FORMALDEHYDE IRIS ASSESSMENT
JANUARY 24, 2018

- FORMALDEHYDE - CURRENT WEIGHT OF THE EVIDENCE
- ENSURING A ROBUST ASSESSMENT OF THE SCIENCE
- MODE OF ACTION - RESEARCH HIGHLIGHTS
- SCIENTIFIC EXPECTATION

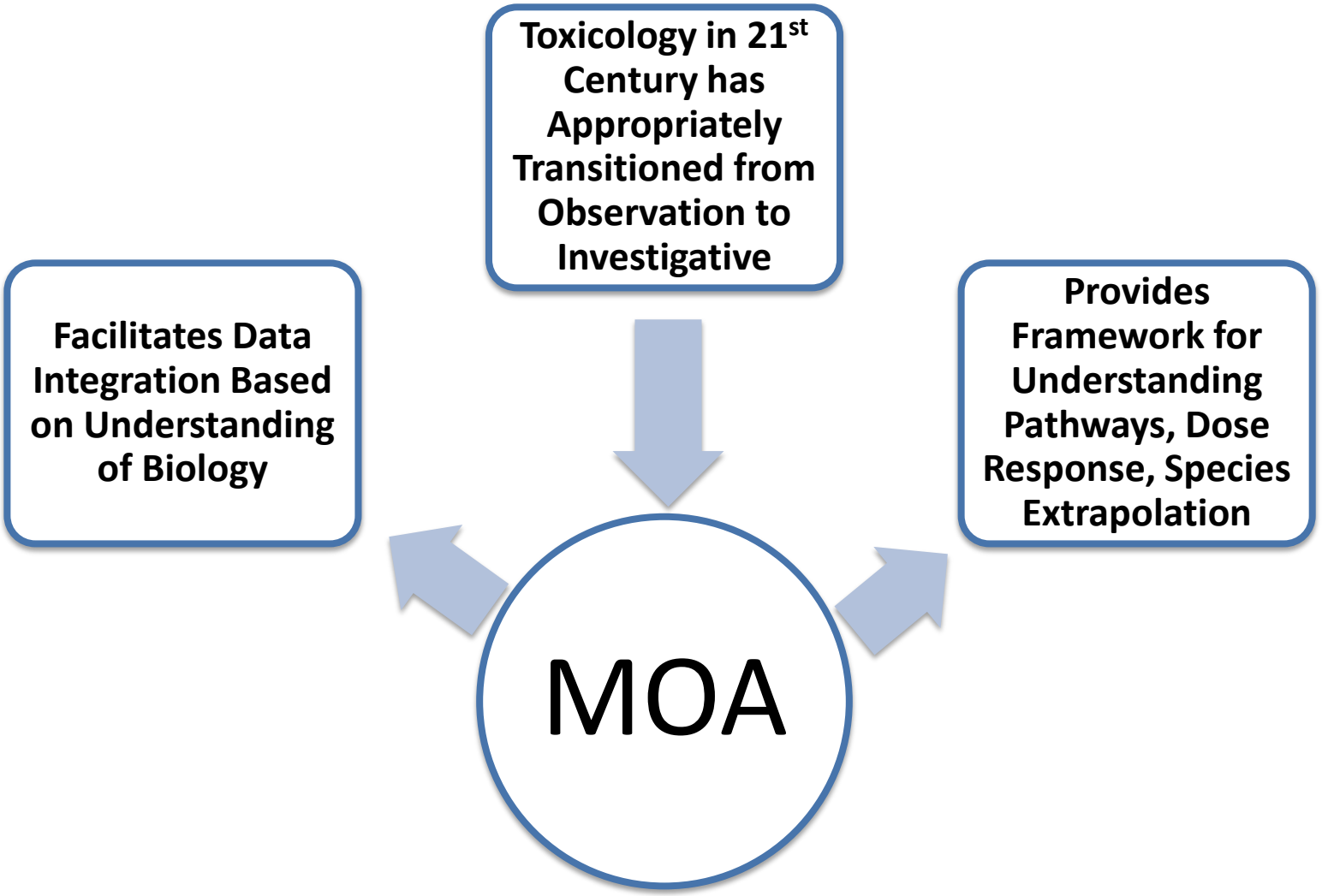
FORMALDEHYDE - CURRENT WEIGHT OF THE EVIDENCE

FORMALDEHYDE – USING BEST AVAILABLE SCIENCE

Use of a weight of evidence approach to integrate lines of evidence using mode of action as the organizing principle. This science-based approach illustrates:

- ✓ Lack of a causal association between exogenous formaldehyde exposure and leukemia
- ✓ A clear threshold for safe exposures to formaldehyde and application of a non-linear dose-response model and/or mode of action framework to best characterize risk for rodent nasal tumors
- ✓ Lack of biological plausibility for exogenous formaldehyde to move beyond the portal of entry and cause effects at distal sites in the body.

