%) 0.29 (45%) Rats are also responsive to DEB	
30 days	VCH					ED50 for follicle loss in mice = 2.7 mmol/kg, rats were unresponsive Smith et al. (1990); Doerr et al. (1995) mmol/kg (mouse follicle count % control) 7.5 (13%)	
30 days	VCM					ED50 for follicle loss in mice = 0.5-0.7 mmol/kg, for rats = 1.4 mmol/kg (Smith et al. 1990)	

5 days	VCD				Rats exposed to 240 mg/kg-day VCD for 5 days showed altered mRNA expression, increased oxidative stress and apoptosis (Sen Halicioglu et al., 2021)	Rats exposed to 240 mg/kg-day for 5 days showed significantly reduced follicle counts accompanied by increased hemorrhage and congestion, follicular cell degeneration, vacuolization, and increased collagen fibers (Halicioglu et al., 2021)	
5-15 days	VCD				Mice exposed to 160 mg/kg-day showed IGF1R/AKT/mTOR signaling pathway by down-regulating the expression of IGF1R in ovarian granulosa cells and induced autophagy in ovaries (Niu et al., 2025).	Mice exposed to 160 mg/kg-day showed significantly reduced follicle counts (Niu et al., 2025). Mice exposed *in utero* from days 6.5 to 18.5 post coitus to 80, 160, or 240 mg/kg-day VCD via ip injection exhibited decreases in follicle counts by approximately 70%, 95%, and 97% on ppd 3; and by approximately 80%, 95%, 98% of controls on ppd 21 (Song et al. 2023b)	

15 days	VCD				Rats exposed to 80 mg/kg-day for 15 days there was reduced expression of miR-144 in the ovary, increased expression of autophagy proteins (Zhou et al., 2023) Rats exposed to 160 mg/kg-day for 15 days showed reduced m6A levels and increased apoptitic cells (Liu et al. 2023)	Rats exposed to 80 mg/kg-day VCD for 15 days exhibited follicular damage (Zhou et al., 2023); Rats exposed to 160 mg/kg-day VCD for 15 days exhibited significantly reduced follicle counts and reduced cell viability (Liu et al. 2023); Mice exposed to 160 mg/kg-day VCD for 15 days showed near complete loss of follicles by day 30 (Li et al., 2024)	
28 days	VCD				Rats were orally exposed to 100, 250, or 500 mg/kg-d and exhibited dose-dependent increases in ovarian apoptosis (as indicated by caspase-9 and -3 expression). These changes were accompanied by increased malondialdehyde, catalase, glutathione peroxidase, and glutathione S-transferase activities, as well as increased		

					ovarian superoxide dismutase activity, and depleted uterine SOD activity and ovarian glutathione (Abolaji et al.., 2016).		
30 days	VCD					In mice and rats administered VCD at doses ranging from 0.07 to 7.4 mmol/day, ED50 for follicle loss in mice was 0.2 mmol/kg, and in rats was 0.4 mmol/kg (Smith et al. 1990a)	
13 weeks	BD						Bevan et al. (1996) assessed ovarian failure in mice and rats exposed to BD for 13 weeks. In mice, incidence was increased as follows: ppm (incidence) 0 (0/10) 1000 (10/10); Rat were nonresponsive

40 weeks	BD						NTP (1993) assessed ovarian atrophy in mice and rats exposed to BD for 40 weeks. In mice incidence was increased as follows: ppm (incidence) 0 (0/10) 6.25 (0/10) 20 (0/10) 62.5 (0/10) 200 (9/10) 625 (8/8) Rat were nonresponsive
13 weeks	VCH					NTP (1986) mg/kg-d (incidence decreased follicles) 1200 10/10	Bevan et al. (1996) assessed ovarian failure in mice and rats exposed to VCH for 13 weeks. In mice, incidence was increased as follows: ppm (incidence) 0 (0/10) 50 (0/10) 250 (0/10) 1000 (5/10) In rats, incidence was increased as follows: 0 (0/10) 250 (0/10) 1000 (0/10) 1500 (2/10)
13 weeks	VCD						NTP (1989) assessed ovarian failure in mice and rats dermally exposed to VCD for 13 weeks. In mice, incidence was increased as follows:

							mg/kg dermal (incidence) 0 (0/10) 2.5 (0/10) 5 (4/10) 10 (10/10) Rats were not responsive
61 weeks	BD						NTP (1984) assessed ovarian failure in mice exposed to BD for 61 weeks. Incidence was increased as follows: ppm (incidence) 0 (2/49) 625 (40/45) 1250 (40/48)
65 weeks	BD						NTP (1993) assessed ovarian failure in mice exposed to BD for 65 weeks. Incidence was increased as follows: ppm (incidence) 0 (0/10) 6.25 (0/10) 20 (1/10) 62.5 (9/10) 200 (7/10) 625 (2/2)

104 weeks	BD						NTP (1993) assessed ovarian failure in mice exposed to BD for 104 weeks. Incidence was increased as follows: ppm (incidence) 0 (4/49) 6.25 (19/49) 20 (32/48) 62.5 (42/50) 200 (43/50) 625 (69/79)
104 weeks	VCD						NTP (1989) assessed ovarian failure in mice and rats exposed dermally to VCD for 104 weeks. In mice incidence was increased as follows: ppm (incidence) mg/animal dermal (incidence) 2.5 (12/50) 5.0 (43/49) 10 (47/49) Rats were nonresponsive

Table S2. Detailed Support for Key Events in the MOA for General Toxicity of BD (e.g., reduced weight gain)

Duration	Test Agent	KE1: Metabolism of BD to Reactive Epoxide Metabolites	KE2: Distribution of Epoxide Metabolites to Maternal and Fetal Tissues	KE3: Epoxide Conjugation with GSH Resulting in GSH Depletion	KE4: General Toxicity (cytotoxicity *in vitro*; reduced weight gain/growth *in vivo*)
In Vitro	BD, EB, DEB, EBD	In microsomes exposed to 600-25000 ppm BD in air or 20-200 ppm EB in air, mice exhibit higher rates of oxidation to epoxides than rats (Csanady et al. 1992; Nieusma et al., 1998) In liver S9 fractions exposed to 20 uM-2 mM EB, DEB, EBD, oxidation enzyme efficiencies were higher in mice than in rats (Motwani and Tornqvist, 2014);		Nieusma et al. (1998) reported that 5 mM of EB (2 enantiomers), DEB (3 enantiomers), EBD (4 enantiomers) depleted GSH levels (DEB>EB>EBD) in a time-dependent manner over 240 min	Nieusma et al. (1997, 1998) reported time-dependent (over 360 min) cytotoxicity in rat hepatocytes exposed to 1 mM EB, DEB, and EBD enantiomers. Cytotoxic potency of the epoxide enantiomers (DEB>EB>EBD) mirrored their ability to deplete cellular GSH levels. Clerici et al. (1995) reported an LD50 value for *in vitro* growth of 5 uM for mouse embryos exposed directly to DEB (limited detail provided).

1x	BD		Wide distribution of BD's epoxide metabolites (EB, DEB) has been reports in multiple tissues including reproductive tissues (uterus, ovary) following exposures to 62.5-1250 ppm in air (Thornton-Manning et al., 1995, 1997, 1998; Himmelstein et al. 1995). Consistent with chemical partitioning, measured epoxide concentrations in all tissues were generally within a factor of 2-3 for concentrations measured in blood. Measured epoxide concentrations are higher in mice than in similarly exposed rats.	Acute exposures to 2,000 ppm produces a nearly complete depletion (by 80% after 7 hours, by 96% after 15 hours) of hepatic non-protein sulfhydryls (NPSH) in mice (Kreiling et al., 1988). In comparison, NPSH in similarly exposed rats was only moderately reduced (to approximately 65-80% of control levels). In mice exposed to 10-2000 ppm BD for 7 hours, NPSH levels exhibited a concentration-dependent decrease in lung, liver, and heart beginning at 250 ppm, with up to 80% depletion at the two highest exposures (Deutschman and Laib, 1989). In similarly exposed rats, a major reduction in NPSH was only observed at the highest concentration. In mice exposed to 62.5-1250 ppm BD and rats exposed to 62.5-8000 ppm for 6 hours, GSH was significantly lower in lungs at 62.5 ppm from mice compared to rats (Himmelstein et al., 1995). GSH reductions were generally more	The NPSH depletion reported by Kreiling et al., (1988) following exposure to 2000 ppm for 7 hours was accompanied by signs of acute toxicity in exposed mice, but were absent in rats.

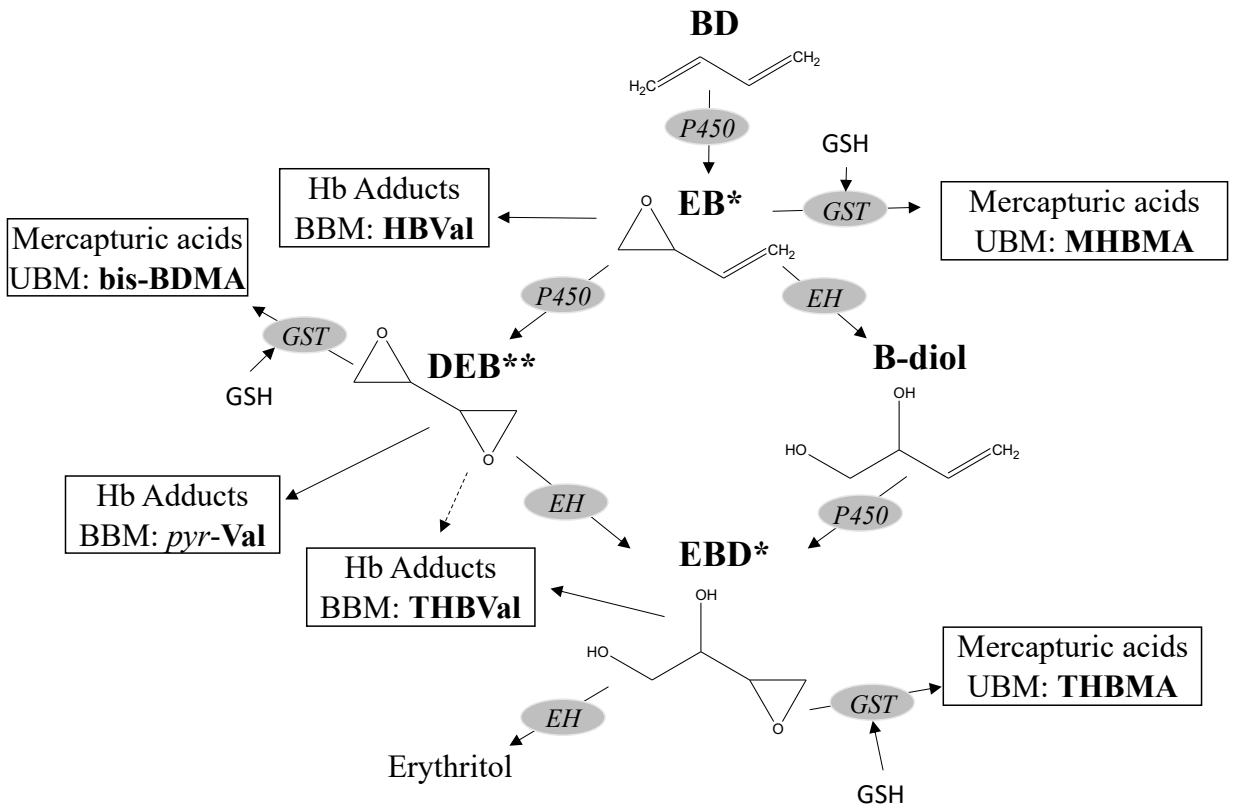
				pronounced in mouse lung and liver compared to rats. The authors noted that GSH depletion was associated with epoxide tissue levels.	
1x	DEB	NA (test agent is a diepoxide)			Clerici et al. (1995) reported an LD50 value for *in vivo* growth of 16 mg/kg DEB for mouse embryos (limited detail provided)
10-20 days	BD	Protein adducts (EB, THB, and pyr-Val hemoglobin adducts) which reflect blood concentrations of BD epoxide metabolites are detected in a concentration-dependent manner in mice exposed to 0.1-625 ppm BD in air (Georgieva et al., 2010). Hemoglobin adduct levels in similarly			

		exposed rats are lower than mice.			
3-5 days	BD				Maternal weight gains were reduced in mice in a concentration-dependent manner at the end of the gestation period (gd 11-16; Hackett et al.,1987a). ppm (% control) 40 (95%) 200 (86%) 1000 (80%) Body weights for mature (i.e., not growing), nonpregnant mice were unaffected by BD exposure (weights remained at ~30 g for all treatment groups and time points). Weight gains in similarly exposed pregnant rats was unaffected by BD (Hackett et al., 1987b).

					Hazleton (1981) reported significant reductions in rat maternal body weight gain compared to controls (+13 g), particularly on gd 6-9 with BD exposures of 1000 ppm (+1 g) and 8000 ppm (-1 g), and a nonsignificant reduction at 200 ppm (+9 g).
10 days	BD				Fetal body weight gains were reduced in a concentration-dependent manner (Hackett et al., 1987a); ppm (% control) 40 (96%) 200 (84%) 1000 (78%) Similarly exposed rats were unaffected (Hackett et al., 1987b) Hazleton (1981) reported a small but significant reduction in rat fetal body weight gain compared to controls (+3.3 g) from dams exposed to 8000 ppm (+3.1 g), and nonsignificant reductions at 200 and 1000 ppm (+3.2 g for both treatment groups).
5-30 days	EB				Body weight gains were reduced in a dose-dependent manner in growing, nulliparous mice exposed to EB across doses ranging from 0.005-1.43 mmol/kg, by approximately 10% at the highest dose (Doerr et al., 1996); Similarly exposed growing rats were unaffected by EB.

5-30 days	DEB				Body weight gains were reduced in a dose-dependent manner in growing, nulliparous mice exposed to DEB across doses ranging from 0.002-0.29 mmol/kg, by approximately 15% at the highest dose (Doerr et al., 1996); In similarly exposed growing, nulliparous rats, the effects of DEB on body weight gains were even more pronounced (50%) than in mice, and were accompanied by increased mortality at the highest dose.
12 days	VCD				In pregnant mice exposed directly to VCD (80, 160, 240 mg/kg-day via ip injection) body weights of F1 animals at birth were significantly reduced (by ~20%) at the highest dose (Song et al 2023b)

Figure 1. Metabolism of BD to Reactive Epoxide Metabolites



*monofunctional alkylating agent; **bifunctional alkylating agent

Figure 2. Proposed MOA for the Ovarian Effects of BD

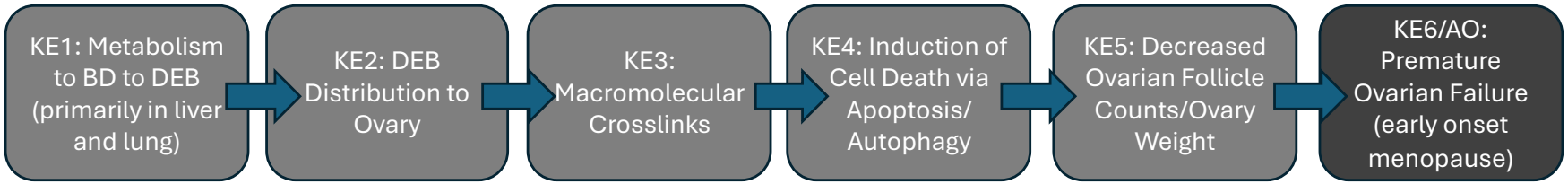


Figure 3. Proposed MOA for the General Toxicity of BD (e.g., reduced weight gain)

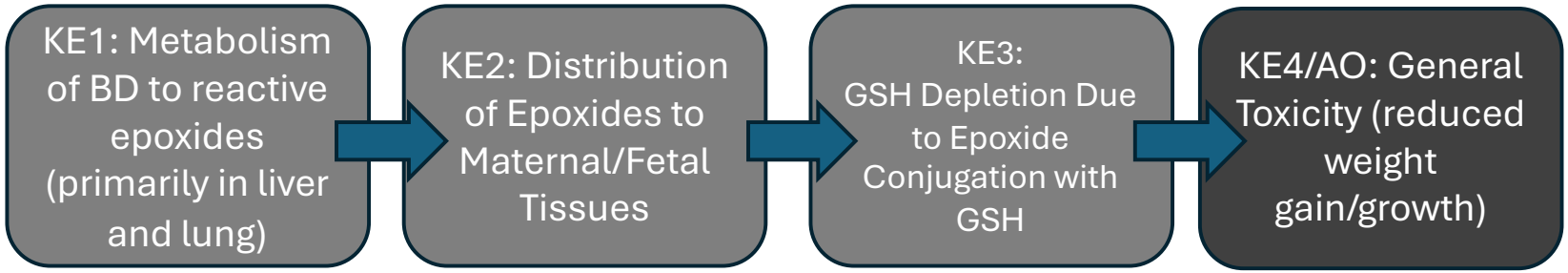


Figure 4.Maternal Weight Gain vs Fetal Weight Gain in Mice Exposed to BD (Hackett et al., 1987a)

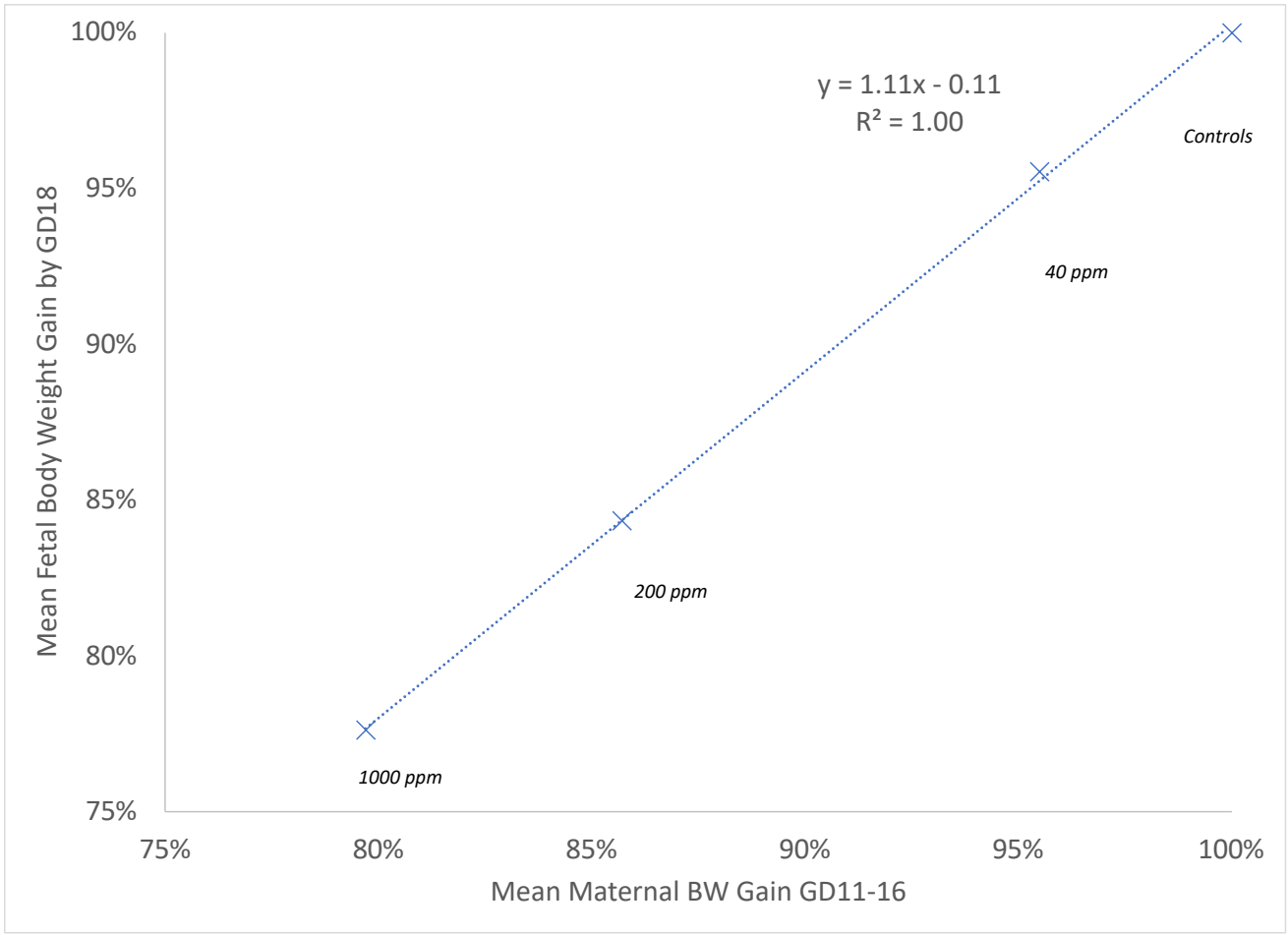


Figure 5. Mean Adduct Burdens for BD Metabolites in Exposed Workers (Georgieva et al., 2025). Symbols = Arithmetic mean; Error bars = SD; Solid circles = Male workers from Study 1; Hollow circles = Male workers from Study 2; Solid triangles = Female workers from Study 2.

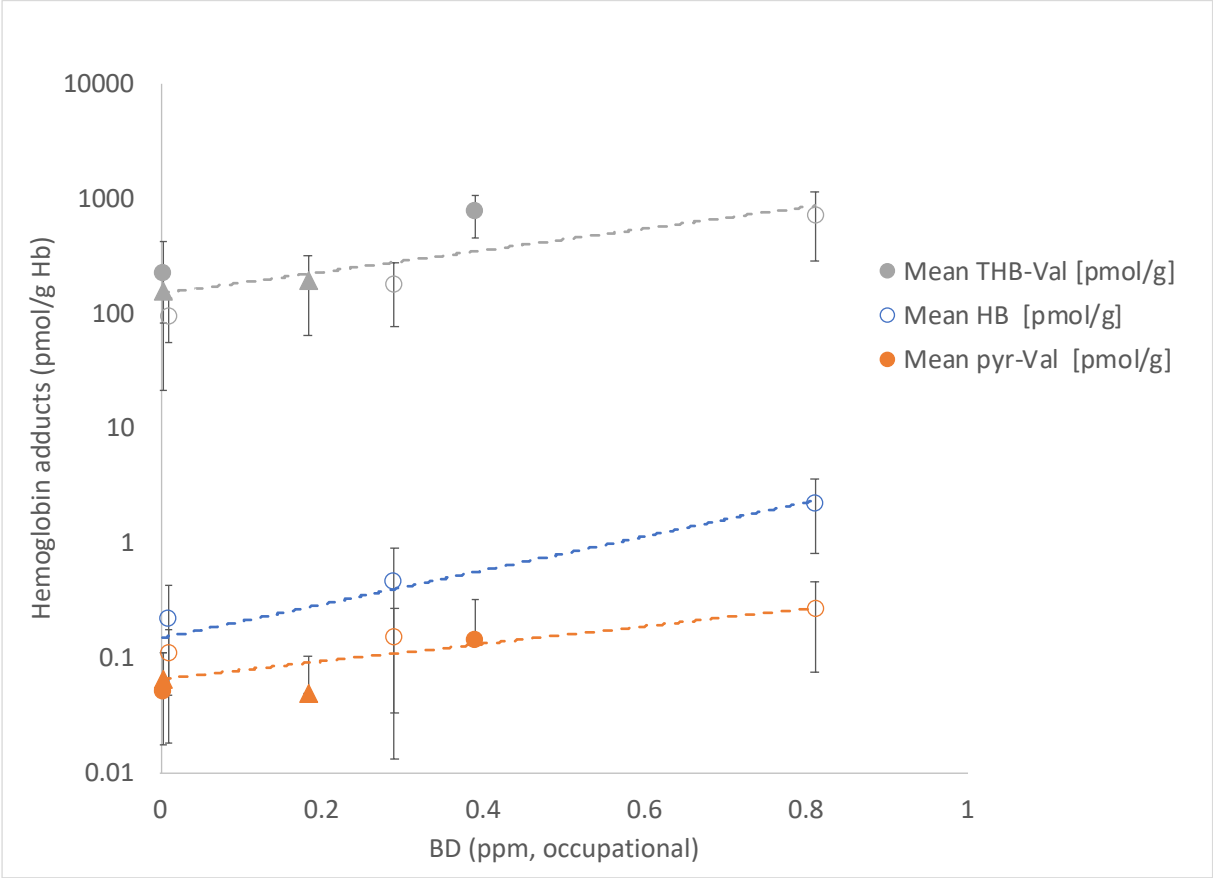


Figure 6. Species Differences in pyr-Val Adducts (reflects DEB internal dose) as a Function of BD Exposure in Mice, Rats, and Humans (Georgeieva et al., 2010, 2025)

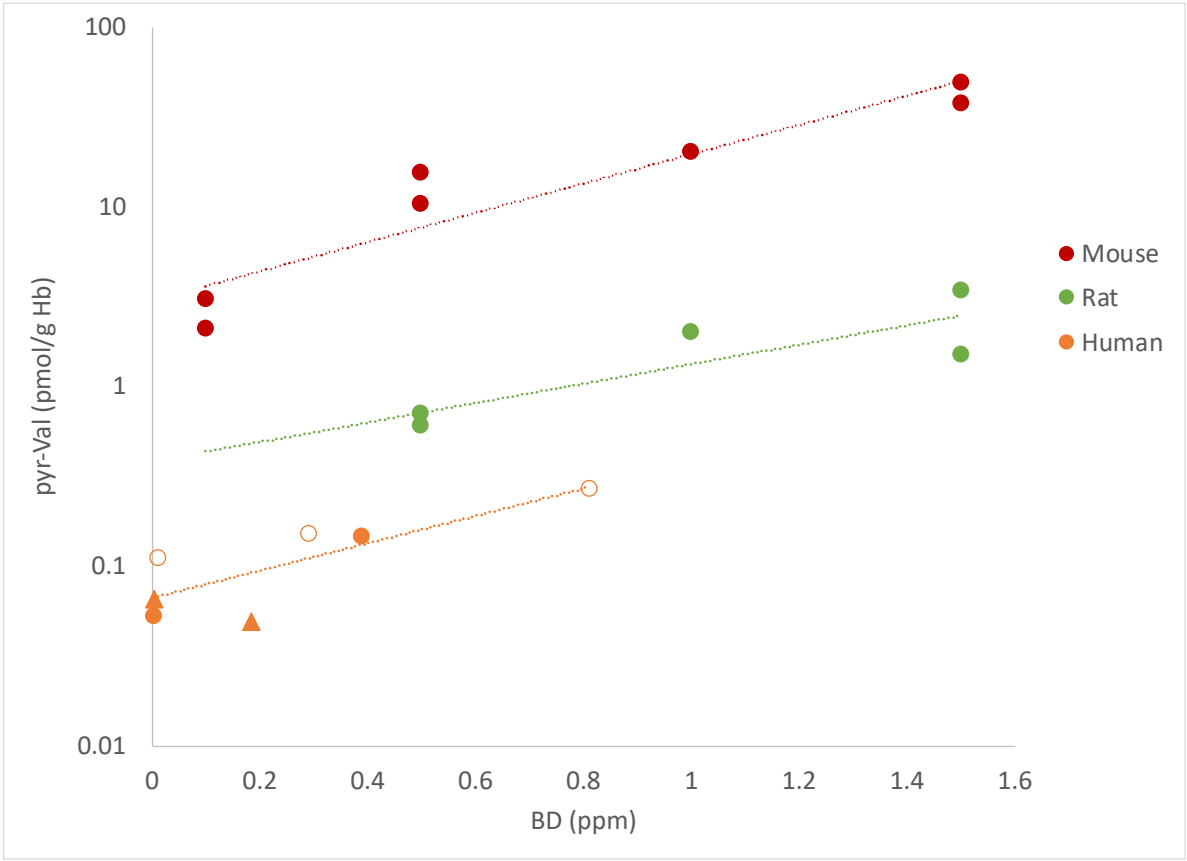


Figure 7. Panel Conclusions on the MOA for BD Noncancer Endpoints and Human Relevance (Appendix A, Questions 1.2-1.4, 2..2-2.4)

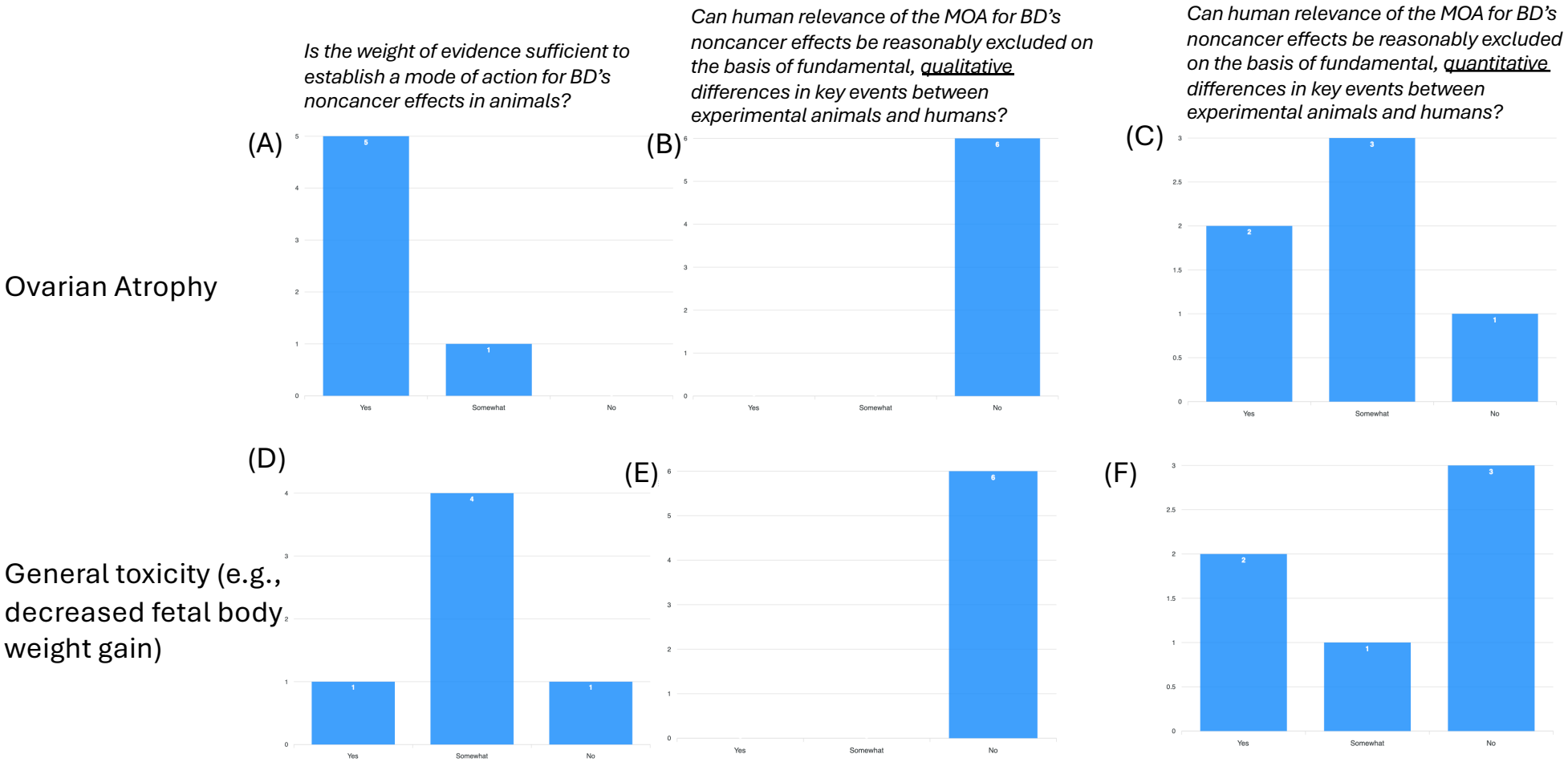


Fig. 8. Panel Confidence in the Noncancer MOAs for BD. MOA for Premature Ovarian Failure: mean=8.2±0.8 out of 10 ; MOA for General Toxicity (e.g., reduced fetal body weight gain): mean=5.2±1.7 out of 10

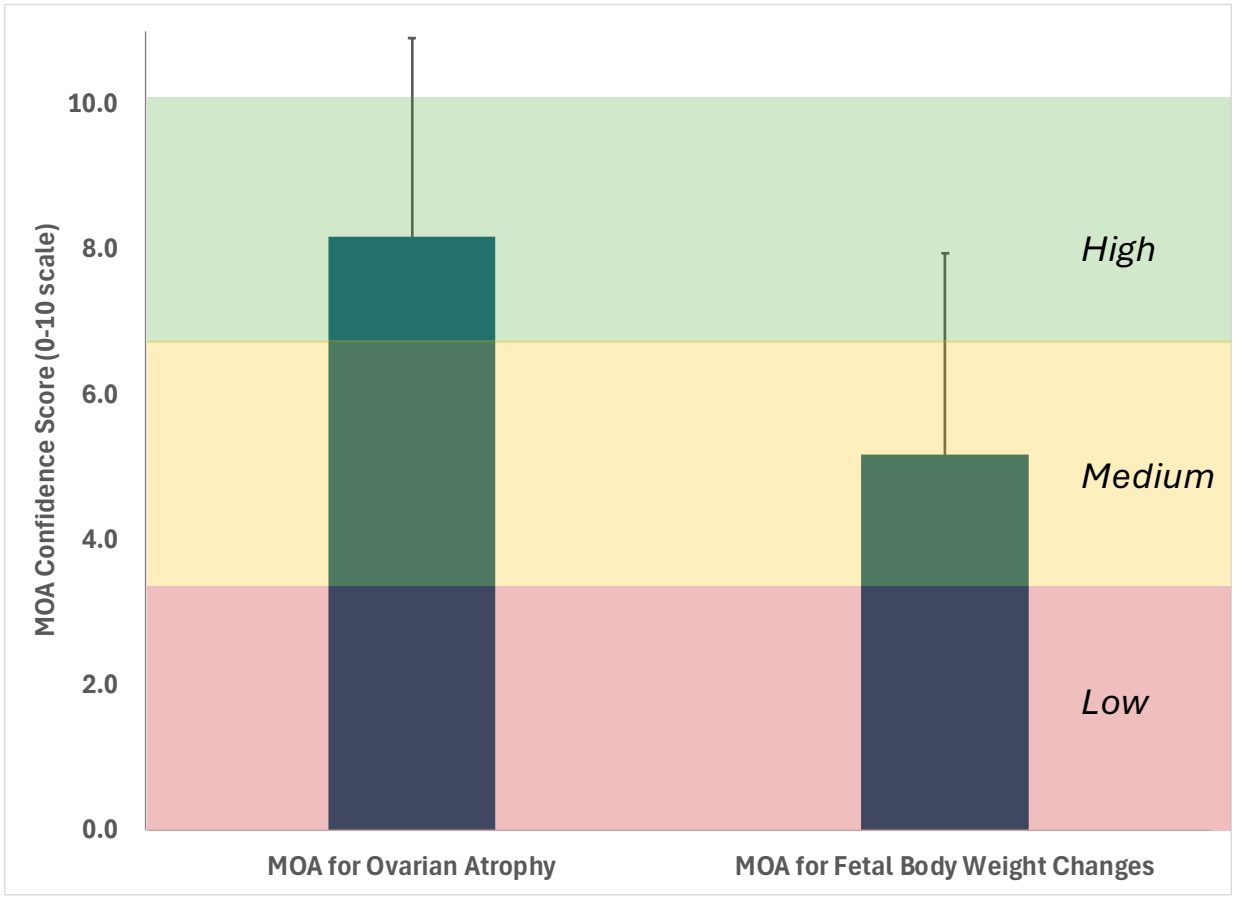


Fig. 9. Panel Confidence in Dosimetry Decisions to Account for Species Differences BD Toxicokinetics

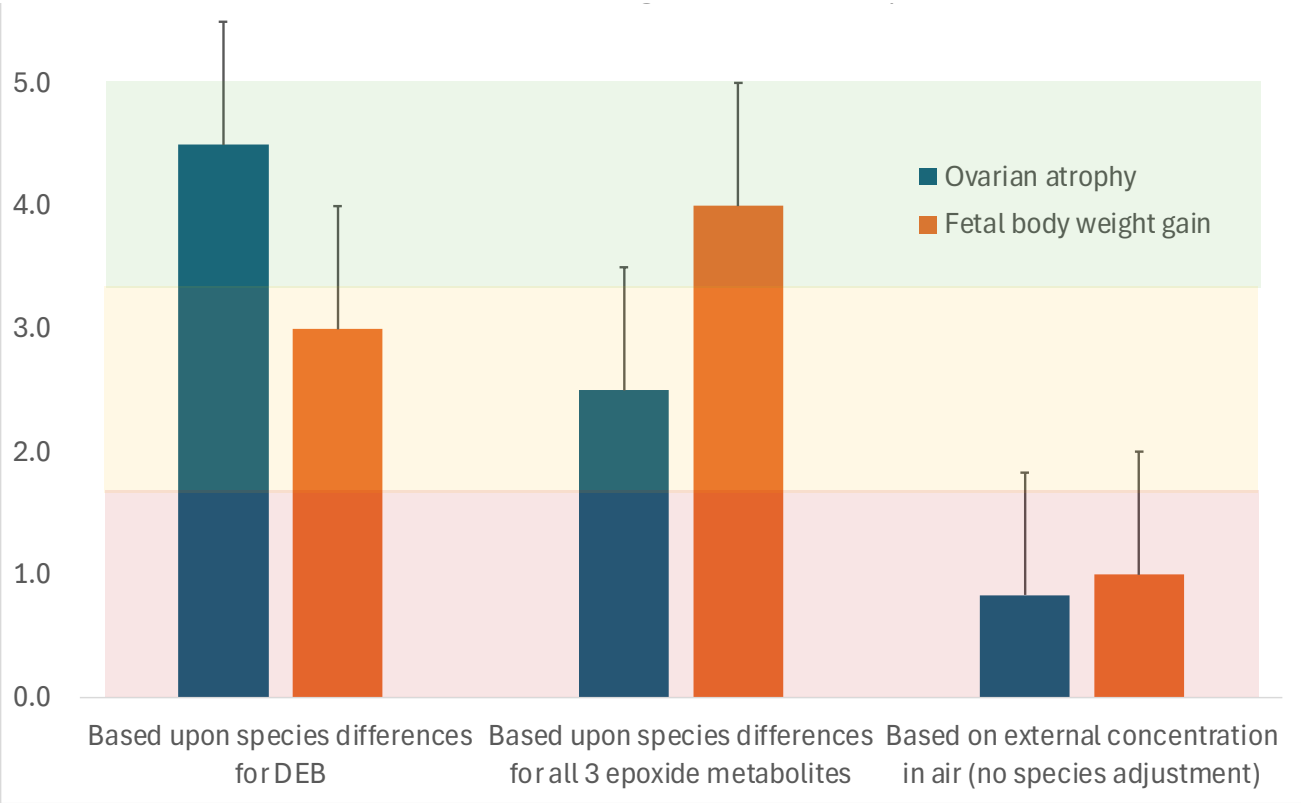
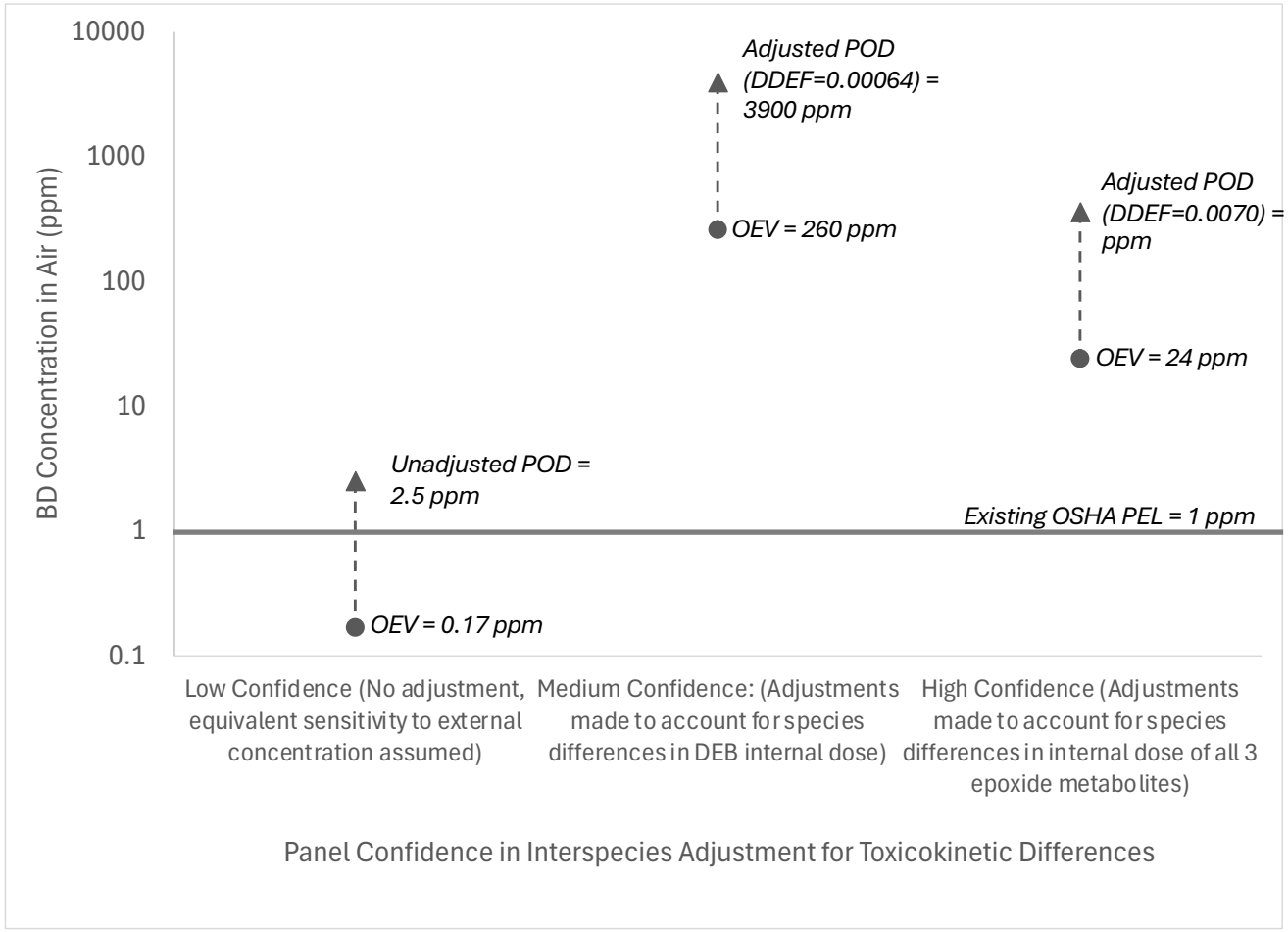


Fig. 10. Calculation of Occupational Exposure Values for BD Based Upon Lower Fetal Body Weight in Mice Using Different Approaches for Interspecies Extrapolation (Triangles = human equivalent concentration for the point of departure; dashed lines = application of an uncertainty factor of 30; Circles = OEV values)



SciPinion

SciPi #814

October 14, 2025

Appendix A: Revisit Mode of action Review for Reproductive and Developmental Effects in Mice

PREPARED FOR:

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Introduction: SP814 Expert Panel Engagement

SciPinion engaged an independent panel of experts to serve on a science advisory panel (SAP) using methods described in Kirman et al. (2019). The process was designed with the goal of maximizing the pool of ideal panelists, defined as the intersection of four populations, people who have expertise in the subject matter, are objective, are available to participate, and are willing to participate. Six experts in developmental/reproductive toxicity, mode of action (MOA), and dosimetry in human health risk assessment were identified to participate in this panel. The process for recruiting, selecting, and engaging the expert panel is described below.

