Integration of Mechanistic Data to Evaluate Proposed MOAs: A Case Study with Formaldehyde and Leukemia

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FORMALDEHYDE EXPOSURE AND LEUKEMIAS

- Inhalation exposure to exogenous formaldehyde has been proposed to be associated with an increased risk of leukemia, specifically acute myeloid leukemia (AML), in humans (IARC 2006; NTP 2016; Zhang et al. 2009; Zhang 2018).
- Challenges to these conclusions
  - NRC (2011) – Conclusions based on selected epidemiological studies, with little consideration of other streams of evidence such as animal bioassays, dosimetry, or mode of action (MOA) studies
  - Gentry et al. (2013); Checkoway et al. (2015); Mundt et al. (2017) - significant methodological and/or analytical concerns with the studies that are the basis of the conclusions
- Mechanisms by which formaldehyde exposure could result in the key events leading to the development of leukemia remain elusive.
  - Decades of dosimetry research indicate that inhalation of formaldehyde does not result in systemic delivery or elevation of the levels of formaldehyde naturally present in the blood (Lu et al. presentation).
- **In drawing any conclusions regarding the potential for leukemia or developing any postulated MOA for the development of leukemia following formaldehyde exposure, it is important to understand the relative dosimetry of exogenous and endogenous formaldehyde exposures.**

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POSTULATED MOAS FOR LEUKEMIAS

- To date, four MOAs have been postulated for the development of leukemia following formaldehyde inhalation (Zhang et al. 2009, 2010; Zhang 2018)
  - MOA 1: Initiation of leukemia by direct DNA damage to hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) in bone marrow
  - MOA 2: Direct induction of mutations or toxicity to circulating HSCs and HPCs in the blood
  - MOA 3: Direct DNA damage or toxicity to pluripotent nasal/oral cells
  - MOA 4: Direct induction of mutations or toxicity to HSCs and HPCs in the lungs

POSTULATED MOAS FOR LEUKEMIAS

- Common to each of the four postulated MOAs:
  - direct, DNA-reactive mutagenic damage to the target cells
    - hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPCs) or pluripotent stem cells to initiate leukemic stem cells (LSCs).
- Evidence in contrast:
  - Formaldehyde *in vitro* clearly shown to have genotoxic potential (e.g. SCEs, micronuclei) in all systems tested ranging from plasmids and bacteria to mammalian cell cultures; however, differences have been reported in the genotoxicity literature for *in vivo* systems (USEPA 2010; Albertini and Kaden 2017).
  - Notably, human volunteer studies that have attempted to measure genotoxicity (micronuclei) in human volunteers exposed to formaldehyde by inhalation in controlled settings reported no changes in genotoxic endpoints in buccal cells (Speit et al. 2007) or peripheral blood cells and nasal epithelial cells (Zeller et al. 2011).
- **Understanding dosimetry and systemic delivery are critical in the evaluation of the biological plausibility of these MOAs.**

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EVALUATION OF THE BIOLOGICAL PLAUSIBILITY OF POSTULATED MOAS

- Framework
  - World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) MOA Framework
    - method for integration of mechanistic data and the application of modified Bradford Hill criteria to evaluate causality; considers consistency, concordance of dose response relationships across key events, coherence of the database, and biological plausibility.
- Systematic Review
  - comprehensive review and determination of study quality and relevance have become increasingly important in the evidence integration process (NRC 2011; NTP 2015; USEPA 2018),
    - evaluation of the quality of the mechanistic studies and their relevance to the postulated MOAs was also conducted using USEPA (2018) guidance
- Formaldehyde Dosimetry:
  - Consideration of endogenous production and whether studies can differentiate between endogenous and exogenous formaldehyde.
    - European Commission SCENIHR criterion (SciRAP) used to assess the relevance of data to the postulated problem

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MECHANISMS IMPACTING SYSTEMIC DELIVERY