Why Mode of Action (MOA) is Critical



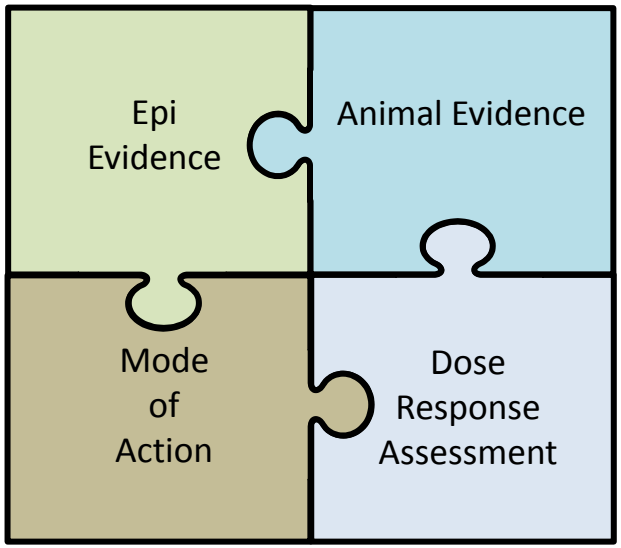
MOA shouldn't be relegated to an add on after the assessment is largely complete: it should form the framework for assessment

Understanding the Formaldehyde Science

Drawing conclusions regarding the potential for human health risk requires a balanced weight of evidence analysis

MOA is critical for

- ✓ Establishing biological plausibility of selected cancers
- ✓ Understanding how inhalation of formaldehyde may impact normal processes.



ORGANIZATION	POPULATION	APPROACH	RISK LEVEL	Basis of Decision
EU/ECHA	General	Qualitative but not low-dose linear	No convincing evidence of a carcinogenic effect at distant sites	Causes tumors above a threshold concentration by mechanisms that are initiated by the cytotoxic effects but ...data does not allow firm conclusion on a threshold-mode of action
Health Canada	General	Threshold Carcinogen DSL Low priority substance	2.3×10^{-10} at 1 ppb	Carcinogenic hazard to humans "...under conditions that induce cytotoxicity and sustained regenerative cell proliferation."
Occupational Standards from various bodies In the US and EU	Workers	Threshold Carcinogen	Exposure standards: TWAs with STELs 0.1 ppm ACGIH; 0.016 pp NIOSH; NIOSH; 3ppm MAK and SCOEL	Varied: from MAK - Cancer classification 4: non-genotoxic; cell proliferation important to MoA to ACGIH's "cancer classification A1: confirmed human carcinogen "
World Health Organization	General	Threshold Carcinogen	Short- and long-term exposures 0.1 mg/m3 (0.08 ppm)	Guideline value is considered to prevent all portal-of-entry effects, including nasal cancer, and potential systemic cancers. Even though the potential systemic cancer effects are considered not to be relevant with regard to setting an indoor guideline.