Panel Recruitment

Potential candidates were identified as having relevant experience using a variety of sources, including: (1) SciPinion's internal database; (2) searches for authors of recent publications on the topic of interest in online databases (e.g., Pubmed, Google Scholar); (3) searches of profiles on social media databases (e.g., LinkedIn); (4) general internet searches; and (5) referrals. Email addresses were obtained for as many potential candidates as possible. An email invitation was sent to all potential candidates, requesting interested candidates to volunteer on https://app.scipinion.com, upload a copy of their CV, and provide a brief application statement (*i.e.*, *what makes you qualified for this panel?*). SciPinion received CVs from a total of 143 applicants, 3 of which were excluded for failing to upload their CV, leaving 140 candidates to go through the next step of the process.

Panel Selection

A triple blinded process was used: (1) candidates were blinded to the review sponsor; (2) the review sponsor was blinded to the candidates and played no role in selection; and (3) those selected for the panel were blinded to one another. Expertise data provided by the applicants and extracted from their CVs were used to rank the candidates with respect to general expertise metrics (e.g., academic degree, number of years of experience, number of publications) and topic-specific expertise metrics (e.g., CV key word counts). Six panel members were selected by SciPinion from the available candidates based upon expertise metrics. Additional candidates were identified as potential alternates in case a panelist is unable to complete their participation. The demographics and expertise metrics for the 6 panelists in the panel are summarized in **Table 1**.

Table 1. Demographics and Expertise Summary for Panel Members

Name	Employment Sector	Years Post-Degree	Advanced Degree	Country	Publications	Qualifications
Dr. John Lipscomb	Consulting; former EPA	33	PhD	US	89	- Toxicologist and Toxicokinetics Expert - Former Toxicologist for EPA ORD - Former FDA Biologist, Division of Reproductive and Developmental Toxicology - Co-author of EPA guidelines for DDEFs - Co-author of multiple MOA papers - Responsible for decisions among dose metrics to use in EPA risk assessments
Dr. Bette Meek	Academia; former Health Canada	43	PhD	Canada	250	- Toxicologist and Risk Assessment Expert - Former Manager Health Canada Bureau of Chemical Hazards - Internationally recognized expert in MOA & AOP evaluation - Author of multiple MOA framework papers including those for non-cancer endpoints
Dr. John Rogers	Consulting; former EPA	42	PhD	US	147	- Developmental Toxicologist - Former EPA Chief of the Perinatal Toxicology Branch and Director of the Toxicity Assessment - Past President of the Teratology Society - Past President of SOT Reproductive and Developmental Toxicity Specialty Section - Editor in Chief of Birth Defects Reseach
Dr. Rita Schoeny	Consulting; former EPA	47	PhD	US	97	- Risk Assessment Expert - Former Senior Science Advisor EPA OSP & Acting Director EPA RAF - Co-author of EPA guidelines for DDEFs, weight of evidence in MOA, & approaches to mixtures risk assessment - Co-author of multiple MOA & AOP papers

Dr. Jennifer Seed	Consulting; former EPA	37	PhD	US	44	- Developmental Biologist/Toxicologist - Former Senior Science Advisor, Deputy Division Director, Branch Chief EPA OPPTS - Co-author of EPA test guidelines for developmental toxicity & reproductive toxicity - Co-author of multiple EPA guidelines on MOA and risk assessment - Involved in multiple international working groups on MOA for noncancer effects - Co-author of multiple MOA papers - Co-authors of multiple developmental toxicity papers
Dr. Babasaheb (Bob) Sonawane	Academia; former EPA	53	PhD	US	137	- Developmental Toxicologist - Former EPA NCEA Supervisory Interdisciplinary Toxicologist & Acting Group Chief Quantitative Risk Management Group - Co-author of EPA guidelines for reproductive and developmental toxicity risk assessment

Panel Engagement

The 6 panel members were placed under contract. Email addresses corresponding to their SciPinion user accounts were verified as belonging to the experts (i.e., associated with their publication record, with their place of employment, or verified by personal communication). Charge questions were developed by SciPinion.

During the application process and throughout the peer review, panel members were blinded to the identities of their fellow panel members (identified online only by their display names of “Expert 1”, “Expert 2”...). Individual responses to the charge questions are linked to the experts anonymized display names, and not to their identities, an effort intended to provide psychological safety.

The primary review material consisted of a summary document (see Section 2 of the paper), and the following key references from the published literature:

- Aboloji et al 2016.pdf
- Boobis et al 2008.pdf
- Boysen-2007.pdf
- Deutschmann Laib 1989.pdf
- Doerr et al 1995.pdf
- Doerr et al 1996.pdf
- Georgieva et al 2010.pdf

- Georgieva et al 2025.pdf
- Goggin et al 2011.pdf
- Goggin-2009.pdf
- Hackett et al 1987a.pdf
- Hackett et al 1987b.pdf
- Halicioglu et al 2021.pdf
- Hazleton 1981.pdf
- Himmelstein-1995.pdf
- Jelitto-1989.pdf
- Kirman et al 2022.pdf
- Kreiling-1988.pdf
- Motwani and Tornqvist 2014.pdf
- NTP-1993.pdf
- Owen-1987.pdf
- Sangaraju et al 2012.pdf
- Thornton-Manning-1995.pdf
- Thornton-Manning-1997.pdf
- Thornton-Manning-1998.pdf
- Vangala et al 1993.pdf
- Zhou et al 2023.pdf

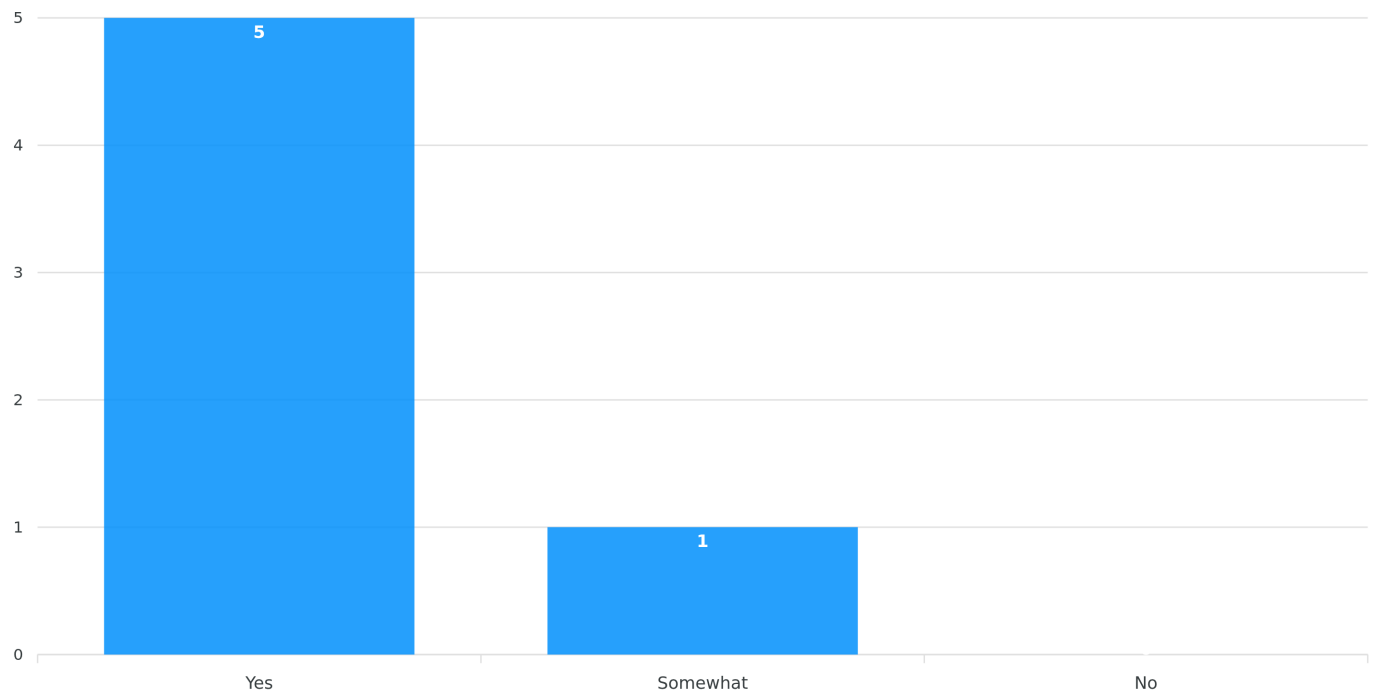
Panel members were also permitted to request additional publications and reports as needed to support their participation. The expert panel engagement was structured to have 3 rounds using a modified Delphi format (start in August of 2025, completion in October of 2025):

- *Round 1* – Panel members worked independently to read the review material and answer Round 1 charge questions. All 6 panel members completed their assignment as scheduled.
- *Round 2* – Panel members worked deliberatively to review and comment on each other's responses to Round 1 questions. All participation was conducted online (app.scipinion.com) in an anonymous manner (i.e., experts were randomly assigned display names "Expert 1", "Expert 2"...). A total of 67 comments were received during Round 2, with all panel members participating.
- *Round 3* – Panel members worked independently to revise their Round 1 responses as needed. All panel members completed this round as scheduled.

All charge questions and panel member responses, explanations, and comments from this engagement are provided below.

Is it appropriate to rely upon MOA information for structurally related chemical VCH and its diepoxide (VCD) to help characterize KEs (particularly KE4) in the MOA for BD?

Results | 6 answers



It is generally appropriate to rely on MOA information from VCH and VCD to characterize key events (particularly KE4) in the BD MOA, with most experts expressing strong agreement. The consensus centers on several key points:

• VCH/VCD provides valuable supporting evidence for BD's MOA due to structural similarities and comparable metabolic pathways leading to diepoxide formation
• Both chemicals demonstrate similar species-specific ovarian toxicity patterns (particularly in mice vs. rats)
• The rich database available for VCH/VCD offers insights into cellular processes that may be lacking for BD

Some limitations were noted, with Expert 6 pointing out that while the approach is defensible, the extension based solely on structural similarity may be insufficient without additional supporting evidence. Expert 4 suggested that while using data from structurally related chemicals is reasonable for hypothesis development, specially designed mechanistic studies for BD would provide higher confidence in the proposed MOA.

AI generated summary content

ANSWER EXPLANATIONS

Expert 1 | Yes | It is certainly appropriate to rely upon currently available published MOA information based on the overall weight of evidence of bioactivation of of 1,3-Butadiene to DEB and other epoxide metabolites such as EB in mice and rats and oxidative metabolism is much more active in mce compred to rats or humans. The blood DEB levels are estimated to be 40 to 100 times higher in mice compred to rats and 100 to 300 times or more higher in mice compred to humans for the same administerd dose of 1,3- Butadiene. An analog of 1,3- Butadiene, 4-vinylcyclohexene (VCH) induces ovarian atrophy only in mice but not rats and is metabolized into either a mono-or di-epoxide form. The di-epoxide form of VCH was 2 to3 times more potent at inducing ovarin follicle loss than mono-epoxide form, and both epoxides were 2-3 times more active in mice compared to rats(Hyer and Sipes, 2007). Therfore,MOA information for structurally related chemical VCH and its di-epoxide (VCD) makes an excellent supporting argument to characterize MOA key events including for KE4 for BD. There are several multiple studies showing that complex responses occur in ovary follicles on exposure to VCD (and by analogy to DEB), which includethe induction of oxidative stress,autophagy,inhibition of c-kit signling, decrese in expression of miR-144, and alterd expression of many other genes.Although,multiple mechanisms may contribute to the follicule destruction leading to premature ovarian failure,data on VCH clearly demonstrates that connection between follicule loss,and ovarian failure leading to atrophy (Liu et al,2015;Hoyer and Sipes, 2007).

Expert 2 | Yes | I would make more explicit use of the VCD/ VCH observations in a revised dose / time concordance table. Please note suggestions under 1.3. I would also make clear early in the document the relevance of such information, at the beginning of section 2.1. I think that the VCD observations can provide important support for the proposed intermediate KE.

Note added in round 3. The latest draft makes more (and appropriate) of the VCD / VCH data in supporting the MOA.

Expert 3 | Yes | Well-studied structural analogs are an important source of data for developing MOAs. VCH is particularly relevant in that it is a dimer of BD, forms a diepoxide, and produces similar ovarian toxicity.

Expert 4 | Somewhat | It's reasonable to hypothesize key events based on information available for structurally related chemicals. Indeed, this is the premise for AOPs, characterizing toxicodynamic key events for relevant biological pathways normally based on collective data for a range of prototypical chemicals. To have higher confidence in assessing the mode of action of BD, however, it's desirable to have specially designed mechanistic studies where the key events at the various levels of biological organization (i.e., ,subcellular, cellular, organ, etc.) in the hypothesized mode of action are documented - essentially verifying that BD acts principally via the hypothesized MOA. Studies in specialized (e.g., null) experimental models documenting the essentiality of the hypothesized key events also contribute to confidence in the hypothesized MOA.

Expert 5 | Yes | Yes, it is appropriate to use MOA information for VCH and its diepoxide (VCD) to help characterize the KEs for BD. VCH is a dimer of BD, and ovarian toxicity has been shown to be due to formation of the diepoxide VCD, analgous to the formation of DEB from BD. Other structural analogues that do not form diepoxides (ethylcyclohexene oxide, vinylcyclohexane oxide, cyclohexene oxide) do not cause ovarian toxicity. There is a fairly rich database available for VCH and VCD providing valuable information on the MOA for ovarian toxicity. VCH (via VCD) shows the same species differences in ovarian toxicity as BD (via DEB), and within mice it shows a similar pattern of KEs. There is limited information on KE4 (perturbation of cellular processes) for BD, and it is entirely appropriate to use data for VCH and VCD.
No change to this answer for round 3.

ANSWER EXPLANATIONS, CONTINUED

Expert 6 | Yes | VCD is called a "dimer of BD" at lines 101 and 116 and but I can't agree with that characterization based on molecular structure.

It is appropriate to rely on information and data from VCH to help characterize the Key Events in the BD MOA. But, I'm not certain that such reliance in this instance is completely sufficient as presently described and justified. In particular, information regarding the bifunctional alkylating agents and the use of VCH/VCD as a research tool that induces ovarian atrophy is proposed based on an extension of the studied molecular events (mechanistic steps) identified for VCH/VCD (lines 139, etc) that have evidently not been identified for BD/DEB. No evidence is presented that other bifunctional alkylating agents demonstrate the same molecular events is presented – such might be considered. It should be noted that (at line 257) apoptosis is specifically mentioned relative "diepoxides" in studies by Springer et al., 1996 and Hoyer and Sipes, 2007 (but, those studies may only address VCH. Mentioning these other diepoxides and specifically relating them to apoptosis would provide valuable support to the implication of apoptosis as a mechanistic aspect of follicular damage as a Key Event.

If it is possible to find such, a quantitation of doses or adduct levels in VCH/VCD-exposed animals relative to the animal dose-response function for VCH/VCD-induced ovarian dysfunction would be of substantial value. Further supportive value would come from a comparison of the dose response data from VCH/VCD to dose response data from BD/metabolites when a common endpoint can be found. The description of KE4 (on lines 139 etc) makes it clear that the specific events (i.e., oxidative stress, induction of autophagy, inhibition of IGF1R/AKT/mTOR pathway, etc) have not been identified in studies of BD/DEB. This represents a shortcoming (but perhaps not a fatal limitation) in the extension of VCH/VCD/s mode (really, "mechanism" – see line 256) of action to BD/DEB. Is it true that none of these events have been observed following BD/DEB exposures? Has it been shown that other bifunctional alkylating agents besides DEB cause such effects? [please see Doerrer et al., 1996, top of second column, page 129: "Structure activity studies of these olefins indicate that a diepoxide metabolite is essential for the depletion of pre-antral ovarian follicles in mice (Table 1) ....."]. Can some other basis for a quantitative comparison of VCH/VCD to BD/DEB be identified?

I find the basing of the extension of molecular events from VCH/VCD to BD/DEB solely on the basis of structural similarity of parent molecules (which is where the comparison of similarity stops in the present description of KE4) represents a target for criticism. Perhaps some thought should be given to characterizing KE4 as something a bit more general (e.g., more "mode-like") such as "depletion of pre-antral ovarian follicles" (Doerrer et al., 1996, second column, page 129) or "accelerated oocyte depletion" (line 258). I believe that such a proposition may be supportable by the data available. Doing so would move the point of attention a bit away from a step/process that might be considered more "mechanistic" than "mode" and shift attention to an event more directly aligned with "mode" than the effects identified at lines 139, etc for VCH/VDC.

In sum, I believe the MOA as proposed is defensible. And that it will have to be defended. There will be those that discount it based on potential weaknesses at KE4, and those that believe the structurally-based extension among bifunctional alkylating agents is sufficient.

This aspect, the subject of this comment is directly, clearly and unambiguously addressed in the Uncertainty Section, lines 266-272. I believe that this is sufficient.

Mechanistic effects associated with KE4 are discussed at lines approximately 190, etc. References Liu et al., 2023 and Halicioglu et al., 2021 do not appear in the bibliography.

Debate | 6 comments

Expert 1 | 9/02/2025 18:53 SCORE: **1**

I think we have an excellent agreement that it is appropiate to rely upon MOA information for structurally chemical VCH and its diepoxide (VCD) to help characterize KEs in the MOA of BD. I kind of also agree with the Expert 4 that to have a higher level of confidence in the proposed MOA of BD, desirable to have specically designed mechanistic studies for each of the key events. However, there is a sufficient information to support weight of evidence for the proposed key events for the MOA of BD. The argument further strengthned by using VCH as dimer of BD and its epoxide to charecterize the KEs for BD. VCH (via VCD) have shown the similar species dffrerences in ovarian toxicity as BD via DEB in mice.

Expert 6 | 9/04/2025 8:34 SCORE: **0**

It does seem that there is rather broad agreement. It is noteworthy that several comments offer suggestions for strengthening the presentation, including Expert 4's mentioning of specially designed studies and Expert 5''s identification of mono-functional analogues that do not produce ovarian toxicity.

Expert 2 | 9/04/2025 10:08 SCORE: **0**

There is general agreement on the utility of the information on VCH and VCD. I also saw excellent suggestions for describing data gaps and how to address them (Expert 4). I would certainly recommend the document authors consider and incorporate the extensive discussion provided by Expert 6.

Expert 4 | 9/05/2025 8:43 SCORE: **0**

I agree very much with the query/suggestion of expert 6 to consider whether additional quantitative analysis of the VCH/VCD data (including in vitro) can additionally inform inference for BD/DEB. I posed a similar question in response to Question 1.5 but agree also that the lack of mechanistic underpinning for the intermediate key events is complicating. In vitro data to provide a better mechanistic understanding of these KEs would add confidence to the VCH/BD analogy.

Expert 4 | 9/05/2025 8:49 SCORE: **0**

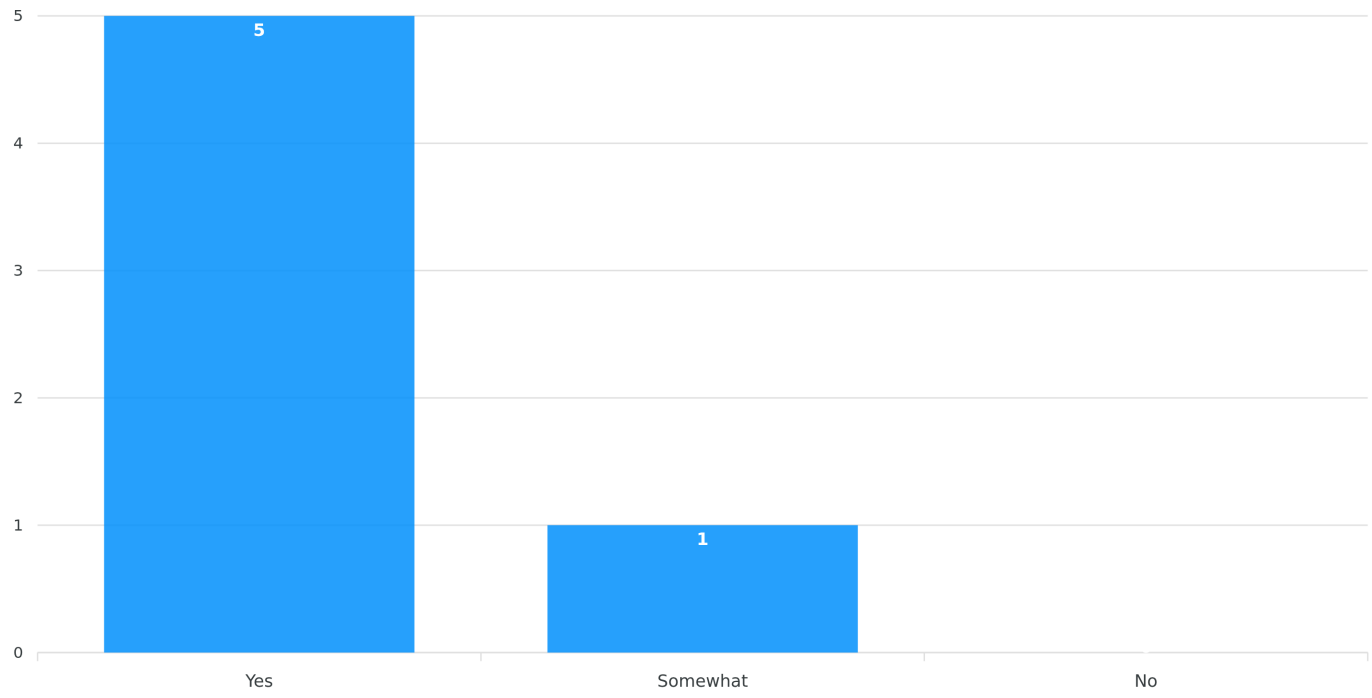
I agree also, that a dose-response concordance table for the VCD/VCH observations would be extremely helpful. There are separate dose-response concordance tables for each of the prototypical chemicals (which serve as analogues for MOA) in AOP development.

Expert 3 | 9/09/2025 17:52 SCORE: **0**

The general agreement that VCD/VCH data are relevant to supporting the MOA. Also agree that a significant data gap centers on Key Event 4 "perturbation of cellular processes". Although this is true, it is vague as stated. The VCD/VCH data on specific cellular processes affected are especially valuable here, but some similar experiments on BD/EB/DEB would address this gap. A KE should be measurable and measured in animal and in vitro models where possible. The current KE 4 is broad and not a specifically measurable event. Additional data focusing on the most important measurable cellular processes affected would strengthen the MOA.

Key Question #1 (Boobis et al., 2008): Is the weight of evidence sufficient to establish a mode of action for ovarian atrophy in animals?

Results | 6 answers



The weight of evidence for establishing a mode of action for ovarian atrophy in animals is considered sufficient by most experts, with five experts answering "Yes" and one answering "Somewhat."

Areas of agreement among experts include:

- The role of BD's bioactive epoxide metabolites (particularly diepoxide) in causing ovarian toxicity
- Species differences in sensitivity between mice and rats due to metabolic differences
- The overall strength of evidence from multiple studies with various exposure durations and dose levels

Areas of concern or refinement noted by experts:

- Expert 4 highlighted that the description doesn't conform well with accepted practice for describing AOPs and chemical-specific MOAs
- Several experts suggested better documentation of dose-response relationships between key events
- Expert 3 noted that key events 4 and 5 could be more explicit, particularly regarding follicle cell death via apoptosis
- Expert 6 suggested including more specific data on species differences in metabolism

AI generated summary content

ANSWER EXPLANATIONS

Expert 1 | Yes | **The ovarian toxicity of BD have been examined in multiple published studies in mice and rats with a wide range of exposre durations and dose levels.Exoposre to 1,3- Butadiene results in ovarian atrophy in mice and it's bioactive epoxide metabolites are likely be responsible for this toxic effect.Furthermore, mice appears to be more sensitive to 1, 3-Butadiene -induced ovarian toxicity compared to rats due to greater oxidative metabolism and potentially increased toxicodynamic sensitivity.Although, multiple mechanisms of ovarian toxicicity are plausible , the weight of evidence supporting KEs for the ovarian atrophy effects of 1,3-Butadiene from studies BD itself , its epoxide metabolites(EB and DEB) including that of VCH(a dimer of BD) and its epoxide metabolites is certainly sufficient and strong to establish MOA for ovarian toxicty in animals.**

Expert 2 | Yes | I think that the hypothetical pathway (call it an AOP) is strong for ovarian atrophy in animals. The lines of evidence that BD produces the adverse outcome are much better defined in this draft than in other versions I have seen.

The authors make good points in the introduction about the utility of understanding a MOA in proposing data derived extrapolation factors. I think the point that MOA informs DDEF selections is well taken. I suggest that the authors lean more on the thought that the hypothetical pathway is well supported by science and that the evidence is "good enough" to say that this AOP is the mode of action by which BD causes ovarian atrophy.

Note added round 3. The latest document makes a stronger case for the MOA in general. I found the discussion of and support for apoptosis to be stronger as well.

Expert 3 | Yes | The MOA is supported by both in vivo and in vitro studies and across species. The granularity of the MOA could be better. For example, KEs 4 and 5 could be more explicit, especially KE 5 given evidence of induction of follicle cell death, likely via apoptosis. This more specific KE is supported also by the gene expression findings that include genes related to cell death.

ANSWER EXPLANATIONS, CONTINUED

Expert 4 | Somewhat | The description of the mode of action doesn't conform well with accepted practice for describing generic AOPs and chemical specific MOAs nor with the intended focus of weight of evidence evaluations for the IPCS Human Relevance Framework. Assessment of the weight of evidence for both AOPs and MOA relate to toxicodynamic key events, including metabolic key events but do not include ADE aspects. Rather, these aspects are addressed quantitatively in the subsequent dose-response analysis (through, for example, development of DDEFs based on toxicokinetic parameters or PBK modelling). "Diepoxide Distribution to the Ovary" is not a key event, though its documentation is important in understanding and quantitating ADE in dose-response analysis for consideration of interspecies differences and intraspecies (human) variability . Also, as per the response to Question 1.1, ideally, for higher confidence, documentation of the key events based on experimental observation following exposure to BD or its active metabolites and consideration of the essentiality of key events based on null models is desirable. As illustrated in Table 1, data for several of the key events for BD are missing (proposed KE4) or limited (proposed KE3). By definition, key events are those observed (measured). Inclusion of the dose-response concordance table is extremely helpful in clarifying the extent of the information available at various levels of biological organization. There has been no attempt, however, to consider the patterns of quantitative (empirical) support across studies; this would require additional tabular presentation by increasing dose and time across the various studies separately for BD, its analogues and metabolites. This enables consideration of the complete dataset to address whether or not the data support generally the patterns that we anticipate, consistent with the hypothesized mode of action, namely, for example, whether the incidence of early key events exceeds that of later key events (i.e., concordance between key events). Alternatively, there may be outliers which reasonably detract from the weight of empirical support for the hypothesized mode of action or are a legitimate function of limitations of experimental design or other. Benchmark doses facilitate this comparison.

Expert 5 | Yes | Yes, there is sufficient information to establish the MOA for ovarian toxicity in animals. There is ample information demonstrating that the rate limiting step is formation of the diepoxide both for DB (DEB) and VCH (VCD). There are data demonstrating the distribution of DEB to the ovary and the formation of the DEB specific adduct pyr-Val. Information on the KE4, perturbation of cellular processes, is lacking for BD, but there are data available for VCH (VCD). There are robust data demonstrating ovarian toxicity for both BD and VCH, and onset of premature menopause. The revised document provides a good summary of the temporal associations of the KEs, the strength, consistency and specificity of the MOA, and the biological plausibility of the proposed MOA. The discussion on the dose relationships focuses mainly on the ovarian toxicity. It is lacking in a discussion of the dose relationships among the KEs. Some of that discussion is actually provided in the strength, consistency and specificity section (for example the discussion of adduct formation), and is provided in Table 1. However, it would improve the document by providing a more detailed discussion in the text, rather than leaving it up to the reader to thoroughly digest Table 1.

Additional comments for round 3:

The answer is still yes! The addition of the metabolism section in the revised document for round 3 is great and provides a good overview for the reader. The dose-response section has been expanded, and Figures 3 and 4, as well as Table 2, have been added. These are very helpful. The discussion sections for dose-response and temporal relationships have also been expanded, but the focus is still on the relationship between the specific KE and the final outcome, premature ovarian failure; both sections would benefit by expanding the discussion to include the dose and temporal relationship between KE1 and KE2, KE2 and KE3, etc.

ANSWER EXPLANATIONS, CONTINUED

Expert 6 | Yes | Yes. However, some additional points for refinement may be considered.

Regarding the WOE, the first point is the dose response relationship between species based on exposure concentration. This demonstrates appreciable mouse vs rat differences. The section relates this to differences in metabolism, but no specific data are presented on species differences in metabolism. Some data regarding species differences in metabolism should be presented. This becomes important in that the comparison (presented at this level) is one of toxicokinetics only. An extension into toxicodynamics could be done and would add value. Such an extension would compare rat-mouse differences in ovarian or follicular effects on the basis of metabolite formed. Making a comparison at this level (the level of the metabolite formed) would "correct-for" toxicokinetic differences and would allow an evaluation of similarities/differences between rats and mice at the level of toxicodynamics. If it can be shown that the metabolism-based responses are similar, this would avoid or reduce the argument that "humans are so much more toxicodynamically sensitive that species differences in metabolism (toxicokinetics) may be overshadowed by differences in sensitivity (toxicodynamics)".

However, this has in effect been shown to some extent by Doerrer et al. (1996). Figures 2 and 5 demonstrate a lesser sensitivity or rats compared to mice when receiving equivalent doses of diepoxybutane, when assessed as ovarian or uterine weight. This may be interpreted to indicate that rats are not the most toxicologically sensitive species for reasons in addition to differences in chemical metabolism (and it should be noted that this criticism applies only to ovarian and uterine weight, not to any follicular effect). This finding might be used to sharpen the focus on identifying the specific endpoint to be addressed when conducting a species comparison. Further, the real "importance" of this comment relates to anticipating a criticism of the work following a more broad examination, and so may be considered "beyond the scope" of the charge given.

Debate | 9 comments

Expert 1 | 9/02/2025 19:06 SCORE: **0**

Again, on review of answers of we have no disagreement that the weight of evidence is sufficient to establish a MOA for ovarian atrophy in animals.

Expert 1 | 9/03/2025 9:18 SCORE: **1**

In general, I agree with the comments of Expert 4, however the proposed MOA is well supported by both in vivo and in vitro studies in animals. The ovarian toxicity of BD have been examined in multiple published studies in mice and rats with a wide range of exposre durations and dose levels.Exoposre to 1,3- Butadiene results in ovarian atrophy in mice and it's bioactive epoxide metabolites are likely be responsible for this toxic effect..Although, multiple mechanisms of ovarian toxicicity are plausible , the weight of evidence supporting KEs for the ovarian atrophy effects of 1,3-Butadiene from studies of its epoxide metabolites(EB and DEB) including that of VCH(a dimer of BD) and its epoxide metabolites is certainly sufficient and strong to establish MOA for ovarian toxicty in animals.Certainly, addtional discussion of KEs 4 and 5 coud be improved by considering comments of Experts 3,4 and 6.

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Expert 2 | 9/03/2025 11:10 SCORE: **0**

I appreciated the comments of Expert 6 on toxicokinetic vs. toxicodynamic aspects of the KE.

Expert 2 | 9/03/2025 11:12 SCORE: **0**

While I agree with the majority of Expert 4's comments above, I suggest that the use and development of AOP and MOA have moved beyond exclusion of toxicokinetic KE.

Expert 6 | 9/04/2025 8:46 SCORE: **0**

There is broad agreement that the WOE is sufficient, and there are several recommendations for improving the documentation of the support for specific elements of that proposal. Those recommendations seem to be communicated in adequate detail. And, I agree that moving into the development of an AOP runs beyond the charge. But I am not advocating a dismissal of these issues because those issues may arise in later discussions. I believe the shortcomings associated with proposed KE4 may fall into this category. As Expert 2 wrote, I believe the proposed MOA for ovarian atrophy is "good enough" to move forward. And, I agree with Expert 4 in that the description of the MOA does not presently conform to the norms of write-ups.

Expert 5 | 9/04/2025 11:13 SCORE: **0**

There seems to be general agreement that the WOE is sufficient. Including the metabolism step is appropriate since it is a requisite step in the MOA. It is also the critical piece for developing the DDEF.

Expert 4 | 9/05/2025 8:57 SCORE: **0**

As indicated by Expert 2, I also very much appreciate the suggestion of Expert 6 for potential extension into toxicodynamics for interspecies comparison. I agree that this would add value.

Expert 4 | 9/05/2025 9:02 SCORE: **0**

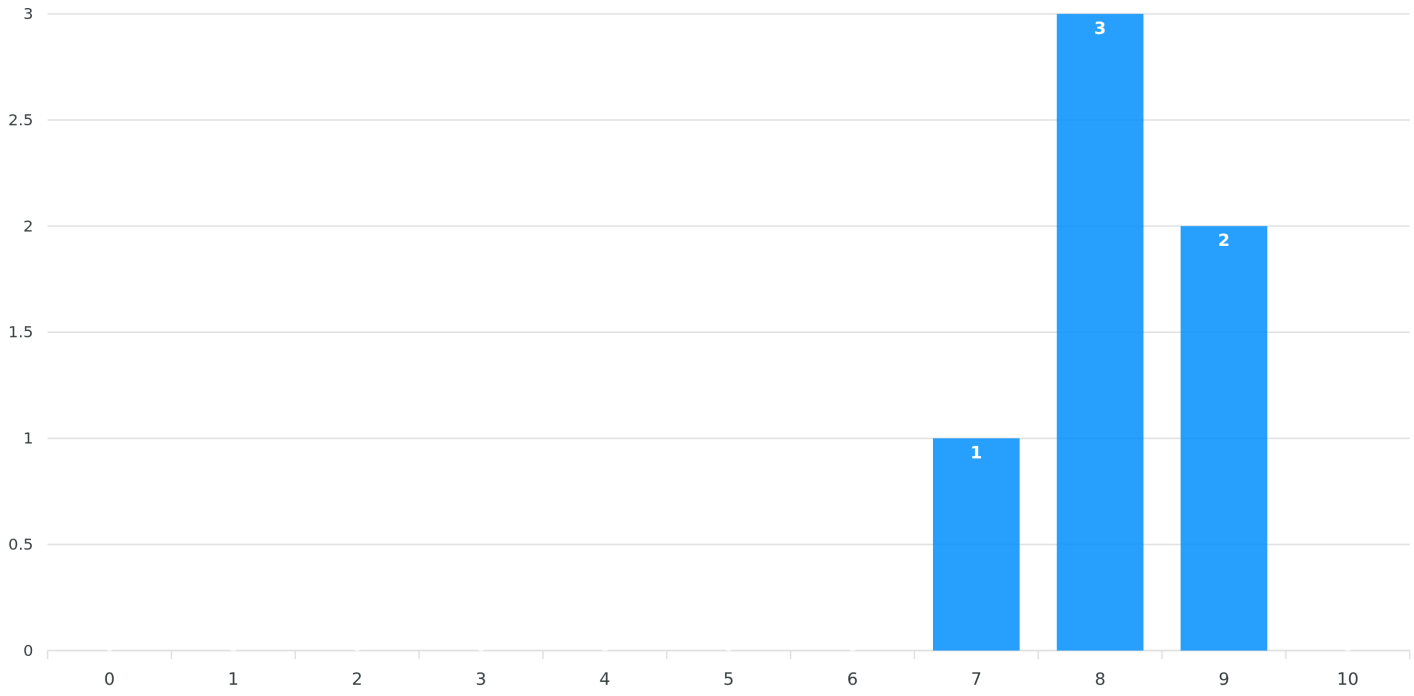
The perspective of Expert 2 in the comments, that the use and development have moved beyond exclusion of toxicokinetic parameters (i.e., ADE) is inconsistent with all recently developed guidance with which I'm familiar (or to which I have contributed). It appears to be based more on wishful "drift" in the application community.

Expert 3 | 9/09/2025 18:24 SCORE: **0**

I have worked with and understand the structure of AOPs, which are first chemically agnostic. Therefore, kinetics and metabolism of a chemical are not considered. But we are evaluating and developing a MOA that explicitly includes and depends on metabolism and distribution of a specific chemical. That is established up front and is critical to understanding, especially, species differences in sensitivity to ovarian toxicity of BD. However, once metabolism to the active metabolite and its distribution, are described (KE1 and KE2), the rest of the model can be considered a chemically agnostic and toxicodynamic AOP. The AOP is perhaps somewhat better supported for VCH/VCD than for BD/EB/DEB, but that data for these analogs are mutually supportive of KEs 3-5 strengthens the MOA/AOP. comparing the toxicodynamics of the analogs on specific measurable cellular functions and measurable ovarian adverse outcomes would be the next step.

What is your degree of confidence in the proposed MOA for the ovarian atrophy effects of BD? (0-10 scale; 0=no confidence; 10=highest confidence). Please explain your answer

Results | 6 answers



Expert consensus on the proposed mode of action (MOA) for ovarian atrophy effects of BD is relatively high, with confidence ratings ranging from 7-9 out of 10. Most experts (4 of 6) rated their confidence at 8 or higher.

Areas of agreement:

- Strong support exists for KE1 (metabolism) and the adverse outcome
- The evidence is sufficient to conclude the proposed MOA is operational in rodents
- Mice are particularly sensitive to these effects
- The addition of MOA diagrams was helpful for understanding the process

Areas of concern:

- KE4 (Perturbation of Cellular Processes) was identified as the weakest point by multiple experts
- Limited data exists for intermediate key events compared to metabolism and adverse outcomes
- Dose-response and temporal relationships could be better described
- More robust discussion on excluding alternative modes of action would strengthen the case

Several experts suggested that specially designed studies documenting the complete sequence of key events would further strengthen confidence in the proposed MOA.

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ANSWER EXPLANATIONS

Expert 1 | 8 | The degree of confidence in the proposed MOA for the ovarianrian effects upon BD exposure itself,its epoxides(EB and DEB), a structral analog 4- vinylcyclohexene (VCH, a dimer of BD)and atrophy effects of BD exposure in mice is high.The evidence is sufficient to conclude that the propose MOA is operational in rodents and mice are particularly sensitive. The evidence supporting the key events (KEs) is available from published studies for the proposed MOA (Figure 1 and Table 1) for the ovaits epoxide metabolites.

ANSWER EXPLANATIONS, CONTINUED

Expert 2 | 8 | The MOA discussion is much stronger in this draft. The addition of the MOA diagrams is very useful, as it greatly helps the reader focus on what is entailed in each KE.

I think that the MOA is reasonably well supported for some KE. The observations described in the document show strong support for KE 1 (metabolism), and the adverse outcome. The support for intermediate KE is not as strong, but it is good enough.

It's almost there but the description of dose response and temporal relationships can be improved. The description of the dose response data in particular focuses on just AO and jumps back to metabolism. The species differences in AO and metabolism are important for the POD and DDEF selection. But they have still not been described in a manner that shows that early KE (KE3: Molecular Crosslinks; KE4:Perturbation of Cellular Functions; KE5:Ovarian Toxicity are observed at lower doses (and earlier times) than the AO. I think the information is is in the document to show that KE2 (distribution to ovarian tissue) takes place and is necessary for the pathway to result in the AO.

I suggest that the arguments can be helped greatly by revising Table 1 (and Table 2 for the same reasons). These tables are very hard to read, and it doesn't really reflect dose /time concordance for the KEs proceeding from metabolism **through the intermediate steps** (bold intentional) to the AO. I would suggest that Table 1 (and 2) be revised to be simple summaries of dose response data, with notes on the time of exposure /observation in the table (instead of just "acute"). The next table would be a summary of the data supporting dose / time concordance for each KE. It's useful to construct an ideal example table for the hypothetical AOP and then fill in the blanks with the BD-specific information. Where there is no information for the KE, note in the table that there is none, thus identifying data gaps. (BTW, identifying a data gap does not invalidate a MOA. Nor does one have to fill the data gaps if there is not sufficient value of information to be gained by doing so). Please see comments under 3.1.

Below are some of the less editorial marginal comment to the revised document on ovarian atrophy.

47 48 Was this observation discussed re ovarian toxicity MOA?

121 May note here also that unlikely to be EB metabolism in the ovary.

137 – 141 Note that some MOA mavens will insist that these responses (oxidative stress, autophagy, etc). are all separate MOA that converge at KE1, KE2, KE3, KE5. I would suggest adding that perspective, but that for purposes of this assessment a vague (or inclusive) KE4 is sufficient.

144 – 147 Cite this as dose response observation that provides support for KE5. The point is not the species difference per se, but the differences in target organ dose resulting in the effect or lack thereof.
158 Are there data for VCH and VCD that would support the existence of earlier KE. This information would contribute to bio plausibility, consistency criteria.

Lines 162 -- 184.
The data presented here are only for the AO, not the KE. The point is to show that the early KE are observed at lower doses than are the later KE. Unless there are no data. In which one assembles a dose / time concordance table for a hypothetical MOA (more like a demonstration of a chemical agnostic AOP). For the BD MOA, fill in the boxes where there are observations.
Further flogging the dead equine, for the MOA discussions the species differences are important in that they demonstrate effects or lack thereof at different doses. In this section the emphasis ought be on (e.g.) "KE 3 is observed at this dose range, but not at lower doses". Or similarly KE would be expected to occur at doses lower than x, but there are no data.