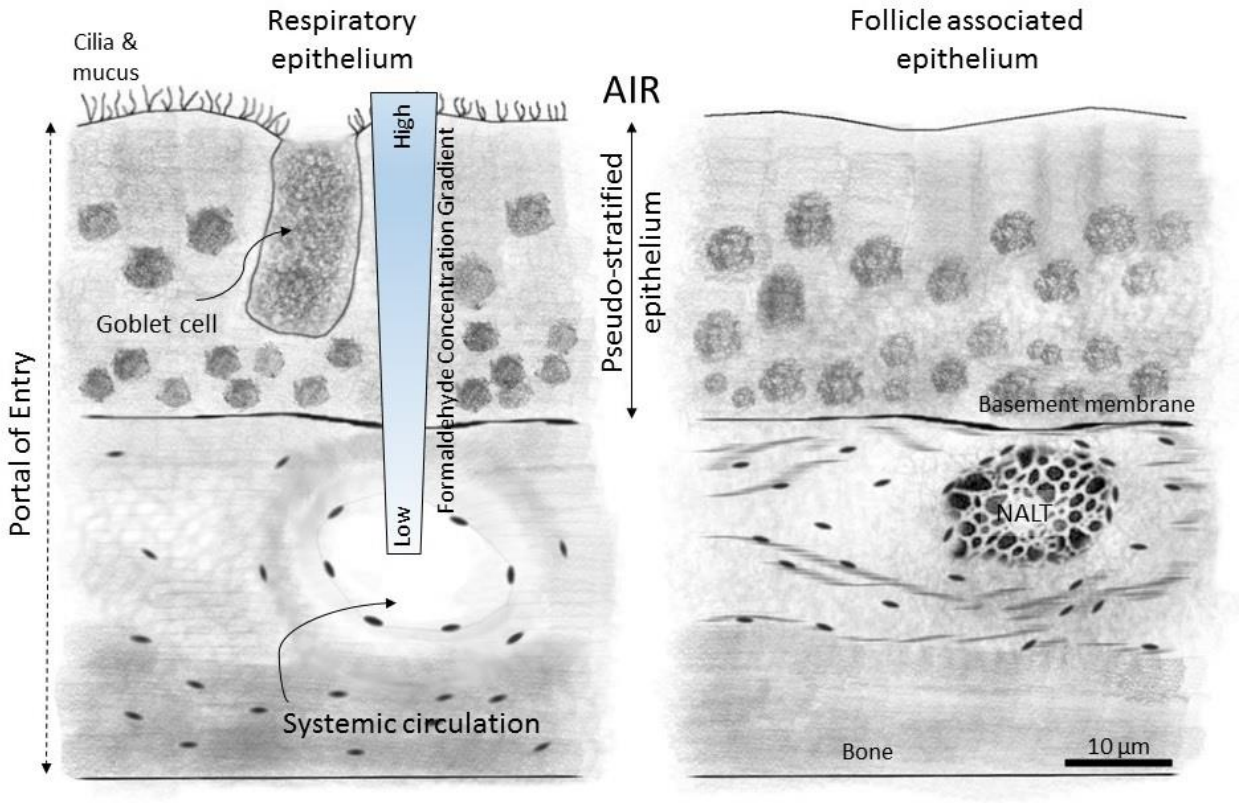

Figure reproduced from NRC (2011)

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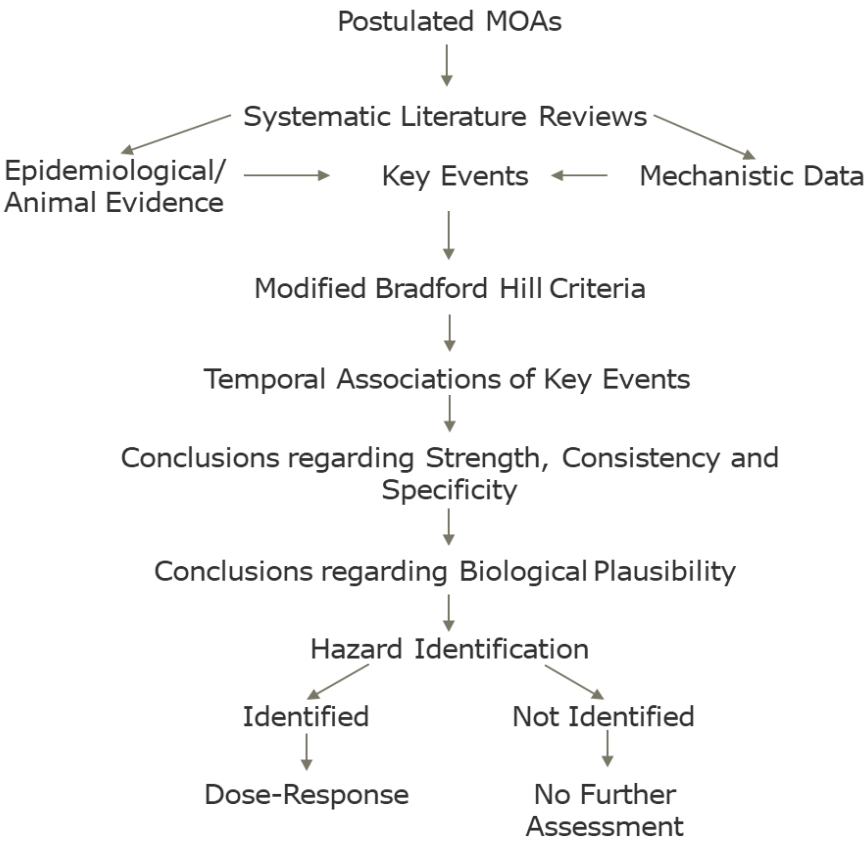
IPCS FRAMEWORK