ENSURING A ROBUST ASSESSMENT OF THE SCIENCE

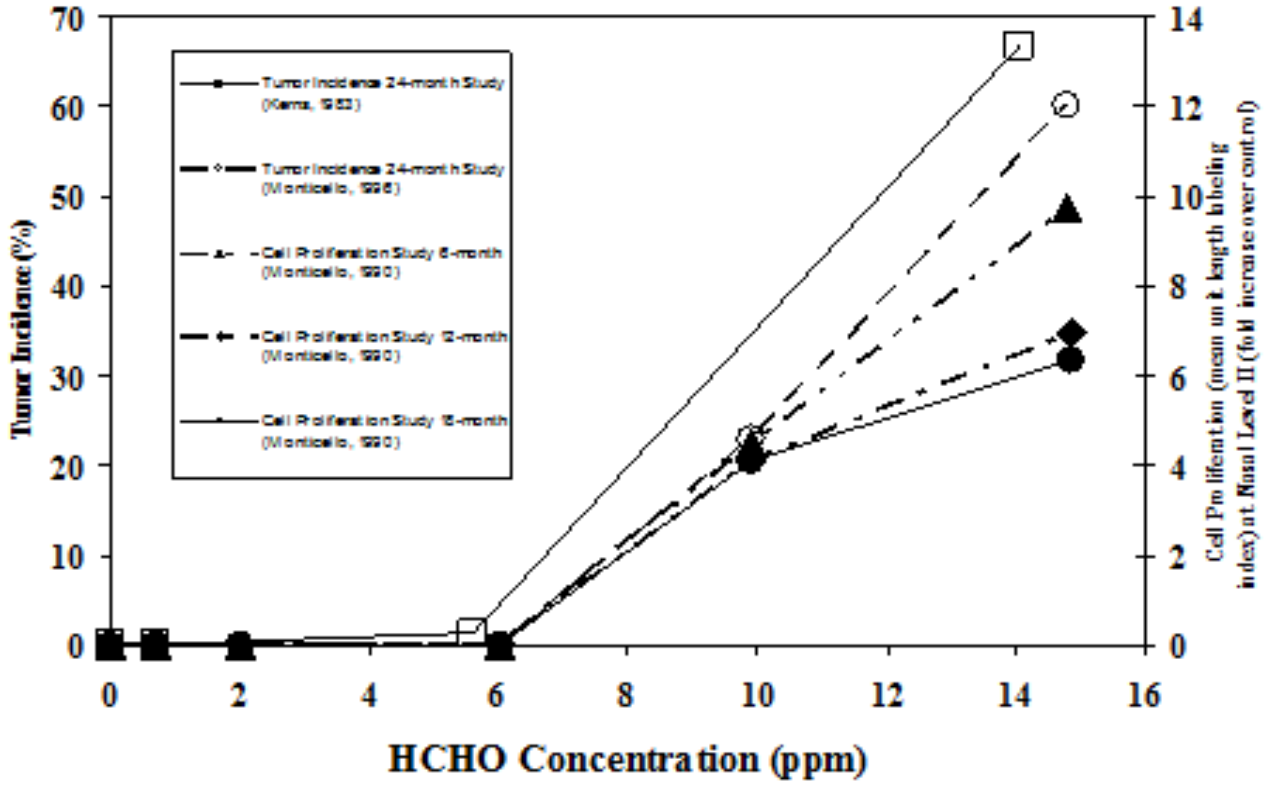
NOTABLE NAS RECOMMENDATIONS

- Select outcomes on the basis of available evidence and understanding of mode of action.
- Revisit arguments that support determinations of causality for specific LHP cancers
- Use the BBDR model for formaldehyde in its cancer assessment, compare the results with those described in the draft assessment, and discuss the strengths and weaknesses of each approach.
- More fully evaluate the utility of using computational fluid dynamic (CFD) models to extrapolate to low concentrations.
- The draft assessment needs to discuss more fully the methods of the assessment. This should include clear concise statements of criteria used to exclude, include, and advance studies for derivation of the RfCs and unit risk estimates.
- All critical studies need to be thoroughly evaluated for strengths and weaknesses by using uniform approaches.
- The weight-of-evidence descriptions need to indicate the various determinants of "weight." The reader needs to be able to understand what elements (such as consistency) were emphasized in synthesizing the evidence.

MODE OF ACTION - RESEARCH HIGHLIGHTS

Threshold for Safe Exposures – Animal Evidence

Tumor Incidence and Cell Proliferation in Rats Exposed to Formaldehyde



Swenberg, James A., Benjamin C. Moeller, Kun Lu, Julia E. Rager, Rebecca C. Fry, and Thomas B. Starr. "Formaldehyde Carcinogenicity Research 30 Years and Counting for Mode of Action, Epidemiology, and Cancer Risk Assessment." Toxicologic Pathology (2013): Feb;41(2):181-9.