199 200 Note that it may be useful to include some VCH, VCD in the data summary table, and to include observations on dose / time by analogy.

242 Important in human relevance discussions.

271 So it is important to show the information for observation of KE after VCD exposure.

Note for round 3. I did not check specifically to see if the points above were addressed. Again, I find the latest document to be much improved.

Expert 3 | 9 | Again, more specific key events would be better, but none of the KEs are wrong, and keeping them more generic is a more conservative representation of the data.

Expert 4 | 7 | My degree of confidence in the proposed mode of action for BD induced ovarian effects is relatively high, similar to that included in the previous Scipinion poll. The concordance table summarizes nicely the extent and limitations of the available data, though additional analysis might contribute to better understanding of temporal and dose-response concordance across studies. The results of specially designed studies documenting the KEs for BD in addition to investigation of the essentiality of KEs following exposure to BD if supportive of the hypothesized MOA, would contribute to confidence. While the contribution of other metabolites to ovarian effects cannot be excluded, the extent of the supporting data on the critical role of DEB (proposed KE 1) is considerable (e.g., Doerr et al., 1995, 1996), in addition to mechanistic inference based on its potential to cause crosslinks as a bifunctional alkylating agent. Supporting data for KE's 3 and 4 are less convincing. Data from specially designed short term mechanistic studies in which the incidence of key events at the various levels of biological organization (i.e., ,subcellular, cellular, organ, etc.) in the hypothesized mode of action is assessed at several dose levels of BD in small groups of animals would add to confidence in the hypothesized MOA, as would additional investigation of the recently hypothesized additional bifunctional metabolites. More robust discussion of the exclusion of other potential modes of action (e.g., follicle dysfunction/depletion vs. apoptosis) would also increase confidence. The basis for the current rationale concerning proposed alternatives seems inadequate, given the current availability of potentially relevant pathway descriptions in repositories such as the AOP wiki.

Expert 5 | 9 | I would give the proposed MOA a score of 9. As stated in question 1.2, there are substantial data demonstrating the necessity of formation of the diepoxide, DEB, distribution to the ovary, formation of the pyr-Val adduct, and ovarian toxicity. Data for KE4 is sparse for DB, but is provided for VCH. The weaknesses are simply the lack of data for KE4 on BD, and the necessity of piecing together data from a variety of studies of different doses, durations, and routes of exposure. In an ideal world, there would be studies designed to demonstrate the entire sequence of KEs in a single study.

No change for round 3.

Expert 6 | 8 | I believe that all points of the Key Effects are satisfactorily addressed. The weak point is KE4, Perturbation of Cellular Processes, as addressed previously, and treated in the document as an uncertainty. Support is provided by results from VCH/VCD, for which the clarity is acceptable. The MOA document does not identify any specific event among the several effects resulting from VCH/VCD. No experimental results are presented in which the prevention of any of these events has been shown to effectively prevent the end effects, thus it has not been conclusively shown that any of these events, alone or in combination are causative. As communicated in the document, only one bifunctional alkylating agent is effectively discussed; bringing in more agents that either produce the KE or the health endpoint would be valuable. The suggestion to identify another effect, less like a mechanistic process and more like a mode process, for KE4 has been made previously.

Debate | 5 comments

Expert 1 | 9/03/2025 9:57 SCORE: **0**

Among the Experts, the degree of confidence in the proposed MOA for the ovarian atrophy effects is pretty high (range 7-to 9). There are sufficient published data demonstrating the formation of the diepoxide, DEB, distribution to the ovary, and the pyr-Val adducts across species and, ovarian toxicity observed in mice and rats. Inspite of lack of data for the entire sequence of KEs, specifically KE4 in experimental animal studies, the overall weight evidence supporting the key events is sufficient for the proposed MOA as presented in Figure 1 and Table 1 of the document.The comments Expert 4 are highly relevant and needs to be considered in the revised draft document.

•

Expert 5 | 9/04/2025 11:16 SCORE: **0**

There is general agreement that the confidence is relatively high. There is also agreement that the inclusion of Table 1 is helpful, but the discussion around the Table needs to be expanded and particularly address the dose-relationships between each of the KEs - not just KE1 and the final outcome.

Expert 4 | 9/05/2025 9:08 SCORE: **0**

I agree with Expert 6 concerning the datagaps - e.g., essentiality of the KEs, that if addressed, might contribute to additional confidence in the hypothesized MOA. I suspect that in any manuscript, we should be able to be pretty explicit re these datagaps.

Expert 4 | 9/05/2025 9:12 SCORE: **1**

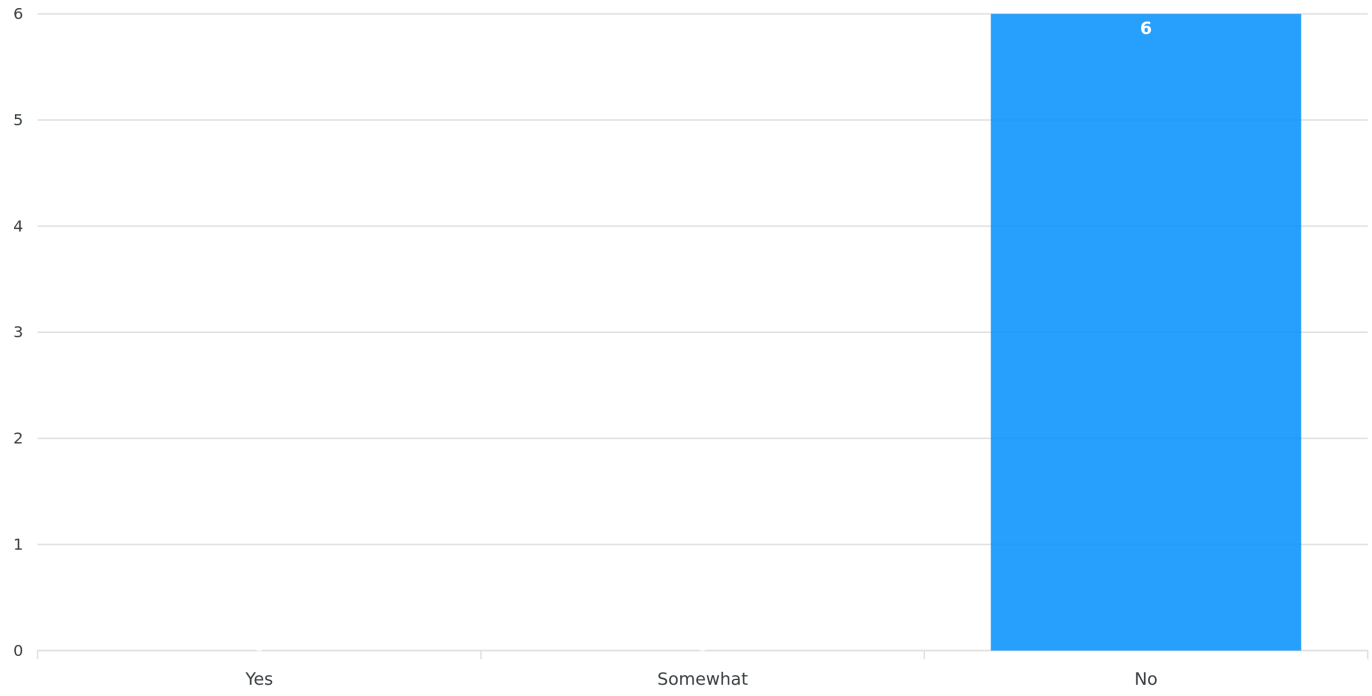
I agree also with Expert 2 on the limitations of the dose-response concordance analysis. There are relatively standard formats for these tables which would facilitate observing the relevant dose-time-severity-incidence patterns supportive of the MOA (separately for different analogues)

Expert 3 | 9/09/2025 18:39 SCORE: **0**

My comments are similar to those of the other experts in that the MOA is strongly supported by the data in hand for BD as well as the analog. So, confidence is pretty high. Developing a MOA or an AOP generally starts with the first and last steps and filling in the middle KEs is always the most difficult. I think the VCD/VCH data are highly supportive, although similar data with BD/EB/DEB would, obviously, be more directly applicable.

Key Question #2 (Boobis et al., 2008): Can human relevance of the MOA for ovarian atrophy be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

Results | 6 answers



All experts unanimously agree that the mode of action (MOA) for ovarian atrophy cannot be reasonably excluded from being relevant to humans based on qualitative differences between experimental animals and humans.

The experts cite several supporting lines of evidence:

- Metabolism pathways are qualitatively similar across species and DEB forms in humans
- Ovarian toxicity was observed in nonhuman primates exposed to VCD
- In vitro studies show VCD produces increased intracellular ROS, DNA damage, and altered gene expression in human ovarian cells
- C-kit receptor and its ligand have been detected in human ovaries
- The effect is not species-specific

While Expert 6 and Expert 2 noted the brevity of the discussion in the document, they still agreed with the conclusion. Expert 5 pointed out that while qualitatively relevant, there may be quantitative differences in diepoxide formation that would make humans less sensitive to ovarian toxicity.

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ANSWER EXPLANATIONS

Expert 1 | No | **The IPCS Framework for Analyzing the Relevance of a Noncancer MOA for Humans (Boobis et al, 2008) in considering how to interpret the human relvance of ovarian atophy is applicable . The general steps and the proposed key events as decribed in the "Revised MOA Summary ofr Noncancer Effects of 1, 3 -Butadiene (BD)" are sufficienty supported by the evidence to the proposed MOA for ovarian toxicity in rodents , especially mice. The metaolism pathway s are qqualitatively similar across species and DEB does form in humans.Ovarian toxicity was observed in nonhuman primates exposed to VCD and in vitro studies show that VCD produces increased intracelluar ROS, DNA damage and alterd expression of genes and oxidative stress ,resulting in increased apoptosis in human ovarian cells (Song et al; 2023).Additionally, the c-kit receptor and its ligand have been detected in human overies(Tuck et al: 2015) indicating that potential mechanism supporting the relevance of the proposed MOA for ovarian atrophy in animals to humans is applicable and can not be excluded.**

Expert 2 | No | The discussion in the revised document is brief, but it appears sufficient to me.

Expert 3 | No | the KEs in the MOA can qualitatively occur in humans as well as in rodents

Expert 4 | No | In my view, the rationale provided in the revised draft summary describes well the considerations relevant to the conclusion that the ovarian effects are qualitatively relevant to humans. This includes the observed ovarian toxicity in rats and mice exposed to DEB and in rats exposed via direct administration to the analogue VCD including in human granulosa cells in vitro.

Expert 5 | No | No, there are no qualitative data suggesting that the MOA could not occur in humans. Ovarian toxicity is noted when DEB or VCD are administered to rats or when VCD is administered to nonhuman primates, demonstrating that the effect is not species specific. VCD also causes perturbation of cellular processes (increased intracellular ROS, DNA damage, and gene expression) in human ovarian (granulosa) cells. Therefore, from a qualitative standpoint the MOA is plausible in humans. However, there are quantitative differences in the formation of the diepoxide which would make humans less sensitive to the ovarian toxicity.

No change for round 3.

ANSWER EXPLANATIONS, CONTINUED

Expert 6 | No | On the surface, and based a lot on information not presented in this document, it seems that the MOA cannot be ruled out on the basis of qualitative considerations. However, the text addressing the qualitative aspect of human relevance is a mere 12 lines long. This section should be expanded to include important points in a more detailed manner.

Debate | 3 comments

Expert 1 | 9/03/2025 10:22 SCORE: **1**

There is an unanimous agreement among the experts that the human relevance of MOA for ovarian atrophy can not be excluded on the basis of fundamental,qualitative differences in the key events between experimental animals and humans. I agree with the comments of the Expert 4 the text addressing the section should be expanded to include important points in a more detailed manner.

Expert 5 | 9/04/2025 11:16 SCORE: **0**

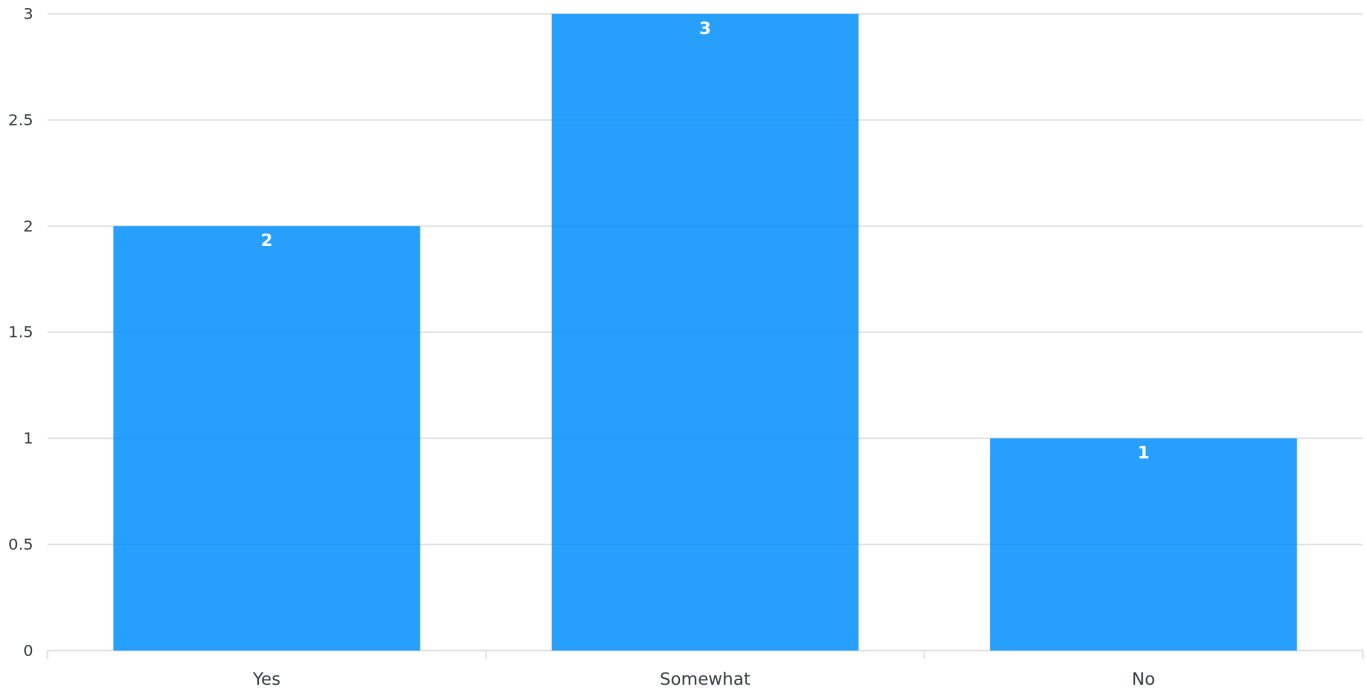
Seems to be total agreemnt!

Expert 3 | 9/09/2025 18:43 SCORE: **0**

All agree, and no specific qualitative species differences have been raised for the KEs in the MOA.

Key Question #3 (Boobis et al., 2008): Can human relevance of the MOA for ovarian atrophy be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

Results | 6 answers



Expert opinions on whether human relevance of the MOA for ovarian atrophy can be excluded based on quantitative differences show mixed conclusions:

• Agreement points:
• Most experts acknowledge significant quantitative differences in metabolism between species (mice > rats > humans) regarding DEB production.
• Several experts note that humans likely produce much lower levels of DEB than mice for equivalent BD exposures.

• Disagreement points:
• Only one expert (Expert 6) firmly answered "Yes" that human relevance can be reasonably excluded.
• Expert 1, despite answering "Yes," concluded that human relevance "cannot be excluded."
• Expert 2 explicitly answered "No," stating that human relevance has not been dismissed based on quantitative considerations.
• Three experts (3, 4, and 5) answered "Somewhat," indicating uncertainty about completely excluding human relevance.

• Key uncertainties cited:
• Potential contribution of other metabolites besides DEB
• Need for full risk assessment with human exposure data
• Whether quantitative differences make the MOA irrelevant or just indicate low risk

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ANSWER EXPLANATIONS

Expert 1 | Yes | There are significant quantitative differences between across species (mice>rats> humans) with respect to circulating levels of DEB upon exposure to BD and must be considered in quantative health risk assessment of BD environmental exposure scinarios.Ovarian toxicity is observed in mice and rats in a dose-and duration -responsive manner in multiple published studies. A strong evidence indicates that mice are more sensitive than rats ,and humans, both toxicokinetically and toxicodynamically based on relative levels DEB levels. Studies of hemoglobin biomarkers demonstrate that for a given exposure to BD, estimated DEB blood levels in humans are several orders of magnitude lower than corresponding DEB blood levels in mice (Motwani and Tornquist, 2014). Furtermore, recently published data (Georgieva et al, 2025) which includes female workers, suggests that women may show lower internal doses of DEB than men following exposures to BD. Therfore, it is very likely that humans (females) will not be producing levels of DEB that are sufficient to cause ovarian atrophy. Therfore, any DEB -mediated MOA for ovarian atrophy in humans would likely require orders of magnitude higher environmental exposure to 1, 3- Butadiene to produce ovarian atrophy.Since, ovarian toxicity cannot be explicitly roued out for humans, the IPCS framework (Boobis et al; 2008) recommend bringing forward and accounting for any quantitative differences into dose-response analysiis.In conclusion, human relevance of the proposed MOA for ovarian atrophy can not excluded given the quantitative differences in either kinetic or dynamic factors between mice, rats and humans .

Expert 2 | No | "For this reason, it is possible that humans are not capable of producing 331 levels of DEB that are sufficient to produce ovarian toxicity (i.e., above a threshold for 332 this endpoint)." I don't disagree with this statement, but I also don't feel that the human relevance of the MOA has been dismissed based on quantitative considerations. It would be up to a risk assessor to determine the likelihood of a specific BD exposure scenario resulting in human toxicity. This is the risk characterization step of the process, which will certainly consider the MOA as well as any health benchmark informed by it.

Round 3. I still find that the human relevance cannot be dismissed based on qualitative considerations.

ANSWER EXPLANATIONS, CONTINUED

Expert 3 | Somewhat | The quantitative aspects of the MOA are key for interspecies extrapolation. While the kinetic differences across species render human risk to be extremely low based on internal dose, this does not make the MOA irrelevant, it just requires quantitative KE relationships that together show that the adverse outcome is unlikely in humans. While the species comparisons of kinetics are adequate to indicate de minimus risk for humans, this does not render the MOA irrelevant. I do realize that this is somewhat of a semantic argument, but the kinetics do nothing to suggest that the MOA is incorrect.,

Expert 4 | Somewhat | Based on the extent of the available data indicating that DEB is the principal metabolite inducing the ovarian (and other) effects in mice and rats and the documented quantitative variations in metabolic conversion between rats, mice and humans, the likelihood that ovarian atrophy would be observed in humans exposed to BD is seemingly, negligibly low. However, there are notable uncertainties including the potential contribution of other metabolites. The observation of Motwani et al. (2014) in this context is interesting, namely :"Because of the relatively high AUC of EBDiol in humans, this metabolite is expected to contribute to more than 90% of the total genotoxic dose, despite its low genotoxic potency". Also, I wondered if it is at all possible to relate quantitatively the observations by Song et al. (2023) in in vitro human ovarian (granulosa) cells of increased intracellular ROS, DNA damage, and altered gene expression related to apoptosis and oxidative stress following exposure to the analogue diepoxide (VCD) to estimated human levels of the relevant metabolite(s) and by analogy with respect to relative potency, to BD?

Expert 5 | Somewhat | Somewhat. There are data suggesting that there are species differences in the metabolism of BD to the reactive metabolite DEB. This is a requisite step in the MOA and has to be accounted for in the DDEF. This suggests that humans would be less sensitive, but a full risk assessment involving human exposure data would be needed to actually state that the MOA is or is not operative in humans.

No change for round 3.

Expert 6 | Yes | I believe it can be ruled out, but the document in its present form does not support such. This question is directly addressed to this point in the document only at lines 319-335. Much of the information briefly presented in this section would carry more weight if introduced and expanded in earlier sections of this document. I anticipated the presentation of threshold value for metabolite production that must be passed to produce the effect and then a more complete toxicokinetic presentation that culminated with the presentation of results that showed something along the lines of “this level of metabolites would be possible in humans only when exposure was to lethal levels”, or the like. In its present form, this section does not support the conclusion that quantitative differences can serve to rule out the MOA. A simple reorganization and expansion of the contents of the document would be worthwhile.

Debate | 9 comments

Expert 1 | 9/03/2025 11:18 SCORE: **0**

It seems there is a fairly good agreement among the experts that the human relevnce of the proposed MOA for ovarian atrophy can not be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans.Request to Expert 2 to be explicit about the comment "For this reason, it is possible that humans are not capable of producing 331 levels of DEB that are sufficient to produce ovarian toxicity (i.e., above a threshold for 332 this endpoint)." I agree with the comments of Experts 3, 4 & 5 that the extent of the available data indicating that DEB is the principal metabolite inducing the ovarian (and other) effects in mice and rats and the documented quantitative variations in metabolic conversion between rats, mice and humans, the likelihood that ovarian atrophy would be observed in humans exposed to BD but the human risk likely to be extremely low based on internal dose of reactive metabolites. This important point needs to be better articulated and clearly stated in the revised draft of the document.

•

Expert 6 | 9/04/2025 9:37 SCORE: **0**

The responses are interesting. My read is different from that of Expert 1 (above), in that I think the general agreement is that "Yes", the MOA can be excluded based on the basis of quantitation. However, it must be acknowledged that the basis for the difference in the "read" is documented in the individual responses, which I believe call for a more definitive presentation of quantitative data, especially the dose response data. Those data should be evaluated to identify doses/conditions that do and that do not result in the endpoint. With that threshold identified, not just alluded-to, a more definitive argument can be made regarding the importance of quantitative differences. I feel that the response from Expert 3 is "closest to the mark" for this query. A reliable quantitative analysis would be useful, and may reveal a DDEF for interspecies differences so low that the value alone would lead to criticism - but having a reliable quantified value is acceptable over reliance on a default value. The concept of DDEF, the value of the DDEF and the human relevance of the MOA should be kept as separate issues, though.

Expert 4 raises an important consideration regarding other metabolites. While that issue is relevant, I'm not sure where that issue should be discussed because I don't see its impact on a quantitative difference regarding the proposed DEB-based metabolite. It is most assuredly an uncertainty and should be captured.

Expert 5 | 9/04/2025 11:19 SCORE: **1**

Again, I agree that the metabolic differences make if unlikely that humans would be susceptible. However, theoretically if exposure was high enough, humans might be affected. A full risk assessment would be needed to determine this.

Expert 4 | 9/05/2025 9:32 SCORE: **1**

My sense in reading the comments and responses is that there's agreement that while unlikely in humans, additional quantitation to support this inference would be helpful. Since exposure is not addressed in the MOA framework, quantitative differences would need to be rather extreme to conclude yes to this question.

Expert 4 | 9/05/2025 9:36 SCORE: **1**

I agree also with Expert 6 that Expert 3 has best captured the (collective?) sense based on good understanding of the Human Relevance Framework.

Expert 2 | 9/07/2025 9:17 SCORE: **0**

In response to Expert 1's query above. I was tacitly agreeing with lines 331 - 332 (quoted in my remarks above). That is, its is possible that humans are not capable of generating active BD metabolites sufficient to result in ovarian toxicity. It also has not been demonstrated that this is likely. My point is that I saw no rationale in the revised document to exclude the proposed MOA based on quantitative considerations. Whether a specific BD exposure scenario is likely to exceed some health benchmark (TBD) is determined in the risk characterization analysis.

Expert 6 | 9/07/2025 9:36 SCORE: **1**

What Expert 2 just wrote is, I believe, on-the-mark. His/her comment extends into the value of the uncertainty (extrapolation, adjustment, whatever) factor for interspecies differences. EPA went through great pains to include in the DDEF guidance (EPA, 2014) that DDEF values computed on the basis of relevant data, and pertaining to animal-to-human differences can be lower than 1. Thus, if it is possible that data can reliably show that the likelihood of humans reaching a metabolite load that is lower than that responsible for developing the effect, there are two options. The first is that the MOA can be excluded, the second is that the MOA cannot be excluded and a DDEF value can be developed. This is an issue that will have to be faced. As far as EPA is concerned, this may likely be a policy option, and a good topic for inclusion in a risk characterization effort. If the MOA is to be excluded on the basis of quantitative considerations, these considerations should be rather well-documented. The present draft of the MOA summary does not rise to that standard, in my opinion (referring back to my original response). However, I believe that the available data would support such an argument, but I do not presuppose the outcome.

Expert 4 | 9/08/2025 8:14 SCORE: **1**

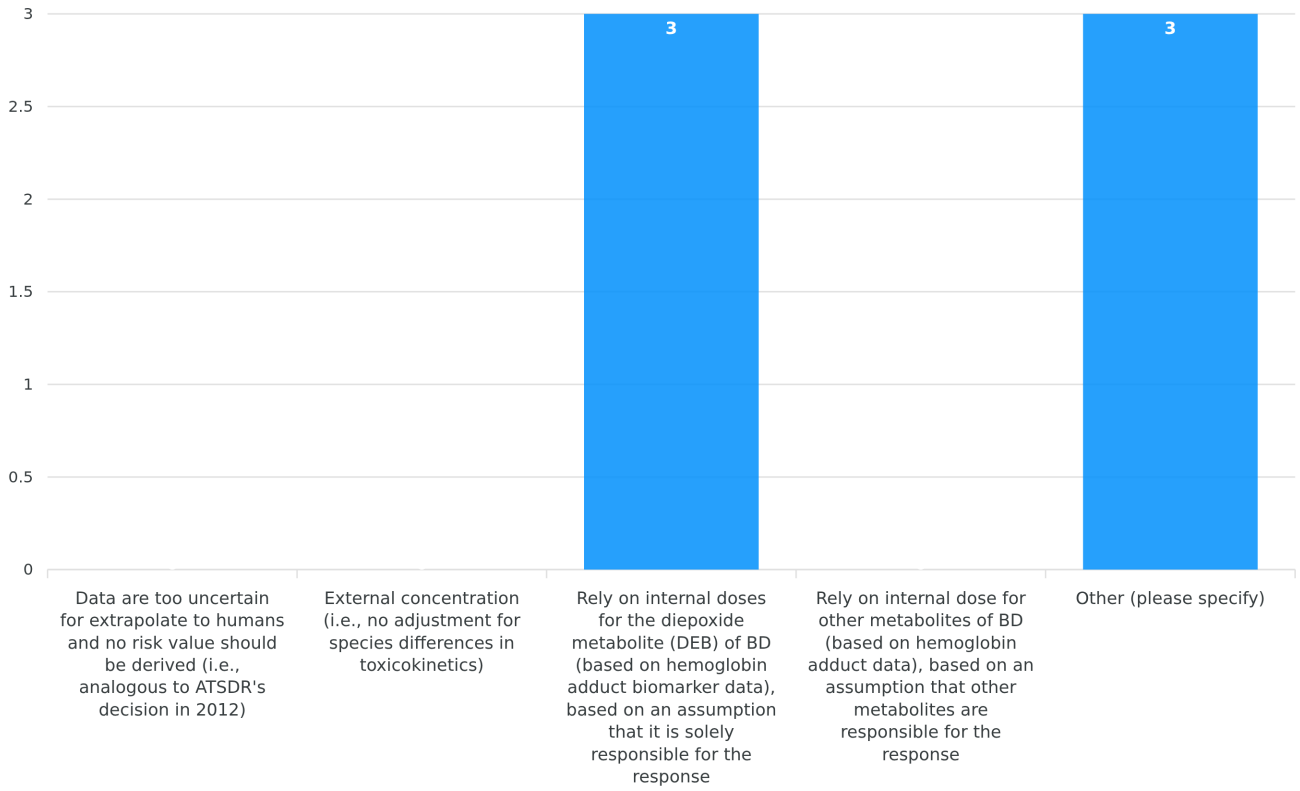
I'm aware of very few cases where we have been able to exclude human relevance based on quantitative variations, since exposure is not addressed in the human relevance framework (rather it's part of subsequent risk characterization). This was a point of confusion in the development of the framework with some suggesting, for example, that melamine might fall within this category, i.e., that potential exposure levels would not approach any "threshold" for the renal effects!!! (and the rest is history). As per the view of Expert 6, the pertinent question is really whether the data are considered sufficient for the development of a DDEF (CSAF) and how best to address associated uncertainties, characterized in responses to Question 1.6 on the relevant dose metric.

Expert 3 | 9/09/2025 19:02 SCORE: **0**

This is from Boobis, 2008 (journal page 93) and is reflected in the overall discussion of this question by the panel: "Only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences. Since quantitative exposure assessment is part of the subsequent risk characterization rather than the HRF [Human Relevance Framework], the difference would have to be of such a magnitude that human exposure could not possibly be envisaged to reach such levels. In most cases, it will not be possible to reach such a conclusion without undertaking formal exposure assessment in the subsequent risk characterization. Hence, the answer to the question will be no, but it may still be concluded that the risk is negligible in the subsequent risk characterization."

Based on the proposed MOA for ovarian atrophy and your degree of confidence, what is your recommendation for the dose measure that should be used in the risk assessment to extrapolate across species?

Results | 6 answers



Expert consensus indicates strong support for using internal doses of the diepoxide metabolite (DEB) as the primary dose measure for cross-species extrapolation in BD risk assessment. Three experts (1, 5, and 6) explicitly recommend DEB-based measurements using hemoglobin adduct biomarker data, citing evidence that DEB is the principal agent responsible for ovarian atrophy.

The remaining experts (2, 3, and 4) suggest more comprehensive approaches while still acknowledging DEB's importance. Expert 4 recommends using all three dose measures (DEB, total metabolites, and external concentration) with DEB ranked as most reliable. Expert 3 leans toward DEB but suggests including other metabolites may be more comprehensive. Expert 2 sees value in multiple approaches.

Key considerations mentioned include:

- Significant species differences in BD metabolism to DEB (mouse > rat > human)
- Potential gender differences in metabolism, with Expert 5 suggesting female-specific data may be more appropriate
- The similarity between BD/DEB and VCH/VCD patterns of toxicity across species

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ANSWER EXPLANATIONS

Expert 1 | Rely on internal doses for the diepoxide metabolite (DEB) of BD (based on hemoglobin adduct biomarker data), based on an assumption that it is solely responsible for the response | There is strong evidence that ovarian atrophy is mediated by the formation of diepoxides, such as Bd diepoxide metabolite DEB and the diepoxide metabolite of VCH (VCD) and ovarian toxicity was observed follwing exposure to diepoxides.There are large species differences in the metabolic activation of BD to diepoxide metabolite, DEB (mouse>rats) and thresholds for in producing ovarian atrophy. It is the dose of DEB deliverd to the overies that is a key determinant of ovarian response. Quantitative differences in the in vivo production of BD metaboltes (DEB) are also reflected in their in vivo accuulationsas hemoglobin adducts The difference between species is dose dependent.Humans have been shown to form less of the DEB than rats(Boysen et al: 2012 ; Motwani and Torquist, 2014)Furthermore, the pattern for effects and species differences obsreved for BD and DEB are similar to as reported to VCH and VCD.. Therefore, based on current understanding and well recognized species differences in metabolic activation of BD and internal dose estimates of DEB based upon hemoglobin adduct biomarkers data are highly relevant and could be used for the dose measure in human health risk aseessment of BD to extrapolate across species.

ANSWER EXPLANATIONS, CONTINUED

Expert 2 | Other (please specify) | I think the authors have presented good information and arguments in support of extrapolation. This reading of the document seemed more pointed to DEB as the metabolite most likely to be responsible for the observed effects. However, I still see utility in doing both approaches 3 and 4.

lines 650 - 658.

I tend to concur with the last point made by the authors of the revised document re the data points in figure 4.

Note also that Georgieva et al (2025) also state " These findings suggest that human males may be at a higher risk for BD-induced toxicity at elevated exposure levels. However, it is essential to consider that previous studies in rodents have indicated females may be more susceptible to BD-induced carcinogenesis based on metabolism, mutagenesis, and tumorigenesis data."

I would describe (in section 4?) the metabolism by males vs. females as an area of uncertainty and probable variability.

663 - 665
I agree with this statement, but would again describe the male vs. female capacity for metabolism as an area of uncertainty rather than giving the impression that human females do not metabolize BD to reactive forms.
Future studies may provide better support for quantifying within human (particularly gender-specific) variability.

Expert 3 | Other (please specify) | Somewhat torn on this one, I think using DEB internal dose will do the job and I lean toward this choice. However, using DEB plus other metabolites may be more comprehensive.

Expert 4 | Other (please specify) | I'd suggest to use all three dose measures and rank order them in relation to relative confidence (uncertainty). In my view, DEB is the most certain dose measure for extrapolation based on the rather extensive evidence that it is principally responsible for the ovarian effects (e.g., Doerr et al., 1995, 1996) followed by total metabolites followed by external concentration.

Expert 5 | Rely on internal doses for the diepoxide metabolite (DEB) of BD (based on hemoglobin adduct biomarker data), based on an assumption that it is solely responsible for the response | There are adequate data to demonstrate that formation of the diepoxide, DEB, is a rate limiting step in the MOA for ovarian toxicity, and that there are profound species differences in metabolism leading to the formation of DEB. Calculating DDEF values for the interspecies toxicokinetic differences using the pyr- Val biomarker measurements in mice, rats and humans is certainly appropriate. Georgieva et al., (2025) have provided some data that suggests that human males might form more *pyr*-Val at higher exposure levels than do females. If this is valid, then it may be more appropriate to use only the female data for the DDEF derivation of a female endpoint.

No change for round 3.

Expert 6 | Rely on internal doses for the diepoxide metabolite (DEB) of BD (based on hemoglobin adduct biomarker data), based on an assumption that it is solely responsible for the response | I believe that the data support the formation of bifunctional metabolites of butadiene as the measure of dose to be used.

Debate | 5 comments

Expert 1 | 9/03/2025 12:05 SCORE: **0**

I think, based on current understanding and well recognized species differences in metabolic activation of BD and the internal dose estimates of DEB , and , hemoglobin adduct biomarkers data are highly relevant and could be used for the dose measure in human health risk aseessment of BD to extrapolate across species.In general, I agree with the comments of Expert 5 and 6 that there are adequate data to demonstrate that formation of the diepoxide, DEB, it is a rate limiting step in the MOA for ovarian toxicity, and that there are profound species differences in metabolism leading to the formation of DEB. It is certainly appropriate calculating DDEF values for the interspecies toxicokinetic differences using the biomarker measurements in mice, rats and humans. I have a great difficulty in understanding the comments of Experts 3, and 4, requesting to be explicit about their answers to Question 1.6.

Expert 6 | 9/04/2025 9:55 SCORE: **0**

I appreciate the comment from Expert 1, above. Calculating an interspecies DDEF is an acknowledgement that the MOA is relevant in humans. If, in moving forward, a DDEF is to be calculated, some clear presentation of the proposal for the human relevance of the MOA should be communicated to avoid an unnecessary distraction. I'm interested to read follow-up comments.

Expert 4 | 9/05/2025 9:45 SCORE: **1**

In relation to the query from Expert 1 re clarification of my response, our role as risk assessors is to provide a range of estimates to inform risk management with clear communication of the extent of relative uncertainty associated with various approaches. There are uncertainties associated with each of the options. While I have greatest confidence in the diepoxide being the most appropriate dose measure for the development of DDEFs/CSAF for interspecies differences, there are associated uncertainties. "Bounding" of the variability in DDEFs ceveloped based on reliance on the total metabolites and the external concentration is additionally informative to risk managers.

Expert 4 | 9/05/2025 9:52 SCORE: **1**

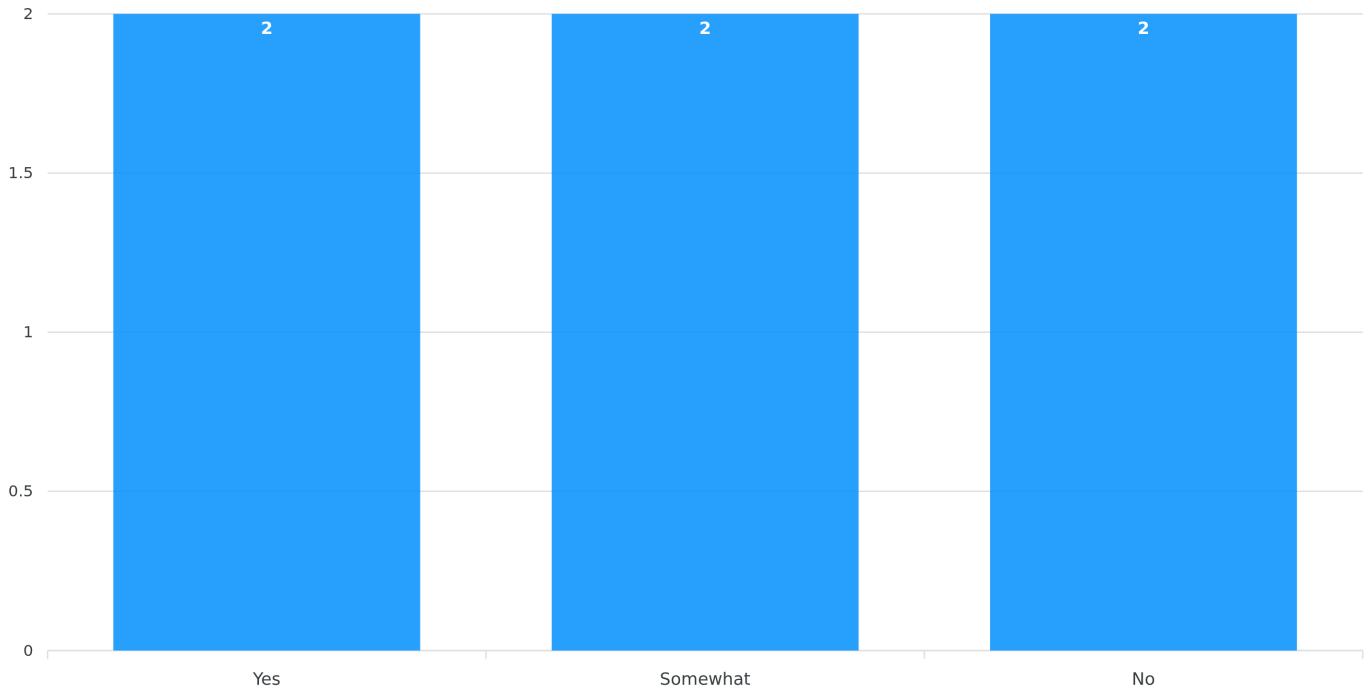
Though we haven't been asked to address specifically, relevant to the discussion on whether to use the data for males and/or females, there is precedent in developing DDEF/CSAF to use the relevant sex (females in the case of both ovarian and developmental effects considered in this poll); I think it's a question of whether or not the noted variation represents a meaningful difference, in view of limited sample size.

Expert 1 | 9/09/2025 13:16 SCORE: **0**

I agree with the comments of Expert 4 responding to my query, specifically as related to uncertainty's associated with each of the options.

Is it appropriate to rely upon information for body weight gain effects in nulliparous animals to support the MOA for maternal weight gain/fetal body weight changes?

Results | 6 answers



Expert opinions on using nulliparous animal data to support the MOA for maternal weight gain/fetal body weight changes are divided:

• Supporting (Yes):Experts 1 and 2 believe it is appropriate to use available nulliparous animal data, with Expert 1 noting the well-described key events in the MOA document.
• Partially Supporting (Somewhat):Experts 4 and 5 acknowledge the data is helpful but insufficient. They suggest that information on non-pregnant females helps support the premise that fetal effects may be secondary to maternal toxicity, but more direct mechanistic studies are needed.
• Opposing (No):Experts 3 and 6 reject this approach. Expert 3 emphasizes that maternal weight gain in pregnancy differs fundamentally from weight changes in non-pregnant females. Expert 6 provides detailed evidence that fetal body weight changes may occur independently of maternal weight effects, citing studies where fetal effects were observed without corresponding maternal weight changes.

The key disagreement centers on whether fetal effects are secondary to maternal toxicity or result from direct action of butadiene metabolites on fetal tissues.

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ANSWER EXPLANATIONS

Expert 1 | Yes | As reviewed and very well described in the " Revised Summary "document ,the proposed key events in the MOA for body weight changes upon exposure to BD and the weight of evidence (WOE) in rodents within the context of the modified Bradford - Hill critera is very appropriate. For noncancer risk assessment of Bd, fetal body weight changes, besides ovarian atrophy in animals have served as a primary endpoint of concernby regulatory agencies and risk assessors.

Expert 2 | Yes | Not my area of expertise. But I suggest that in the absence of a strong indication to the contrary that the assessors use what is available; that is data from nulliparous animals.

Expert 3 | No | In developmental toxicity studies (e.g, Segment 2, OECD TG 414), females that do not mate successfully are not included in assessment of maternal weight or weight gain. Maternal weight gain is primarily due to the weight of her litter, but also due to changes in the mother, including larger blood volume and hypertrophy of her organs. Effects of dosing on weight gain in females that did not get pregnant is not really useful in assessing the role of maternal weight gain on fetal weight.

Expert 4 | Somewhat | The (indirect) information on doses which induce effects on body weight gain in non-pregnant females in toxicity studies is helpful in supporting the premise that effects in the fetus may be secondary to maternal toxicity. The Doerr et al. (1996), Clerici et al. (1995) and Chi et al. (2002) data are also helpful in identifying that it is the metabolites that are influential in this context (in addition to the consistent available data on the role of metabolites in acute and repeated dose toxicity and carcinogenicity). However, as per ovarian effects, direct data from specially designed mechanistic studies where the key events at the various levels of biological organization (i.e., ,subcellular, cellular, organ, etc.) in the hypothesized mode of action are documented following exposure to the active metabolite(s) would provide more reasonable support. Key events are based on measured (rather than hypothesized) perturbations.

ANSWER EXPLANATIONS, CONTINUED

Expert 5 | Somewhat | Exposure to DB results in a significant reduction in maternal body weight in pregnant mice, but not in pregnant rats. The question that arises from this result is whether this difference is due to metabolism of DB to the epoxides. No data are available on the effects of the metabolites in pregnant rodents. Therefore, studies which examined the effects of DB metabolites, EB and DEB, on body weight gain in nulliparous mice and rats were considered. This is appropriate to help gain an understanding of the potential responsible compound(s) for the maternal toxicity. However, it does not directly address the question of the active metabolites involved in the fetal toxicity.

