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Considerations for refining the risk assessment process for formaldehyde: Results from an interdisciplinary workshop

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ABSTRACT

Anticipating the need to evaluate and integrate scientific evidence to inform new risk assessments or to update existing risk assessments, the Formaldehyde Panel of the American Chemistry Council (ACC), in collaboration with the University of North Carolina, convened a workshop: "Understanding Potential Human Health Cancer Risk - From Data Integration to Risk Evaluation" in October 2017. Twenty-four (24) invited-experts participated with expertise in epidemiology, toxicology, science integration and risk evaluation. Including members of the organizing committee, there were 29 participants. The meeting included eleven presentations encompassing an introduction and three sessions: (1) "integrating the formaldehyde science on nasal/nasopharyngeal carcinogenicity and potential for causality"; (2) "integrating the formaldehyde science on lymphohematopoietic cancer and potential for causality; and, (3) "formaldehyde research-data suitable for risk assessment". Here we describe key points from the presentations on epidemiology, toxicology and mechanistic studies that should inform decisions about the potential carcinogenicity of formaldehyde in humans and the discussions about approaches for structuring an integrated, comprehensive risk assessment for formaldehyde. We also note challenges expected when attempting to reconcile divergent results observed from research conducted within and across different scientific disciplines - especially toxicology and epidemiology - and in integrating diverse, multi-disciplinary mechanistic evidence.

1. Background

Formaldehyde is a high-volume industrial chemical with production of 52 million tons in 2017. It is used in the manufacture of numerous products including urea-formaldehyde, phenol-formaldehyde, and melamine-formaldehyde resins, and serves as an adhesive in the production of particle board, medium density fiberboard, and plywood. As expected from its chemistry, airborne formaldehyde is a potent eye and respiratory tract irritant. In a 2-year inhalation study conducted almost 40 years ago, formaldehyde increased the incidence of squamous cell cancer in the front of the rat nose following lifetime exposures – 6 h/day, 5 days/week - at or above 6 ppm, with incidence reaching 50% at 15 ppm (Kerns et al., 1983). The extensive and still growing body of work on the mode of action (MOA) of formaldehyde in the rodent nose

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is discussed in detail later in this report (Swenberg et al., 2013).

In addition to toxicology studies, there have been several large epidemiological cohort studies examining nasopharyngeal cancer (NPC) (Beane Freeman et al., 2009; Coggon et al., 2014; Marsh et al., 2016) or lymphohematopoietic (LHP) cancers (Hauptmann et al., 2003, 2004, 2009). While these epidemiology studies have generally shown weak, inconsistent relationships between formaldehyde exposures and NPC or LHP cancers, one human study reported cross-sectional differences in several blood biomarkers, including aneuploidies, between a group of formaldehyde-exposed workers and a comparison group (Zhang et al., 2010). A more detailed analysis of the same study data identified a number of problems that challenge the findings (Mundt et al., 2017). Furthermore, these biomarker results stand in contrast with studies in multiple species of animals (Lu et al., 2010, 2011; Edrissi et al., 2013; Moeller et al., 2011; Lai et al., 2016; Swenberg et al., 2011, 2013) that demonstrate formaldehyde exposures do not increase formaldehyde concentrations in tissues beyond the portal of entry.

Between 2001 and 2012, various regulatory and advisory bodies conducted risk assessments for formaldehyde arriving at very different estimates of carcinogenic risk of formaldehyde exposures for general population, commercial (e.g. building materials) and occupational exposures (USEPA, 2010; ECHA, 2011; SCOEL, 2016; Health Canada, 2001). A proposed USEPA risk assessment regarded formaldehyde as a known human carcinogen, based on the epidemiological studies, and used a low dose linear model to estimate general population risks (USEPA, 2010). That risk assessment underwent review resulting in significant criticism from the National Academy of Sciences (NRC, 2011) and remains in revision. In finalizing the draft, it will be necessary to account for the diverse and sometimes divergent estimates of the risks of exposure to low levels of formaldehyde and reconcile different expectations for risk arising from the detailed toxicological evaluations or from the epidemiology.