Dose and Temporal Association of Key and Associative Events for Nasal Tumors

Temporal Association

Days | Weeks | Months | Years

Dose Response Concordance

Concentration (formaldehyde ppm)	Overwhelm Intracellular Detoxification Mechanisms	x-links (% bkg)	DNA Adducts (HO-Me, % bkg)	Cytotoxicity	Epithelial Regenerative Hyperplasia	Metaplasisa	Rat Nasal Carcinoma (Monticello)
0.001 – 0.029							-
0.03- 0.29							-
0.3 - 0.82							-
0 - 0.83							0/90
0.84 – 2.3							0/90
2.4 – 7.1							0/96
7.2 - 11							1/90
12 - 17							20/90
18							69/147

[a]Concentration ranges are provided to align with concentrations used in carcinogenesis bioassays (lower bound values in range) and succinctly compare results from multiple studies

Lack of Exogenous Formaldehyde Beyond Portal of Entry - Animal Evidence

TABLE 1. Formation of N^2-HOMe-dG Mono-Adducts (mean ± SD) in Rat Nasal Epithelium, Bone Marrow, and White Blood Cells Exposed to 2 ppm Labeled Formaldehyde for 28 Days

Exposure Period	Rat Nasal Epithelium			Rat Bone Marrow			Rat White Blood Cells		
	N^2-HOMe-dG (adducts/10^7 dG)			N^2-HOMe-dG (adducts/10^7 dG)			N^2-HOMe-dG (adducts/10^7 dG)		
	Endogenous[a]	Exogenous	n	Endogenous[a]	Exogenous	n	Endogenous[a]	Exogenous	n
7 days	2.51 ± 0.63	0.35 ± 0.17	5	3.37 ± 1.56	n.d.	6	2.62 ± 1.12	n.d.	4
14 days	3.09 ± 0.98	0.84 ± 0.17	5	2.72 ± 1.36	n.d.	6	2.26 ± 0.46	n.d.	4
21 days	3.34 ± 1.06	0.95 ± 0.11	5	2.44 ± 0.96	n.d.	6	2.40 ± 0.47	n.d.	4
28 days	2.82 ± 0.76	1.05 ± 0.16	6	3.43 ± 2.20	0.34[b]	12	2.49 ± 0.50	n.d.	4
28 days + 6 h postexpo	2.80 ± 0.58	0.83 ± 0.33	9	2.41 ± 1.14	n.d.	6	2.97 ± 0.58	n.d.	4
28 days + 24 h postexpo	2.98 ± 0.70	0.80 ± 0.46	9	4.67 ± 1.84	n.d.	5	2.57 ± 0.58	n.d.	4
28 days + 72 h postexpo	2.99 ± 0.63	0.63 ± 0.12	9	5.55 ± 0.76	n.d.	6	1.75 ± 0.26	n.d.	4
28 days + 168 h postexpo	2.78 ± 0.48	0.67 ± 0.20	10	2.78 ± 1.94	n.d.	4	2.61 ± 1.22	n.d.	4
Air control	2.84 ± 0.54	n.d.	8	3.58 ± 0.99	n.d.	6	2.76 ± 0.66	n.d.	6

[a]No statistically significant difference was found using the 2-sided Dunnett's test (multiple comparisons with a control) (Dunnett, 1964).
[b]The amount of exogenous N^2-HOMe-dG adducts that was found in only 1 bone marrow sample analyzed by AB SCIEX Triple Quad 6500.
n.d., not detected.

TABLE 2. Formation of N^2-HOMe-dG Mono-Adducts (Mean ± SD) in Rat Thymus, Tracheal Bronchial Lymph Nodes, Mediastinal Lymph Nodes, Trachea, Lung, Spleen, Kidney, Liver, and Brain Exposed to 2 ppm Labeled Formaldehyde for 28 Days

Rat tissues	Exposure Period	N^2-HOMe-dG (adducts/10^7 dG)			Exposure Period	N^2-HOMe-dG (adducts/10^7 dG)		
		Endogenous	Exogenous	n		Endogenous	Exogenous	n
Thymus[a]	28 days	0.63 ± 0.06	n.d.	4	Air control	0.78 ± 0.04	n.d.	4
TBLN		3.01 ± 0.71	n.d.	4		3.46 ± 1.24	n.d.	4
Lymph nodes		2.80 ± 1.38	n.d.	4		2.99 ± 0.85	n.d.	4
Trachea		2.63 ± 0.92	n.d.	4		3.18 ± 0.72	n.d.	4
Lung		2.13 ± 0.26	n.d.	4		2.29 ± 0.24	n.d.	4
Spleen		1.83 ± 0.25	n.d.	4		2.18 ± 0.19	n.d.	4
Kidney		1.99 ± 0.09	n.d.	4		2.17 ± 0.60	n.d.	4
Liver		1.80 ± 0.02	n.d.	4		1.97 ± 0.38	n.d.	4
Brain		2.35 ± 1.00	n.d.	4		2.13 ± 0.17	n.d.	4