No change for round 3.

Expert 6 | No | Whether the fetal body weight changes are dependent on general maternal toxicity as measured by decreased maternal body weight gain or, alternately are the result of a direct action of epoxide metabolites within the fetal tissues cannot be conclusively ascertained from the information available. One interpretation is that the overall data favor a direct action of butadiene metabolites on fetal tissue over an impact of maternal weight effects impacting fetal weight gain, as explained below.

The correlation between butadiene exposure on maternal body weight and fetal body weight data from Hackett are shown in the MOA Summary, Figure 3. The MOA Summary uses this as a point supporting the impact of maternal toxicity.

Doerr et al. 1996 Figures 1 and 2 show that the extent of epoxidation impacts ovarian weight, uterine weight and body weight, regardless of species. It is unfortunate that the study design did not include butadiene administration. Other results generally show increased metabolism (in mice) correlates with increased effects (over rats), specifically in that the mono-epoxide metabolite has a greater impact in mice than rats, which can be explained by increased metabolism in mice versus rats. And that finding relates to maternal weight, ovarian weight and uterine weight. So, metabolism and metabolites correlate with decreased fetal body weight. But, is a decrease in maternal body weight required? I don't think so.

The MOA summary argues for maternal weight alterations for KE4, but omits some important information, which might have been included at line 403. Hackett et al (page 12) write, "Exposures to 1,3-butadiene did not significantly affect the body weights of nonpregnant [CD-1 strain] mice at any time during gestation (Table 5)". This comes from the same, authors, lab and study in which decreased fetal body weight in CD-1 mice was demonstrated. An additional finding is worth mentioning, which also separates fetal effects from maternal effects. Hackett's study concentrations were 40, 200 and 1000 ppm. Hackett et al (page 19) write important text in the second paragraph, text beginning, "The reduction ...." This passage indicates that body weights of male fetuses were reduced at 40 ppm but decreases in maternal body weight and extragestational weight were only observed for the 200- and 1000-ppm exposure groups. These two passages from Hackett, who studied both pregnant and nonpregnant mice in the same study argue strongly for the separate effects of butadiene on the maternal unit (weight gain in maternal and non-pregnant mice) and the fetus. This substantially complicates and may remove from consideration the necessity of maternal body weight changes on the fetal body weight endpoint. Passages elsewhere in the MOA summary report the presence of butadiene metabolites in cord blood, confirming the possibility of a fetal exposure, regardless of whether the metabolites were formed in the maternal unit and transferred to the fetus, or formed in the fetal unit from butadiene transferred across the placenta. Obviously, adducted hemoglobin formed in the maternal unit cannot be transferred across the placenta.

Debate | 7 comments

Expert 1 | 9/03/2025 16:50 SCORE: **0**

In general , there is a good agreement among the experts (4 out of 5) that it is appropiate to rely upon for body weight gain effects in nulliparous animals for the maternal weight gain/ fetal body weight changes. The information on doses which induce effects on body weight gain in non-pregnant females support that the effects in the fetus may be secondary to maternal toxicity. Certainly, the studies of Doerr et al. (1996), Clerici et al. (1995) and Chi et al. (2002) data are also important in identifying that the metabolites that are likely to play role in addition to the consistent available data on the role of metabolites in acute and repeated dose toxicity .The overall data support a potential direct action of reactive metabolites of of BD on fetal tissue over maternal weight effects.The correlation between butadiene exposure on maternal body weight and fetal body weight data from Hackett as shown in the MOA Summary, Figure 3. and the MOA summary support the impact of maternal toxicity.

•

Expert 6 | 9/04/2025 10:01 SCORE: **0**

As the lone dissenter, and relying on information identified in my comment above, I'd like to hear from Expert 1 how the information from Hackett can be discounted. I have few hang-ups in accepting the proposal to accept data from nulliparous animals, but Hackett's findings seem to be from a well-designed and valid study.

Expert 1 | 9/04/2025 11:25 SCORE: **0**

Hackett et al (1987) reported that in mice, exposure to BD during gestation (GD 5-15) resulted in decreased maternal body weight gain and no efects were observed on maternal weight gain in similarly exposed rats.Fetal body weight gains have been reported to be reduced in a dose dependent manner following exposure to BD.. Therefore , I agree with the comments of Expert 6 that the effects of BD on maternal weight gain and fetal body weights are considered to reflect the general toxicity of BD to dam and fetus, The information from Hackett et al can not be discounted.

Expert 6 | 9/05/2025 10:34 SCORE: **0**

I appreciate the response from Expert 1. But the research findings themselves point out that relying on body weight findings from nulliparous animals in this instance unnecessarily introduces a complication that only distracts from the objective.

It is not appropriate to rely on weight loss effects in nulliparous animals to inform the MOA for butadiene, especially without acknowledging the full suite of data. Hackett 1987a clearly demonstrates **no weight gain effects in nulliparous mice** (page 12, Table 5).

These findings of no weight loss reported by Hackett 1987a contradict and overshadow findings from Doerr et al 1996, a study from a different lab, and a different mouse strain. In contrast, Hackett et al., 1987a reported their "no-effect" findings from the same lab, same study, same strain of mice in which fetal weight reductions were noted and relied upon in this evaluation. The issue of including findings from nulliparous animals offers no advantage to the MOA considerations; it only adds a distraction.

The issue of weight gain in nulliparous animals requires some thought. The inclusion of findings from nulliparous animals only complicates the issue, for reasons including that the MOA document does not address the fact that BD did not alter weight gain in nulliparous mice, shown by Hackett 1987a in the same study that showed fetal effects.

To add some context, the key issue for fetal effects may be the designation of whether BD is a developmental toxicant or not. If the fetal effects are dependent (only) on maternal effects, then BD is not a developmental toxicant, and not subject to issues and constraints of "being" a developmental toxicant. Findings of developmental effects would be "off limits" for IRIS etc risk value determination. The issue is not trivial. (BD does seem to be a reproductive toxicant.) If fetal effects are *only* seen at doses that produce maternal toxicity, then EPA assumes that the fetal effects may be brought about by maternal effects. If, however, fetal effects are observed in the absence of maternal effects (typically categorized by a reduction of, often 10%, in weight gain), then the chemical is considered to have a direct effect on the fetus, and is considered a developmental toxicant. The MOA document, section 3.1, line 419 addresses this writing, "...the effects of BD on maternal weight gain and fetal body weights are considered to reflect the general toxicity of BD on the dam and fetus". Thus, the authors of the MOA document, themselves, acknowledge the possible/likely/potential effect of BD on the fetus.

The MOA document, section 3.1, lines 380 ... infer the distribution of BD metabolites to the fetus, citing findings of hemoglobin adducts from other reactive chemical species in cord blood (i.e., in the fetal circulation). When it is understood that hemoglobin does not pass the placenta, the explanation is either that the bioactivated metabolites were formed in the dam and passed, unbound, to the fetus, or that parent chemical was metabolized in the fetus (which is not devoid of metabolic activity). Either way, the fetus, in these cases, has been exposed to reactive metabolites, including epoxides. If this is accepted, it seems that the path to ruling out fetal effects from fetal exposures to BD metabolites is a difficult one.

The MOA document's section 3.1 indicates no effect on weight loss in **non-pregnant rats** (Hackett 1987b), contrasted with weight loss in nonpregnant B6C3f1 mice (Doerr et al., 1996). That section of the report cites Hackett (1987a) as the basis for findings of fetal effects in CD-1 mice, a different strain from the strain Doerr et al used. Section 3.1 discusses (lines 416...) the effects on maternal and fetal weight gain in Hackett 1987: "Inspection of the data for maternal body weight gain (KE4) and fetal body weight changes (KE5) indicates a high degree of correlation between these two Kes (Figure 3), which when expressed as a percentage of control values, these two dose-response trends are essentially identical. For this reason, the effects of BD on maternal weight gain and fetal body weights are considered to reflect general toxicity of BD to the dam **and fetus** [emphasis added]". Section 3.1 quotes from the NAS 2009 report which did not consider growth impacts on nulliparous animals, but focused only on the findings form Hackett 1987a. It should be noted that the NAS report is a technical summary document that supported the development of Acute Exposure Guideline Level (AEGL) values, which characterize the severity-specific effects of inhalation exposures up to 8 hour durations. The NAS report was prepared for a purpose removed from mode of action determination, and its conclusions should be weighed with respect to programmatic differences when considered in the context of these discussions. Hackett et al. 1987a present several findings in male offspring (e.g., male fetal body weight was significantly reduced at 40 ppm, Hackett et al., 1987a, Table 8) and their placentas at the lowest concentration tested (40 ppm). Hackett characterized these effects (page 19), "The reduction in weight for male fetuses was statistically significant at all butadiene concentrations, and for female fetuses at the two higher exposure levels, 200 and 1000 ppm." Hackett et al., 1987a Table 4 demonstrate a significant impact on dam weights at 200 and 1000 ppm, but not at 40 ppm. However, a careful and detailed investigation of that point seems to move beyond the scope of the present

Expert 4 | 9/05/2025 11:10 SCORE: **0**

I appreciate the perspective of Expert 6 based on thoughtful, more critical consideration of the data in relevant studies. Based on his/her observations, I tend to agree that it's likely best not to include the findings from nulliparous animals in the mode of action discussion. It isn't helpful in any case, in supporting the significant uncertainties in the hypothesized mode of action for fetal effects, the seeming focus here (more relevant to the question as to whether the observed effects are secondary to maternal toxicity - i.e., whether BD causes devleopmental effects, which hasn't really been addressed).

Expert 5 | 9/06/2025 14:34 SCORE: **0**

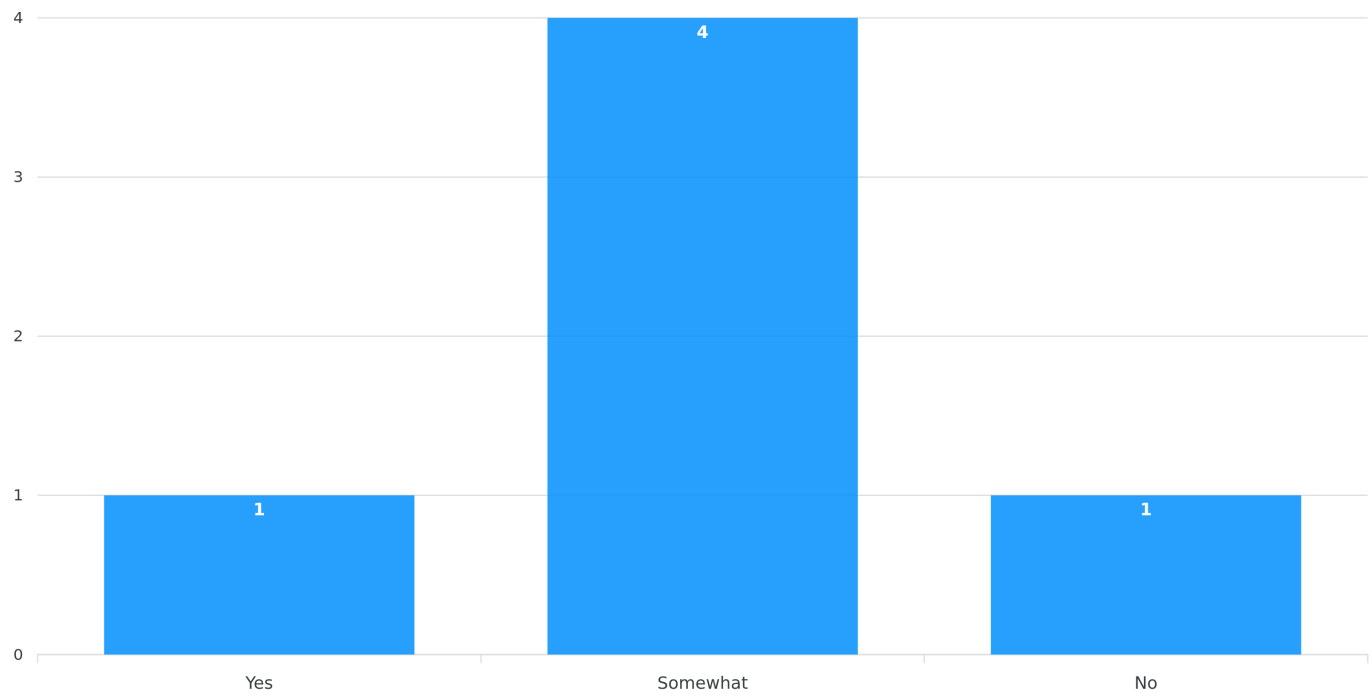
In my opinion, the data can be used to address the question of whether BD or its metabolites are involved in the maternal toxicity. The data do not directly address the question of whether BD or its metabolites are involved in the fetal body weight reductions.

Expert 3 | 9/09/2025 20:11 SCORE: **0**

I must not have entered my answer to this question. The effects on the nonpregnant mice should not be considered and I agree with Expert 6 and others that that they are a distraction. Pregnancy affects a multitude of physiological processes in the dam, increasingly as pregnancy proceeds. It affects internal dose in inhalation studies through its effects on respiratory rates and volumes. The great demands of pregnancy on the body of the pregnant animal can make the more sensitive to toxicant exposure. Extragestational weight gain in the pregnant mice is the best measure overall, although other incremental weight data should be considered, but only in pregnant animals. In Hackett et al. 1987a, extragestational weight gain is affected at 200 and 1000 ppm, with a trend including the lowest dose, 40 ppm. (6.99 g vs 7.60 g in controls). Male (but not female) fetus weight was significantly lower at all doses. Female fetus weight was significantly lower only at 200 and 1000 ppm. However, the trend across all doses in very similar in males and females. The fact that statistical analysis suggests that the male fetuses were more sensitive than the mothers, I think this is splitting statistical hairs, as the dose response for extragestational weight gain, male fetus weight and female fetus weight are very similar and suggest to me that they are similarly if not equally sensitive. I disagree with statements concerning attribution of developmental toxicity to maternal toxicity when they occur at the same dose. In fact, developmental toxicity guidelines EPA, FDA, and OECD all state that the presence of maternal toxicity at a given dose does not negate the finding of developmental toxicity at the same dose and that the test article should be considered a developmental toxicant unless it can be shown that some specific aspect of maternal toxicity was causing the developmental toxicity. Showing this usually requires additional experiments. An exception is when the maternal toxicity is extreme (e.g., >10% mortality), which can make the study uninterpretable. In practice, a weight of evidence approach is used and considers the severity and incidence of both maternal and developmental toxicity.

Key Question #1 (Boobis et al., 2008): Is the weight of evidence sufficient to establish a mode of action for fetal body weight changes in animals?

Results | 6 answers



Expert consensus on whether there is sufficient evidence to establish a mode of action (MOA) for fetal body weight changes in animals exposed to 1,3-Butadiene is mixed, with varying degrees of confidence.

One expert firmly answered Yes, citing multiple studies showing concordant dose-response effects in mice with supporting findings in rats, along with mechanistic evidence of fetal toxicity from epoxide metabolites.

Most experts (4 of 6) responded Somewhat, indicating partial agreement with reservations:

- Several noted the MOA lacks specificity in the key events
- One highlighted that the revised MOA in Round 3 was more convincing
- Another pointed out uncertainties in dose-relationships between proposed key events
- One questioned including maternal toxicity as a key effect

One expert firmly answered No, criticizing that the MOA description doesn't conform with accepted practice for describing adverse outcome pathways (AOPs) and chemical-specific MOAs, noting key events are non-specific with limited supporting information.

Areas of agreement include recognition that epoxide metabolites likely play a role in toxicity, while disagreement centers on whether the evidence sufficiently establishes the specific mechanisms and relationships between key events.

AI generated summary content

ANSWER EXPLANATIONS

Expert 1 | Yes | Several studies have investigated the effects of 1,3- Butadiene exposure on maternal and developmental toxicity in laboratory animals (Battelle PNL,1987;Hazelton Labs, 1981; Wil Research, 2003).Experimental animal evidence based on these studies show concordant and dose response effects in mice and is supported by qualitatively similar findings in rats at higher doses. Although limited, mechanistic evidence in rodents also demonstrate fetal and and embryonic toxicty of DEB alone and may be in combination with other epoxide metabolites (Chi et al; 2002; Clerici et al; 1995 Doerr et al; 1996) Substantial species differences in the metabolism of BD result in humans and ridents for internal doses of reactive metabolites that are qualitatively similar exibiting large quantitative differences.

Expert 2 | Somewhat | Note that I have made some suggestions in the marked-up document and below that I hope will improve the discussion. That this MOA is somewhat vague, lacking in details (per paucity of KE data) is probably okay for the purposes of this assessment.

Round 3 note. The latest document is much improved, and as with the version used for debate, I find the evidence provided to be less convincing. The latest draft seems to be more diffuse regarding whether fetal effects are attributable to general maternal toxicity. But the MOA is still good enough for the stated purpose.

Expert 3 | Somewhat | While the MOA is likely generally correct, the key events are not specific enough to help understand the mode of action. What is the specific toxic effect that is affecting maternal weight and how does that translate to lower fetal weight? I don't think the MOA is there yet. (In Round 3 the MOA for reduced fetal weight is revised and more specific, so my answer for the revised MOA is yes.)

ANSWER EXPLANATIONS, CONTINUED

Expert 4 | No | As for ovarian toxicity, the description of the mode of action doesn't conform well with accepted practice for describing generic AOPs and chemical specific MOAs nor with the intended focus of weight of evidence evaluations for the IPCS Human Relevance Framework. Assessment of the weight of evidence for both AOPs and MOA relate to toxicodynamic key events, including metabolic key events but do not include ADE aspects. Rather, for MOA, these aspects are addressed quantitatively in the subsequent dose-response analysis (through, for example, development of DDEFs or PBK modelling). "Diepoxide Distribution to the Ovary" is not a key event, though its quantitation is important in understanding ADE. It's unfortunate, as well, that there are no studies of the distribution of BD metabolites to maternal and fetal tissues to directly inform this quantitation (it seems surprising that these data have not been generated) though reported inference of distribution to the fetus seems reasonable, based on determination of BD metabolites in related tissues in a range of available studies and epoxide metabolites of analogues in cord blood. The descriptions of the key events in the hypothesized mode of action are non-specific (e.g., KE3 - "reaction of epoxides with cellular macromolecules" and KE 4 "general toxicity - growth reduction in dams) with essentially, no direct supporting information and limited mechanistic underpinning.

ANSWER EXPLANATIONS, CONTINUED

Expert 5 | Somewhat | Somewhat. There are data to support the metabolism of DB to the epoxides (KE 1). KE 2, distribution of epoxide metabolites to maternal and fetal tissues, is supported by distribution data on nulliparous animals, chemical partitioning information, and other epoxide metabolites. KE 3, binding to cell molecules, is supported by hemoglobin adduct data from a variety of studies. KE 4, toxicity to the dam, has been demonstrated. Hackett (1987) showed that administration of DB to pregnant mice resulted in a significant decrease in maternal body weight gain, but similar effects were not observed when DB was administered to pregnant rats. Given the species differences in DB metabolism, as well as the role of DEB in ovarian toxicity, the question arose as to whether the effects on maternal body weight gain are due to the epoxide metabolites. No data are available for pregnant rodents. So body weight gain data from nulliparous mice and rats exposed to EB or DEB has been used to support the proposal that the epoxide metabolites are responsible for the effects on maternal body weight gain. KE 5, reduced fetal body weight, is supported by the Hackett studies of DB in mice.

Application of the Bradford-Hill criteria, however, shows uncertainties in the proposed MOA. First, Hackett showed that administration of DB resulted in reductions in maternal and fetal body weight in mice, but not in rats. Studies on nulliparous rodents have shown that exposure to the epoxide metabolites may be responsible for the reductions in maternal body weight gain. Similar studies are not available on pregnant rodents so it has not actually been demonstrated that the epoxide metabolites are responsible for the reductions in fetal body weight. In addition, the link between KE 3, 4 and 5 has not been established. If KE1 is required for the maternal and fetal body weight effects, and KE 3 is viewed as responsible for the reduction in maternal body weight, then KE3 could just as easily be responsible for the reductions in fetal body weight without bringing in the argument of a role of maternal toxicity. The current data also support a MOA that involves bifurcation after KE3 – one path leading to maternal toxicity and the other leading to fetal toxicity.

In conclusion, although, the proposed MOA has some biological plausibility, there are weaknesses in the dose-relationships between the proposed key events and in the temporal relationships of the key events. There are only data showing a dose relationship between exposure and the final outcome. There are no data linking each of the proposed key events in a dose or temporal fashion. It would be interesting to know whether VCH also results in fetal body weight reductions.

For round 3:

The proposed MOA has been changed. KE3 is now GSH depletion and KE4 is a lumped category for all indicators of general toxicity. These changes get around discussions of the potential role (or not) of maternal toxicity leading to fetal toxicity which is commendable. However, lumping all of these together also highlights the uncertainties in additional KEs between KE 3 and 4. There are data supporting the potential role of KE1, KE2 and KE3, but there are substantial uncertainties in the proposed MOA. The author(s) provide a good discussion of many of the uncertainties in the proposed MOA. Although it is highly likely that the epoxide metabolite(s) are responsible for the general toxicity (KE 4), there are actually no data that directly assess the potential toxicity of the metabolites in pregnant mice. In addition, there are no data examining the essentiality of the KEs. More importantly, there may be additional KEs between KE3 and KE4, and KE 4, which currently is a lumped category, may actually split into several final outcomes (e.g. maternal toxicity, fetal toxicity, etc.).

In addition, the dose concordance depicted in Table 5 highlights some uncertainties in that KE3 is noted at doses lower than KE2, and mouse body weight changes are noted at doses lower than KE2. This is probably due to lack of data at appropriate doses, but should be discussed in the discussion. As noted for the MOA for ovarian toxicity, the discussions of dose and temporal concordance should focus on the relationships between KE1 and KE 2, KE2 and KE3 etc, not just the final outcome.

Expert 6 | Somewhat | It is, but I can't agree that the MOA includes the impact on maternal weight or impact of maternal toxicity as presently described as a key effect.

Debate | 8 comments

Expert 4 | 9/04/2025 9:06 SCORE: **0**

Apologies for the error. "Diepoxide distribution to the ovary" should be distribution of epoxide metabolites to maternal and fetal tissues.

Expert 1 | 9/04/2025 9:35 SCORE: **0**

Multiple studies have demonstrated species -specific differences in 1,3- Butadiene metabolism. Allthough, specific role of the BD reactive metabolites during gestation and early pos-natal periods may not be clear, however it is reasonable to assume that BD epoxy metabolites are likely to contribute to the observed maternal and developmental toxicity in experimental animals. Two studies both mice and rats demonstrated that DEB is toxic to developing fetuses and embryos (Chi et al; 2002; Clerici et al; 1995). Temporal association data for KEs in the MOA for the fetal body weight changes as summarized in Table 2 of the Summary document show a consistent observations of reduced maternal weight gain and fetal body weight at the end gestation period. The data from Doerr et al (1096) strongly support for the role of BD metabolites, particularly DEB , in causing body weight changes in mice.DEB is consistently identified as the most toxic metabolite of BD in multiple test systems (in vivo and in vitro). Maternal body weight gain and fetal body weights were reduced in mice exposed to a structrally similar chemical, isoprene, whose toxicity is also attributed to the formation of reactive metabolites (see Anderson , 2001) Therefore , there is evidence to support the importance of BD metabolism in MOA for producing fetal body weight changes, with some evidence of a specfic role for DEB in toxicity. Although a strict application of Bradford_Hill criteria shows some uncertainties in the proposed MOA, however based on the overall weight of evidence , avalable dose-response data for maternal and developmental toxicity , dose response analysis should be considered appropiate ..Expert 4 comments are reasonable but need to recognize that two studies referred above ,in both mice and rats demonstrated that DEB is toxic to developing fetuses and embryos.

Expert 5 | 9/04/2025 11:25 SCORE: **0**

As written, the MOA basically states that the fetal body weight effect is due entirely to maternal body weight. This suggest that BD or its metabolites really do not directly affect the fetus. And if one removed maternal body weight effects, the fetus would have normal weight. The data do not support that. Showing reduced fetal and maternal body weight does not demonstrate cause and effect.

Expert 4 | 9/05/2025 11:21 SCORE: **0**

I'm always a bit sceptical in hypothesized modes of action with such limited mechanistic underpinning that it would be difficult to design appropriate studies to more robustly consider the weight of evidence or for which there is precedent (e.g., documentation of relevant pathways for the nonspecific endpoint in available respositories).

Expert 4 | 9/05/2025 11:22 SCORE: **0**

I particularly like Expert 5's question concerning whether VCH results in fetal body weight reductions. Consideration of this question in the summary would add value, in my view.

Expert 5 | 9/06/2025 14:39 SCORE: **1**

I would add that the current data also support a MOA that bifurcates after KE3 with maternal toxicity going in one direction and fetal toxicity in another. Many health effects have related early KEs but then diverge. If there is a belief that maternal toxicity always results in fetal toxicity (which it doesn't), then in the current case the maternal toxicity might also be viewed as a modifying effect, not a true KE. There are data showing that the fetus can be affected by BD without maternal toxicity. In my opinion, the current proposed MOA does not fully support a MOA for fetal toxicity

Expert 6 | 9/07/2025 15:51 SCORE: **0**

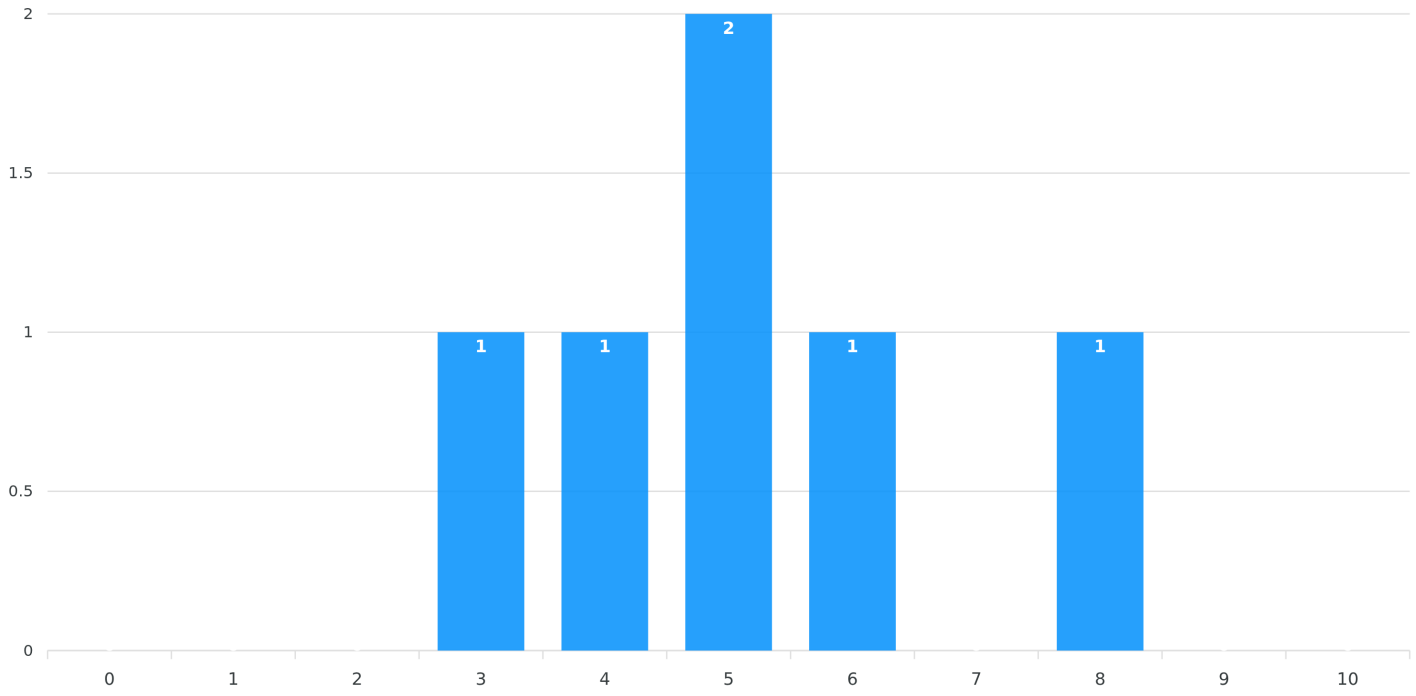
In considering the responses and comments to date (especially that of Expert 5 immediately above), I think it is fair to say that a successfully describing a MOA for fetal body weights may be possible. Two alternatives (or a combination of the two) seem to emerge as the most critical effects, and they relate to either 1) fetal effects from the impact on the maternal system (which I don’t favor), and 2) fetal effects from the impact of BD/metabolites in the fetal system (which I think requires additional attention). Regarding the latter, the same impact on cellular signaling pathways, etc, as proposed for BD’s ovarian toxicity should be considered as an alternative KE to maternal toxicity. It seems that the same (weak) evidence for this KE in ovarian toxicity might also be proposed for fetal effects.

Expert 4 | 9/09/2025 17:54 SCORE: **0**

Based on the available data, I'm still not confident that there is enough to hypothesize a credible MOA for such a non-specific endpoint for which there is limited documentation generically concerning possible pathways to effect (e.g., as in the AOP wiki) and almost no mechanistic underpinning (with the exception of data relevant to consideration of the relevant metabolite). The bar for acceptance of hypothesized MOAs for which there is limited prior hypothesis or acceptance is necessarily higher than well known pathways, as was noted in the publications on the IPCS framework.

What is your degree of confidence in the proposed MOA for the fetal body weight effects of BD? (0-10 scale; 0=no confidence; 10=highest confidence). Please explain your answer

Results | 6 answers



Expert confidence in the proposed mechanism of action (MOA) for 1,3-butadiene's effects on fetal body weight varies considerably, with scores ranging from 3 to 8 on a 10-point scale.

Areas of agreement:

• Most experts acknowledge that BD metabolism to epoxide metabolites likely plays a role in the observed effects (KE1-3)
• Species differences in metabolism (mouse > rat > human) are recognized as important

Areas of disagreement:

• Confidence in Key Event 4 (KE4) is particularly low, with Expert 6 expressing skepticism
• Experts 3, 4, and 5 cite insufficient data on the specific toxicodynamic events between metabolism and the adverse outcome
• Expert 2 notes the MOA demonstration is "good enough" for assessment purposes despite limitations
• Expert 1 stands out with high confidence (8/10), citing clear published evidence of epoxide metabolites contributing to developmental toxicity

Several experts suggest the relationship between maternal toxicity and fetal effects needs further clarification, with Expert 5 proposing a bifurcated pathway after KE3.

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ANSWER EXPLANATIONS

Expert 1 | 8 | The degree of confidence in the proposed KEs for MOA for the fetal body effects of BD exposure is high. Published studies clearly show that 1,3- Butadiene epoxide metabolites , both in mice and rats demonstrate that they are likely to contribute to the observed maternal and developmental toxicity (fetal body weight changes). The species differences (mouse >rat>human) in bioactivation of BD to reactive epoxide metabolites is important key dterminant of BD toxicity. Therefore, the proposed MOA is consistent when extended to toxicokinetic and toxicodynamic events.The approach for using biomarker data across species (metabolite-specific hemoglobin adducts) to quantify species differences in the internal doses of BD metabolites in mice, rats and humans makes sense . The use of hemogobin adducts for BD is consistent with USEPA's practice in assessment of other chemicas such as acrylmide(IRIS, 2010.

ANSWER EXPLANATIONS, CONTINUED

Expert 2 | 5 | I find that the revised document has greatly improved the discussion of the proposed BD MOA for fetal body weight effects. And (perhaps ironically) it has decreased my confidence in the demonstration of the MOA. I think the MOA demonstration is good enough for the purpose of this assessment; that is, in informing the preparation of DDEFs.

I have made some marginal notes on the document with suggestions for improvement in the tables and MOA discussions. Some of these I have pasted below. Note also that the comments under 1.3 and 3.1 are largely applicable here as well.

Some marginal notes from the revised document on fetal body weight changes are below.

47 48 Was this observation discussed re ovarian toxicity MOA?

121 May note here also that unlikely to be EB metabolism in the ovary.

137 – 141 Note that some MOA mavens will insist that these responses (oxidative stress, autophagy, etc.). are all separate MOA that converge at KE1, KE2, KE3, KE5. I would suggest adding that perspective, but that for purposes of this assessment a vague (or inclusive) KE4 is sufficient.

144 – 147 Cite this as dose response observation that provides support for KE5. The point is not the species difference per se, but the differences in target organ dose resulting in the effect or lack thereof.
158 Are there data for VCH and VCD that would support the existence of earlier KE. This information would contribute to bio plausibility, consistency criteria.

Lines 162 -- 184.The data presented here are only for the AO, not the KE. The point is to show that the early KE are observed at lower doses than are the later KE. Unless there are no data. In which one assembles a dose / time concordance table for a hypothetical MOA (more like a demonstration of a chemical agnostic AOP). For the BD MOA, fill in the boxes where there are observations.
Further flogging the dead equine, for the MOA discussions the species differences are important in that they demonstrate effects or lack thereof at different doses. In this section the emphasis ought be on (e.g.) "KE 3 is observed at this dose range, but not at lower doses". Or similarly KE would be expected to occur at doses lower than x, but there are no data.

187 188 See comments in response to question 1.3. In short, have a data summary table, and a separate dose / time table. The latter can clearly show where there are data gaps.

199 200 Note that it may be useful to include some VCH, VCD in the data summary table, and to include observations on dose / time by analogy.

242 Important in human relevance discussions.

271 So it is important to show the information for observation of KE after VCD exposure.

Expert 3 | 4 | While the MOA is likely generally correct, the key events are not specific enough to help understand the mode of action. What is the specific toxic effect that is affecting maternal weight and how does that translate to lower fetal weight? I don't think the MOA is there yet. (In Round 3 the MOA for reduced fetal weight is revised and more specific, so my answer for the revised MOA is yes.)

ANSWER EXPLANATIONS, CONTINUED

Expert 4 | 3 | There is some support for assuming that the fetal effects are caused by BD metabolites (and hence a very tentative basis for the development of DDEFs in dose-response analysis) based on the studies of Doerr et al.. Consideration of the correlation between the effect on maternal and fetal body weight gain is also helpful as a basis for assumption that fetal effects appear to be secondary to those on the exposed mothers. However, the very limited (almost complete lack of) direct mechanistic information on the subsequent toxicodynamic key events between metabolism and the non-specific adverse outcome precludes developing a credible mode of action. In relation to weight of evidence, as per Table 2 (for example), available data to support consideration of empirical support (temporal and dose response concordance across key events) are minimal. Confidence in the proposed MOA is also diminished due to the non-specific nature of the adverse outcome and associated lack of documentation of potentially relevant biological pathways in repositories such as the AOP wiki.

Expert 5 | 5 | I would give the proposed MOA a score of 5. As stated in question 2.2, the proposed MOA is somewhat biologically plausible. Data support the metabolism of BD to the epoxide(s), but there are no direct data showing that fetal body weight reductions are due to the epoxide metabolite(s) or which may be responsible. In addition, there are no data demonstrating the dose-relationship or temporal relationship between the proposed key events. Conclusions of the NAS (2009) for AEGLs may not be appropriate as support for this proposed MOA as the criteria used for AEGLs is different than the criteria used for a MOA analysis. Current data also supports a MOA that bifurcates after KE3, one arm leading to maternal toxicity and the other leading to fetal toxicity.

For round 3:

I would still give the newly proposed MOA a score of 5. There is substantial evidence that KEs 1-3 are involved in the MOA, but it is not clear exactly how those relate to the maternal or fetal toxicity. The rat versus mouse data supports the importance of metabolism to the epoxides and a role for the epoxide metabolites in the causal pathway to general toxicity.

Expert 6 | 6 | The relatively low score reflects my skepticism in KE4; I do not believe that the impact of metabolites can be ruled out, but the impact on maternal toxicity can be ruled out as a KE.

Debate | 3 comments

Expert 4 | 9/04/2025 9:02 SCORE: **0**

Apologies, here. In my final review, I had intended to upgrade my degree of confidence to a 2 or 3, based on a read through of the responses. But for some reason, when I "saved" the final revisions after review of the responses to Section 1 on ovarian effects, all responses were submitted. My low score here reflects the very limited mechanistic underpinning and empirical support for the hypothesized mode of action for a comparatively non specific adverse outcome compared to moderate and high confidence datasets with which I am familiar.

Expert 1 | 9/04/2025 15:45 SCORE: **1**

It seems that the majority of experts (4 out of 5) have a medium to high confidence (Score range 5-8) in the proposed MOA for the fetal body weight effects of BD exposure in experimental animals. Expert 4 scored it 0, but agrees that there is " some support for assuming that the fetal effects are caused by BD metabolites (and hence a very tentative basis for the development of DDEFs in dose-response analysis) based on the studies of Doerr et al.. Consideration of the correlation between the effect on maternal and fetal body weight gain is also helpful as a basis for assumption that fetal effects appear to be secondary to those on the exposed mothers." However, Expert 4 further commented that " there is very limited (almost complete lack of) direct mechanistic information on the subsequent toxicodynamic key events between metabolism and the non-specific adverse outcome precludes developing a credible mode of action In relation to weight of evidence, as per Table 2 (for example), available data to support consideration of empirical support (temporal and dose response concordance across key events) are minimal. Confidence in the proposed MOA is also diminished due to the non-specific nature of the adverse outcome and associated lack of documentation of potentially relevant biological pathways in repositories such as the AOP wiki." I believe the comments of Expert 2 are highly relevant and needs to be carefully addressed as they relate to key events,specifically for KEs 3&4 as well as including discussion of VCH, VCD data in support of the MOA argument for the fetal body effects which may may convince the Expert 4 to change the score to high level. To my knowledge., there is no perfect data set for each key steps or events for MOAs or mechanisms of toxicity of any environmental agent 's exposure .Acknowledging the uncertanties and idenfying data gaps, I think, there is sufficient information tor the proposed MOA for the fetal body effects considering well recognized differences in kintec and dynamic events across species.

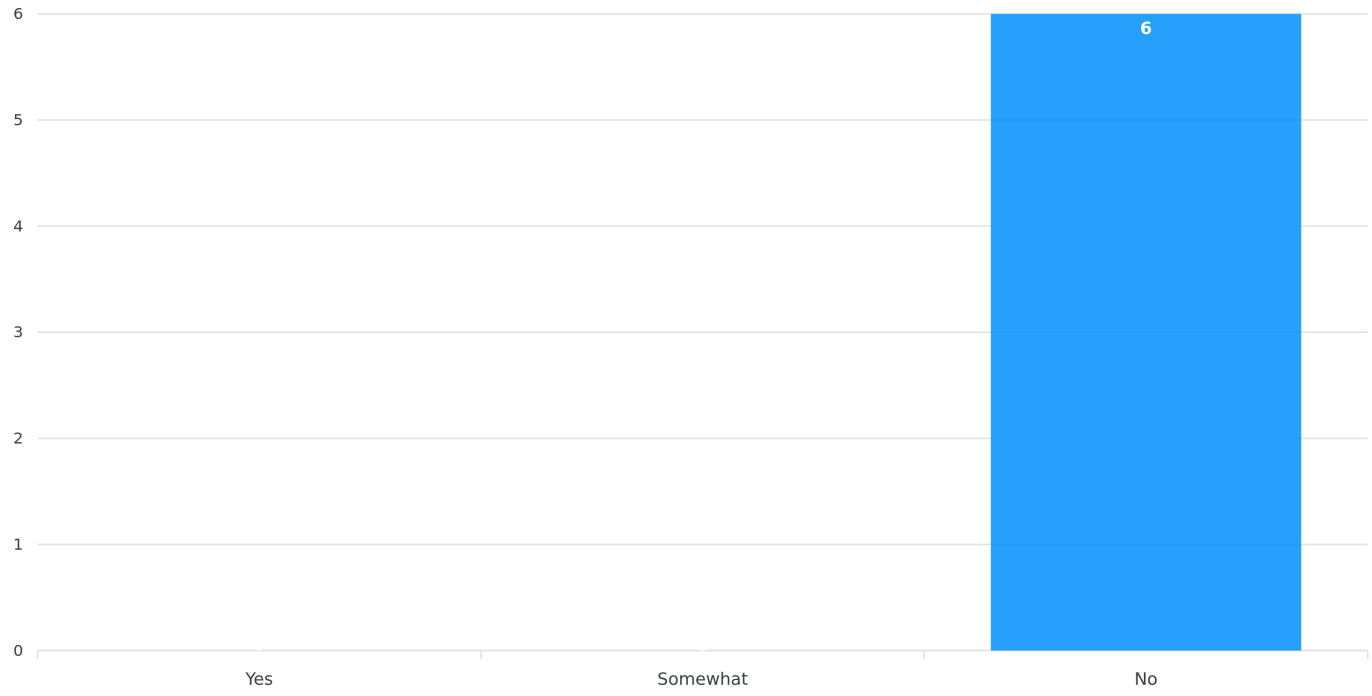
•

Expert 4 | 9/05/2025 11:34 SCORE: **0**

Please see my additional comments relevant to the previous question. While necessarily subjective, based on experience in evaluating AOPs and MOAs, mechanistic information on a relatively nonspecific endpoint here is extremely limited compared to other datasets, with which I'm familiar, for which hypothesized MOAs are well supported. This doesn't imply that DDEFs should not be developed, if there is sufficient confidence in the relevant dose metric for the effect but this doesn't require a documented mode of action.

Key Question #2 (Boobis et al., 2008): Can human relevance of the MOA for fetal body weight changes be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

Results | 6 answers



All experts unanimously agree that the human relevance of the mode of action (MOA) for fetal body weight changes cannot be reasonably excluded based on qualitative differences between experimental animals and humans.

Key points of agreement:

• The reactive epoxide metabolites of butadiene (BD) likely contribute to toxic responses through similar mechanisms across species (Expert 1)
• There is insufficient evidence to dismiss the relevance of decreased maternal weight gain leading to decreased fetal weight in humans (Expert 2)
• No fundamental maternal or developmental differences exist between rodents and humans that would render the MOA irrelevant (Expert 3)
• Limited data precludes excluding human relevance (Expert 4)
• No data suggests the MOA would not be operative in humans (Expert 5)

Expert 5 notes that the question itself may not be relevant if the weight of evidence is insufficient to establish a mode of action for fetal body weight changes in animals.