To address the need to evaluate and integrate currently available scientific evidence that would be relevant for a new formaldehyde cancer risk assessment, the formaldehyde panel of the American Chemistry Council (ACC), in collaboration with the University of North Carolina, convened a day and a half, invited-expert workshop: “Understanding Potential Human Health Cancer Risk - From Data Integration to Risk Evaluation,” in Chapel Hill, North Carolina, on Oct 10 and 11, 2017. The co-chairs of the workshop were Dr. James A. Swenberg, University of North Carolina, and Dr. Kenneth Mundt, Ramboll. Invitations were offered to 30 scientists who had conducted research on formaldehyde, performed risk assessments for the compound or had participated in the interpretation of toxicological and epidemiological evidence on formaldehyde. Of these, 24 accepted the invitation including 4 USEPA scientists, 1 state agency scientist, 9 members of academia, 9 independent scientists and 5 scientists employed by industry. Attendee expertise included biostatistics, epidemiology, toxicology, science integration and risk evaluation. Counting invited participants and members of the organizing committee, there were 29 participants.

The meeting included eleven (11) presentations, providing background information on the toxicology, epidemiology and risk assessment of formaldehyde. Table 1 shows the list of presentations from the workshop, identifying the individual who developed and presented the material and the order in which the material was discussed at the meeting. Presentations were complemented by both formal and informal discussion sessions, providing opportunities for contributions from all participants on each topic. The meeting agenda, list of participants and a file with all presentations are provided in the supplemental materials (Supplemental Files 1–3). An introductory session was followed by three technical sessions: (1) “integrating the formaldehyde science on nasal carcinogenicity and potential for causality”; (2) “integrating the formaldehyde science on lymphohematopoietic cancer and potential for causality; and, (3) “formaldehyde research-data suitable for risk assessment”. A set of charge questions was developed before the meeting both to ensure coverage of key issues associated with developing an up-to-date risk assessment for formaldehyde and to keep the discussions focused on the most important issues (Table 2). These questions were intended to focus, but not constrain, the discussions that followed each session, and are discussed in more detail at various places in this paper. This workshop overview organizes the material from the presentations in textual format and also provides summary points from the talks in Tables and in Supplemental Files 4A - 4F.

2. Introductory session

The present workshop was intended, in part, to build on a meeting held by the USEPA in April 2014: “State-of-the-Science Workshop to Discuss Issues Relevant for Assessing the Health Hazards of Formaldehyde Inhalation”. The USEPA workshop focused on three topics: (1) evidence pertaining to the influence of endogenous formaldehyde on the toxicity of inhaled formaldehyde and implications for the health assessment; (2) mechanistic evidence relevant to inhaled formaldehyde and LHP cancers (leukemia and lymphomas); and, (3) epidemiological research on formaldehyde exposures and these lymphohematopoietic cancers. The agenda for that workshop is available at https://www.epa.gov/iris/formaldehyde-workshop. No report has been published and none has been posted on the USEPA website. In contrast to the targeted focus of the USEPA workshop, the goal of our 2017 workshop was to provide a broadly collaborative opportunity to discuss the existing data streams for formaldehyde; outline possible approaches to integrating toxicological, mechanistic and epidemiological evidence on potential formaldehyde carcinogenicity; and advance the understanding of how these contributions could guide a comprehensive risk assessment.

The current risk assessment landscape: With different emphases placed on various information streams, e.g. the toxicology and MOA studies in animals or the epidemiological and biomarker studies in humans, the current hazard classifications and quantitative risk estimates for formaldehyde vary widely across regulatory agencies and authoritative bodies (Table 3). Quantitative risks estimated for exposures to 1 ppb formaldehyde range over 6 orders of magnitude from 2.3×10^{-10} (Health Canada) to 1×10^{-4} (USEPA, 2010 Draft Assessment). These differences arise largely from decisions regarding the manner in which formaldehyde causes cancer in rats. Several authoritative bodies have concluded that formaldehyde is a known human carcinogen for NPC and LHP cancer (IARC, 2012; NTP, 2011), while other authoritative bodies and regulatory agencies take the position that formaldehyde would only be expected to cause tumors at sites of contact if concentrations were sufficient to cause cytotoxicity and that effects are unlikely in any tissues beyond those, such as the portal of entry, in which there is reported cytotoxicity (ECHA, 2011; Nielsen et al., 2017; WHO, 2010; Health Canada, 2001).