[a]Statistically significant difference was found using the 2-sided unpaired Student's t-tests.
TBLN, tracheal bronchial lymph nodes.
n.d., not detected.

Yu, Rui, Yongquan Lai, Hadley J. Hartwell, Benjamin C. Moeller, Melanie Doyle-Eisele, Dean Kracko, Wanda M. Bodnar, Thomas B. Starr, and James A. Swenberg. "Formation, accumulation, and hydrolysis of endogenous and exogenous formaldehyde-induced DNA damage." Toxicological Sciences 146, no. 1 (2015): 170-182.

Lack of Exogenous Formaldehyde Beyond Portal of Entry - Animal Evidence

Table 1

Formaldehyde-induced dG-Me-Cys in nose, peripheral blood mononuclear cells (PBMC), bone marrow, and liver of primates exposed to air control vs. 6 ppm of [$^{13}CD_2$]-formaldehyde (6 h per day).

Tissue	Targeted [$^{13}CD_2$]-Formaldehyde Concentration (ppm)	Exposure period (days)	dG-Me-Cys (crosslink/10^8 dG)	
			Endogenous	Exogenous
Nose	Air Control	2	3.59 ± 1.01 (n=5)	ND *
	6 ppm	2	3.76 ± 1.50 (n=5)	1.36 ± 0.20
PBMC	Air Control	2	1.34 ± 0.25 (n=5)	ND
	6 ppm	2	1.57 ± 0.58 (n=4)	ND
Bone Marrow	Air Control	2	2.30 ± 0.30 (n=4)	ND
	6 ppm	2	1.40 ± 0.46 (n=5)	ND
Liver	Air Control	2	15.46 ± 1.98 (n=6)	ND
	6 ppm	2	11.80 ± 2.21 (n=6)	ND

* ND, Not Detected.

Table 2

Formaldehyde-induced dG-Me-Cys in nasal tissue, peripheral blood mononuclear cells (PBMC), and bone marrow of rats exposed to Air Control verses 15 ppm of [$^{13}CD_2$]-formaldehyde (6 h per day).

Tissue	Targeted [$^{13}CD_2$]-Formaldehyde Concentration (ppm)	Exposure period (days)	dG-Me-Cys (crosslink/10^8 dG)	
			Endogenous	Exogenous
Nasal	0 - Air Control	4	6.50 ± 0.30 (n=5)	ND
	15.0	1	4.42 ± 1.10 (n=6)	5.52 ± 0.80
	15.0	2	4.28 ± 2.34 (n=6)	4.69 ± 1.76
	15.0	4	3.67 ± 0.80 (n=6)	18.18 ± 7.23
PBMC	0 - Air Control	4	4.98 ± 0.61 (n=5)	ND
	15.0	1	3.26 ± 0.73 (n=4)	ND
	15.0	2	3.00 ± 0.98 (n=5)	ND
	15.0	4	7.19 ± 1.73 (n=5)	ND
Bone Marrow	0 - Air Control	4	1.64 ± 0.49 (n=4)	ND
	15.0	1	1.80 ± 0.47 (n=4)	ND
	15.0	2	1.84 ± 0.61 (n=4)	ND
	15.0	4	1.58 ± 0.38 (n=4)	ND

ND, Not Detected.

Lai, Yongquan, Rui Yu, Hadley J. Hartwell, Benjamin C. Moeller, Wanda M. Bodnar, and James A. Swenberg. "Measurement of endogenous versus exogenous formaldehyde-induced DNA-protein crosslinks in animal tissues by stable isotope labeling and ultrasensitive mass spectrometry." Cancer Research (2016): 2016 May 1;76(9):2652-61.