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ANSWER EXPLANATIONS

Expert 1 | No | **The human relevance of the proposed MOA for fetal body weight changes observed in experimental animals upon exosre to BD can not be excluded on the basis of fundamental qualitative differences in key events for MOA between experimental animals and humans.When developmental and maternal studies are evaluated gestational exposure to BD in mice (Battle PNL, 1987) and three in rats (WIL Research, 2003; Battle PNL,1987; Hazelton ,1981) , these endpoints of toxicity are relevant to both intermediate and chronic potential exposure of BD to humans. There is reasnably sufficient mechanistic information avialable supporting the proposed MOA for fetal body weight changes following gestational BD exposure and to estimate role of reactive epoxide metabolites of BD. These metabolites of BD (EB,DEB, EBD) are likely to contribute to the toxic response based on a common mode of action (ie;cytotoxicty and alkylation of cellular micromolecules across expermental animals (mice and rats) and humans.**

Expert 2 | No | I don't think the MOA can be excluded unless there is hard evidence that decreased weight gain in human pregnancy does not result in decreased fetal weight. Even though it is vague, the MOA cannot be dismissed as irrelevant on qualitative grounds.

Expert 3 | No | There are no fundamental maternal or developmental differences between rodents and humans that would render the MOA not relevant to humans.

Expert 4 | No | The limited available data to support a hypothesized mode of action for the fetal body weight changes precludes excluding the adverse outcome on the basis of qualitative differences in key events between experimental animals and humans.

Expert 5 | No | This question is not relevant if in fact the weight of evidence is not sufficient to establish a mode of action for fetal body weight changes in animals. However, if the weight of evidence was sufficient in animals, there are no data to suggest that qualitatively the MOA would not be operative in humans.

No change for round 3.

ANSWER EXPLANATIONS, CONTINUED

Expert 6 | No | This answer would apply to the MOA, regardless of whether KE4 (maternal toxicity) was or was not changed to a fetal effect dependent on butadiene metabolites.

Debate | 3 comments

Expert 2 | 9/03/2025 11:35 SCORE: **0**

Oops. Looks like I hit the incorrect button for this question. My response should be **NO** human relevance of the MOA for fetal body weight changes **CANNOT** be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans.

Thus, it appears that we are unanimous that the human relevance of the MOA can't be excluded for qualitative reasons.

I am sending this note to SciPinion in the message center, and I ask that my faulty response to 2.4 be corrected.

Expert 1 | 9/04/2025 10:05 SCORE: **0**

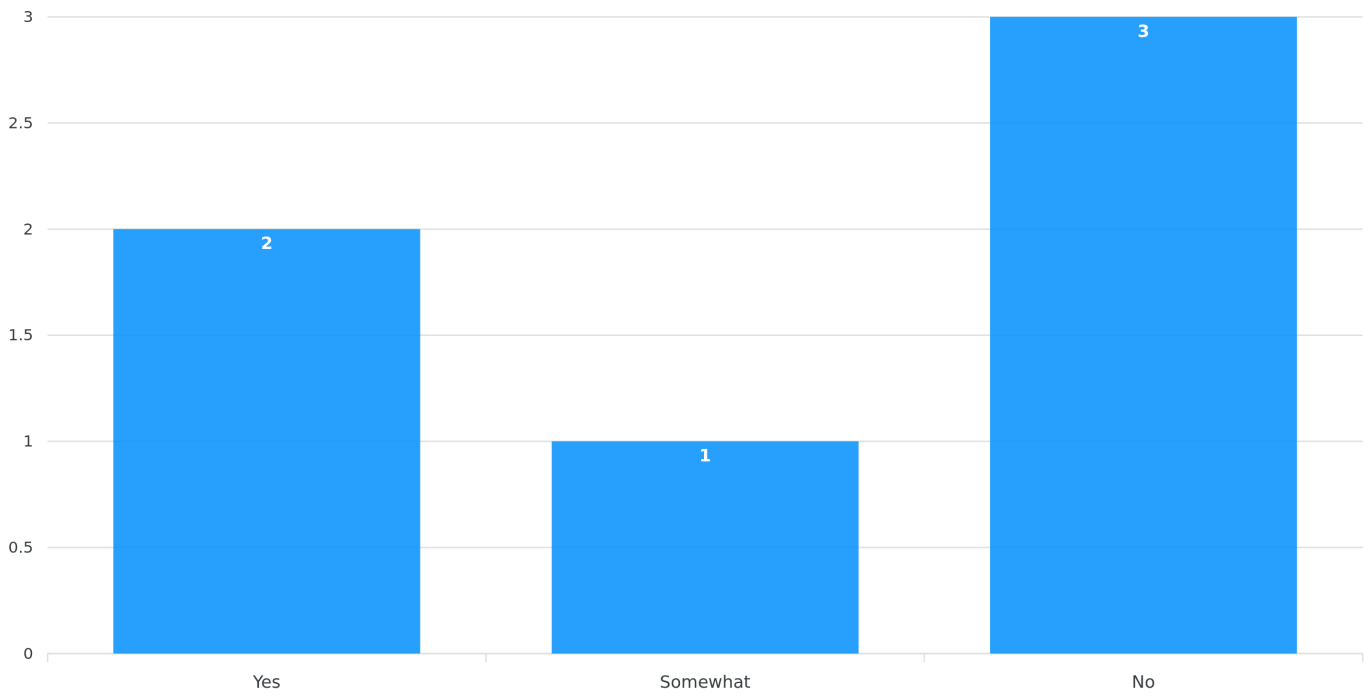
It seems that all Experts agree that the human relevance of the MOA for the fetal body changes can not be reasonably excluded on the basis of fundamental qualitative diffrernces in key events between experimental animals and humans.

Expert 1 | 9/09/2025 13:45 SCORE: **0**

I think the comment of Expert 2 " Even though it is vague, the MOA cannot be dismissed as irrelevant on qualtative grounds" is kind of supports a hypothesized (proposed) mode of action for the fetal body weight changes on the basis of qualitative differences in key events between experimental animals and humans.There are no data to indicate that qualitatively the MOA potentially not applicable to humans.

Key Question #3 (Boobis et al., 2008): Can human relevance of the MOA for fetal body weight changes be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

Results | 6 answers



Expert opinions on whether human relevance of the MOA for fetal body weight changes can be reasonably excluded based on quantitative differences between animals and humans are divided:

• Experts supporting exclusion (Yes):Experts 1 and 6 cite well-demonstrated quantitative differences in BD metabolite levels between species, noting humans have substantially lower internal doses of epoxide metabolites than rodents. Expert 6 states it's "doubtful that human butadiene exposures would be sufficient to cause maternal toxicity."
• Experts opposing exclusion (No):Experts 2, 4, and 5 disagree, with Expert 4 citing "limited available data" to support the hypothesized MOA. Expert 2 argues that "at sufficiently high BD exposure levels, human females could experience general toxicity." Expert 5 notes that while humans would likely be "far less sensitive than mice," the MOA itself hasn't been sufficiently established.
• Middle position (Somewhat):Expert 3 acknowledges species differences in metabolism suggest "the risk of fetal body weight changes in humans is very low" but believes the MOA remains relevant.

A key point of discussion is recent data from Georgieva et al. (2025) suggesting women may experience lower internal doses of BD metabolites than men, though the implications of this finding are interpreted differently among experts.

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ANSWER EXPLANATIONS

Expert 1 | Yes | **The quantitative differences between mice, rats, and humans for circulating levels of epoxide metabolites upon exposure to BD have been well demonstrated (Swenberg et al; 2011;Boysen et al; 2012; Motwani and Tornquist; 2014) showing that for a given exposure to BD , its epoxy metabolite levels in humans are substanially lower than rats and mice. Furthermore , recently Georgia et al (2025) reported that women workers may have lower internal doses of DEB and EBD than men follwing exposure to BD. Therefore, human relevance of the proposed MOA on the basis of quantitative differences in either kinetic or toxicodynamic factors between experimental and humans can not be excluded.**

Expert 2 | No | I'm proposing that at sufficiently high BD exposure levels, human females could experience general toxicity, leading to decreased weight gain and decreased fetal weight gain. It's a risk characterization question as to whether such exposures are likely to be attained.

I don't think the statement below disqualifies the MOA on quantitative grounds. I made some notes on section 4 of the revised document. "Newly published data (Georgieva et al., 2025; discussed further in Sec on 4), which 609 includes female workers, suggests that women may experience lower internal doses of 610 DEB and EBD than men following exposures to BD."

Marginal notes on section 4 of the revised document. lines 650 - 658.
I tend to concur with the last point made by the authors of the revised document re the data points in figure 4.

Note also that Georgieva et al (2025) also state " These findings suggest that human males may be at a higher risk for BDinduced toxicity at elevated exposure levels. However, it is essential to consider that previous studies in rodents have indicated females may be more susceptible to BD-induced carcinogenesis based on metabolism, mutagenesis, and tumorigenesis data."

I would describe (in section 4?) the metabolism by males vs. females as an area of uncertainty and probably variability.

Round 3. Please note additional discussion of this issue.

ANSWER EXPLANATIONS, CONTINUED

Expert 3 | Somewhat | Species differences in metabolism indicate that the risk of fetal body weight changes in humans is very low. However, that is best left to the exposure assessment and risk management processes, the MOA is still relevant

Expert 4 | No | As per the response to Q's 2.2 to 2.4, the limited available data to support a hypothesized mode of action for the fetal body weight changes precludes excluding the adverse outcome on the basis of quantitative differences in key events between experimental animals and humans.

Expert 5 | No | Again, this question is not relevant if in fact the weight of evidence is not sufficient to establish a mode of action for fetal body weight changes in animals. However, the species difference in fetal body weight reductions in mice versus rats combined with the knowledge of the species differences in metabolism of DB strongly suggests that humans would be far less sensitive than mice.

No change for round 3, except the authors have also provided data on the species differences in GSH depletion which also would be considered if the MOA had been established.

Expert 6 | Yes | This answer is the same whether KE4 remains as the maternal effect or is changed to a metabolite effect. It is doubtful that human butadiene exposures would be sufficient to cause maternal toxicity, but this aspect is not yet adequately covered. If KE4 is revised to the effect of metabolites directly on fetal tissue, the answer is more solidly "yes". Again, however, additional comparisons of the metabolite formation between mice, rats and humans should be digested and included. Motwani contains some important passages (page 279, left column regarding rate data), which might be combined with rate data in humans reported in Georgieva. Some attention to converting both animal and human rates into equivalent units is warranted. Such a direct comparison should be sufficient to exclude the MOA from serious quantitative consideration and clearly establish a basis for DDEF quantitation.

Debate | 4 comments

Expert 1 | 9/04/2025 12:05 SCORE: **0**

The quantitative differences between mice, rats, and humans for circulating levels of epoxide metabolites upon exposure to BD have been well demonstrated showing that for a given exposure to BD , its epoxy metabolite levels in humans are substanially lower than rats and mice. Furthermore , recently Georgia et al (2025) reported that women workers may have lower internal doses of DEB and EBD than men follwing exposure to BD. Therefore, human relevance of the proposed MOA on the basis of quantitative differences in either kinetic or toxicodynamic factors between experimental and humans can not be excluded. I think the weight of evidence is reasonably sufficient to establish the proposed mode of action for fetal body weight changes in animals. However, the species difference in fetal body weight reductions in mice versus rats combined with the knowledge of the species differences (mice> rat> human) in metabolism of BD strongly suggests that humans are likely to be far less sensitive based on toxicokintetc and toxicodynamic factors. The comments of Experts 2,4 , and 5 essentially questin that the WOE is limited or not sufficient to establish a MOA for for fetal body weight changes in animals. I think there are clear quantitative differences between mice, rats, and humans with respect to circulating levels of epoxy metabolites upon exposure to BD which needs to be considered in evaluting BD risk assessment.

Expert 4 | 9/05/2025 11:37 SCORE: **0**

As per my comments on the previous question, lack of a credible hypothesis for the mode of action does not preclude devleoping a DDEF, if there is sufficient knowledge on the relevant dose metric.

Expert 5 | 9/06/2025 14:42 SCORE: **1**

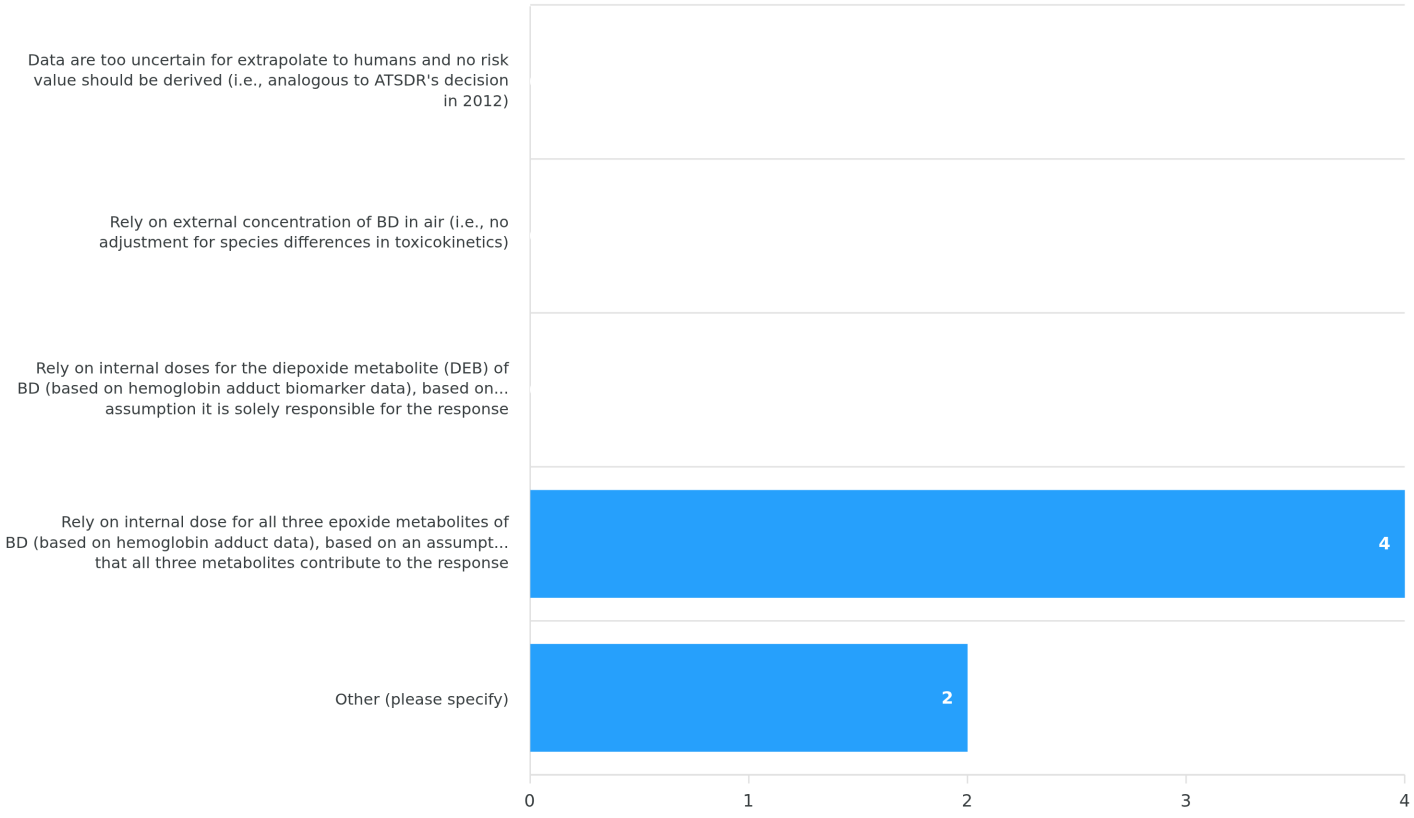
I agree that a complete MOA is NOT needed in order to develop a DDEF. But that is a separate issue from whether the current proposed MOA is not relevant to humans based on quantitative considerations. It is likely that humans are less sensitive, but a full risk assessment would be needed to establish potential risk.

Expert 6 | 9/08/2025 7:56 SCORE: **0**

My initial Comment mirrors what I read in the comments from Experts 4 and 5 above. While my Response was "yes", that it can be excluded, I do not agree that the MOA for fetal effects is acceptably established in this draft. So, while I believe the relevance "can" be excluded, I do not agree that the present draft presents an acceptable argument, beginning with the proposed MOA being dependent (solely?) on maternal effects.

Based on the proposed MOA for fetal body weight changes and your degree of confidence, what is your recommendation for the dose measure that should be used in the risk assessment to extrapolate across species?

Results | 6 answers



Expert consensus suggests using internal dose measurements for all three epoxide metabolites (based on hemoglobin adduct data) for cross-species extrapolation in risk assessment. Four experts (1, 3, 5, and 6) explicitly recommend this approach, noting that while the diepoxide metabolite may be most toxic, all three metabolites likely contribute to fetal body weight effects.

Areas of disagreement center on confidence in the proposed mode of action (MOA). Expert 4 expresses lower confidence in the established MOA and recommends considering multiple dose measures (external concentration, diepoxide metabolite, and all three epoxides). Expert 2 suggests using both approaches that consider the diepoxide alone and all three metabolites together.

Several experts highlight specific uncertainties, including gender-specific metabolism differences and the relative contribution of each metabolite to the observed effects.

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ANSWER EXPLANATIONS

Expert 1 | Rely on internal dose for all three epoxide metabolites of BD (based on hemoglobin adduct data), based on an assumption that all three metabolites contribute to the response | In my opinion, based on currently published information , the use of hemoglobin adducts data to quantify species differences in the internal doses of reactive metabolites data in rodents and humans reasonabally support dose-responce risk assessment and could be considered more relevant for the fetal body weight changes observed in experimental animals.There is sufficient mechanistic information supporting the proposed MOA for the fetal body effects following gestational exposure with relevant quantitative data on epoxide metabolites of BD and the propsed key events of MOA for the endpoint of concern.Therfore, relying on use of defalt dosimetric adjustments is inappropriate. The species differences in DEB internal dose and adducts (pyr-Val adducts) as a function of BD exposure in mice, rats, and humans (Georgieiva et al; 2010 , 2025) could be estimated and used in dosimetric adjustments appying a DDEF approach (U.S. EPA, 2014), given a good understanding of of the toxicokinetis and exposure -response associated with the fetal body weight effects upon BD exposure.

ANSWER EXPLANATIONS, CONTINUED

Expert 2 | Other (please specify) | I think the authors have presented good information and arguments in support of extrapolation. I am less convinced that DEB is the sole metabolite responsible for changes in fetal body weight. Thus, I still see utility in doing both approaches 3 and 4. I think my comments on 1.6 are relevant here, so I have pasted them below.

lines 650 - 658.

I tend to concur with the last point made by the authors of the revised document re the data points in figure 4.

Note also that Georgieva et al (2025) also state " These findings suggest that human males may be at a higher risk for BD-induced toxicity at elevated exposure levels. However, it is essential to consider that previous studies in rodents have indicated females may be more susceptible to BD-induced carcinogenesis based on metabolism, mutagenesis, and tumorigenesis data."

I would describe (in section 4?) the metabolism by males vs. females as an area of uncertainty and probable variability.

663 - 665
I agree with this statement, but would again describe the male vs. female capacity for metabolism as an area of uncertainty rather than giving the impression that human females do not metabolize BD to reactive forms.
Future studies may provide better support for quantifying within human (particularly gender-specific) variability.

Expert 3 | Rely on internal dose for all three epoxide metabolites of BD (based on hemoglobin adduct data), based on an assumption that all three metabolites contribute to the response | See previous answer, but here the other metabolites may be more important than for ovarian atrophy where the role of DEB is clearer. Also, this is even more important in the revised MOA for fetal weight effects in Phase 3, where GSH depletion is a KE that can be caused by all 3 metabolites.

Expert 4 | Other (please specify) | My personal preference here, given the lack (in my view) of relevant information to develop a credible mode of action, would be to consider a range of dose measures to extrapolate across species, namely external concentration, the diepoxide metabolite and all three epoxide metabolites. I would then qualify the estimates based on relative confidence indicating that it is likely greatest for BD (relying principally on data for other effects), followed by all three epoxide metabolites followed by external concentration of BD in air.

Expert 5 | Rely on internal dose for all three epoxide metabolites of BD (based on hemoglobin adduct data), based on an assumption that all three metabolites contribute to the response | Regardless of whether the actual MOA for fetal body weight has been established, it is likely that metabolism to the epoxides is requisite. However, it is not known whether one or all of the metabolites are involved. I would therefore, base the DDEF on the internal dose for all three epoxide metabolites of BD (based on hemoglobin adduct data), based on an assumption that all three metabolites contribute to the response.

No change for round 3.

Expert 6 | Rely on internal dose for all three epoxide metabolites of BD (based on hemoglobin adduct data), based on an assumption that all three metabolites contribute to the response | While the bi-alkylating metabolite might be the most toxic, it is not the only metabolite capable of important molecular interactions. The quantitative data available from Motwani and from Georgieva should be converted to the same unit of expression and used to develop a quantitative comparison between mice and humans.

Debate | 4 comments

Expert 4 | 9/04/2025 9:18 SCORE: **0**

Sorry, another error that I picked up in my final review that wasn't transmitted due to submission rather than save. For the sentence on relative confidence "BD" should be DEB.

Expert 1 | 9/04/2025 10:47 SCORE: **0**

I think that relying on internal dose of all three epoxy metabolites of BD based on hemoglobin adduct data with the assumption that they are likely to contribute the observed biological response (fetal body weight changes) makes sense. Given the comments of Expert 4 about lack of relevant information to develop a credible MOA , considering a range of dose measures to extrapolate across species based on external exposure concentration in air could be calculated and presented.However, still rely on internal dose of all three metabolites of BD based on hemogobin adduct data for the dose-respnse analysis to extrapolate across species.

Expert 6 | 9/04/2025 10:51 SCORE: **1**

Experts 2 and 4 have raised an issue that I will describe as uncertainty regarding the active chemical moiety. WHO/IPCS' (2005) Chemical Specific Adjustment Factor guidance addresses uncertainty directly, advocating for the development of multiple CSAF values when uncertainty about the active chemical moiety (parent versus metabolite) exists. EPA's (2014) DDEF guidance does not address this uncertainty directly, but indicates that the dose metric (the expression of dose including identification of the active moiety and the nature of the time-normalization process) be determined using a weight-of-evidence approach. It is reasonable to apply WOE considerations to evaluating whether/which metabolites are responsible, and then (subsequent to that) developing DDEF values from that understanding. While CSAF guidance says in the face of uncertainty to develop alternate CSAF values and then choose the most conservative of them for application in the assessment, DDEF guidance intentionally does not take this approach to avoid the criticism of "value-shopping". Thus, I think it is very important to decide first what the dose metric should be and then embark on a DDEF quantitation. This perspective places a high level of importance on selecting the active chemical moiety(ies). Risk characterization will address the assumptions, extrapolations and uncertainties in the risk assessment, and provides input to Risk Management decisions. This (Risk Characterization step) is where the impact of alternate choices should be evaluated and discussed. The responses to Query 2.6 and the comments clearly identify the separation of Risk Assessment from Risk Management, and it is critical that we stick to the Risk Assessment side of the coin. So, while it may be inviting to "punt" on the decision of the active chemical moiety (here, the choice between the active metabolite), I'd push for a little more discussion.

The process Expert 4 advocates is a valid one, but care should be placed into documenting the method used to select the most reliable value before the process is started.

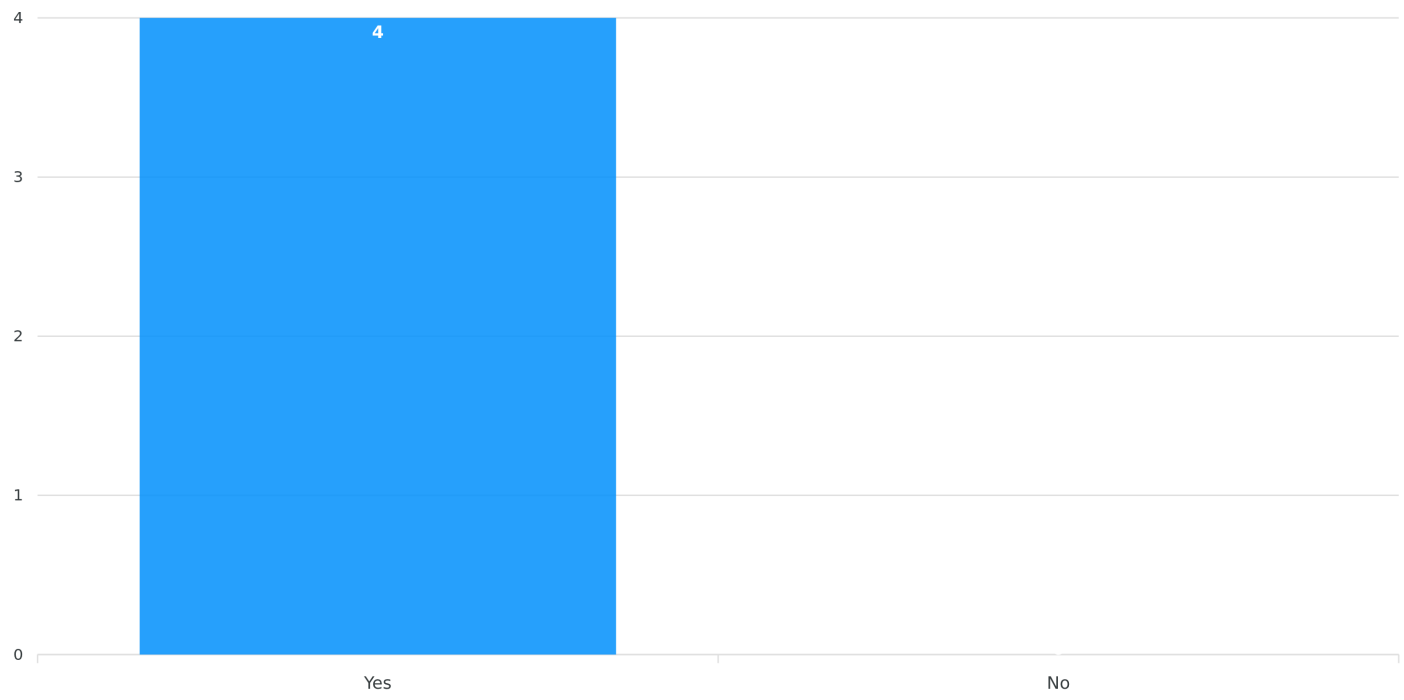
I believe that additional effort may be expended to improve/expand the discissions in the document (using more details from the source references) regarding the selection of the active chemical moiety - and this relates to both ovarian effects (section 1) and fetal effects (section 2).

Expert 4 | 9/05/2025 11:41 SCORE: **1**

Clarification on my response to Question 1.6 also applies here. There is no single "right" choice for the dose metric in the development of DDEF (CSAF). Rather, some choices are more certain than others (depending on the extent of the evidence). Understanding the variability in estimates based on this uncertainty is extremely informative.

Do you have any recommended changes to the revised MOA summary document? Please describe below or you may email a marked-up version of the document via the message center.

Results | 6 answers



The experts unanimously recommend changes to the revised MOA (Mode of Action) summary document, with several specific suggestions for improvement:

• Increased detail neededfor the Ovarian effects MOA, particularly regarding:
• Better linkages between molecular responses and outcomes
• More supporting evidence from bi-alkylating agents (VCH and isoprene)
• Clearer relationship between molecular changes and adverse outcomes

• Tables 1 and 2 require revisionas they are currently difficult to read and interpret, with Expert 2 suggesting alternative formatting approaches based on published examples
• Bradford-Hill criteria need expansion, particularly regarding dose-relationships and temporal relationships between each key event
• Data consistency concernswere raised about using VCH data for ovarian toxicity but not for fetal toxicity
• Further clarification neededon whether the MOA for fetal and maternal toxicity potentially bifurcates after KE3

AI generated summary content

ANSWER EXPLANATIONS

Expert 1 | *Did not answer* | *No explanation provided.*

Expert 2 | Yes | I did not edit the document. However, I have made some comments on a mark up to be emailed to SciPinion. I have also copied some marginal comments on the document into responses to the questions.

I found the current Tables 1 and 2 to be very difficult to read, particularly in the context of providing support or showing lack of support for the proposed KE for both MOA. Data rich chemicals such as BD can be tricky to evaluate for MOA. There is a plethora of studies but few that address what is happening at a cellular level that is relevant to identifying KE -- that is, those changes that are necessary but not sufficient to result in an adverse outcome. This is the major reason I suggest a large summary table of data that may be relevant to the MOA, followed by a table of what the ideal set of observations would be in a dose / time concordance analysis, followed by a fill in the blanks table. Different approaches to untangling the data and presenting it in tabular or graphic form can be seen in these papers, which might provide some useful templates.

Clifford R. Elcombe, Richard Peffer, Douglas C. Wolf, Jason Bailey, Remi Bars, David Bell, Russell Cattley, Stephen Ferguson, David Geter, Amber Goetz, Jay Goodman, Susan Hester, Abigail Jacobs, Curtis Omiecinski, Rita Schoeny, Wen Xie and Brian G. Lake. Mode of Action and Human Relevance Analysis for Nuclear Receptor-Mediated Toxicity: A Case Study with Phenobarbital as a Model Constitutive Androstane Receptor (CAR) Activator. Critical Reviews in Toxicology. 44(1):64-82, 2014.

Martha Moore, Rita Schoeny, Richard Becker, Kimberly White, Lynn Pottenger. Development of an Adverse Outcome Pathway (AOP) for a Mutagenic Mode of Action for Chemically Induced Hepatocellular Carcinoma Illustrated by the Data-Rich Chemical Aflatoxin B1 (AFB1). Crit. Rev. Toxicol., 48(4):312-337. doi: 10.1080/10408444.2017.1423462. Epub 2018 Feb 12. 2018.

Jordan S. Kozal, Heather N. Lynch, Joanna Klapacz, Rita S. Schoeny, Paul A. Jean, Andrew Maier. Mode of Action Assessment for Propylene Dichloride as a Human Carcinogen. Chem Biol. Int. Feb 6;110382. doi: 10.1016/j.cbi.110382. (2023).

Corton, J. C., Cunningham, M. L., Hummer, B. T., Lau, C., Meek, B., Peters, J. M., … Klaunig, J. E. (2013). Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPAR⍰) as a case study. Critical Reviews in Toxicology, 44(1), 1–49. https://doi.org/10.3109/10408444.2013.835784

ANSWER EXPLANATIONS, CONTINUED

Expert 3 | Yes | I will email a marked-up version with edits and comments, but nothing major.

Expert 4 | *Did not answer* | Most of the important changes that I'd recommend are captured in the responses to the other questions. This includes modifying the MOA descriptions consistent with convention, ensuring that all key events have been measured (as per the definition of key event), more clearly distinguishing aspects of mode of action from subsequent dose-response analysis, extending the temporal and dose-response concordance analysis to include cross study comparisons and providing more robust rationales for eliminating modes of action other than those hypothesized.

Expert 5 | Yes | I recommend expanding the discussions of the Bradford-Hill criteria of dose-relationships and temporal relationships. So far the discussions of dose-relationships for bot proposed MOAs focuses on metabolism and the final outcome. This section should also discuss the dose-relationships between each of the key events. In addition, VCH data is used for the MOA for ovarian toxicity, but there is no mention of VCH data in the section on the fetal toxicity. Are there data available for VCH? And are there data that refute the possibility that the MOA for fetal and maternal toxicity actually bifurcates after the proposed KE3?

Expert 6 | Yes | Revisions should include increased detail in the MOA for Ovarian effects, regarding the linkages between molecular responses including those identified at lines 139 etc, and the outcome. Increased detail communicating the pertinent findings from the two identified bi-alkylating agents (VCH and isoprene) should be included to provide support for the molecular changes identified. Increased detail describing the relationship between these changes and actual adverse outcomes including weight changes should be included, irrespective of the causative chemical. My comments regarding the treatment of KE4 for fetal effects should result in increased detail in the MOA summary. Specific points include the counterpoints made against the potential role of maternal toxicity as a KE for fetal effects.

Debate | 1 comment

Expert 1 | 9/04/2025 10:52 SCORE: **0**

I plan to email a marked -up version with edits and comments. No major comments or recommendations to change the document.

Example points of departure for premature ovarian failure in mice under different assumptions for interspecies extrapolation are provided in Table 3 of the MOA summary document. Based upon the proposed MOA for premature ovarian failure, what is your degree of confidence in each method for extrapolating ovarian effects in mice to humans for risk assessment purposes (0=lowest confidence; 5=highest confidence)

Results | 6 answers

	0	1	2	3	4	5	TOTAL
Based upon species differences for DEB	0.0% 0	0.0% 0	0.0% 0	0.0% 0	50.0% 3	50.0% 3	6
Based upon species differences for all 3 epoxide metabolites	0.0% 0	33.3% 2	16.7% 1	33.3% 2	0.0% 0	16.7% 1	6
Based on external concentration in air (no species adjustment)	50.0% 3	33.3% 2	0.0% 0	16.7% 1	0.0% 0	0.0% 0	6
Other	100.0% 1	0.0% 0	0.0% 0	0.0% 0	0.0% 0	0.0% 0	1

Expert consensus strongly favors using species differences for DEB as the most appropriate method for extrapolating ovarian effects from mice to humans. Five experts rated this approach with high confidence (4-5 out of 5), citing compelling evidence that DEB is the primary metabolite responsible for ovarian toxicity.

There was moderate support for using all three epoxide metabolites, with ratings ranging from 1-5, but most experts considered this less reliable than focusing on DEB alone.

Experts unanimously rejected using external air concentration without species adjustment, giving this approach the lowest confidence ratings (0-1). They emphasized that ignoring the significant species differences in BD metabolism would be inappropriate and introduce substantial error.

Several experts specifically noted that experimental data, including metabolite-specific studies across species, provides conclusive support for DEB's role in ovarian toxicity, making it the most relevant point of departure for human risk assessment.

AI generated summary content

ANSWER EXPLANATIONS

Expert 1

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	1	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	1	0	0
Based on external concentration in air (no species adjustment)	0	0	0	1	0	0
Other	0	0	0	0	0	0

• The degree of confidence based on the proposed MOA for premature ovarian failure using hemogolbin (Hb) adducts of DEB as primary dosimeters for relative internal dose across mice, rats, and humans (mouse > rat □ human) , is high for mouse, moderate for rat, and moderate to high for human. Mouse studies consistently show a very good exposure-dependent formation of hemoglobin (Hb) adducts for BD reactive metabolites ,especially the diepoxide (DEB) that helps to explain mice's higher sensitivity. DEB Hb adducts are reliable for internal dose estimates and for scaling across -species for exposures to BD. Rats form markedly fewer DEB-specific Hb adducts than mice at the same airborne BD exposure, reflecting lower activation to DEB. Hb adducts of DEB have been detected with exposure to BD in human workers and are useful for relative internal dose estimation, but given their interindividual variability, they may result in less certainty. Male worker and controlled-exposure data show detectable EB/EBD adducts and much lower DEB-specific Hb adducts levels than in mice that is consistent with lower human DEB formation. Hemoglobin (Hb) adducts, therefore are highly relevant for comparing internal dose across species. Hb adducts integrate exposure and reflect in vivogeneration of the reactive epoxides. The DEB-specific pyr-Val adduct is particularly informative for the
• enabling cross-species internal dose comparisons of metabolits.Multiple lines of evidence show a clear exposure-response in DEB Hb adducts therfore, their analyses could be used to quantify species differences in internal dose metrics. Airborne BD exposure does not necessarily mean to be equal for internal dose of reactive metabolites such as DEB..

Expert 2

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	0	1
Based upon species differences for all 3 epoxide metabolites	0	0	0	0	0	1
Based on external concentration in air (no species adjustment)	0	1	0	0	0	0
Other	0	0	0	0	0	0

I appreciate the comparison of the PODs from Kirman et al (2022) in the current document Table 3. (Please note that BMDSD should be written out in a footnote to the table. And isn't it BMDL1SD per Kirman?). The human equivalent exposure with no adjustment could be chosen by some as the most conservative approach. The clear species differences in epoxide metabolism production argue against this. Furthermore, the revised document makes a stronger case for DEB as the major metabolite responsible for the adverse outcome, as well as KEs 3 and 4. A choice of the POD based on benchmark modeling of the DEB data alone would be reasonable. A choice of the POD for all epoxides may be suggested as more conservative; however both POD are within an order of magnitude.

ANSWER EXPLANATIONS, CONTINUED

Expert 3

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	0	1
Based upon species differences for all 3 epoxide metabolites	0	1	0	0	0	0
Based on external concentration in air (no species adjustment)	1	0	0	0	0	0
Other	1	0	0	0	0	0

Data clearly point to DEB as the active metabolite for effects on the ovary. Including other metabolites introduces error into the calculations, and species differences in metabolism preclude simply using exposure levels.

Expert 4

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	1	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	1	0	0
Based on external concentration in air (no species adjustment)	0	1	0	0	0	0
Other	0	0	0	0	0	0

Due to the consistent and rather convincing evidence including metabolite specific studies, my confidence is greatest for extrapolating ovarian effects in mice to humans based on species differences for DEB, followed by all 3 metabolites followed by the external concentration in air. The basis for my ratings of relative confidence is outlined in my Round 2 responses . As noted in earlier rounds, however, there are still issues with the description of the supporting hypothesized mode of action which does not conform with evolved description convention for MOA/AOPs (e.g., the inclusion of "distribution to the ovary" as a key event; the lack of recognition of VCD as a "protoypical stressor" and referencing of weight of evidence considerations as "criteria".

Expert 5

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	0	1
Based upon species differences for all 3 epoxide metabolites	0	1	0	0	0	0
Based on external concentration in air (no species adjustment)	1	0	0	0	0	0
Other	0	0	0	0	0	0

The ovarian toxicity has been shown to be due to DEB so that is the most relevant point of departure. Given the species differences in metabolism of DB, it is not appropriate to ignore species adjustments.

ANSWER EXPLANATIONS, CONTINUED

Expert 6

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	1	0
Based upon species differences for all 3 epoxide metabolites	0	0	1	0	0	0
Based on external concentration in air (no species adjustment)	1	0	0	0	0	0
Other	0	0	0	0	0	0

I believe that the data provided, including data from VCH/VCD are compelling. While an extrapolation based on all three epoxides is somewhat supportable, the experimental data especially including metabolite administration across species provides conclusive support for the role of DEB. Metabolism (TK) data and experimental data from metabolite administration are more than sufficient to warrant an advanced species dosimetric adjustment for BD.

Example points of departure for reduced fetal body weight gain in mice under different assumptions for interspecies extrapolation are provided in Table 6 of the MOA summary document. Based upon the proposed MOA for general toxicity/reduce weight gain, what is your degree of confidence in each method for extrapolating ovarian effects in mice to humans for risk assessment purposes (0=lowest confidence; 5=highest confidence)

Results | 6 answers

	0	1	2	3	4	5	TOTAL
Based upon species differences for DEB	0.0% 0	0.0% 0	33.3% 2	33.3% 2	33.3% 2	0.0% 0	6
Based upon species differences for all 3 epoxide metabolites	0.0% 0	0.0% 0	0.0% 0	33.3% 2	33.3% 2	33.3% 2	6
Based on external concentration in air (no species adjustment)	33.3% 2	50.0% 3	0.0% 0	16.7% 1	0.0% 0	0.0% 0	6
Other	100.0% 1	0.0% 0	0.0% 0	0.0% 0	0.0% 0	0.0% 0	1

Experts show strongest confidence in extrapolation based on all three epoxide metabolites for reduced fetal body weight gain in mice, with scores ranging from 3-5 (average ~4). Most experts rated this approach higher than using DEB alone.

For DEB-based extrapolation, confidence was moderate with scores ranging from 2-4. Several experts noted that while DEB is important, other metabolites likely contribute to the effect.

There was strong consensus against using external air concentration without species adjustment, with most experts giving this approach very low confidence scores (0-1), with only one expert rating it a 3.

Key rationales included:

- Metabolic pathways are qualitatively similar across species but with quantitative differences
- GSH depletion due to epoxide conjugation involves all three metabolites
- Mice produce higher levels of reactive metabolites than humans
- Some uncertainty remains about the exact contribution of specific metabolites

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ANSWER EXPLANATIONS

Expert 1

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	1	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	1	0	0
Based on external concentration in air (no species adjustment)	0	0	0	1	0	0
Other	0	0	0	0	0	0

- Butadiene exposure in mice and rats consistently shows reduced body weight gain and general systemic toxicity at relatively very high exposure levels. These effects could be linked primarily to metabolic activation of butadiene to reactive epoxides (eg; DEB). The metabolic pathways leading to these systemic effects are qualitatively similar in rodents and humans, but quantitative differences in metabolism,notably higher production of reactive metabolites in mice make them much more sensitive to butadiene exposure for these effects than humans.I think there is a high confidence for a systemic general toxicity like weight loss and reduced body weight gain, since the underlying metabolic and physiological processes are belived to be broadly conserved across species.The degree of confidence for extrapolating general systemic toxicity (weight changes) is moderate-to-high.

Expert 2

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	0	1	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	0	1	0
Based on external concentration in air (no species adjustment)	0	1	0	0	0	0
Other	0	0	0	0	0	0

For this endpoint, as well as for ovarian effects, the more scientifically sound approach uses interspecies extrapolation for derivation of a human equivalent exposure. I feel that the information is less convincing that fetal body weight changes are solely due to the DEB metabolite. I would recommend the POD derived from BMD modeling based on all epoxide metabolites.

Expert 3

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	1	0	0	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	0	1	0
Based on external concentration in air (no species adjustment)	0	1	0	0	0	0
Other	1	0	0	0	0	0

In the revised MOA, GSH depletion due to epoxide conjugation is a key event and can occur with all three metabolites. While DEB is still predominant, using all three should give a better estimate.