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STUDY QUALITY

- Study quality was evaluated using methods from USEPA (2018), which includes pre-defined criteria for each type of data or information source.
  - Numerical scoring system used to inform the characterization of the data
  - Includes the evaluation of domains, metrics and criteria
  - Each domain is comprised of a unique set of metrics to assess the methodological conduct of the data.
  - Each metric contains criteria for assessing confidence to guide the identification of study strengths and limitations scored from 1 to 4 and are assigned a weighting factor of 1 (less critical) or 2 (most critical).
  - Overall quality scores are calculated for each data source

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STUDY QUALITY

Confidence Level	Score	Definition
High	1	No notable deficiencies or concerns are identified in the domain metric that are likely to influence results.
Medium	2	Minor uncertainties or limitations are noted in the domain metric that are unlikely to have a substantial impact on results.
Low	3	Deficiencies or concerns are noted in the domain metric that are likely to have a substantial impact on results.
Unacceptable	4	Serious flaws are noted in the domain metric that consequently make the data/information source unusable
Not Applicable	NA	Rating of this metric is not applicable to the data source being evaluated. Therefore, no score is given.

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STUDY RELEVANCE/PROBLEM FORMULATION

1. Does inhalation exposure to exogenous formaldehyde add to increased amounts of endogenous formaldehyde at sites distant from the portal of entry such as the circulatory system? If so, were the analytical techniques utilized specific for exogenous formaldehyde?
2. Does inhalation exposure to formaldehyde lead to binding of exogenous formaldehyde to macromolecules in primary cells of the bone marrow and/or blood? If so, did the analytical techniques utilized measure specifically for the binding of exogenous formaldehyde?
3. Does inhalation exposure to formaldehyde lead to genotoxicity in primary cells at sites distant from the portal of entry? If so, did the analytical techniques utilized measure specifically for the effects of exogenous formaldehyde?
4. Based on the integration of the different streams of evidence (i.e. epidemiological, in vivo animal and mechanistic) are the postulated MOAs for increased risk of leukemia after inhalation of formaldehyde biologically plausible?

OVERVIEW OF POSTULATED MOAS

MOA Key Events:	MOA 1: Initiation of leukemia by direct damage to hematopoietic stem cells in bone marrow	MOA 2: Toxicity to circulating blood stem cells and progenitors	MOA 3: Targeting pluripotent nasal or oral cells	MOA 4: Targeting blood stem cells and progenitors in the lung tissue
Event 1:	Conversion of formaldehyde to methanediol for transport through portal of entry	NP	NP	NP
Event 2:	Systemic delivery and transport of formaldehyde through blood	Systemic delivery and transport of formaldehyde through blood	NP	NP
Event 3:	Macromolecular binding to hematopoietic stem cells and progenitor blood cells residing in the bone marrow	Macromolecular binding to hematopoietic stem cells and progenitor blood cells in the circulatory system	NP	NP
Event 4:	Formaldehyde induced genotoxicity in the hematopoietic stem cells and progenitor blood cells derived from bone marrow or residing in the bone marrow	Formaldehyde induced genotoxicity in the hematopoietic stem cells and progenitor blood cells derived from bone marrow or residing in the bone marrow	Formaldehyde induced genotoxicity in primitive pluripotent cells in the nasal or oral tissue	Formaldehyde induced genotoxicity in hematopoietic stem cells and progenitor blood cells present in lung tissue (extravascular)
Event 5:	NP	NP	Damaged cell release into systemic circulation	Damaged stem cell release into systemic circulation
Event 6:	NP	Incorporation of initiated stem cell into the bone marrow	Incorporation of initiated pluripotent cell into the bone marrow	Incorporation of initiated stem cell into the bone marrow
Event 7:	Initiation of Leukemia	Initiation of Leukemia	Initiation of Leukemia	Initiation of Leukemia
NP – Not postulated				