Integrating the science on nasal/nasopharyngeal carcinogenicity of formaldehyde and potential for causality:

The interpretation of formaldehyde epidemiology in workers should be informed by the more detailed understanding of the effects of higher concentration, life-time exposures in the rodent nasal passages and the overall information on the MOA of formaldehyde. Toward this end, the session provided an overview on (1) the toxicology of formaldehyde in the rat nose, (2) toxicology of other compounds whose metabolism produces formaldehyde in the body, (3) current occupational standards and (4) a discussion of the NPC epidemiology studies. A summary of the information presented is provided in the following sections.

MOA-related Toxicology Studies with Formaldehyde and Formaldehyde Generating Substances: The MOA for carcinogenesis (Boobis et al., 2006) was defined as: “a biologically plausible sequence of key events leading

Table 1
List of presentations.

Presentation Title	Presenter	Section in Paper
1. Understanding Potential Human Cancer Risk From Data Integration to Risk Evaluation	Dr. Kenneth Mundt	Introduction
2. Understanding the Formaldehyde Science and Putting the Pieces Together	Dr. P. Robinan Gentry	Introduction
3. Summary of Global Risk Assessment Approaches for the Formaldehyde Science	Dr. James Bus	Looking at the Current Risk Assessment Landscape
4. European Approach for Evaluating the Formaldehyde Science: OEL, Nasal Impacts and Threshold Assessments	Dr. Hermann Bolt	Session 1: Integrating the science on nasal/nasopharyngeal carcinogenicity
5. Formaldehyde and Nasopharyngeal Cancer: What have we learned from Epidemiology Studies?	Dr. Gary Marsh	Epidemiological studies on Nasopharyngeal Cacner
6. Key Events and Considerations for LHP Cancers	Dr. Kenneth Mundt	Session 2 - Integrating the formaldehyde science on LHP cancer and potential for causality:
7. Epidemiological Evidence for Associations between Formaldehyde and Lymphohematopoietic Malignancies	Dr. Harvey Checkoway	Epidemiological evaluations of possible LHP in occupationally exposed populations
8. Overview of Animal Science for Plausibility of Formaldehyde to Induce LHP	Dr. Chad Thompson	Other data streams pertinent to an integrated assessment
9. Keeping up with Knowledge from Formaldehyde and Genetics	Dr. James Swenberg	Modern assessment of formaldehyde-DNA reaction products in rodents and non-human primates1
10. Looking Across Data Streams to draw Conclusions regarding Causality: Key Considerations in the Formaldehyde Science	Dr. Harvey Clewell	Moving toward data integration for the risk assessment.
11. Formaldehyde MOA Framework for Pursuing Integrated Risk Assessments Based on all Available Information	Dr. Melvin Andersen & Dr. James Sherman	Section 3: Formaldehyde - Data Rich chemical ripe for risk assessment?

to an observed effect supported by robust experimental observations and mechanistic data. It describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed carcinogenicity—in a logical framework. MOA contrasts with mechanism of action, which generally involves a sufficient understanding of the molecular basis for an effect and its detailed description, so causation can be established in molecular terms."