ANSWER EXPLANATIONS, CONTINUED

Expert 4

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	1	0	0	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	1	0	0
Based on external concentration in air (no species adjustment)	0	1	0	0	0	0
Other	0	0	0	0	0	0

In my view, as per my responses in previous rounds, current data are inadequate to credibly support a hypothesized mode of action for general toxicity/reduced body weight gain. The proposed, modified description of the MOA also does not conform with accepted practice for the description of key events, including "distribution to maternal/fetal tissues" as a key event, the separation of "metabolism of BE to reactive epoxides" and "GSH depletion" as separate key events and reference to "lumped events" (umbrella KEs?). While this does not preclude the development of a DDEF (which don't require an elaborated MOA), the extent of the data supporting the role of specific BD metabolites in induction of reduced fetal body weight gain is considerably less than that for ovarian toxicity with the nature of the important evidence gaps and associated uncertainties being pretty clear (also indicated in previous rounds). Probably, the most convincing evidence for the likely role of DEB relates to the consistent observation of its importance across endpoints (and knowledge concerning the role of bifunctional alkylating agents); however, in my view, this is insufficient as a basis for high confidence or conviction in its application in the development of a DDEF for this endpoint. Given that we have some relevant data, I'm reluctant, however, to advocate reliance on the external concentration in air which could be (and often is) predicated on the basis of the need for prudence for conservatism to protect public health, in view of the considerable uncertainties.

Expert 5

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	1	0	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	0	0	1
Based on external concentration in air (no species adjustment)	1	0	0	0	0	0
Other	0	0	0	0	0	0

The general toxicity has been shown to require metabolism, but it is not clear whether one or all metabolites are involved. The rat data demonstrate that DEB is probably involved so I give that a score of 3. Again, given the species differences in metabolism it is not appropriate to include no species adjustment.

ANSWER EXPLANATIONS, CONTINUED

Expert 6

Human Equivalent Exposure Assumption	0	1	2	3	4	5
Based upon species differences for DEB	0	0	0	1	0	0
Based upon species differences for all 3 epoxide metabolites	0	0	0	0	0	1
Based on external concentration in air (no species adjustment)	1	0	0	0	0	0
Other	0	0	0	0	0	0

While DEB seems to be the most potent of the metabolites, data indicate a role for other epoxide metabolites, as acknowledged in the report, e.g., lines 592-598, 708-713 and 732-736. The decision regarding which data set to use will impact DDEF quantitation, but not necessarily human relevance.

Based upon male worker hemoglobin data, quantitative species differences between mice and humans have been estimated to be approximately 1100x for DEB alone and approximately 180x for all three expoxide metabolites combined (Kirman et al., 2022). These differences may be even larger if female worker data are used in the calculations. In reference to the quote from Boobis et al. (2007), in your opinion what magnitude of difference would be needed to support a conclusion of "not relevant to human health"?

Results | 6 answers

Expert 1

Boobis et al (2007) human relevance framework is a weight-of-evidence approach/process where human relevance is judged by whether the animal mode of action (MOA) similarly operates in humans, do key MOA events occur at human internal doses, and how large the toxicokinetic (TK) and toxicodynamic (TD) differences are and what uncertainties exist. Boobies et al (2007) did not suggest any specific numeric cutoff point. In my poinion, a MOE (margin of exposure) of ≥10,000 from a suitable point of departure which is typically considered of low level of effects but *not*necessarily of "no risk." Kirman et al (2022). estimate ≈1100× (DEB alone) and ≈180× (combined epoxides) lower human internal doses vs mice based on Hb-adduct biomonitoring makes a practical and reasonable scientific argument . However , there are large toxicokintec gaps, and they are likely to increase using female worker data to be "relevant" I think, there is a need to show that animal MOA is not likely to be occur in humans at real-world exposures (key events, concordance, toxicodynamic differences, sensitive subpopulations, etc;) of BD in ambient air. An argument could be articulated showing a strong evidence that one or more *key human MOA step(s)* is/or absent quantitatively and likely implausible at human internal doses (e.g., negligible DEB formation at relevant BD exposures,and lack of or very limited evidence for downstream key events). Based upon male worker hemoglobin data, quantitative species differences between mice and humans have been estimated to be approximately 1100x for DEB alone and approximately 180x for all three expoxide metabolites combined (Kirman et al., 2022) is justifiable. A convincing toxicodynamics evidence and margin of exposure (MOE), one could plausibly argue for a "low level concern" in humans given very low leves of DEB-specific adducts are observed in exposed workers (male)..

Expert 2

I hesitate to offer an order of magnitude recommendation. But I certainly think it would need to be higher than 1x103 in order to pass muster with any authoritative body. One could use 1x106, but that is as arbitrary as the original one in a million that has been used for "acceptable risk".

I think the more solid approach is to calculate the margins of exposure (MOE) between the proposed RfV and the BD workplace exposure example of 0.089 ppm. The document in lines 481 - 487 notes "Due to these species differences, some of the human equivalent concentration (HEC) values calculated for corresponding higher test concentrations in mouse studies exceed 1x105 ppm (Kirman et al., 2022), levels which exceed the lower explosive limit for BD and potential

> for oxygen displacement become of concern. For this reason, it is possible that humans are not capable of producing levels of DEB that are sufficient to produce ovarian toxicity (i.e., above a threshold for this endpoint)." The chosen POD is presumably at or near the threshold for observation of effects (here adverse outcome), and the RfV is one's best guess at a threshold for adverse effects in humans. So flagging the highest human equivalent levels tested in mice as greater than 1x105 doesn't make an argument that humans couldn't possibly be exposed to BD concentrations that would result in sufficient reactive metabolites to reach an effect level.

Expert 3

> I don't think the MOA is the right place to rule out human relevance based on quantitative species differences. This should be done after quantitative exposure assessment, as stated in the quote. If the species differences are so large that human exposures would have to be at levels that might be explosive or deplete oxygen availability, that's an easy exposure assessment, but doesn't make the MOA irrelevant.

Expert 4

> This is necessarily dependent upon envisaged exposure and even then, the likelihood of adverse impact cannot be precluded (Witness our experience with melamine as a case study in the development of the original MOA/human relevance framework where it was the contention of some at that time that exposure of some portion of the human population would never be sufficient to exceed the level associated with adverse effect. Unfortumately, history has proven them wrong). So, there is no "magic number" concerning an acceptable magnitude of difference and I believe that our state of thinking on the possibility of excluding human relevance due to quantitative variations has moved on. The question relates more to focussing attention on the relevant (often kinetic) data to ensure that quantitative variations are taken into account in the subsequent risk assessment.

Expert 5

> This question is only relevant for ovarian toxicity since the animal MOA for general toxicity has not been established. The only data that would allow one to definitively support a conclusion of not relevant to human health based on quantitative data would be if metabolism to DEB does not occur in humans. Otherwise, one would need an estimate of human exposure to determine whether the ovarian toxicity was likely or not likely to occur.

Expert 6

> In my opinion, there is no numerical ratio of animal to human exposures that can be used as the sole basis to reliably exclude human relevance. The quote from Boobis et al., 2007 acknowledges the role of exposure assessment. Concisely stated, only when the exposure that is sufficient to drive a level of internal dose to a benchmark level (be it a human equivalent concentration, a human equivalent dose, a reference concentration, a reference dose, etc) is not feasible or compatible with life, can the MOA be ruled out on a quantitative basis.
>
> In that the MOA for the chemical may be agnostic to route of exposure, both oral and inhalation exposures should be considered relevant. While some exposure levels may not be likely/reasonable/possible via inhalation, there may be oral exposures that can result in higher internal doses than are possible via inhalation. This (oral exposure issue) is not explored in this document. It is my opinion that arguments CAN BE MADE about given numerical ratios regarding whether they are sufficient to result in a quantitative disqualification of the animal mode of action, and I believe such would be potentially successful for inhalation exposures to BD. Such an argument would need to include quantifying the exposure concentration/duration necessary in humans to drive the internal dose/concentration of (here) toxic metabolites necessary to produce the effect. Such arguments may be substantially supported by PBPK models, which can address issues like the saturation of metabolic activation pathways at high exposures. Thus, there may well be value in advancing a PBPK model to support this argument. Using the classic modeling to date and the ppm-based mouse and human rates of formation to estimate a human exposure concentration that produces human adduct levels equivalent to the mouse adduct concentrations at the risk assessment point of departure would be a very valuable step.
>
> As opposed to a numerical value of a ratio, the argument surrounding quantitative relevance should be based on the likelihood that humans can be exposed to conditions that may lead to the (here) formation of an internal threshold-

concentration of metabolites necessary to produce the effect observed in test species. One issue that should be faced in such an evaluation is whether the comparison should be based on either the point of departure concentration or the end-level risk value (i.e., the uncertainty-factor-adjusted value). I would favor an initial exercise that used the Human Equivalent Concentration as the basis for determining the likelihood of a non-lethal human exposure that may produce the effect. I should note that under EPA's non-cancer risk guidance, the Human Equivalent Concentration is that exposure that is adjusted from animals to human to account only for toxicokinetic differences, not toxicodynamic differences. Comparisons to LEL values are not relevant in this situation because (1) exposures can exceed the Lower Explosive Limit and (2) do not cover potential oral human exposures to BD.

It should be noted that whether and how the quantitative aspect of the HRF is addressed will be quite important. If the MOA cannot be excluded, then species differences must be quantified under DDEF guidance (EPA 2014). That guidance specifically states that animal to human toxicokinetic DDEF values can be lower than 1. SO, it follows that UF A- TK (in DDEF vernacular, EFAK) could be 0.010 - or lower, as shown in Table 8.

Expert opinions vary on what magnitude of species difference would support a conclusion of "not relevant to human health" for butadiene metabolites.

Areas of agreement:

• Most experts reject establishing a specific numerical cutoff for human relevance
• Several experts (1, 3, 4, 6) emphasize that the Boobis framework is about weight-of-evidence rather than specific numerical thresholds
• Multiple experts note that exposure assessment is critical in determining relevance

Areas of disagreement:

• Expert 2 suggests a threshold higher than 1000x would be needed, while Expert 1 indicates the current estimates (1100x for DEB, 180x for combined epoxides) could be reasonable
• Expert 6 argues no numerical ratio alone can exclude human relevance, while Expert 5 suggests human relevance could be ruled out if DEB metabolism doesn't occur in humans
• Expert 3 believes MOA isn't the right place to rule out human relevance based on quantitative differences

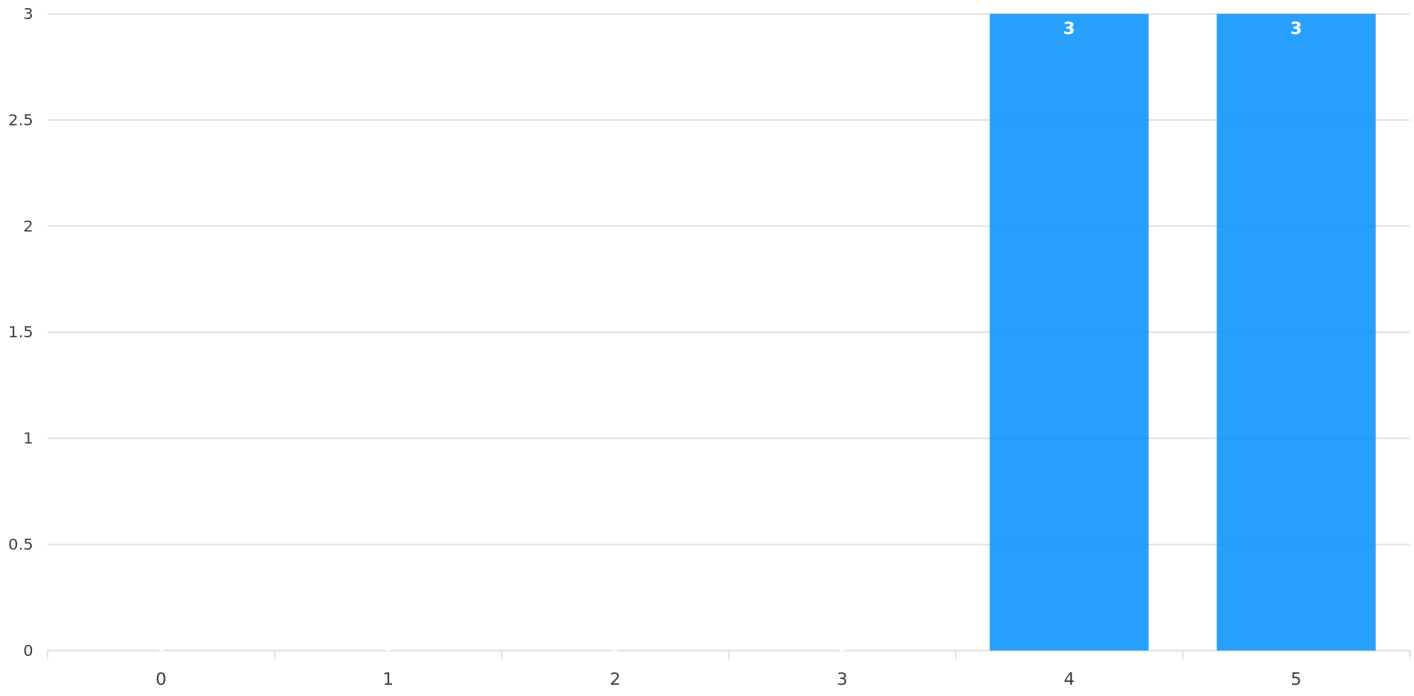
Key considerations mentioned:

• Whether humans could realistically be exposed to levels that produce sufficient reactive metabolites
• The need to consider both oral and inhalation exposure routes
• The value of PBPK models in supporting quantitative arguments
• Consideration of toxicokinetic and toxicodynamic differences

AI generated summary content

In the absence of a fully developed PBPK model for BD, what is your degree of confidence in using hemoglobin adduct data to estimate internal doses of BD metabolites in mice, rats, and humans? (0=lowest confidence; 5=highest confidence)

Results | 6 answers



Expert consensus shows high confidence in using hemoglobin adduct data to estimate internal doses of BD metabolites across species. Four experts rated their confidence at the maximum level (5), while three experts rated it slightly lower (4).

Areas of strong agreement include:

- Hemoglobin adducts provide reliable quantitative measures of internal reactive epoxide dose
- The empirical data comes from actual animals and humans using reliable analytic methods
- Species-specific differences in BD metabolites correlate with differences in hemoglobin adduct accumulation

Minor reservations expressed by some experts include:

- Need to account for metabolic variability in humans
- Questions about exposure assessment in relating human occupational adducts to underlying exposure
- Concerns about comparability of exposure periods and sampling times
- Preference for direct metabolite measurements (though adducts were still considered highly reliable)

AI generated summary content

ANSWER EXPLANATIONS

Expert 1 | 4 | Generally, In mice rats and humans; hemoglobin (Hb) adducts give a reliable, and good-quantitative measure of internal reactive epoxide dose (DEB); While in humans they'are good for a group-level dosimetry unless control for metabolic variability is accounted for adduct formations without develping a full PBPK model (see also comments under 4.1). I think there is strong link between BD exposure and Hb - Val adducts. Mice genterate much more reactive metabolites and they are reasonable as biomarker/ surrogate of internal dose , especially at veyr low environmetal exposures with a very high level of confidence.

Expert 2 | 5 | The document lays out the strong lines of evidence supporting the use of hemoglobin adducts as a measure of BD metabolites generated in the three species.

Expert 3 | 4 | The adducts are stavble and seem to work well. only actual metabolite values would be better so I did not go to 5.

Expert 4 | 4 | The availability of the haemoglobin adduct data as a cumulative measure of exposure to BD metabolites facilitates the interspecies comparisons. I have some residual reservation concerning the comparability of exposure periods and associated sampling times, as a basis to characterize the interspecies DDEF.

Expert 5 | 5 | There is ample evidence that the quantitative differences in the BD metabolites are also related in the quantitative accumulations of metabolite-specific hemoglobin adducts, and both the metabolites and adducts show similar species specific differences. In addition, as summarized in the document "Motwani and Tornqvist (2014) estimated internal dose (i.e., blood AUCs per unit exposure) for
BD metabolites in mice, rats, and humans using two approaches: (1) estimating blood dose
from hemoglobin adduct data using second-order rate constants for adduct formation and
erythrocyte half-lives; and (2) scaling up metabolite clearance rates from in vitro studies. For
DEB, both approaches yielded consistent results in which large differences are estimated across
species (mice>rats>humans)." The use of the adduct data is entirely appropriate.

ANSWER EXPLANATIONS, CONTINUED

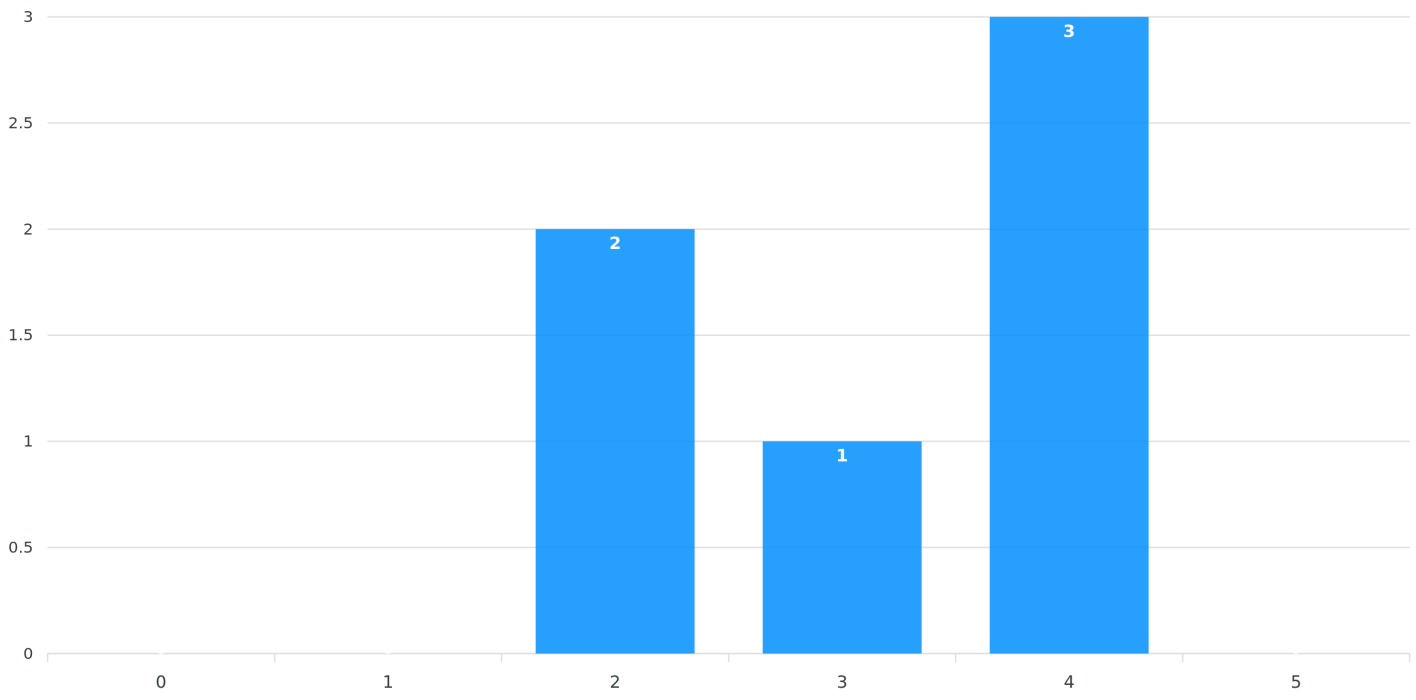
Expert 6 | 5 | The data available are empirical data from actual animals and humans exposed to actual concentrations (known or estimated) of the chemical of interest. They are developed using reliable analytic methods and derived from peer-reviewed publications. Data of this type are recognized by EPA (DDEF guidance, etc) are valid data upon which to quantify species differences. The approaches used in the MOA document follow EPA's DDEF guidance for interspecies comparisons. While there are some limitations in the data set (data are for females, not necessarily pregnant females, there are certain non-linearities that are dealt with in an acknowledged conservative manner, etc), the data re valid and reliable and have been reliably interpreted.

There is no reason to exclude reliable TK data just because they have not been translated into a "full" model such as a PBPK model. This is because of the "fit for purpose" aspect of risk assessment. Valid, relevant TK data form the basis of DDEF quantitation, just as can predictions for PBPK models. Empirical data, such as those available here, are without many of the complications, assumptions, extrapolations and uncertainties as those accompanying PBPK model development, validation and implementation. My only reservation here (reflected in "4" versus "5") is the nature of the exposure assessment used in relating the human occupational hemoglobin adducts to the occupational exposure underlying their development.

Further, in the context of this risk assessment, the metabolic differences between mice and humans are characterized to the point that a PBPK model is not (in my opinion) required to refine the dose response aspect. To avoid potential arguments to the contrary, dose response data on BD-related effects in mice can be very directly, unambiguously presented as threshold values (i.e., no-effect levels of circulating metabolites) in mice. These threshold values could be directly compared to metabolite values (blood concentrations of adduct levels) in occupationally exposed humans.

What is your degree of confidence in using the ratio of internal doses (see Section 4) obtained from nonpregnant rodents and humans to estimate the ratio of internal doses for pregnant animals and humans for the fetal body weight endpoint? (0=lowest confidence; 5=highest confidence)

Results | 6 answers



Expert confidence in using non-pregnant rodent/human dose ratios to estimate pregnant animal/human dose ratios for fetal body weight endpoints varies considerably, with scores ranging from 2 to 4 out of 5.

Areas of agreement:

• Most experts acknowledge that pregnancy-specific data would be preferable but is currently limited
• Several experts note that metabolic changes occur during pregnancy that could affect internal dose ratios

Areas of disagreement:

• Higher confidence experts (scoring 4) believe the lack of pregnancy-specific data is not a critical limitation and that current approaches are scientifically sound
• Lower confidence experts (scoring 2-3) emphasize that mice generate more reactive epoxide metabolites than humans, and that pregnancy-related physiological changes may alter these species differences
• Experts disagree on whether these uncertainties represent a significant limitation in risk assessment

Expert 5 specifically notes that fetal metabolism of BD appears minimal, suggesting maternal metabolism is the key factor, while Expert 1 highlights concerns about maternal-fetal transfer dynamics during pregnancy.

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ANSWER EXPLANATIONS

Expert 1 | 2 |

• Mice generate reactive epoxide metabolites more than rats or humans which suggest that mice have higher sensitivity. Extrapolating a fixed non-pregnant rodent□human ratio across gestation ignores how those differences may change/multify through maternal-fetal transfer during pregnancy. The US EPA's current draft (2004) considered fetal body-weight data for BMD modeling; but analogous human developmental toxicokinetics (TK) data are lacking. ATSDR noted that PBPK models exist but are limited for key metabolites; default TK ratios could mis-characterize human health risk because mice are "many-fold more sensitive" due to metabolic activation of BD to reactive metabolites (DEB) .Therfore, uncertainty will increase when one considers physiology. and metabolic aspects during pregnancy of humans. Although, EPA's 2024 draft hazard/BMD documents identify the correct endpoint of toxicity but still rely on animal (mouse) data. For 1,3-butadiene exposure and the fetal-body-weight endpoint, substituting a non-pregnant rodent□human internal-dose ratio to estimate pregnant animal□human internal-dose ratios is fine but I will rate that the overall confidence is low for estimating the ratio of internal doses for pregnant animals and humans for the fetal body weight changes, since pregnancy-specific PBPK + adduct biomonitoring information in humans is not cuurently available.

Expert 2 | 4 | Data from pregnant animals and humans would be preferable, but I don't think it is critical. The document notes these uncertainties in using data from non-pregnant subjects: "but it is recognized that there are changes during pregnancy (e.g., increases in volume of distribution, changes in P450 and GST activity) that can have quantitative impacts on these KEs [1-3]."
Data to address these uncertainties would be nice to have, but their lack does not preclude a scientifically sound assessment using the information at hand.

Expert 3 | 3 | Metabolism changes during pregnancy but most changes are quantitative rather than qualitative. The degree of quantitative change from nonpregnant to pregnant is likely not the same in humans and rodents, so the ratio will vary. I think it is still somewhat useful extrapolation method, but confidence is not high.

ANSWER EXPLANATIONS, CONTINUED

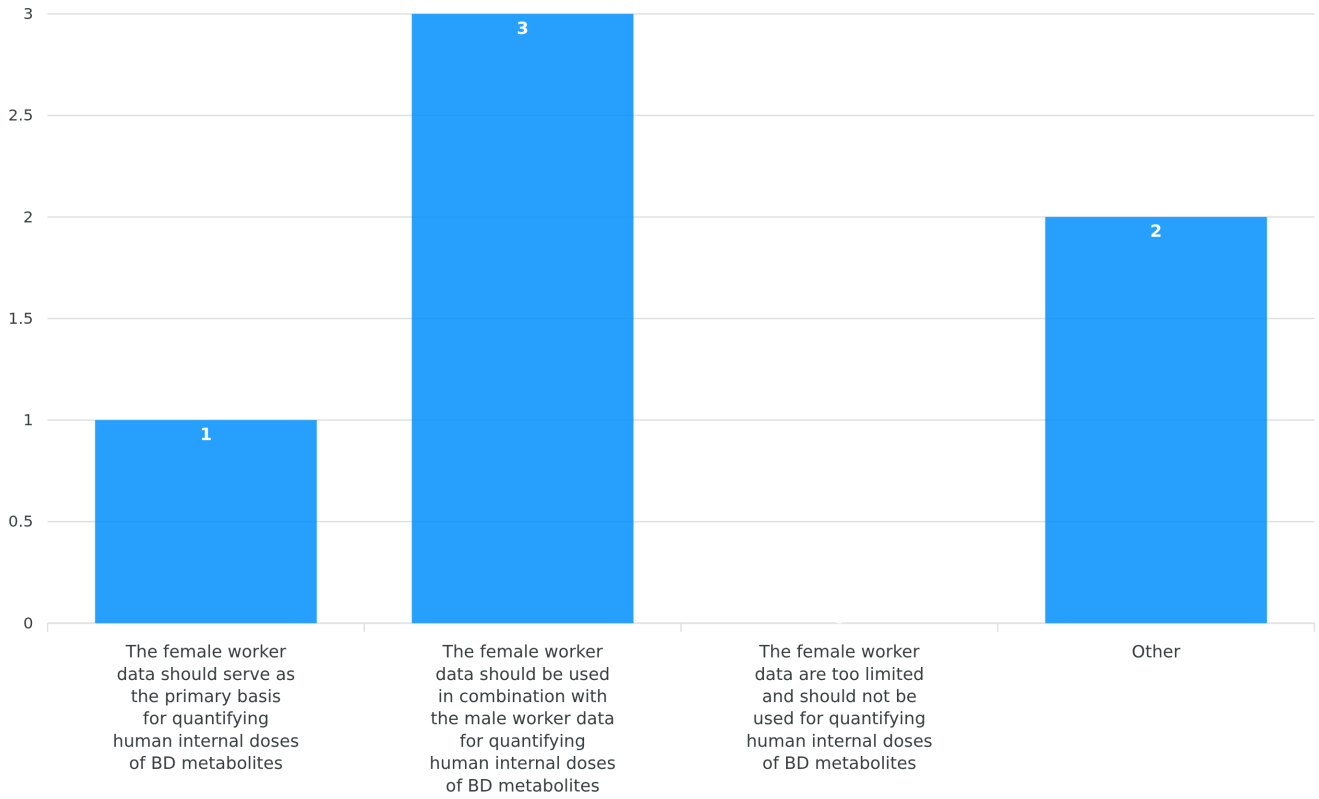
Expert 4 | 2 | See response to Question 4.2.

Expert 5 | 4 | There are data showing that the fetus does not metabolize BD which indicates that the dam is responsible for the generation of the epoxide metabolites. Use of the data from nonpregnant rodents and humans assumes that any changes during pregnancy that affect internal dose are similar in rodents and humans. Data for acetaminophen and its conversion to N-acetyl-p-benzoquinone imine (primarily via CYP2E1) apparently changes only slightly during pregnancy in women. However, it is not known whether this is also true for the conversion of BD which introduces some uncertainty.

Expert 6 | 4 | There are no data that indicate that pregnancy can alter rates of metabolism for BD or its metabolites that I know if. The use of data from non-pregnant animals and humans is addressed as an uncertainty, and appropriately so. It is not a limiting uncertainty. The use of a PBPK model developed, validates and implemented for pregnant animals and humans would add a level of specificity to the assessment, but it must be recognized that doing so would also add further uncertainty specific to PBPK models, including the human variability of values for parameters, and the uncertainty of estimating values for parameters that cannot be measured, etc. The passages around lines 993-999 should be revised to indicate that using a PBPK model is not required under EPA's DDEF guidance, and that empirical data from classical PK models, like those values used in this assessment are most certainly deemed by the DDEF guidance as a valid approach for developing DDEF values. There is no value in mentioning this use as a "limitation", because it is not such.

As noted in Section 4, there are recently published hemoglobin adduct for female workers that could be used to support interspecies extrapolation calculations, but the data have limitations. How should the data for female workers be used to support quantifying species differences for BD's noncancer endpoints (premature ovarian failure, reduced fetal body weight gain)?

Results | 6 answers



The expert panel generally agrees that female worker data should be combined with male worker data for quantifying human internal doses of BD metabolites, rather than used alone. Three experts (1, 2, and 3) explicitly recommend this combined approach.

Key points of agreement:

• While female-specific data is relevant for the noncancer endpoints in question (premature ovarian failure, reduced fetal body weight gain), most experts believe the combined dataset provides more robust estimates
• The Georgieva et al. (2025) study shows potential gender differences in hemoglobin adduct formation, but has limitations including small sample size and limited exposure measurements
• Several experts note that the magnitude of difference between approaches is relatively small compared to the overall species differences

Points of disagreement:

• Expert 6 recommends using female worker data as the primary basis, arguing that since the toxic effect is observed in pregnant females, data from the affected sex is most valid
• Experts 4 and 5 suggest presenting multiple approaches (female-only, male-only, and combined) for transparency, noting uncertainty about whether observed gender differences are real or artifacts

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ANSWER EXPLANATIONS

Expert 1 | The female worker data should be used in combination with the male worker data for quantifying human internal doses of BD metabolites | The recent study (Georgio et al 2025) in female workers confimed measurable DEB adduct formation but at levels consistent with much lower levels compared to mice or rats at comparable BD exposures. Note that it is a cross sectional study that includes non-occupationally exposed controls with a detectable pyr-Val (N,N-(2-3- dihydroxy-1-4 butadiel) valine and had limited personal exposure measurements, and lack of information pregnancy status which may increase quantitative uncertainty.The data show that DEB/ EB/ EBD metabolite levels in exposed adult women , but due to study design and context , they should not be used alone to adjust default uncertainties or to determine/ estmate point of departures (PODs).The use of hemoglobin adducts from female workers alone may not be a replacement for default interspecies factors supporting sensitivity analyses for BD noncancer endpoints. A practical approach could be a dose-metrics by enpoints that may yield a conservative and defensible results . DEB Hb adducts is lkely to drive noncancer endpoints across -species (premature ovarian failure and reduced fetal body weight gain) in mice more efficiently due to DEB Hb adduct formation differences than humans. Given some limitations of the female worker data and uncertainties to serve as the primary basis for quantifying human internal doses of BD metablites supports quantifying species differences of for BD's noncancer endpoints. I also suggest that the female worker data should be used in combination with male worker data for quantifying humam internal doses of BD metabolites, with clear a description of strengths and weaknesses and with a clear short description of uncertaintis.

Expert 2 | The female worker data should be used in combination with the male worker data for quantifying human internal doses of BD metabolites | There appear to be female vs. male differences in hemoglobin adduct formation as reported in Georgieva et al (2025). However, I would not recommend solelyusing data from female workers. Generally speaking, a larger dataset offers advantages over a more limited one. That females may produce fewer BD hemoglobin adducts (particularly at larger exposure) is noted in the latest draft of the document as well as in the primary reference. Georgieva et al (2025) also state the following: "These findings suggest that human males may be at a higher risk for BD-induced toxicity at elevated exposure levels. However, it is essential to consider that previous studies in rodents have indicated females may be more susceptible to BD-induced carcinogenesis based on metabolism, mutagenesis, and tumorigenesis data."

ANSWER EXPLANATIONS, CONTINUED

Expert 3 | The female worker data should be used in combination with the male worker data for quantifying human internal doses of BD metabolites | Female exposures are what drive the noncancer endpoints of concern, and there seem to be some differences in internal doses with similar exposures in females and males. However, given that the differences are not large, using both sexes should give a more robust estimate. If it's a bit off, it will lean toward a more conservative estimate, which is preferred.

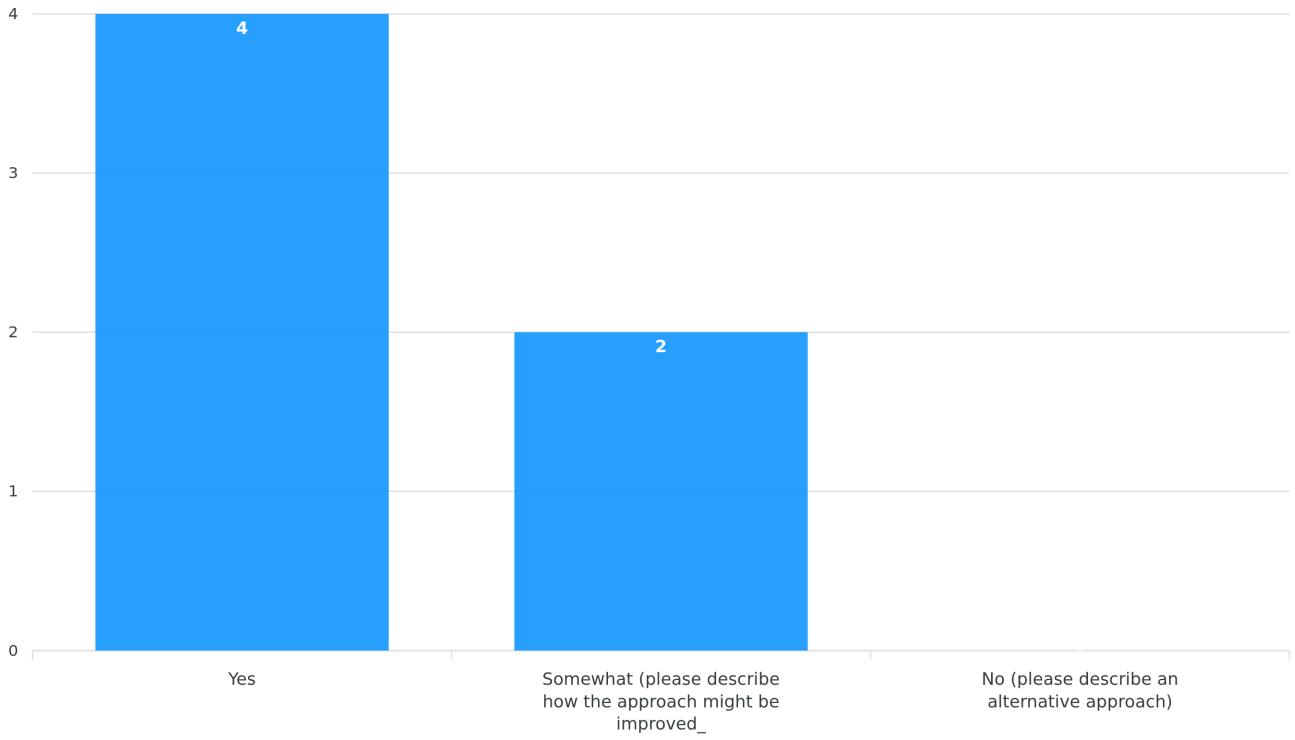
Expert 4 | Other | Due to the acknowledged limitations, it is difficult to ascertain whether or not the difference between males and females is real or an artifact of the study protocol. That said, I think that it's always helpful to present a range of derivations and their associated limitations to inform risk managers.

Expert 5 | Other | Georgieva et al. (2025) reported a potential gender difference. However, other studies have shown slight gender differences in metabolism in rodents and humans, which would not result in a decrease in pyr-Val. It is therefore uncertain whether the apparent gender difference is due to unreported nonoccupational exposures, small sample size etc. Similarly, using the male only data introduces similar uncertainties. Given this uncertainty, it may be appropriate to present the data in three ways: the female only, the male only, and the combined gender data. The magnitude of the differences is not great so why not just be transparent. Either way, the DDEF is very small.

Expert 6 | The female worker data should serve as the primary basis for quantifying human internal doses of BD metabolites | As indicated in the revised MOA description document, the toxic effect is observed in pregnant females. Because there are impactful sex differences in a Key Element of the MOA, the most valid approach seems to use the data for the affected sex. There are no such data from pregnant females. The document does acknowledge some difficulties with the female human data including some nonlinearities, and clearly indicates reliance on the most conservative interpretation of the data on adduct formation in females. As shown in Table 8, the impact of relying on female human exclusively versus male or combined human data results in a roughly four-fold difference in DDEF values. While this may seem like a substantial difference, the magnitude of the quantified DDEF value (EFAK less than or equal to 0.01) should be taken into account - a move from default of this magnitude is unprecedented. A demonstration of linearity in mice around the point of departure concentration would add confidence to the description of the approach in subsequent documents. Likewise, acknowledging the assumption of linearity of human data at increasing exposure concentrations would also be a valuable addition.

In Section 4, an approach was described to provide a conservative estimate of incremental adduct burdens in female workers and avoiding the calculation of a negative number. Is this approach appropriate?

Results | 6 answers



The experts generally agree that the approach described in Section 4 for providing a conservative estimate of incremental adduct burdens in female workers is appropriate. Four experts answered "Yes" while two answered "Somewhat."

Areas of agreement:

- The approach is conservative and health-protective
- Using the upper 90% CI for exposed workers and lower 90% CI for controls is reasonable given the limitations
- Calculating a negative number would not make sense in this context

Concerns raised by experts who answered "Somewhat":

- The approach is "unsatisfying but better" than alternatives (Expert 3)
- Limited data availability may make regulatory acceptance difficult (Expert 4)
- Both experts who answered "Somewhat" noted they couldn't suggest better alternatives with the current data

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ANSWER EXPLANATIONS

Expert 1 | Yes | In risk assessment context, a conservative estimate usually means health-protective upper bound, not a point estimate. There are multiple sources of uncertainty recognized and considered in the DDEF values as calculated and described in section 4 , and presented in Table 8 of the document.The
limitations correctly noted for the female worker data contributing uncertainty to DDEF values. An approach as described in section 4 provide a conservative upper bound estimate for these data as proposed for the incremental adduct burdens and presented in the DDEF calculations is relevant and makes sense to me. In absence of a robust physiologically-based pharmacokinetic (PBPK) model for supporting interspecies extrapolations is not available, the DDEF calculations based upon a simpler approach using hemoglobin biomarkers to quantify species differences in the internal doses of BD metabolites in mice, rats and humans is reasonable and practical. The use of hemoglobin adducts for BD is consistent with the US EPA's practice in the health risk assessment of other chemicals (e.g.,USEPA's IRIS assessment for acrylmide).

Expert 2 | Yes | It looks good to me, but this is out of the realm of my expertise.

Expert 3 | Somewhat (please describe how the approach might be improved_ | The levels in the control and exposed females are not really distinguishable, likely due to a combination of biological and technological variability. Using a negative number does not make sense, and the use of the upper 90%bound of the exposed and lower 90% bound of controls is an unsatisfying but better approach. I don't have a better approach short of getting more data in exposed females.

Expert 4 | Somewhat (please describe how the approach might be improved_ | Given the study observations, it's difficult to envisage an alternative approach to meaningfully interpret the data (save, perhaps, for choosing different percentiles). That said, it's difficult to envisage the derivation being acceptable to regulatory agencies, given the limited availability of relevant data and the associated need to "interpret" the results through reliance on characterization of the variability in the data.

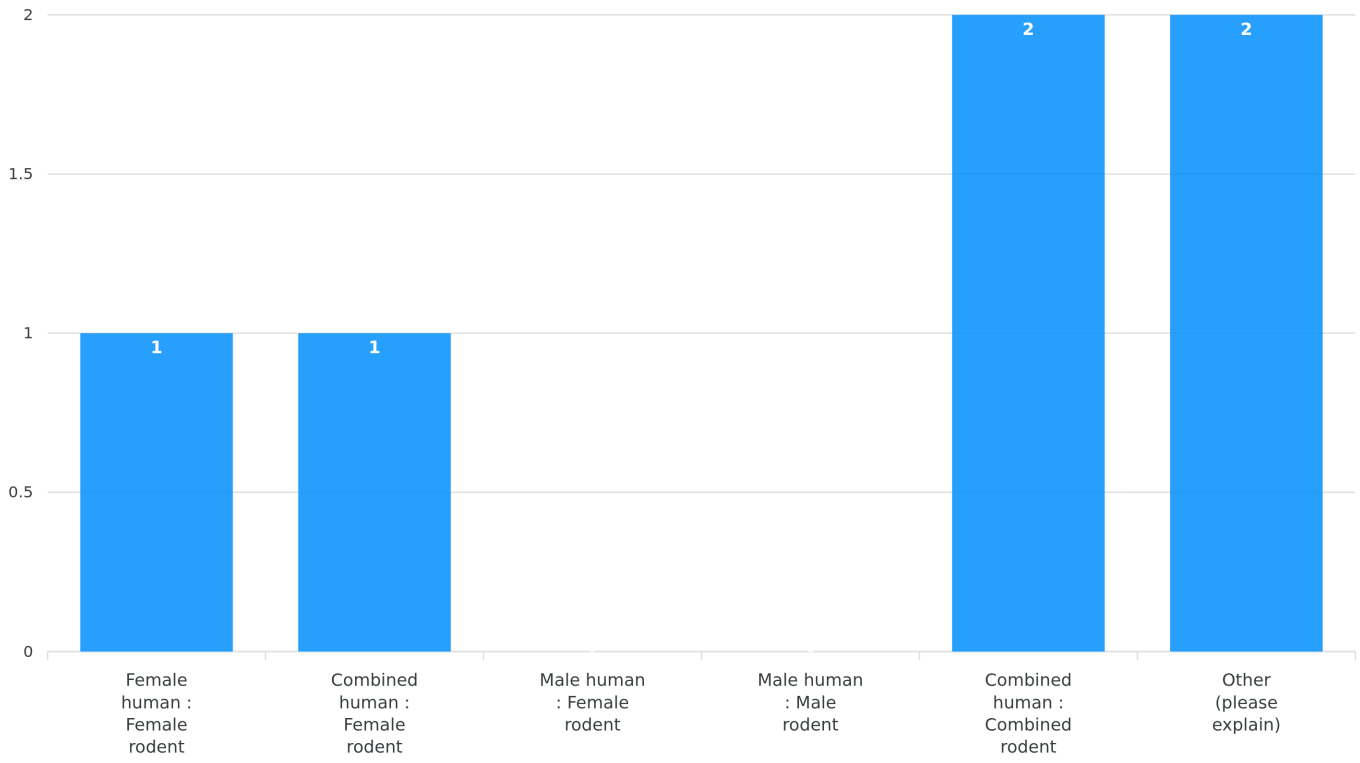
Expert 5 | Yes | Yes. It is not known why the female workers would have a lower value than the controls in the Georgieva et al. (2025) study; it might be due to unreported exposures, small sample size, etc. Therefore, it is appropriate to use a conservative estimate between the difference between the upper 90% CI value for the arithmetic mean pyr-Val in exposed workers and the lower 90% CI value for the arithmetic mean pyr-Val in control workers.