Reality Check for Plausibility of Systemic Effects

- **Human Blood**
  - 2.61 µg/g background
    - No statistically significant increase in average blood concentrations were observed in a group of subjects exposed to 1.9 ppm HCO by inhalation for 40 minutes (Heck et al., 1985)
  - Blood volume approx. 7% b.w. – about 4,500 to 5,700 ml for an adult
  - At steady state there is about 13 mg of HCHO in blood (2.61 µg/g x 5000 g blood)

- **Whole body human production of HCHO/day 878-1310 mg/kg/day (EFSA, 2014)**
  - 52,680 – 91,700 mg/d for a 60-70 kg person
  - Amount of HCO inhaled at WHO Indoor Air Quality Standard (IAQS)
    - 100 µg/m^3 x 20 m^3/day = 2,000 µg/day (2 mg/day; Derived Calculation)

- **HCHO endogenously produced at ADI for aspartame is 4 mg/kg bw/day (EFSA, 2014)**
  - 280 mg for a 70 kg adult (Derived Calculation)

- **Based on the above, the maximum amount of formaldehyde inhaled at the WHO IAQS and available for systemic distribution is over 10,000x less than endogenously produced. The amount of HCHO generated through metabolism of aspartame at the ADI is about 140 times more than the amount of HCHO inhaled per day at the WHO IAQS.**

Lack of a Causal Association between Exogenous Formaldehyde and Leukemia - Epidemiology Evidence

Table 2
Summary of NRC (2011) comments or identified data gaps and new formaldehyde science by lines of inquiry.

NRC (2011) Comment/Identified Data Gap	New Formaldehyde Science
A. Epidemiological Evidence	
Evaluation of the most specific diagnoses available in the epidemiologic data (i.e., acute myeloblastic leukemia, chronic lymphocytic leukemia, and other specific lymphomas). *(NRC, p. 113)*	• New analyses of the NCI formaldehyde workers cohort specifically for AML are reported. Results do not support the hypothesis that formaldehyde causes AML. *See*: Checkoway et al., 2015 • Associations seen between formaldehyde exposure and Hodgkin lymphoma and chronic myeloid leukemia (CML) have not been observed in other studies and are not considered plausible. *See*: Checkoway et al., 2015
Because the draft IRIS assessment relies solely on epidemiologic studies to determine causality, further discussion of the specific strengths, weaknesses, and inconsistencies in several key studies is needed. *(NRC, p. 113)*	• A critical review of the epidemiological literature indicated no consistent or strong epidemiologic evidence that formaldehyde is causally related to any lymphohematopoetic malignancies. The absence of established toxicological mechanisms further weakens any arguments for causation. *See*: Checkoway et al., 2012
Clarification of the basis of its interpretations of the results regarding the various dose metrics (peak versus cumulative) and the various LHP cancers. *(NRC, p. 112–113)*	• Acute myeloid leukemia (AML) was unrelated to cumulative, average or peak exposure, and few deaths occurred within 20 or more years of last peak exposure. Suggestive associations with peak exposure were observed for chronic myeloid leukemia, based on very small numbers. Hodgkin lymphoma relative risk estimates suggested trends for both cumulative (p_{trend} = 0.05) and peak (p_{trend} = 0.003) exposures. However, no other lymphohematopoietic malignancy was associated with either cumulative or peak exposure. *See*: Checkoway et al., 2015
The selection and use of the NCI cohort (Beane Freeman et al., 2009) should be further justified. *(NRC, p. 112)*	• Extended follow-up of a cohort of 14,008 chemical workers at 6 factories in England and Wales, covering the period 1941–2012. Results provide no support for an increased hazard of myeloid leukemia from formaldehyde exposure. *See*: Coggon et al., 2014 • Extended follow-up of 11,098 employees of three garment manufacturing facilities. Results demonstrated limited evidence for formaldehyde exposure and any LHM including AML, based on 14 observed cases. *See*: Meyers et al., 2013

Mundt, Kenneth, Robinan Gentry, Linda Dell, Joseph Rodericks, and Paolo Boffetta. Six years after the NRC review of EPA's Draft IRIS Toxicological Review of Formaldehyde: Regulatory implications of new science in evaluating formaldehyde leukemogenicity. Regul Toxicol Pharmacol. (2017) Nov 20. pii: S0273-2300(17)30363-X.