Over the 35 years since the first report of tumors in the front of the rat nose associated with high concentration chronic exposures to formaldehyde, various groups have examined many aspects of the MOA of formaldehyde and key events in the rat (Andersen et al., 2010; Casanova-Schmitz et al., 1984; Lu et al., 2010, 2011; Moeller et al., 2011; Monticello et al., 1996; Swenberg et al., 2011). The incidence of tumors correlated with increasing levels of cell proliferation in the tumor-bearing regions of the nose (Monticello et al., 1991) and the dose-response for nasal cancer in male rats increased sharply at exposure concentrations above 6 ppm (Fig. 1). In other work, formaldehyde did not increase K-ras or p53 mutations in nasal tissues (Meng et al., 2010), indicating little to no mutagenic effect on these oncogenes *in vivo* at concentrations up to 15 ppm in the target tissue for toxicity and cancer. DNA-reaction products, first measured as radiolabeled DNA-protein crosslinks (Casanova et al., 1994) and more recently as various formaldehyde DNA-adducts, have been quantified in the nasal epithelium and other tissues following formaldehyde inhalation in rats and non-human primates (Swenberg et al., 2013; Yu et al., 2015). Detailed gene expression data from nasal epithelial tissues were also collected for various times of exposure and at formaldehyde concentrations equivalent to those used in the cancer bioassays (Andersen et al., 2008, 2010). These studies indicated dose-dependent transitions with different patterns of gene enrichment for exposures below 6 ppm and signatures related to cytotoxicity, cell proliferation and DNA-damage repair only apparent at concentrations of 6 ppm and above. An extensive effort developing a biologically-based dose-response (BBDR) model for formaldehyde induced cancer predicted the shape of the dose-response curve at exposures below those used directly in the bioassay (Conolly et al., 2003, 2004). The BBDR model was consistent with a highly non-linear dose-response curve for cancer at lower exposures with cancer incidence primarily influenced by enhanced cell proliferation rather than mutation. A National Academy of Sciences (NAS) committee evaluating the 2010 USEPA draft formaldehyde risk assessment strongly recommended the use of this BBDR model in the risk assessment (NRC, 2011).

While exogenous exposures to high concentrations of formaldehyde caused toxicity and cancer in the front of the nose in rats, formaldehyde is a normal product of intermediary metabolism in mammals, formed endogenously from serine, methionine, choline, and glycine by demethylation of N-, O -, and S -methyl compounds, including demethylation of histone proteins in the nucleus Due to these metabolic processes, formaldehyde is present at concentrations near 0.1–0.2 mM in blood and tissues (Heck et al., 1982, 1985) where it is expected to be hydrated or reversibly bound to glutathione (GSH) and other cellular nucleophiles. The presence of background formaldehyde has been included in several pharmacokinetic (PK) models (Casanova et al., 1991; Andersen et al., 2010). Other pharmacokinetic models for formaldehyde uptake and reactivity in tissues were developed and linked to computational fluid dynamic (CFD) modeling throughout the respiratory tract (Schroeter et al., 2014). In addition to endogenous production of formaldehyde throughout the body, a variety of compounds and quite a few drugs are metabolized to formaldehyde, including methanol, methyl chloride, aspartame and caffeine, the latter of which produces 3 mol of formaldehyde per mole of caffeine metabolized. Two-year bioassays with methyl chloride and methanol (ATSDR, 1998; Cruzan, 2009), both extensively metabolized to formaldehyde in the body, showed no evidence for cancer.

Overall, there is a rich, pertinent body of evidence available for understanding formaldehyde-induced squamous cell carcinoma in the front of the rat nose and for assessing the dose-response for mechanistically-linked effects – cell proliferation, histopathology, gene expression and accumulation of DNA-reaction products – in both rats and with some of the measures in non-human primates. These high-quality toxicology studies should be influential in establishing the MOA by which formaldehyde causes squamous cell carcinoma in the rat nose, the appropriate approaches for extrapolation of expected cancer risks below the region of observation and inferences about whether formaldehyde would be expected to reach and cause toxicity and cancer in deeper regions of the nose, such as the nasal pharynx, in either the rat or the human.

Occupational Exposure Guidelines: Occupational exposures to formaldehyde arise in various work environments and the inhaled concentrations at work can be significantly greater than in the general population. The approaches used in setting exposure limits for workers have focused on the evidence regarding nasal/nasopharyngeal carcinogenicity and should be considered an important part of developing a more integrated assessment for much lower concentration exposures that occur in the general population. In 2006, the German MAK commission set a maximum workplace concentration of 0.3 ppm based on several factors: (1) much higher level exposures were necessary to cause DNA-protein crosslinks (DPX) and cell proliferation in rats; (2) risks

Table 2
Charge questions.