ANSWER EXPLANATIONS, CONTINUED

Expert 6 | Yes | It is appropriate in that it is conservative.

In Table 8, potential DDEF values for application to noncancer risk assessment for BD are provided that consider the use of different data sets (male, female, or combined). What is/are your preferred basis for calculating DDEF values (please explain)

Results | 6 answers



Expert opinions on the preferred basis for calculating DDEF values for butadiene (BD) noncancer risk assessment show both agreement and divergence:

• Combined human : Combined rodentwas supported by Experts 1 and 2, with Expert 1 citing the Georgieva et al. (2025) hemoglobin adduct data that includes female workers, while Expert 2 noted minimal male-female differences in human-mouse comparisons.
• Female human : Female rodentwas preferred by Expert 6, specifically female human to female mouse, arguing that since the endpoint is female-specific and mice are the most sensitive species, this comparison is most appropriate.
• Combined human : Female rodentwas favored by Expert 3, who noted that while female data in both species would be ideal, human female data alone are inadequate, but female rodent data are sufficient.
• Experts 4 and 5 suggested presentingmultiple DDEF valuesfor transparency, with Expert 5 recommending three specific comparisons (female human:female rodent, combined human:female rodent, and male human:female rodent) to address limitations in using male data for female endpoints.

Most experts acknowledged the importance of female-specific data given the nature of the key endpoints (ovarian atrophy and fetal body weight changes), with the main disagreement centering on how to handle the limited female human data.

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ANSWER EXPLANATIONS

Expert 1 | Combined human : Combined rodent |
I agree and support noncancer dose-response assessments for BD, as very well articulated by Kirman et al. (2022) based upon the use of hemoglobin adduct data to quantify species differences in internal dose estimates for BD metabolites. Recently published (Georgieva et al. 2025) provide a more complete set of hemoglobin adduct data that includes the data from Study 1 (only male workers), but also includes hemoglobin adduct data from a second study in Czech Republic workers (Study 2; Vacek et al., 2010), including pyr-Val data. This Study 2 is considered important since it also includes data collected from female workers , which could be considered more relevant for the key noncancer endpoints of BD (i.e., ovarian atrophy and fetal body weight changes). The species differences in DEB internal dose and adducts (pyr-Val adducts) as a function of BD exposure in mice, rats, and humans (Georgieiva et al; 2010 , 2025) could be estimated and used in dosimetric adjustments appying a DDEF approach (U.S. EPA, 2014), given a good understanding of the toxicokinetis and exposure -response relationship associated with the noncancer effects upon BD exposure. Please note that EPA (2024) did not apply a DDEF because the metabolite data (DEB vs EBD in humans) and considerd it to be too uncertain and further stated that any plausible DDEF values would likely to be less protective with more uncertainty.This conclusion of the US EPA needs to be systematically addressed in order to advance the proposed DDEF approach and values.

Expert 2 | Combined human : Combined rodent | If one stays within a human to mouse comparison, the male female differences are not big. What would be the reason to compare male human to female mouse?

Expert 3 | Combined human : Female rodent | Female data in both species would be preferred, but the human female data are inadequate to use alone. The female rodent data are adequate, so I see no advantage to combining female and male rodent data. The DDEFs are close either way.

ANSWER EXPLANATIONS, CONTINUED

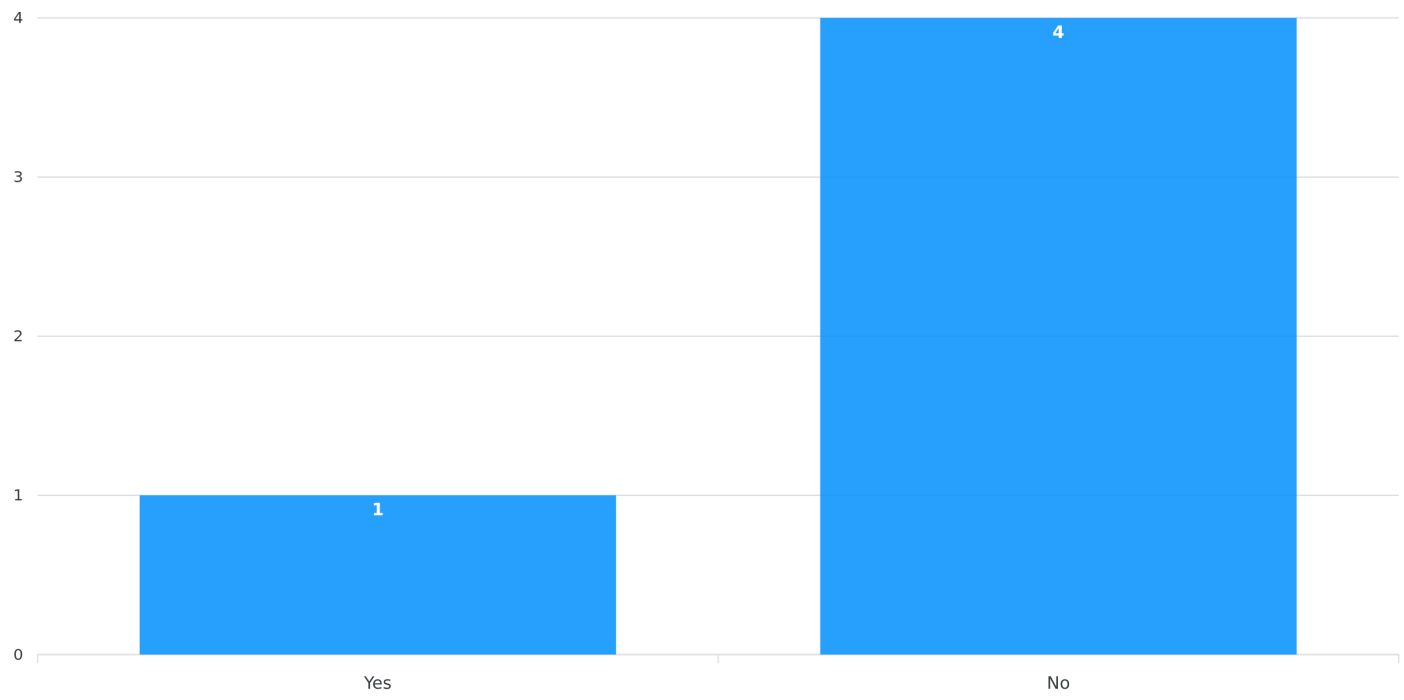
Expert 4 | Other (please explain) | I think that it's important to characterize all of the potential DDEF values to provide an idea of their variability, depending upon various selection considerations. Consistent with the development of CSAF/DDEF for gender specific effects, there is precedent for restricting the DDEF derivation to females (i.e., female human: female rodent). However, given the limited availability of data for females, derivation based on combined human: female rodent is likely well justified.

Expert 5 | Other (please explain) | As stated in question 4.6, I would present 3 DDEF values to be entirely transparent about the limitations in using male data for female endpoints, and the uncertainties with the Georgieva et al. (2025) data. I would not use data for male rodents since the endpoints are for females. I would simply present 3 DDEFs: female human to female rodent; combined human to female rodent; and male human to female rodent. The values are not that different.

Expert 6 | Female human : Female rodent | Specifically, I prefer female human to female mouse. The mouse is the most sensitive species, the EPA risk assessment would not choose the rat as the species demonstrating the point of departure for risk assessment, per EPA risk assessment guidance. Because the endpoint is specific for the female of a species, and because data are available for the female of the species, these data are those that are most appropriate for DDEF calculation.

Based upon the extent of changes to the mode of action summary document, and the new charge questions provided in Round 3, would you like to extend this review to include an additional round comment/debate?

Results | 6 answers



The majority of experts (4 out of 5) do not believe an additional round of review is necessary for the mode of action summary document. Expert 1 was the only one who explicitly supported extending the review, citing the need to develop scientific consensus on the changes made in Round 3.

Among those who voted "No," there were varying degrees of certainty:

• Expert 6 indicated their "No" was closer to a "Maybe," suggesting they would value hearing additional perspectives from other experts
• Expert 5 felt the document improvements were sufficient and did not influence their overall conclusions
• Expert 3 noted reasonable agreement among experts throughout the process
• Expert 4 expressed concern that important observations from earlier rounds were not being addressed and questioned the efficiency of the current review format

While most experts did not support another round, there was an underlying theme that considering diverse perspectives remains important, even as they felt the document had progressed sufficiently.

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ANSWER EXPLANATIONS

Expert 1 | Yes | In my view , the extent of changes made to the mode of action summary document (Round 3) are well described and relevant in response to the reviewers comments but I am not sure if other experts have same opinions ,if any. Therefore, we should consider them and resolve or minimize differnces of opinions and attempt in develping a scientific consesus as much as possible.

Expert 2 | *Did not answer* | That is not really for me to say, as the charge questions largely pertain to areas that are not my particular expertise. But I did see some substantial changes in the document, and I (reluctantly) suggest that a comment / debate round would be useful.

Expert 3 | No | The thinking among the experts has been in reasonable agreement throughout this process, and I don't think an additional round will change that. If others feel differently, I'm happy to participate.

Expert 4 | No | I have the sense that some of the important observations from earlier rounds are not being addressed. I'm also not so sure that the current round of questions added materially to those in earlier rounds. Additionally, I sense that continued review in the Scipinion format is not the most efficient manner to address the outstanding issues.

Expert 5 | No | The addition of the metabolism data and the new figures and tables greatly improve the document. They do not influence my overall conclusions regarding ovarian toxicity. The change in the proposed MOA for body weight also does not influence my overall conclusion about this MOA. It simply highlights the importance of metabolism and the uncertainties in the intervening KEs between the current KE 3 and 4. However, one does not need a complete MOA to justify a DDEF. It is clear that in both cases, metabolism is the key to the species differences. The uncertainties in the appropriate data for the generation of the DDEFs can only be resolved by additional data. The choice in which dataset to use is really a choice of whether one needs a single DDEF or whether one can use a range to be fully transparent.

ANSWER EXPLANATIONS, CONTINUED

Expert 6 | No | My response is No, but only because there is not a "Maybe" choice. While I believe that the present document has no rate-limiting difficulties, it would be helpful to hear the opinions of other experts. It is possible that there are opinions of those experts with different perspectives and experiences (particularly to question 4.3) that should be considered by the group. Only an evaluation of the comments en masse will determine whether this is the case. If so, then an additional round may be valuable.

April 30, 2025

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Re: TSCA Risk Evaluation of 1,3 Butadiene

Dear Dr. Lowit and Ms. Muneer,

The American Chemistry Council[1] (ACC) 1,3-Butadiene TSCA Risk Evaluation Consortium (Consortium) appreciates the opportunity to respond to the April 21, 2025, email request from Ms. Muneer's office to provide information regarding certain issues raised during the meeting of Science Advisory Committee on Chemicals (SACC) to review the TSCA risk evaluation of 1,3-Butadiene (1,3-BD). The Consortium strongly urges the EPA to continue its crucial work of maintaining and improving the integrity, transparency, and effectiveness of the TSCA Risk Evaluation process. Throughout the Agency's risk evaluation process, the Consortium has been committed to open communication and sharing of information. The Consortium has and will continue to be committed to providing the best available data to the Agency so that the final risk evaluation of 1,3-BD is based on the best available science. To this end, the Consortium sponsored studies that were conducted under the highest scientific standards and the Consortium stands by these studies.[2] In addition to all of the information that the Consortium has provided thus far, the Consortium hopes that the following information will assist EPA as it finalizes the risk evaluation.

[1] The American Chemistry Council (ACC) represents the leading companies engaged in the multibillion-dollar business of chemistry. ACC members apply the science of chemistry to make innovative products, technologies and services that make people's lives better, healthier, and safer. ACC is committed to improved environmental, health, safety, and security performance through Responsible Care®; common sense advocacy addressing major public policy issues; and health and environmental research and product testing. ACC members and chemistry companies are among the largest investors in research and development, and are advancing products, processes, and technologies to address climate change, enhance air and water quality, and progress toward a more sustainable, circular economy.

[2] Human health risk assessment for exposures to 1,3-butadiene in the United States with input from an independent science advisory panel - PubMed

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The Consortium members are committed to responsible operations and to the safety and health of the communities in which they operate. Promoting the safe use of the essential products of chemistry is a shared responsibility of manufacturers, the government, and those who use or sell chemical products. Under TSCA, regulations must be based on the best available science and real-world exposure scenarios.

A. Confidence in the Exposure Estimates- The Macaluso et al. (2004) Study

We would like to address two issues regarding the SACC' s discussion of the exposure estimates for the styrene-butadiene rubber (SBR) cohort of Macaluso et al. (2004). First, members of the SACC were incorrect in referring to Macaluso et al. (2004) as relying upon modeled exposure estimates and that previous exposure assessments (i.e., prior to the 2004 paper) of the SBR cohort as relying upon industrial hygiene measurements. As noted in Macaluso et al. (1996), which served as the basis of the historical exposures to the SBR cohort used in EPA's 2002 assessment of 1,3-BD's cancer potency:

> *"The present investigation is the first in which mechanistic models have been used systematically to obtain exposure estimates for a large number of individual workers."*

Therefore, EPA's past estimates of 1,3-BD's cancer potency are also based upon modeled historical exposures to the SBR cohort.

Second, the SACC's discussion of SBR cohort exposure assessment was incomplete. For example, there are significant limitations of the available IH data for the SBR cohort, which were not mentioned by SACC members but were specifically discussed in Macaluso et al. (2004):

> *"Industrial hygiene (IH) data were not suitable for estimating exposure for several reasons. Plant data were limited, as IH monitoring programs began only in the late 1970s at most plants and were not necessarily designed to describe average workplace exposure levels. Some of the IH measurements were made to assess "worst case" exposure scenarios or to document problems, and overestimated average exposure levels. On the other hand, IH sampling done after the installation of new equipment or after scheduled maintenance may have underestimated average exposure conditions. Thus, it is difficult to interpret the published summaries of these data. Furthermore, whereas the design of the two NIOSH surveys was adequate, only a select group of work areas and jobs were evaluated, and the number of measurements taken for specific jobs was relatively small."*

In addition, there are two addition publications by the University of Alabama that increase confidence in the exposure estimates of Macaluso et al. (2004), namely an exposure validation study (Sathiakumar et al., 2006) and an exposure uncertainty analysis (Graff et al., 2009).

In the validation study, Sathiakumar et al. (2006) compared the modeled estimates of exposure to 4978 measurements initiated in 1977. On average, the modeled estimates were slightly lower (~10%) than the measured values, which indicates that the application of the modeled data in estimating cancer potency

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serves to underestimate potency by the same margin on average. For risk assessment purposes, a 10% difference is considered to be a relatively small source of uncertainty.

In the uncertainty assessment, Graff et al. (2009) created 1000 sets of 1,3-BD exposure estimates using job-exposure matrices consisting of exposure values that corresponded to randomly selected percentiles of the approximate probability distribution of plant-, work area/job group-, and year-specific 1,3-BD concentrations. The authors then examined the impact of this uncertainty on resulting relative rates for leukemia. For the relative rate of leukemia, the minimum and maximum values determined based upon uncertainty in the exposure estimates were within 20% of the mean relative rate for the first quartile (considered as a surrogate for a corresponding point of departure value for leukemia). For risk assessment purposes, a 20% difference is considered to be a relatively small source of uncertainty.

In addition, Macaluso et al. (2004) summarizes the limitations of the industrial hygiene data for 1,3-BD: "Industrial hygiene (IH) data were not suitable for estimating exposure for several reasons." Plant data was limited, as IH monitoring programs began only in the late 1970s at most plants and were not necessarily designed to describe average workplace exposure levels. Some of the IH measurements were made to assess "worst case" exposure scenarios or to document problems and overestimated average exposure levels. On the other hand, IH sampling done after the installation of new equipment or after scheduled maintenance may have underestimated average exposure conditions. Thus, it is difficult to interpret the published summaries of these data. Furthermore, whereas the design of the two NIOSH surveys was adequate, only a select group of work areas and jobs were evaluated, and the number of measurements taken for specific jobs was relatively small.

B. Consideration of Sensitive Subpopulations

Existing risk assessments address sensitive subpopulations due to elevated CYP2E1 activity (e.g., due to alcohol consumption, obesity). For example, the SBR cohort is sufficiently large (n=22,785; Sathiakumar et al., 2021a,b) such that these factors are well represented by the population of workers used to estimate the cancer potency. In addition, the use of hemoglobin adduct data to account for species differences in the toxicokinetics of BD (Kirman et al., 2022) relies upon a population of workers from the Czech Republic, which while smaller than the SBR cohort (n>150) is still expected to provide adequate coverage for these factors, particularly since the Czech Republic has (now and historically) one of the highest per capita alcohol consumptions in Europe according to the WHO's annual European Health Report (https://www.who.int/europe/publications/i/item/WHO-EURO-2025-10668-50440-76183).

The absence of a trend in the hemoglobin adduct data for 1,3-BD's diepoxide metabolite (pyr-val) in female Czech Republic workers may be attributed to a number of factors including: (1) contributions from and variation in exposure pathways outside the workplace (e.g., pyr-Val biomarker levels in exposed female workers are not elevated over background levels) ; (2) exposure variation and uncertainty (e.g., air sampling not capturing high-exposure events during the sampling window); (3) analytical variation; and (4) potentially higher variation in BD metabolism in women compared to men. Regardless of the presence or absence of a trend, the primary conclusion of these data is that they are approximately three

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orders of magnitude lower than that measured in exposed mice, and therefore the observation by SACC members does not impact the use of these data for interspecies extrapolation.

C. Consideration of Combined Leukemia and Bladder Cancer Risks

SACC members discussed the inclusion of other cancer types (e.g., bladder cancer, breast cancer) in the quantitative assessment. However, their discussion did not appear to include two important considerations. First, the members did not appear to recognize that a causal relationship between BD exposure and cancers other than leukemia has not been established, and therefore their inclusion in quantitative estimates of cancer potency is not warranted or supported. Second, the SACC's discussion did not recognize that as part of a sensitivity analysis (i.e., what if a causal relationship was assumed now or established in the future?), Valdez-Flores et al. (2022) specifically considered the inclusion of potential bladder cancer risks in an aggregate risk endpoint (leukemia and/or bladder cancer). As shown in Table 11 of this paper, the impact of including bladder cancer risk in the quantitative potency estimate is relatively small (i.e., less than a factor of 2), resulting in an approximate 7% change in the Cox regression slope (0.00002808 vs. 0.0002991) and an approximately 34% change in the lower confidence limit for the point of departure (0.0085 vs 0.0056 ppm), which reflects the impact of including bladder cancer in the life-table calculations in addition to the change in the slope value.

D. Flaring Operations

The Consortium agrees that flaring events are outside the scope of the TSCA risk evaluation. These events are covered by other Federal and State environmental agencies, such as the Texas Commission on Environmental Quality (TCEQ) and the Louisiana Department of Environmental Quality (LDEQ). Nevertheless, it is important to acknowledge that major manufacturing facilities producing 1,3-BD operate continuously, and controlled flaring of hydrocarbons is a necessary and well-established safety measure integral to maintaining safe plant operations.

* *

The Consortium appreciates EPA's consideration of this information as well as comments previously submitted to the OPPT and the SACC Dockets (Docket ID EPA-HQ-OPPT-2018-0451, Docket ID EPA-HQ-OPPT-2024-0425), and via the CDX platform. The Consortium is available to meet with EPA to provide additional clarity and context on technical issues in the letter and other questions that may arise as the Agency finalizes the 1,3-BD draft risk evaluation. If you have any questions, please contact me at (202) 249-6712 or at Neeraja_erraguntla@americanchemistry.com.

Sincerely,

Neeraja Erraguntla

Director, Chemical Products and Technology

cc: Brooke Porter, Sheila Healey, Kathy Dionisio, Tamue Gibson

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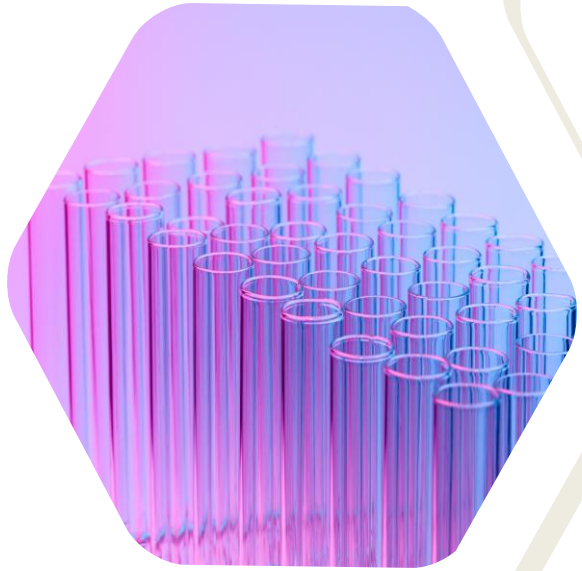
ACC's 1,3-Butadiene TSCA Risk Evaluation Consortium



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1,3-Butadiene TSCA Risk Evaluation Consortium - American Chemistry Council

SciPinion

The Collective Wisdom Company

Presentation to EPA on Final Risk Evaluation for 1,3-Butadiene

03/17/2026

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Overview

- OEVs Calculated for 1,3- Butadiene
  - EPA's Cancer-Based OEV = 0.11 ppm
    - Based upon leukemia and bladder cancer risk in exposed workers (Sathiakumar et al. 2021a,b)
    - BD Hazard Assessment was conducted using a cascade of conservative decisions leading to a worst-case epidemiology-based value
  - EPA's Noncancer-Based OEV = 0.17 ppm
    - Based upon fetal body weight changes in mice (Hackett et al., 1987)
    - Update on MOA/DDEF assessment

- *Note – There is a mathematical error in the cancer assessment*
  - *95%ile value CPH slope of 1.79E-03 for leukemia is incorrect and should be 1.67E-03 (refer to slide 13)*

Hazard Assessment is a Balance of Protective and Predictive Decisions

Animal data-based assessments are well-established with clear guidelines that in practice result in a balance of protective and predictive decisions (see slide 14) that avoid unrealistic, worst-case toxicity values

In contrast, epidemiology data-based assessments are less established and lack clear guidelines, and in practice result in the generation of worst-case toxicity values

The Lack of Epidemiology-Based Dose-Response Guidelines Results in "Worst-Case Assessments": 1,3-Butadiene Assessment is One Example

Decision Point	Nature of conservative decision	Impact on IUR
1) Data set	Trimmed data used; excluding data not justified	~4x increase
2) Endpoint	Inclusion of bladder cancer without causal association	~30% increase
3) Dose measure/ Covariate	High intensity tasks (HITs) Excluded as covariate	~2x increase
4) Dose response model	[Note - Only CPH model has been considered]	
5) Lag assumption	[Note - No impact]	
6) BMR	Benchmark response rate too high and results in point of departure at high end of range of observation	~20-30% increase
7) POD Confidence Limit	Use of 95% lower confidence limit	~60-80% increase
8a) Lifetable: Mortality vs incidence	Incidence used without consideration of appropriateness	~2x increase
8b) Lifetable: Lifetime definition	85 years used in lifetable instead of 78 years	~20-30% increase
8c) Lifetable: Work years definition	69 years used in lifetable instead of 40 years	~20-30% increase
9) Low-dose extrapolation	Low-dose linearity assumed based on genotoxicity	NA
10) ADAF application	Worker IUR: Not applicable; General population: Ignores evidence suggesting early life at lower risk	~70% increase for general population

- *Due to increased complexity, there are more decision points in epidemiology-based assessments than animal-based ones*
- *Ad hoc nature of epidemiology-based assessments (BD is one example) results in protective decisions made at all steps in the process (lack of predictive decisions)*
- *Epidemiology-based assessments like the one conducted for 1,3-butadiene result in a **worst-case** estimate of cancer potency rather than a realistic upper bound estimate*

SciPinion *Yellow = Protective Decision; White = neither protective nor predictive*

Recommended Changes Consistent with Best Available Science

Decision Point	Recommended Change	Impact on IUR
1) Data set	Recommend using full observational worker exposure data (including high exposure and unexposed) vs. trimmed exposure data	
2) Endpoint	Until a causal association between BD exposure and bladder cancer is proven, cancer potency recommended to be based on leukemia, and combined risk considered in sensitivity analysis/discussion	
3) Dose measure/ Covariate	High Intensity Tasks (HITs) excluded as covariate	~2x increase
4) Dose response model	NA	
5) Lag assumption	NA	
6) BMR	To remain consistent with standard practice when using animal studies and BMD guidelines, a BMR of 0.0001 is recommended since it falls at the low end of the range of observation	
7) POD Confidence Limit	Use of 95% LCL	~60-80% increase
8a) Lifetable: Mortality vs incidence	Incidence used without discussion of appropriateness compared to mortality	~2x increase
8b) Lifetable: Lifetime definition	A lifetime of 78 years is recommended as a default value, with other values (e.g., 70, 85) used to support sensitivity analysis/discussion	
8c) Lifetable: Work years definition	A work years of 40 years is recommended as a default value, with other values (e.g., 20, 62) used to support sensitivity analysis/discussion	
Low-dose extrapolation	Low-dose linearity assumed based on genotoxicity	NA
ADAF	Worker IUR: Not applicable; General population: Ignores evidence suggesting early life at lower risk	~70% increase for general population

By including a balance of predictive and protective decisions, an assessment results in a value that is protective but not worst-case, consistent with standard practices for exposure assessment and animal-based toxicity assessment

Yellow = Protective Decision; Blue = Predictive Decision; White = neither protective nor predictive

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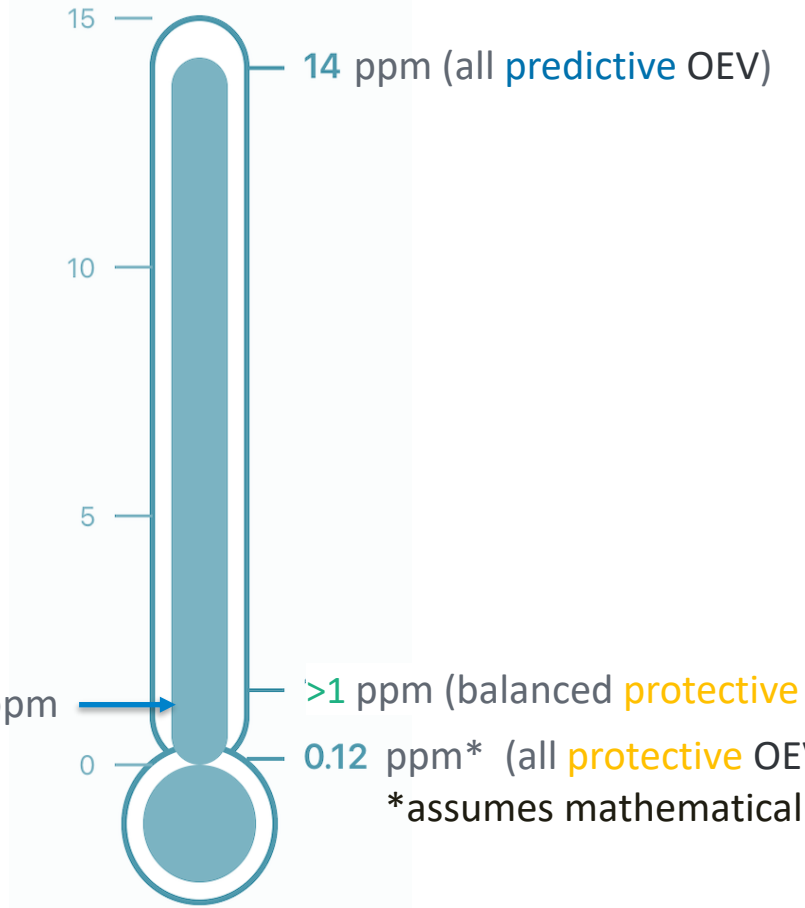
Impact of Predictive vs Protective Decisions on Resulting Inhalation Unit Risk (IUR) and Occupational Exposure Value (OEV)



Using a balance of protective and predictive decisions:

- IUR(worker) = ~0.00046 per ppm
- OEV = >1 ppm

Recommendation: Maintain compliance with the existing OSHA standard (Permissible Exposure Limit = 1 ppm)

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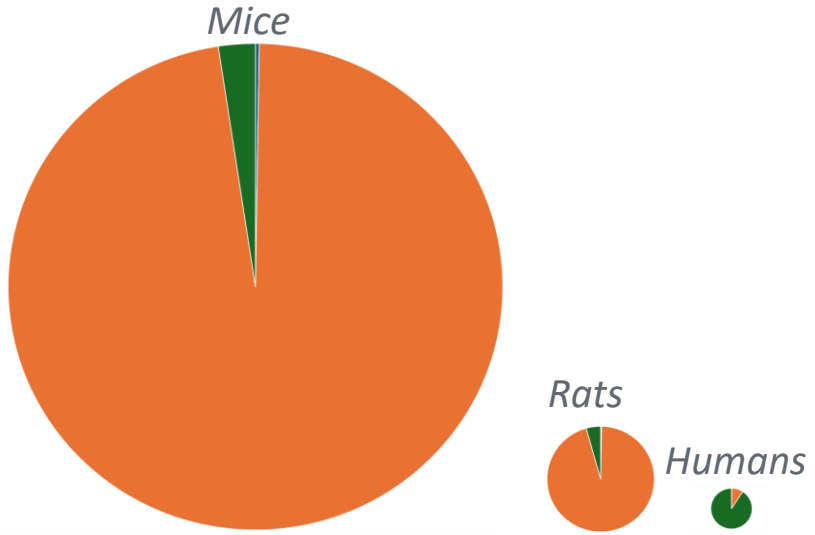
Update on BD Noncancer MOA/DDEF

- EPA Risk Assessment Team raised several concerns regarding the MOA/DDEF:
  - Lack of a PBPK model
  - Lack of data in pregnant humans/animals
  - Uncertainty in the MOA

- As a result of this, we specifically tasked our independent panel to consider the uncertainty associated with these items:
  - Even without a PBPK model for BD, the panel expressed high confidence in use of hemoglobin adduct data to quantify species differences in BD toxicokinetics
  - The independent panel expressed medium confidence in the dosimetry assumption of using the ratio of mouse:human internal doses under nonpregnant conditions to reflect the ratio of mouse:human under pregnancy conditions
  - While uncertainty and data gaps in the noncancer MOA are recognized, the independent panel expressed medium confidence in the noncancer MOA

Noncancer Assessment – MOA/DDEF Conclusions from the Independent Scientific Panel

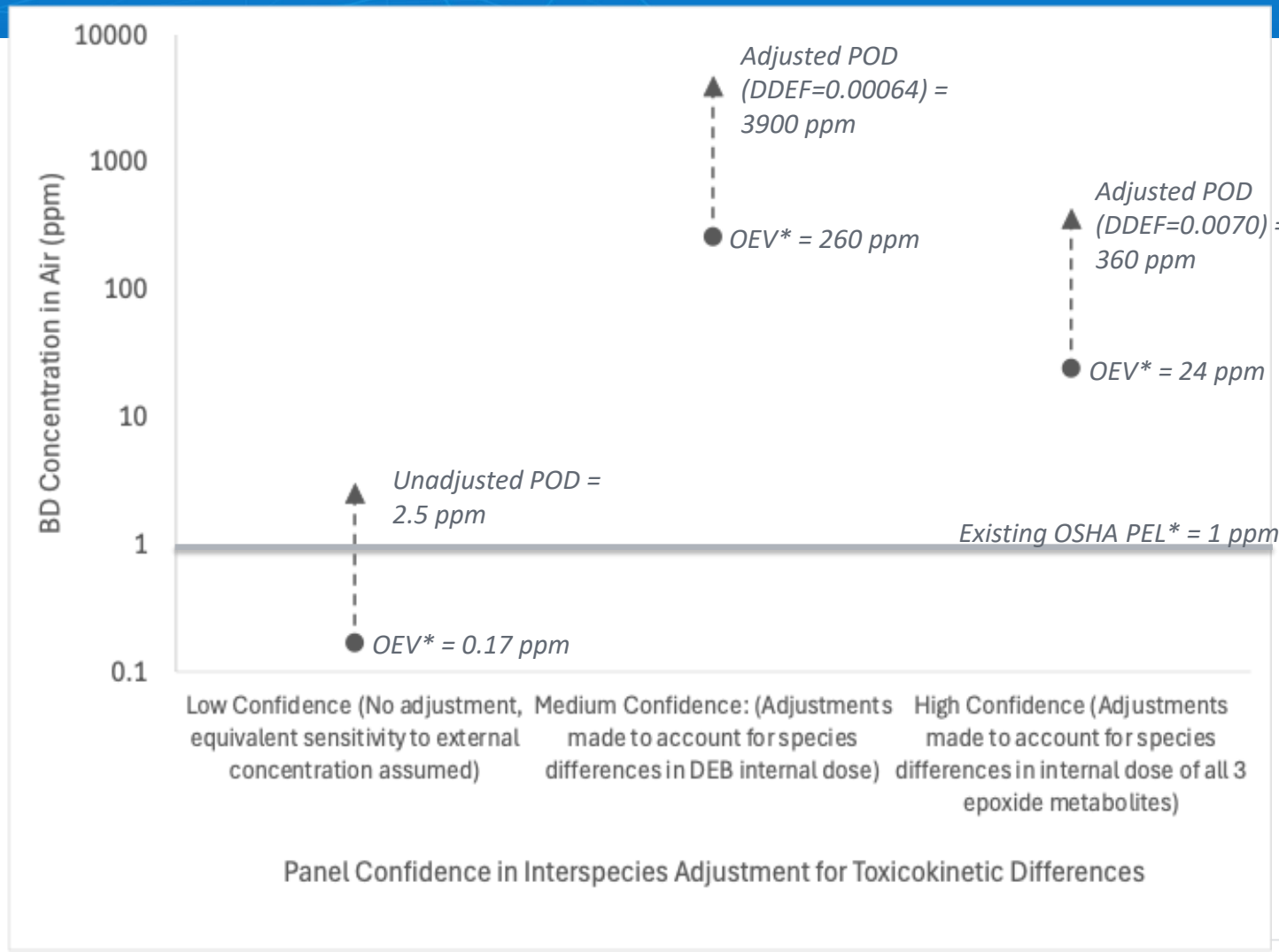
- ATSDR, ECHA: No value calculated due to concerns of overestimating human risk/uncertainty in species differences
- EPA TSCA: Species differences qualitatively recognized but not incorporated in the quantitative assessment --> worst case assumption
- Full quantitative approach with independent panel of subject matter experts' input on best science for MOA and DDEF calculations
  - Kirman et al. 2022: DDEF development
  - Kirman et al. 2025: DDEF application to risk assessment using an independent panel
  - Kirman et al. 2026 (publication in progress): independent panel used to evaluate MOA and DDEF refinement per new data(female workers)



Orange = diepoxide, DEB;
Blue =monoepoxide, EB;
Green = monoepoxide diol, EBD

Best science calls for quantitative consideration of well-documented species differences in metabolic activation

Implications for Independent Panel Dosimetry Recommendations to OEV Calculations for BD (Kirman et al., 2026)



- *By adopting a default policy approach for interspecies extrapolation that has low confidence, the resulting OEV of 0.17 ppm inaccurately suggests that the existing OSHA PEL of 1 ppm for BD is not protective of workers for noncancer endpoints*
- *In contrast, best available science approaches that have medium and high confidence suggest that the existing OSHA PEL of 1 ppm for BD provides a large margin of safety for noncancer endpoints*

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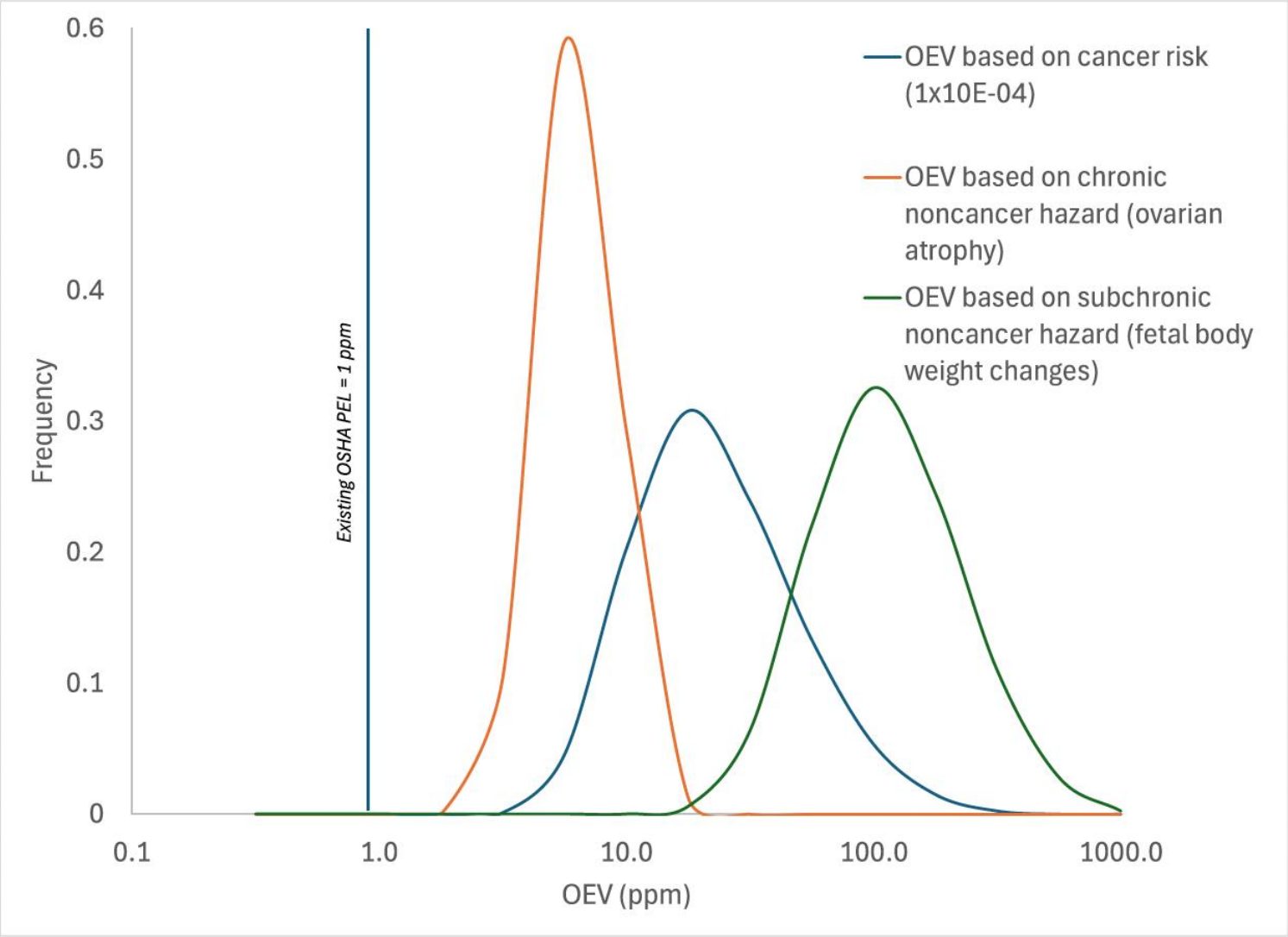
**OEV values reflect health-based values for noncancer endpoints, whereas the OSHA PEL value is a risk-management value that may consider other factors*

Monte Carlo Assessment of BD OEVs Based on Input from and Independent Advisory Panel (Kirman et al., 2025)



- Use of Monte Carlo methods to evaluate protectiveness of existing OSHA PEL
  - Avoids compounded protective decisions (i.e, have low probability)
  - Includes quantification of uncertainty/variation in DDEF values for interspecies extrapolation
- Result show that based on best science the existing OSHA PEL of 1 ppm is protective of cancer and noncancer effects of BD

APPENDIX

Mathematical Errors in Cancer Assessment

- In the final Risk Evaluation for BD 95%ile values for the Cox proportional hazards (CPH) slope were calculated from values published in Sathiakumar et al 2021:
  - Bladder calculations: 95%ile value of **5.56E-04 can be replicated**
  - Leukemia calculations: 95%ile value of **1.79E-03 is incorrect**
    - Value of 1.79E-03 appears in the lifetable calculation spreadsheet (tab 4, cells L29 and L36); the value and calculations are not included in the text (needed for transparency)
    - 95%ile should be 1.67E-03 instead
    - Impact is small; Corrected value yields an LEC01 ~2.2 ppm instead of 2.046 ppm; Worker IUR value of 0.0045 (per ppm) instead of 0.0049 (per ppm)
- Other errors in the lifetable spreadsheet for workers are present but do not have an impact the IUR

Example of Balancing Protective and Predictive Decisions: Standard Practice for Animal Data-Based Dose-Response Assessments

Decision Point	Standard Practice/Policy
Data set	Rely on most sensitive data set relevant to human health
Endpoint	Rely on most sensitive endpoint relevant to human health
Dose measure	Rely on dose measure that is consistent with MOA (not the one generating the lowest human equivalent concentration)
DR Model	Rely on the best fitting model (not the one generating in the lowest POD)
BMR	Rely on a BMR that results in POD near the low end of the range of observation that serves as a good point for extrapolating to lower exposure (not the one generating the highest slope value)
POD Confidence Limit	Rely on 95% LCL
Low-dose extrapolation	Linear or nonlinear based upon consideration of MOA
Adjustments (ADAF)	Rely on an assumption of early life susceptibility, particularly for genotoxic carcinogens

Yellow = Protective Decision; Blue = Predictive Decision

- *Standard practice using animal data results in IUR values that reflect a mixture of predictive and protective decisions at each step in the process*
- *Combining protective and predictive decisions results in protective, realistic upper-bound estimates of potency rather than worst-case estimates*