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FORMALDEHYDE-SPECIFIC DOSE-RESPONSE AND TEMPORAL CONCORDANCE DATA

Dose (ppm)	KE: Formaldehyde reaches systemic circulation*	KE: Macromolecular binding to bone marrow or blood	KE: *In Vivo* genotoxicity[a] in HSCs/HPCs or primitive pluripotent cells	KE: Damaged stem cell released into circulation	KE: Incorporation of initiated cell back into bone marrow
0.2			- [c]		
0.3	-		- [c]		
0.4		- [b] - [c]	+/- [b] - [c]		
0.5	-	- [c]	- [b] - [c]		
0.6			- [c]		
0.7	-	- [b]	- [c] - [d]		
0.8		+ [b] - [c]	+ [b] - [c]		
1	-	- [c]	- [c]		
2	- -	- [b] - [c]	- [c] - [d]		
2.4		+ [b] + [c]	+ [b] + [c]		
3			- [b]		
5			- [c]		
6	- -	- [b] - [c]	- [c] - [d]		
9	-	- [b]			
10	- -	- [b] - [c]	- [c] - [d]		
13.5			- [c]		
14		- [c]			
15	- -	- [b] - [c]	- [b] - [c] - [d]		

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FORMALDEHYDE-SPECIFIC DOSE-RESPONSE AND TEMPORAL CONCORDANCE DATA

+ effect observed; - effect not observed experimentally; +/- effect variably observed

[a] Genotoxicity for supporting evidence is defined by the study authors to be DNA protein crosslinks formation or biomarkers of oxidative stress (i.e. reactive oxygen species (ROS), malondialdehyde (MDA), measured glutathione (GSH), and cytochrome P450 1A1 and glutathione s-transferase theta 1 expression)

[b] bone marrow data

[c] blood data

[d] nasal mucosa

* Requires analytical/biochemical evidence of delivery, not simply an effect reported systemically. Exogenous formaldehyde was reported to not be present in multiple tissues including the lung, liver, bone marrow, blood, spleen, thymus

Green citations – indicate data that provide evidence to support postulated MOA

Blue citations – indicate data that do not provide evidence to support the postulated MOA

Yellow citations – indicated data that provide both evidence to support and evidence that does not support the postulated MOA

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INTEGRATION OF EVIDENCE

Reference	KE: Formaldehyde reaches systemic circulation	KE: Macromolecular binding to bone marrow or blood	KE: *In Vivo* genotoxicity[a] in HSCs/HPCs or primitive pluripotent cells	KE: Damaged stem cell released into circulation	KE: Incorporation of initiated cell back into bone marrow
Evidence to support postulated MOAs	Fox et al. 1985 Walker et al. 1964	Ye et al. 2013 (1.6)[b]	Wei et al. 2017 (1.6) Ye et al. 2013 (1.6) Zhang et al. 2013 (1.5) Murrell et al. 2005	Lefrancais et al. 2017	NIH 2001
Contradictory evidence of postulated MOAs	Edrissi et al. 2013 (1.6) Kleinnijenhuis et al. 2013 (1.2) Lai et al. 2016 (1.2) Lu et al. 2010 (1.6) Lu et al. 2011 (1.3) Moeller et al. 2011 (1.4) Speit et al. 2009 (1.6) Yu et al. 2015a (1.4) Leng et al. 2019 (1.4)	Casanova and Heck 1987 (1.5) Casanova-Schmitz et al. 1984a (1.5) Edrissi et al. 2013 (1.6) Heck et al. 1989 (1.6) Lai et al. 2016 (1.2) Lu et al. 2010 (1.6) Lu et al. 2011 (1.3) Moeller et al. 2011 (1.4) Speit et al. 2009 (1.6) Yu et al. 2015a (1.4)	Dallas et al. 1992 (1.6) Kligerman et al 1984 (1.6) Speit et al. 2009 (1.6) Zeller et al. 2011a (1.1)[c] Meng et al. (2010) (1.1)	No Data	Abkowitz et al. 2003 McKinney-Freeman and Goodell 2004

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INTEGRATION OF EVIDENCE

[a] Genotoxicity for supporting evidence is defined by the study authors to be DNA protein crosslinks formation or biomarkers of oxidative stress (i.e. reactive oxygen species (ROS), malondialdehyde (MDA), measured glutathione (GSH), and cytochrome P450 1A1 and glutathione s-transferase theta 1 expression)

[b] Values in parentheses are overall study quality scores:

Study quality overall score:

High: ≥ 1 and <1.7

Medium: ≥1.7 and <2.3

Low: ≥2.3 and ≤3

[c] Because Zeller et al. (2011a) was a controlled human exposure study it was considered directly relevant to the problem formulation.