SESSION 1: INTEGRATING THE FORMALDEHYDE SCIENCE ON NASAL CARCINOGENICITY AND POTENTIAL FOR CAUSALITY

1 Does the available scientific evidence support a specific MOA and casual association with NPC?
- What mechanistic evidence is available to support the proposed MOA frameworks discussed for NPC? What are the uncertainties?

2 What are the key animal data for characterizing the shape of the dose-response curve for formaldehyde-induced nasal tumors? What are the key epidemiological studies for formaldehyde- induced nasal tumors and how would you reconcile differences between those studies?
- If a causal association can be established for humans, what exposure metrics are associated with evidence of carcinogenicity? Is there evidence of a threshold for NPC in humans?

3 What quantitative methods (e.g., linear and non-linear low dose extrapolation, threshold, PBPK modeling for dose-response assessment) would best characterize the potential for NPC risk in humans?
- Are there uncertainties with any of these quantitative methods that suggest this type of modeling should not be applied?

SESSION 2: INTEGRATING THE FORMALDEHYDE SCINECE ON LHP CANCER AND POTENTIAL FOR CAUSALITY

1 What does the totality of the animal and epidemiology evidence tell us about the potential for a causal association with LHP and what conclusions can be drawn?
- What role dose endogenous production play in drawing conclusions regarding LHP?
- Do the available data support a specific MOA for hematopoietic cancers?

2 What mechanistic data are critical to understanding a causal association between formaldehyde exposure and specific hematopoietic cancers?

3 Do epidemiology studies provide useful dose-response data for LHP?

4 What methods for assessing causality and evidence integration are best applied to the available data for LHP cancer for conducting a hazard assessment (e.g., Bradford Hill criteria, biological systems approach, hypothesis based weight of evidence framework, systematic review, combination of approaches?)

5What uncertainties are important for consideration when integrating the available epidemiological evidence?

SESSION 3: FORMALDEHYDE – DATA RICH CHEMICAL RIPE FOR RISK EVALUATION?

1 What should be considered as the problem formulation and questions to be addressed when conducting a formaldehyde risk evaluation?

2 What are the best available approaches to conduct a robust evaluation of formaldehyde carcinogenic potential?

3 How can the approaches used to evaluate and integrate scientific evidence inform the risk assessment?
- What aspects of the Biological Systems Approach can be used to integrate the formaldehyde data?
- How can hypothesis based weight of evidence approach be used to integrate the data streams for determination of causality?

4 What needs to be added or changed in the draft IPCS MOA framework for nasal carcinogenicity?

5 What is the comparative weight of evidence for each hypothesized MOA for nasal carcinogenicity?

estimated from the BBDR model (Conolly et al., 2003, 2004) were below 1×10^{-6} for 0.3 ppm; and (3) an evaluation that showed a practical no observed adverse effect level (NOAEL) for eye and/or nose sensory irritation in workers at 0.3 ppm (Paustenbach et al., 1997).

A more extensive occupational assessment has recently been completed in Europe (SCOEL, 2016). The Scientific Committee on Occupational Exposure Limits (SCOEL) has developed a report in setting limits for carcinogens and irritant vapors and its application to formaldehyde. The SCOEL has 4 categories of carcinogens - Group A (carcinogens with a non-threshold), Group B (carcinogens unlikely to have a threshold), Group C (carcinogens likely to have a threshold) and Group D (carcinogens with a threshold). The initial evaluation with formaldehyde in 2006 designated formaldehyde as falling in Group C - a carcinogen with a likely threshold. The rationale was the role of nasal tissue irritation in tumor formation and the absence of evidence for systemic effects. Setting exposure limits that avoid local sensory irritation was expected to protect against local tissue irritation and, thus,

Table 3
Current North American and EU risk assessments with formaldehyde.