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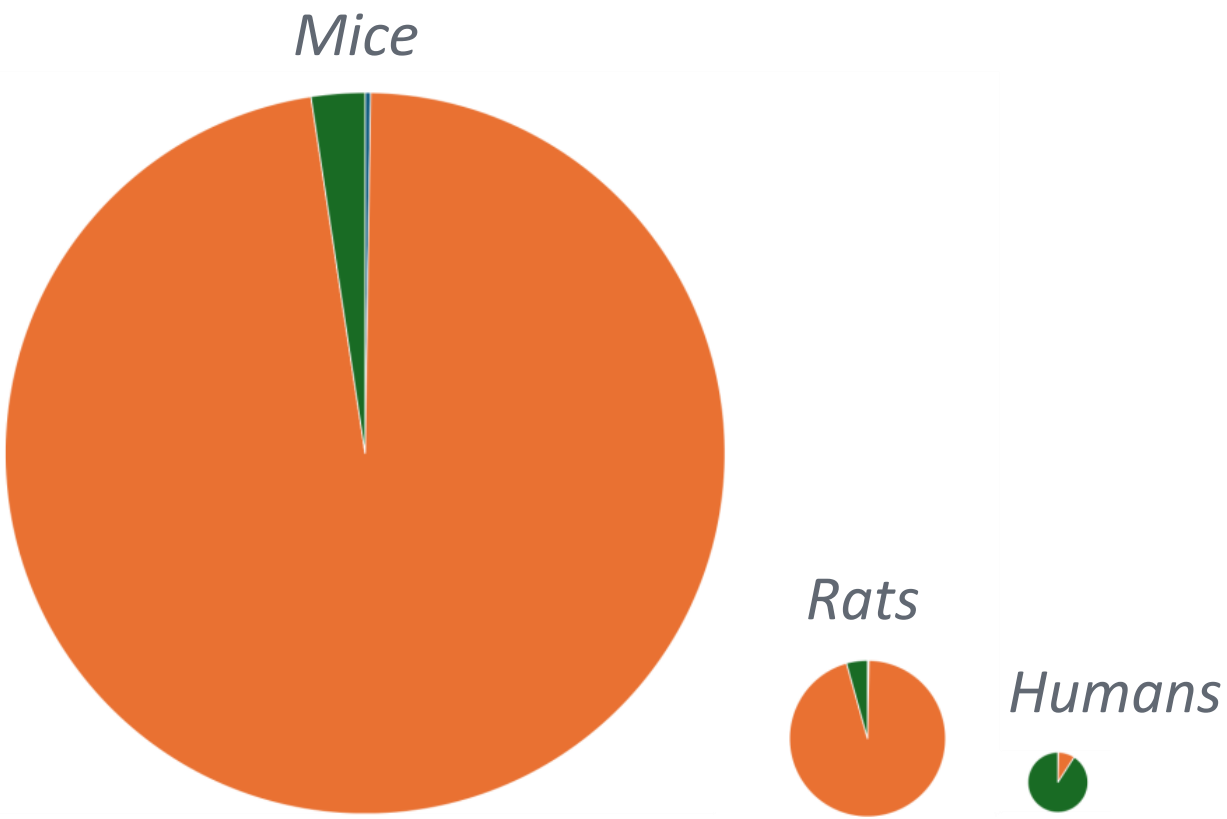
Noncancer Assessment: Ignoring well-documented species differences in metabolic activation results in the calculation of a worst-case OEV for BD that does not reflect best science

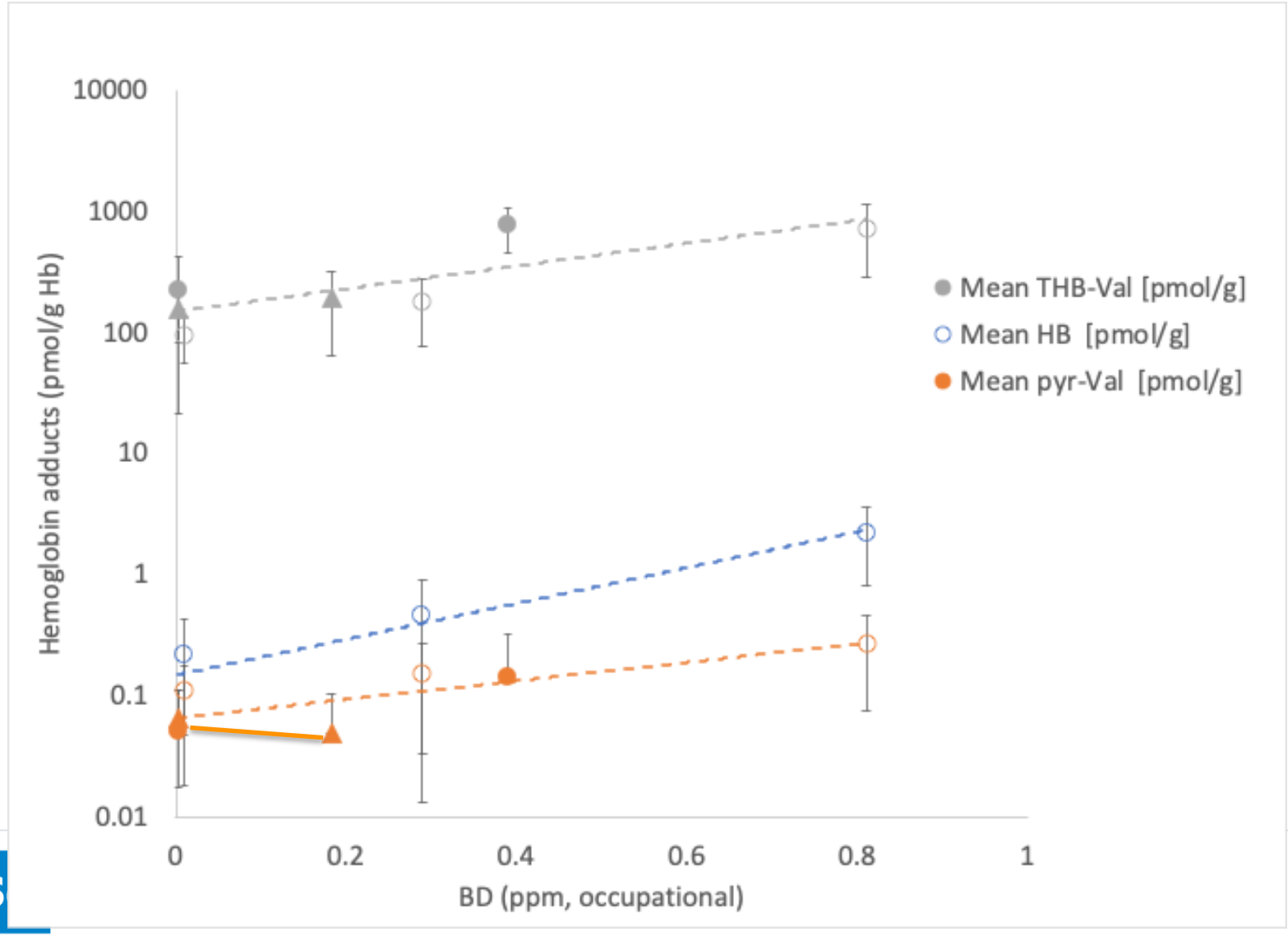


- *Large species differences are well documented across in vitro, in situ, and in vivo studies*
- *For a given exposure to BD mice, rats, and humans experience different internal exposures to BD's toxic metabolites, both in terms of magnitude and composition*
- *Surface area of pies are proportionate to magnitude of internal doses*
  - *Mice>Rats>Humans*
- *Colors indicate contribution of each metabolite to toxicity: Orange = diepoxide, DEB; Blue = monoepoxide, EB; Green = monoepoxide diol, EBD*
- *Assumption Mouse~Humans is not supported by weight of evidence*

In the presence of such large species differences, risk assessors have taken different approaches for BD hazard assessment

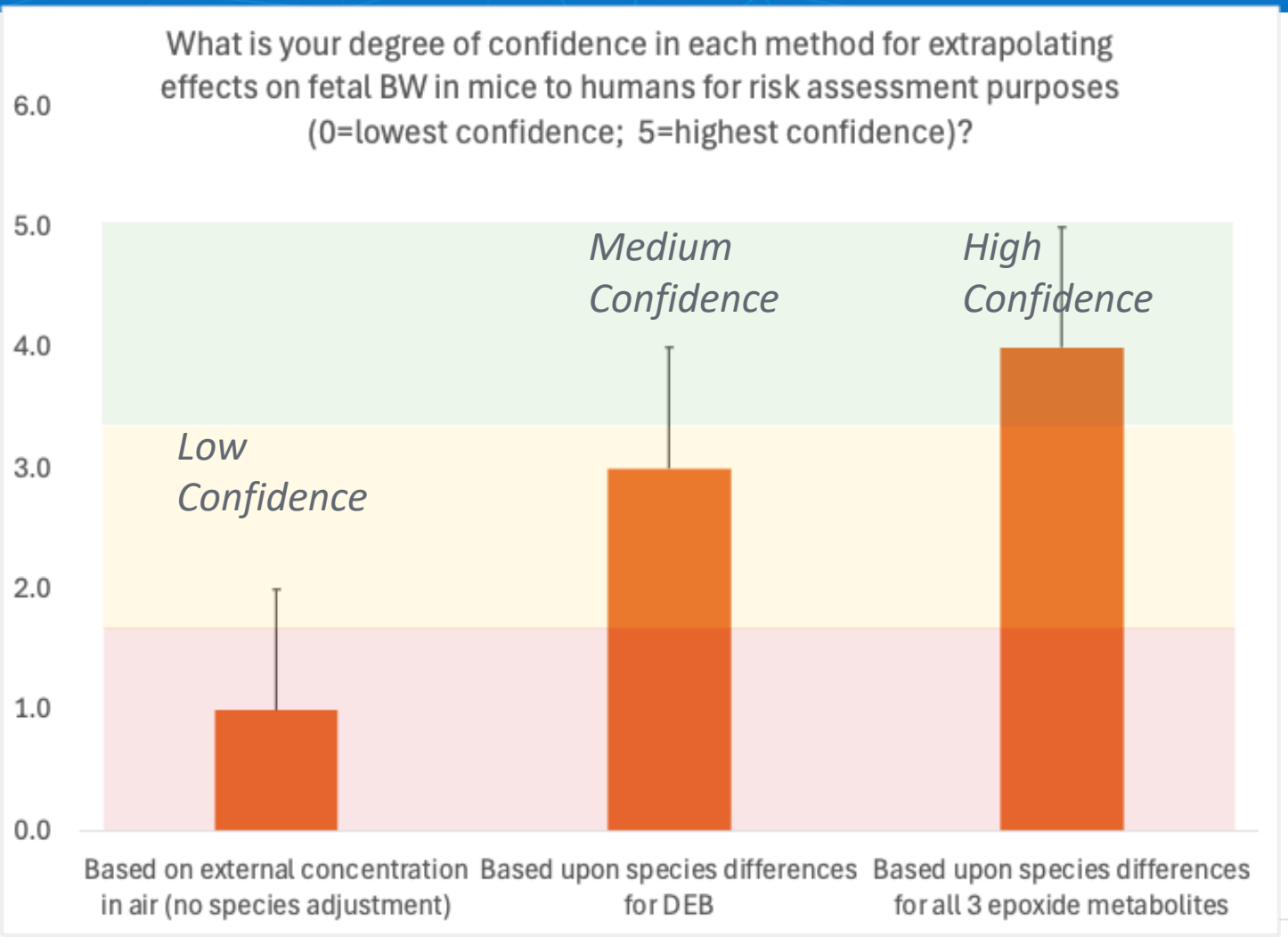
- ATSDR 2012: "*due to the large species differences in the metabolism of 1,3-butadiene and the lack of chemical-specific data to adjust for these differences, which may result in the MRL overestimating the risk to humans.*"
- ECHA 2024: No 8h-TWA value for noncancer effects "*is proposed considering the marked uncertainties in the extrapolation of animal data to humans*"
- EPA TSCA RE: Species differences and uncertainties are recognized in the qualitative discussion, but are ignored in the quantitative assessment (i.e., humans are assumed to be equally sensitive as mice to a give concentration of BD in air) citing uncertainty in the MOA
  - This invokes a worst-case assumption that is not supported by the weight of evidence
- Our approach: full quantification of species differences using approach of Motwani and Tornqvist (2014) with independent panel input on best science for MOA and DDEF calculations
  - Kirman et al. 2022: DDEF development
  - Kirman et al. 2025: DDEF application to risk assessment using an independent panel
  - Kirman et al. 2026: in dependent panel used to evaluate MOA and DDEF refinement per new data (female workers)

Figure NC3: Mean Hemoglobin Adduct Burdens for BD Metabolites in Exposed Workers (Georgieva et al., 2025). Symbols = Arithmetic mean; Error bars = SD; Solid circles = Male workers from Study 1 (Albertini et al. 2003); Hollow circles = Male workers from Study 2 (Vacek et al. 2010); Solid triangles = Female workers from Study 2 (Vacek et al. 2010).



- Hemoglobin adduct available for BD's metabolites are robust and reflect best available science
- Limitations in the human data (e.g., internal inconsistency for pyr-Val in female workers indicated by the solid orange line) do not detract from the key conclusion that the dashed line for pyr-Val adducts in humans is orders of magnitude below the corresponding dashed line for mouse data

Independent Panel Confidence in Dosimetry Decisions to Account for Species Differences BD Toxicokinetics



- For assessing fetal BW changes by BD, the independent panel expressed:
  - Low confidence in the default policy option to rely on external air concentration of BD, which was used in the final RE
  - Medium confidence in quantifying species differences in DEB alone using hemoglobin adducts
  - High confidence in quantifying species differences in all 3 epoxide metabolites using hemoglobin adducts
- Manuscript has been submitted for publication (Kirman et al. 2026)

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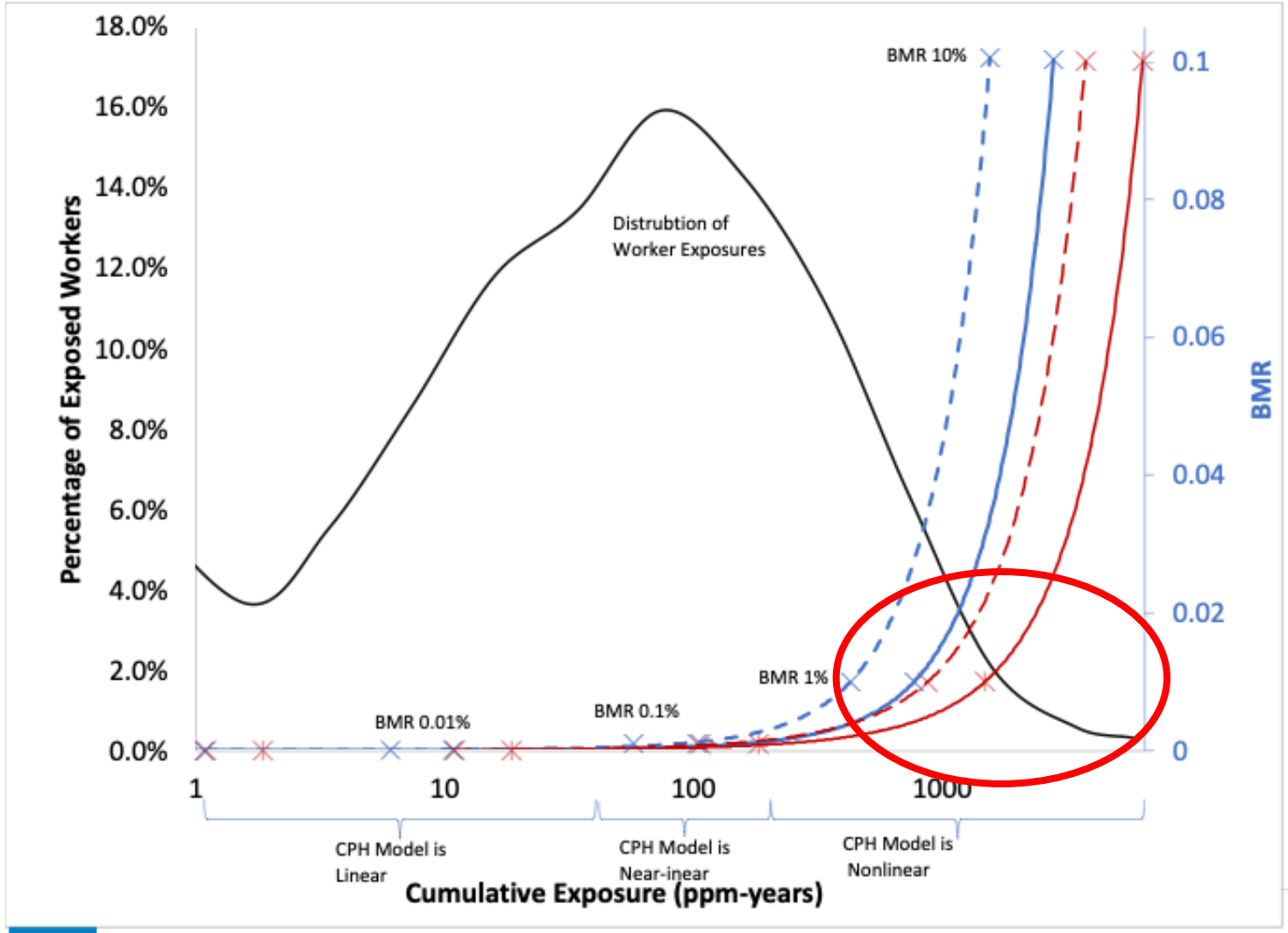
Cancer Decision Point 6: Considering the Range of Observation for the SBR Cohort to Establish an Appropriate BMR



- BMR of 1% results in PODs in the 4th quartile of ROO where CPH is nonlinear
- BMR of 0.1% results in PODs in the 2nd-3rd quartile of ROO where CPH is near-linear
- BMR of 0.01% or lower results in PODs in the 1st quartile of ROO where CPH is linear
- Because EPA has relied upon a "trimmed" data set for leukemia that excludes data from the top 5% (red oval), a BMR of 0.01 should not be used. POD falls near the data that were excluded, and residuals near the POD (an important consideration for assessing model fit; EPA, 2012) cannot be considered
- [Note: Red lines indicate CPH model predictions for bladder cancer; Blue lines indicate CPH model predictions for leukemia (solid = MLE; dashed = LCL); log-linear x-axis used to show lower BMRs & ROO exaggerates nonlinear appearance of CPH model]

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Tox Strategies
A BlueRidge Life Sciences Company

Presentation to EPA for Final Risk Evaluation for 1,3-Butadiene

Review of Occupational Exposure Assessment Conclusions and Application to Risk Management

Julie Panko, CIH, Principal Scientist

3/17/2026

BlueRidge
LIFE SCIENCES

Challenges for translating sentinel exposures in the risk evaluation to risk management in actual workplaces

- Unreasonable risk is concluded for 9 of the occupational exposure scenarios only under the high-end assumptions
  - Site specific risk management measures are regulated and in-use by industry (OSHA's butadiene standard 29 CFR 1910.1051)
- Intermediate exposure scenario is hypothetical; **How does EPA intend to regulate?**

Table_Apx B-1. Parameter Values for Calculating Inhalation Exposure Estimates

Parameter Name	Symbol	Value	Unit
Exposure Duration	*ED*	8	h/day
Breathing Rate Ratio	*BR*	2.04	unitless
Exposure Frequency	*EF*	5–250 [a]	days/yr
Exposure Frequency, intermediate	$EF_{intermediate}$	22	days
Days for Intermediate Duration	$D_{intermediate}$	30	days
Working Years	*WY*	31 (50th percentile) 40 (95th percentile)	years
Lifetime Years, Cancer	*LT*	78	years
Averaging Time, Intermediate	$AT_{intermediate}$	720	hours
Averaging Time, Non-Cancer	*AT*	271,560 (central tendency) [b] 350,400 (high-end) [c]	hours
Averaging Time, Cancer	AT_c	683,280	hours
Body Weight	*BW*	80 (average adult worker) 72.4 (female of reproductive age)	kg

Recommendation: Use an average daily exposure for manufacturing and processing as a reactant COU's.

Assumption Full Shift vs Task Exposure

			Unreasonable Risk Determination (Y/N)	
Conditions of Use	**Employee**	**Exposure Duration**	**Central Tendency**	**High-End**
Manufacturing – importing Processing/Repackaging	Worker, ONU	Full Shift Assumption	Y	Y
	Worker	Task Assumption	N	Y

- Unreasonable risk is concluded for 4 COUs based on task level data provided by this consortium but assumed by EPA to be full shift exposures; despite acknowledging the specific task durations and the uncertainty associated with their assumption (See page 60-61 of Environmental Release and Occupational Exposure Assessment for 1,3-Butadiene)
  - **What is the basis EPA will use to regulate; task or full shift exposure?**
  - **Recommendation: Use task exposure duration for manufacturing and processing as a reactant COU's.**

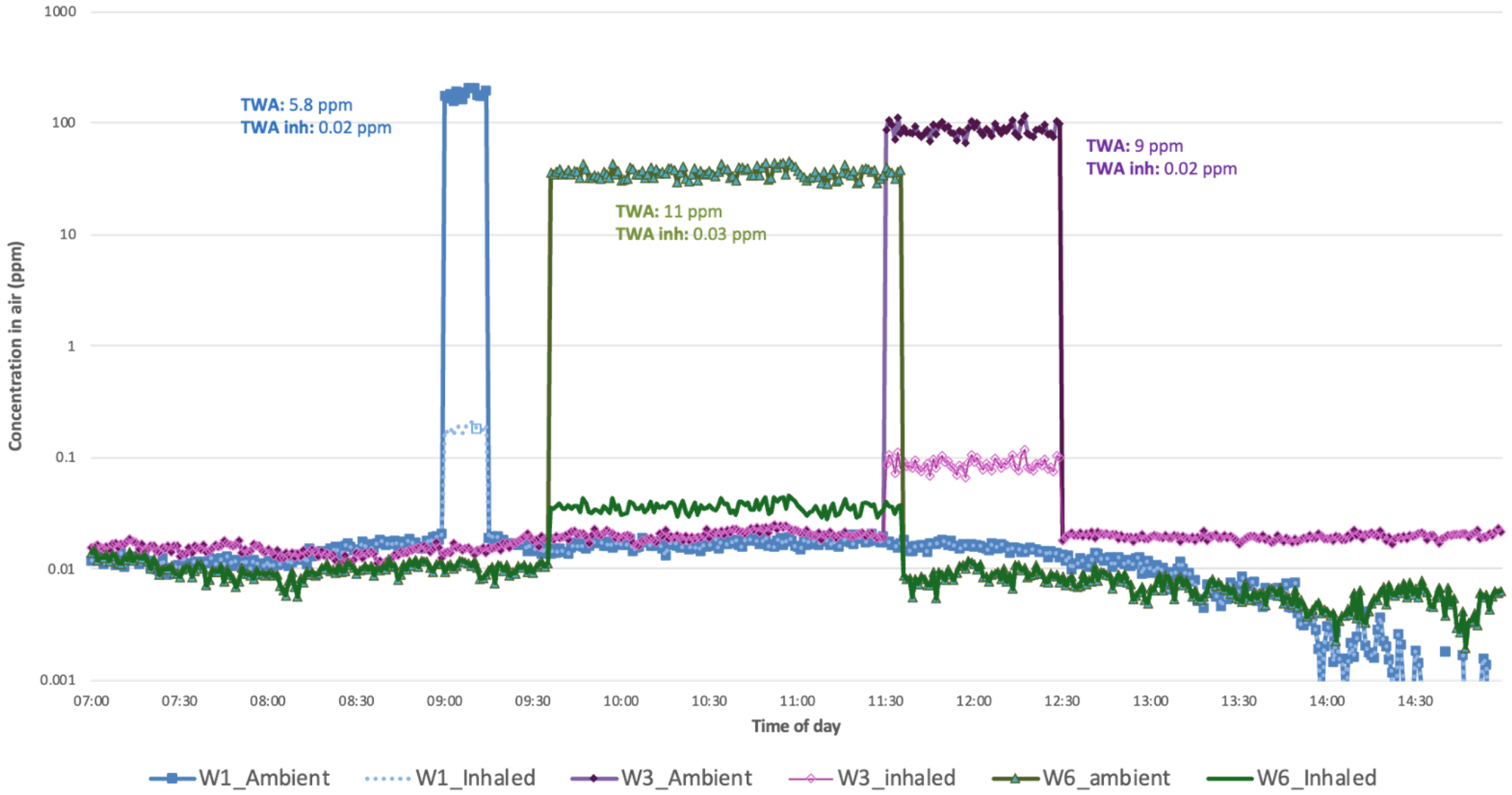
Loading/Unloading Task – Views of Rail and Barges

Typical butadiene rail tank car loading
Task Duration: 15 – 45 minutes
Task Frequency: 2.5 times/day (average)
Exposure time: 10 minutes/connect or disconnect

Typical butadiene barge loading
Task Duration: 10 – 15 hrs
Task Frequency: variable
Exposure time: 15 minutes/connect or disconnect

Photos from Butadiene Product Stewardship Guidance Manual

Impact of Respiratory Protection During High Exposure Tasks



Assumptions for Occupational Non-users (ONUs) Do Not Translate to Actual Workplace Conditions

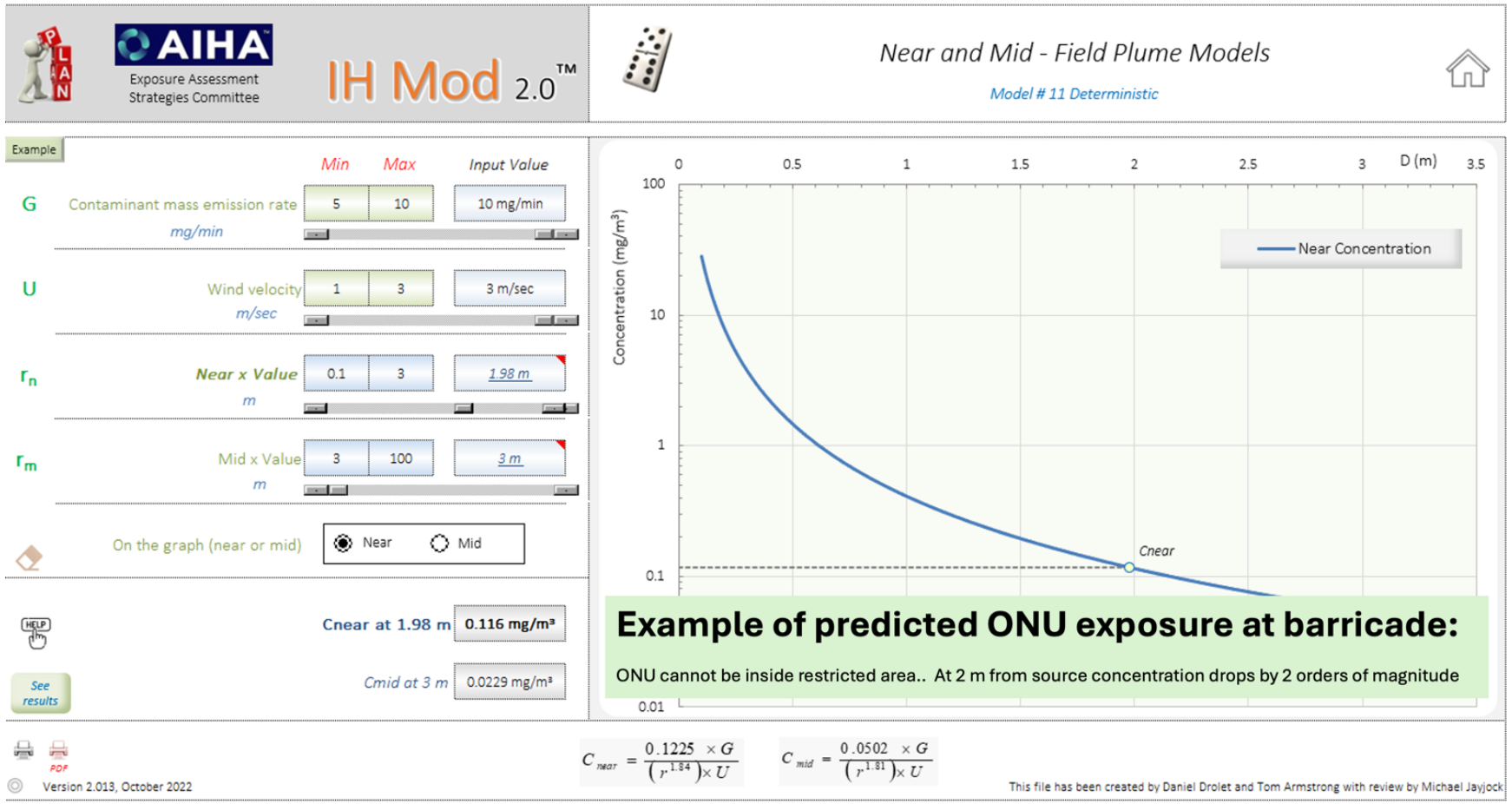
- Unreasonable risk was concluded for ONUs associated with repackaging, recycling and disposal because their exposure was assumed to be equivalent to worker central tendency exposures
- OSHA butadiene standard mandates establishment of regulated areas - therefore ONUs are not expected to be in proximity of these activities for the duration of the task

- **Recommendation: Continue to comply with the existing OSHA 1,3-BD standard as it is protective of ONUs**

https://cgapindustries.com/products/1-3-butadiene-danger-sign-may-cause-cancer-osha-1910-1051-compliant-8x12-aluminum-cgap-safety

Exposure Modeling for Prediction of ONU Exposures

OSHA 1,3-Butadiene standard requires restricted areas to prevent ONU exposures. Simple mathematical model demonstrates that the ONU exposure will not be equal to the worker exposure.



Feasibility of Measurement at Final OEV and Below

- 87% of the Consortium full shift dataset was non-detected
  - Of those detected, only 4% of all air samples had measured concentrations less than 0.11 ppm
- A threshold of 10% of the OEL is indicated as a goal for analytical methods evaluation (NIOSH 2016).
  - With EPA OEV of 0.11 ppm, the lowest limit of quantification should be 0.01 ppm for an 8 hr sample.
- EPA evaluated 3 sampling and analytical methods and only EPA TO-17 would allow for reliable measurement at 0.01 ppm
  - Only 4 AIHA accredited labs in the U.S for TO-17
  - Significant difference in costs to use this method
- **How does EPA transition from Final OEV to ECEL?**

Cost Comparison		
Feature	NIOSH 1024	EPA TO-17
Typical Lab Fee (per sample)	$55-$75	$150 –300+
Analytical Method	GC-FID	GC-MS
Sampling Media	Charcoal Tubes	Multi-bed Sorbent Tubes
Media Cost (per tube)	$5 – $15	uncertain

Processing as a Reactant COU - Polymerization

- Unreasonable risk was concluded for all workers in the Processing as a Reactant COU and the occupational exposure scenario of polymerization process.
  - Exposures will vary by job title and function and therefore exposure estimates used are unlikely to reflect exposures to all workers in this COU/OES
- **Recommendation: Reconsider the exposure literature used for this COU and assess by similar exposure group**

Appendix

ToxStrategies

A BlueRidge Life Sciences Company

Hierarchy of Controls

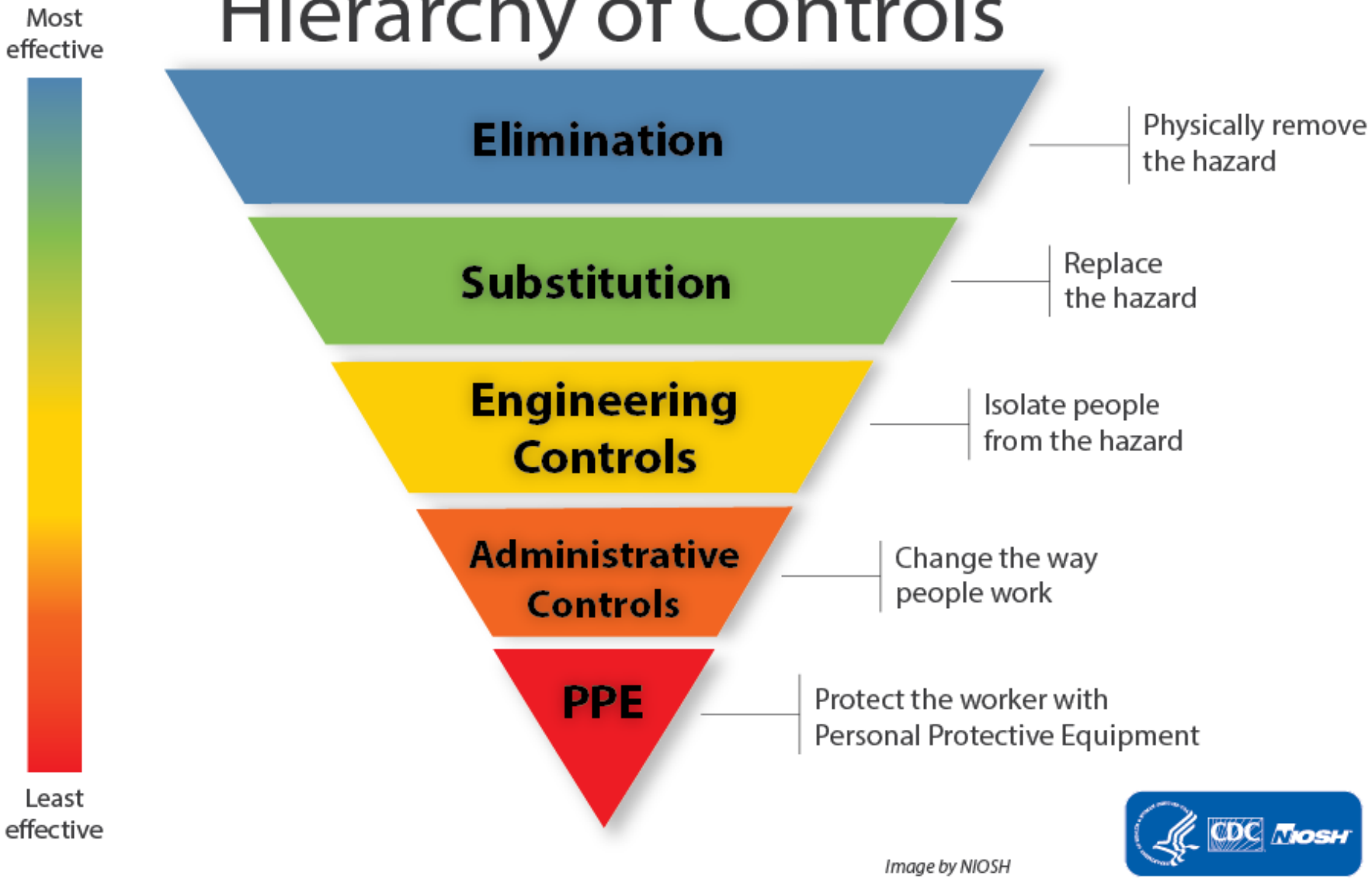


Image by NIOSH

Aspects to consider for ONUs for Risk Management

- ONUs are typically not stationed in process areas
  - Generally perform administrative, support, logistics, or intermittent oversight duties, not prolonged hands-on process work.
  - Work patterns inherently limit time in high-concentration zones.
  - Even short “walk-through” exposures are transient and episodic, not continuous.
- Monitoring Data From Other Worker Categories Are Not Automatically Representative
  - Data collected for production workers or operators represent the highest feasible exposure category, not ONUs, and cannot be directly applied without upward bias.
  - Sampling strategy is often risk-based, targeting areas more likely to show higher exposure.
  - Assumption of steady, unmitigated exposure doesn’t reflect real practice.
- Conservative Assumptions Compound Unreasonably
  - Assuming 100% co-location with high-exposure tasks
  - Assuming no mobility
  - Assuming no variability across days or seasons
  - Assuming full working lifetime exposure at this level
  - Individually, these might be justified as cautious; together, they yield an implausible worst-case scenario that far exceeds credible ONU exposures.

Challenges for translating sentinel exposures in the risk evaluation to risk management in actual workplaces

Conditions of Use	Employee	Exposure Duration	Central Tendency	High-End
Manufacturing – domestic manufacturing	Worker	Intermediate, Chronic (non-cancer and cancer)	N	Y
Manufacturing – importing Processing/Repackaging	Worker, ONU	Intermediate, Chronic (non-cancer and cancer) Full Shift Assumption	Y	Y
	Worker	Intermediate, Chronic (non-cancer and cancer) Task Length Assumption	N	Y
Processing as a reactant – intermediate (adhesive manufacturing; all other basic organic chemical manufacturing; fuel binder for solid rocket fuels; organic fiber manufacturing; petrochemical manufacturing; plastic material and resin manufacturing; propellant manufacturing; synthetic rubber manufacturing; paint and coating manufacturing	Worker	Intermediate, Chronic (non-cancer and cancer)	N	Y
Processing as a reactant – monomer used in polymerization process (synthetic rubber manufacturing; plastic material and resin manufacturing	Worker	Intermediate, Chronic (non-cancer and cancer)	Y	Y
Processing – incorporation into formulation, mixture, or reaction product – monomers (plastic product manufacturing; plastic material and resin manufacturing; synthetic rubber manufacturing	Worker	Intermediate, Chronic (non-cancer and cancer)	N	Y
Processing – incorporation into formulation, mixture, or reaction product – plasticizer (asphalt paving, roofing, and coating materials manufacturing)	Worker	Intermediate, Chronic (non-cancer and cancer)	N	Y
Processing – incorporation into article – monomer (rubber product manufacturing)	Worker	Intermediate, Chronic (non-cancer and cancer)	N	Y
Processing – use-non-incorporative activities – fuel (petroleum refineries)	Worker	Intermediate, Chronic (non-cancer and cancer)	N	Y
Processing – repackaging – (wholesale and retail trade fuel; synthetic rubber manufacturing; petrochemical manufacturing	Worker, ONU	Intermediate, Chronic (non-cancer and cancer) Full Shift Assumption	Y	Y
	Worker	Intermediate, Chronic (non-cancer and cancer) Task Length Assumption	N	Y
Processing – recycling	Worker, ONU	Intermediate, Chronic (non-cancer and cancer) Full Shift Assumption	Y	Y
Disposal	Worker, ONU	Intermediate, Chronic (non-cancer and cancer) Full Shift Assumption	Y	Y
		Intermediate, Chronic (non-cancer and cancer) Task Length Assumption	N	Y

Regulatory Impact Analysis

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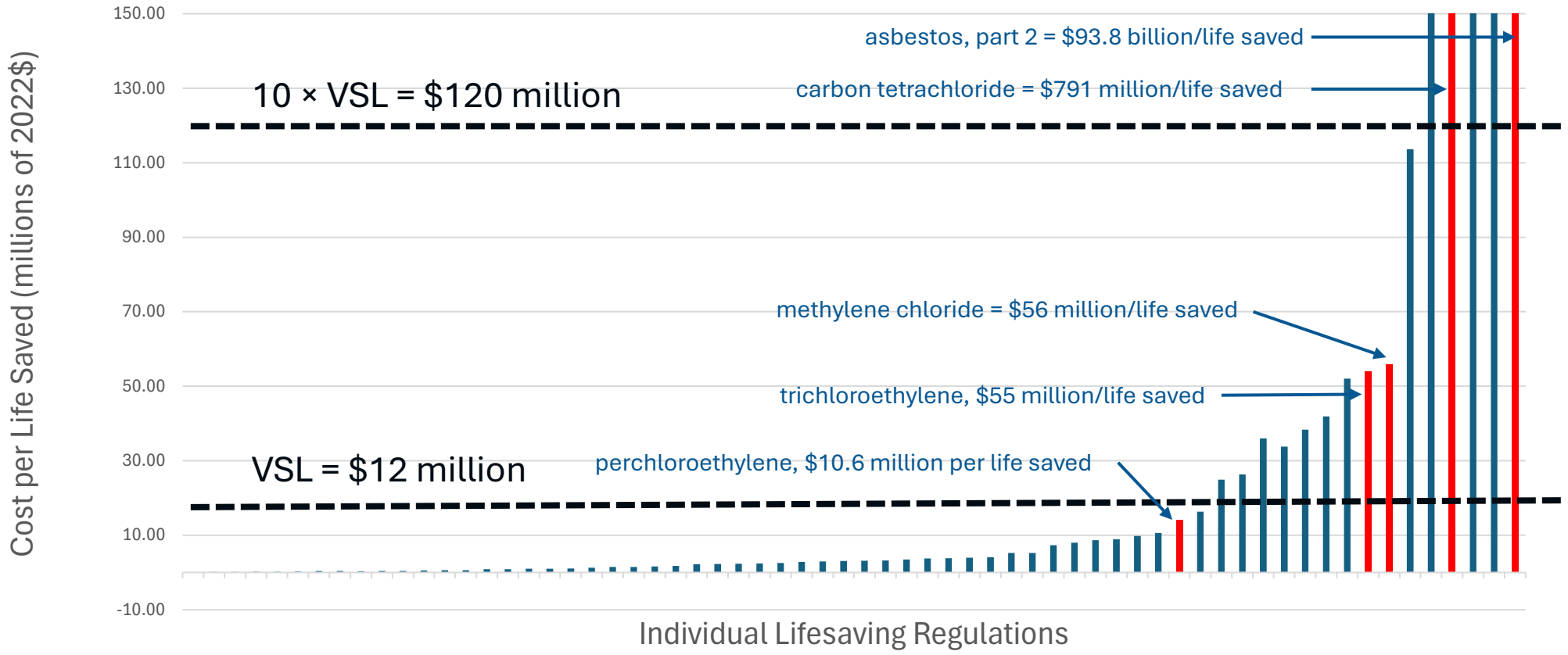
Cost Effectiveness of Federal Lifesaving Regulations, 2009-2024: TSCA Regulations Have Not Been Cost Effective



Source: Belton and Graham, forthcoming
Data: regulatory impact analyses from various federal agencies
Methodology: Morrall III, John F. "Saving lives: A review of the record." *Journal of Risk and Uncertainty* 27, no. 3 (2003): 221-237.

VSL = value of a statistical life

Using Microrisk Reduction to Monetize Benefits: Questions

Previous risk management rules utilize the concept of a microrisk reduction to quantify and monetize benefits in EPA's economic analysis.

Circular A-4 states that health benefits should be valued at the point when they are "observed."

Is a microrisk reduction ever observed? Is its use consistent with OMB Circular A-4?

In the economic analyses conducted to date, the number of microrisk reductions are the same in every year after controls are put in place. This is puzzling.

How is EPA addressing cessation lag when employing the concept of microrisk reductions?