Green citations – indicate studies that provide indirect relevance to the problem formulation questions

Blue citations – indicate studies that provide direct relevance to the problem formulation questions direct evidence to the problem formulation questions because they were conducted in humans

Gray citations - studies that have been used to support the postulated MOAs but do not provide any formaldehyde specific data or were conducted in a compromised animal model. These studies were not scored for study quality or relevance.

POSTULATED MOAS 1/2 – DIRECT DNA DAMAGE TO BONE MARROW/ TOXICITY TO CIRCULATING BLOOD STEM CELLS AND PROGENITORS

Modified Bradford Hill Consideration	Supporting Evidence	Potentially Inconsistent Evidence
Dose-response Temporal concordance	Available evidence provides little, if any, support of a dose-response relationship for any of the key events in the proposed MOA.	Multiple high quality and directly relevant studies from multiple species and across multiple doses, provide evidence indicating macromolecular binding and genotoxicity in the blood do not occur in animals, including humans (i.e. no dose response or temporal concordance demonstrated.)
Consistency, specificity	Available evidence provides little, if any, consistency across studies to support the postulated MOA.	High degree of consistency across studies to demonstrate no macromolecular binding, genotoxicity occurring in the blood.
Biological plausibility	Available evidence provides little, if any, supporting evidence for biological plausibility.	High quality, directly relevant studies in multiple species demonstrate that formaldehyde does not reach the systemic circulation in measurable quantities and, thus, there is no molecular initiating event to start a leukemogenic process. Also, high quality, directly relevant studies indicate there is no evidence that (1) formaldehyde induces genetic damage to HSCs or HPCs in the circulation, or (2) genetically damaged HSCs and HPCs resulting from formaldehyde exposure could re-enter the bone marrow.

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POSTULATED MOAS 3/4 – TARGETING PLURIPOTENT NASAL CELLS OR HSCS/HSPS IN LUNG TISSUE

Modified Bradford Hill Consideration	Supporting Evidence	Potentially Inconsistent Evidence
Dose-response Temporal concordance	No chemical-specific data available to provide support for dose-response or temporal concordance	Dosimetry demonstrates the mass of inhaled exogenous formaldehyde is insignificant compared the body burden of endogenously produced formaldehyde. Temporal concordance of leukemia development has not been identified in animal or human studies.
Consistency, specificity	No chemical specific data available that provides consistent support for the postulated key events.	Dosimetry data demonstrates lack of exposure to target cell populations, and are specific to formaldehyde and consistent across multiple studies
Biological plausibility	Plausible, but no chemical-specific data to support and not generally accepted as a relevant AOP for leukemia development	Pluripotent stem or HSPs/HSC cell data are limited to animal models with compromised systems and are not formaldehyde-specific.

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CONCLUSIONS

Framework for Evaluation and Integration Evidence for Postulated MOAs:

- The IPCS framework provides a method for systematically and critically reviewing mechanistic data and integrating these data with other streams of evidence (epidemiological, animal).
- The framework allows for consideration of study quality as well as considerations that may be specific to the chemical of interest, in this case study, ***endogenous production of formaldehyde and whether methods can determine exogenous versus endogenous formaldehyde.***
- The data quality criteria recommended by USEPA (2018) provides criteria for assessing study quality for data or information source.
- The SciRAP tool provides additional metrics for determining study relevance, using definitions established by the European Commission (SCENIHR 2012), that can be incorporated and establishes the relevance of *in vivo* and *in vitro* study data to the problem formulation.

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CONCLUSIONS

Lack of Biological Plausibility for Postulated MOAs:

- Evaluation of the available evidence indicated a lack of dose-response or temporal concordance and a lack of biological plausibility for the proposed key events based on data reported in high quality studies determined to be relevant to the problem formulation.
- Most of the supporting information for the postulated MOAs were limited to studies conducted in mice from one laboratory or studies conducted in animals with compromised systems that were not specifically exposed to formaldehyde.
- The data that reported inconsistent evidence with the postulated MOAs are from high quality studies considered to be directly relevant in informing the problem formulation questions.
- There is an absence of formaldehyde-specific or directly relevant supporting data for multiple key events, including information to support that formaldehyde reaches systemic circulation, formaldehyde damages circulating stem cells, or initiated circulating stem cells resulting from formaldehyde-induced toxicity migrate back into the bone marrow.

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CONCLUSIONS

- ***Evaluation of the study quality and relevancy of the data indicate the highest quality studies and all the directly relevant studies provide inconsistent evidence to support the postulated MOAs for leukemia.***

- ***This case study also demonstrates that the IPCS framework will work in determining biological plausibility for endpoints for which data from multiple streams of evidence are available to provide supporting evidence for key events in a postulated MOA, as well as those for which the evidence is inconsistent.***

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