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; 3 ppm 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 "
NTP Report on Carcinogens (2011)		Qualitative	Known human carcinogen	Sufficient evidence in humans for nasal tumors and myeloid leukemia
IARC Monographs 10F (2010)		Qualitative	Known human carcinogen	Sufficient evidence in humans for tumors at both sites
IRIS (2010)	General	Low dose linear	1×10^{-4} at 1 ppb	For NPC, mutagenic MoA operating in conjunction with key event of formaldehyde cytotoxicity-induced cell proliferation; sufficient evidence of causal association for NPC and LHP cancer in humans

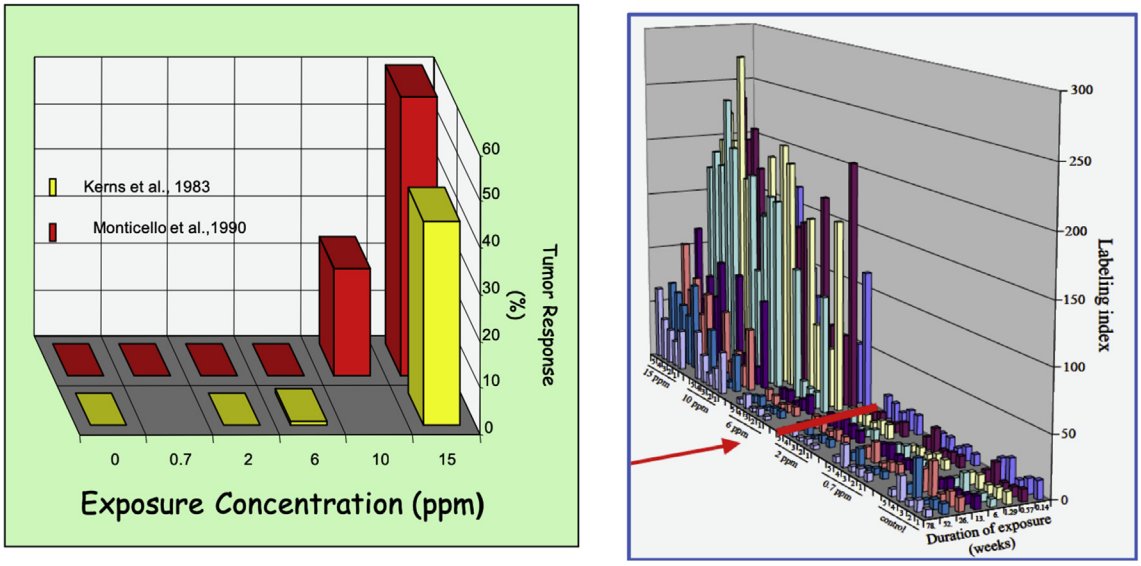

Fig. 1. Non-linear responses for squamous cell carcinoma and induced cell proliferation in the front regions of the rat nose. Two cancer bioassays with formaldehyde in male rats had remarkable consistency. The first had a slight increase in tumors at 6 ppm with an increase to nearly 40% at 15 ppm. In the second, which included an intermediate exposure group (10 ppm) between 6 and 15 ppm, there were no tumors at 6 ppm about 20% at 10 ppm and over 50% at 15 ppm. Groups of rats exposed for various duration were examined for cell proliferation in the tumor-bearing regions of the nose (right side of the figure). There were no increases at the lower concentrations for any duration of exposure. Increases in proliferation took place at the 6 ppm and higher exposures. The increases at 6 ppm persisted through the first 4 weeks and were diminished at longer times. In contrast, proliferation was increased at 10 and 15 ppm, the clearly carcinogenic exposures, for all durations of exposure. These bioassays did not provide evidence for tumors further back in the nose (i.e. the nasopharynx), for LHP cancer or for leukemia.