Lack of a Causal Association between Exogenous Formaldehyde and Leukemia - Animal and MOA Evidence

- No cases of leukemia or lymphohematopoietic neoplasia were seen after formaldehyde inhalation in genetically predisposed C3B6·129F1-*Trp53*tm1Brd mice. *See*: Morgan et al., 2017

- Formaldehyde inhalation did not cause leukemia or lymphohematopoietic neoplasia in genetically predisposed p53-Haploinsufficient mice. *See*: Morgan et al., 2017

- Critical review of the genotoxicity literature found no convincing evidence that exogenous exposures to formaldehyde induce mutations at sites distant from the portal of entry tissue and review of the existing studies of hematotoxicity, likewise, failed to demonstrate myelotoxicity in any species– a probable prerequisite for leukemogenesis. *See*: Albertini and Kaden, 2016

- Additional analyses on the study data obtained from the original study (Zhang et al., 2010a) showed that differences in white blood cell, granulocyte, platelet, and red blood cell counts were not exposure-dependent. No association was observed between individual average formaldehyde exposure estimates and frequency of aneuploidy. *See*: Mundt et al., 2017

Excerpted from - Mundt, Kenneth, Robinan Gentry, Linda Dell, Joseph Rodericks, and Paolo Boffetta. Six years after the NRC review of EPA's Draft IRIS Toxicological Review of Formaldehyde: Regulatory implications of new science in evaluating formaldehyde leukemogenicity. Regul Toxicol Pharmacol. (2017) Nov 20. pii: S0273-2300(17)30363-X.

Ongoing Research - Expected Completion in 2018

Project	Scope
BBDR Modeling – Formaldehyde Case Study	Discusses benefits of the BBDR modeling, potential limitations and key areas where BBDR modeling informs the chemical assessment process using formaldehyde as a case study example
Formaldehyde BBDR Modeling Update	Updates the available formaldehyde BBDR model with new information
Formaldehyde Threshold Research	Evaluates threshold levels of formaldehyde exposure and differences in exogenous and endogenous exposures. Low dose exposures in rats (Air control, 1 ppb, 30 ppb, 300 ppb).
Formaldehyde Leukemia Subtypes Evaluation	Evaluates analytical epidemiology of lymphohematopoietic malignancies, relevant disease etiologies defined according to current classifications and decision-making based on accurate diagnosis and classification of the specific malignancies.
Formaldehyde Peak Exposures Evaluation	Evaluates peak and other exposure metrics in epidemiological research as they pertain to underlying disease mechanisms.

Scientific Expectations

Scientific Expectations

- Structure the chemical assessment for formaldehyde around a MOA framework based on the extensive understanding of cancer causation in the rat nose
- Differentiate carcinogenic potential for point of contact (for which there is affirmative evidence at high concentrations) vs. systemic exposure (for which there are affirmative data that this does not occur)
- Incorporate the role of endogenous formaldehyde into mode of action for carcinogenicity classification
- Incorporate the formaldehyde concentrations in air and tissues associated with postulated effects, the overall evidence for specific modes of action, perform a reality check, and compare and incorporate exogenous to endogenous exposures into the weight of evidence

Cited References

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5. Lai, Yongquan, Rui Yu, Hadley J. Hartwell, Benjamin C. Moeller, Wanda M. Bodnar, and James A. Swenberg. "Measurement of endogenous versus exogenous formaldehyde-induced DNA-protein crosslinks in animal tissues by stable isotope labeling and ultrasensitive mass spectrometry." Cancer Research (2016): 2016 May 1;76(9):2652-61.

6. Swenberg, James A., Benjamin C. Moeller, Kun Lu, Julia E. Rager, Rebecca C. Fry, and Thomas B. Starr. "Formaldehyde Carcinogenicity Research 30 Years and Counting for Mode of Action, Epidemiology, and Cancer Risk Assessment." Toxicologic Pathology (2013): Feb;41(2):181-9.

7. Yu, Rui, Yongquan Lai, Hadley J. Hartwell, Benjamin C. Moeller, Melanie Doyle-Eisele, Dean Kracko, Wanda M. Bodnar, Thomas B. Starr, and James A. Swenberg. "Formation, accumulation, and hydrolysis of endogenous and exogenous formaldehyde-induced DNA damage." Toxicological Sciences 146, no. 1 (2015): 170-182.