tumor formation. In 2016, the SCOEL recommendation was updated following consideration of a new human study with 41 volunteers looking at subjective rating of symptoms and complaints (Mueller et al., 2013) and another study with controlled human exposures in 21 volunteers (Lang et al., 2008). While the two studies applied slightly different exposure regimes, objective signs of sensory irritation were only observed at average concentrations of 0.5 ppm formaldehyde with peaks of 1 ppm. A NOAEL of 0.3 ppm with a peak of 0.6 ppm was consistent across both investigations: this exposure regime was proposed as the basis for an Occupational Exposure Limit (OEL) with a Short-Term Exposure Limit (STEL). The recommendation also noted that systemic toxicity of formaldehyde (such as induction of leukemia in humans) would not be expected at exposures where external formaldehyde does not change internal physiological levels, i.e. at exposures up to 0.4 ppm. This extensive SCOEL documentation should be an important resource in moving to any new risk assessment for formaldehyde exposure in non-occupationally exposed populations.

Epidemiological studies on Nasopharyngeal cancer: An NCI cohort study (reported in Blair et al., 1986; Hauptmann et al., 2004; Beane-Freeman et al., 2013a, 2013b) evaluated mortality outcomes among more than 25,000 workers in relation to formaldehyde exposures estimated retrospectively in 10 plants using or producing formaldehyde. Across the 10 plants, the median average intensity of formaldehyde exposure based on exposed jobs ranged from 0.080 ppm (Plant 7) to 2.799 ppm (Plant 2), although the exposure in Plant 2 may have been overestimated (Marsh and Youk, 2005). Earlier updates of the US NCI Cohort study suggested an association between NPC and formaldehyde that was driven by a statistically significant excess of NPC among workers from only one of the 10 plants (Blair et al., 1986; Hauptmann et al., 2004). This association in a single plant had been relied upon in some of the assessments by authoritative bodies to support designation of formaldehyde as a known human carcinogen (NTP, 2011; WHO, 2010). An independent historical cohort study of this plant included a nested case-control study of NPC, suggesting that the large NPC mortality excess in the single plant may not be due to formaldehyde exposure, but might have been the result of external employment in the ferrous and nonferrous metal industries of the local area with possible exposures to several suspected risk factors for upper respiratory system cancer, e.g. sulfuric acid mists, mineral acid, metal dusts and heat (Marsh et al., 2007).

There are several other studies of formaldehyde-exposed workers (Supplemental File 4A), including a cohort study of 14,408 workers from 6 British chemical plants (Coggon et al., 2014) and a US NIOSH study of 11,039 garment workers from 3 US plants (Pinkerton et al., 2004). The results from the British study provided no support for a cancer hazard of NPC or any other upper airway tumors and neither nasal nor NPC cancers were reported in the NIOSH study. An NCI case-control study (Hauptmann et al., 2009) looked at funeral industry professionals who died between 1960 and 1985. While four case subjects died of NPC, only two of these had performed embalming.

A Finnish cohort (Siew et al., 2012) of 1.2 million economically active men born between 1906 and 1945 that was combined with a Finnish Job Exposure Matrix (JEM) had 149 NPC cases with a relative risk (comparing formaldehyde exposures with no formaldehyde exposure) of 0.87 (i.e. a deficit risk). A Nordic population-based case control study of 1747 NPC cases with five (5) male controls per case had been conducted using a JEM to estimate exposures. No trends were seen across exposure groups - identified broadly as none, low, medium and high exposures. In contrast, in a cohort study of 2750 laminated plastic workers in Italy (Pira et al., 2014) using rough estimates of SMRs there was a suggestion of a possible excess in NPCs; however, formaldehyde concentrations were not measured in this study.

The discussion of status of the evaluation of the epidemiological evidence for NPC cancer (Table 4) noted that the association between NPC and formaldehyde was largely derived from the single plant within the NCI cohort (Marsh et al., 2007), with other studies showing no association. With continuing follow-up and analysis of the remainder of the NCI cohort (Plants 2–10), there seems to be little evidence for any association of NPC and formaldehyde exposure (Marsh et al., 2014, 2016). Several attendees recommend that, for any assessment of risks of NPC from formaldehyde, results from all Plants should be included in a weight of evidence analysis, while clearly noting the limitations - including chance - and possible confounders in Plant 1 of the NCI cohort.

Data integration for an NPC risk assessment: The challenges in

Table 4
Status of evaluation of epidemiological studies related to NPC.

- There are three large studies of Industrial Workers related to NPC cancers:
  - U.S. National Cancer Institute - NCI Blair et al. (1986); Hauptlarge studies of Hauptmann et al. 2003 (LHTC); 2004(Solid-NPC); Beane Freeman et al., 2009 (LHTC); 2013 (Solid-NPC)
  - The British Medical Research Council - MRC(Acheson et al., 1984; Gardner et al., 1993; Coggon et al., 2003, 2014)
  - U.S. National Institutes of Occupational Safety and Health – NIOSH (Stayner et al, 1988; Pinkerton et al., 2004; Meyers et al., 2013)
- The results of NCI cohort study weigh very heavily on all evaluations of the potential carcinogenicity of formaldehyde (e.g., IARC 2004; 2009, NTP 12th RoC; EPA IRIS)

•Updated reanalyses of the NCI cohort data and the independent University of Pittsburg study of NCI's Plant 1 cast considerable doubt on the validity of the NCI findings for NPC

- The cumulative epidemiological evidence for NPC to date, including the NCI cohort study and the University of Pittsburg study of Plant 1, does not support a causal association between FA and NPC

developing a more integrated assessment for NPC unavoidably requires consideration, consolidation and reconciliation of information across four different disciplines: animal toxicology, epidemiology, MOA research and both BBDR and pharmacokinetic modeling. Each of these has high value for the formaldehyde risk assessment and each has its own uncertainties (Table 5). Animal bioassays assist hazard identification and dose-response in the regions of observation; epidemiological studies assess human relevance and dose-response; mechanistic studies inform MOA (mutagenic, cytotoxicity, etc.) and support preferred approaches for low-dose extrapolation; and modeling permits incorporation of MOA into quantitative risk assessment to guide extrapolation across dose, route and species. Consideration of all these data streams will be necessary for a comprehensive risk assessment. However, each data stream carries uncertainties. Animal bioassays have uncertain human relevance and provide little information about dose-response below the experimental region; epidemiology faces problems of small numbers of NPC cases, cofounding and bias (Lash, 2012), artificial linearization of dose-response (Crump, 2005) and inaccurate assessment of exposures. Integration of MOA information in the evaluation of both the animal and epidemiological data may be useful in understanding the observations in animals compared to the absence of strong dose-response signals from the human cohorts. This will also be important in the determination of the type of dose-response modeling needed to describe the available data.

Four main points regarding the mechanistic research with formaldehyde were highlighted in the workshop: (1) transcriptomic analysis showed that tumors occur at concentrations associated with severe cellular disruption (6 ppm and above); (2) mutation analysis shows no evidence of *in vivo* mutagenic activity at concentrations that are clearly toxic and tumorigenic; (3) modeling of endogenous and exogenous formaldehyde-DNA adduct data (discussed further in a later section) supports a nonlinear dose-dependence for tumors; and, (4) the consideration across all these MOA studies strongly supports a non-linear, threshold-like dose-response for formaldehyde carcinogenicity in the rat nose. Nonetheless, the challenge remains: reconciling the extensive, consistent MOA-based toxicology studies of responses in the front of the nose with the epidemiological associations between NPC and formaldehyde exposures seen in a limited group of cohorts. A particular challenge with the broader epidemiological results on NPC is how to incorporate results from the large numbers of non-positive studies reporting no cases or non-positive results and also the inferences from CFD modeling of uptake throughout the nasal passages that indicate little penetration of formaldehyde into the back of the nose in either rodents or humans. In fact, the NAS review of the draft IRIS assessment (NRC, 2011) highlighted challenges that arise in the risk assessment related to the need to consider background levels of formaldehyde and the inferences from CFD and BBDR models in the integrated analyses. The NAS committee directed the USEPA to present results from the BBDR and CFD models alongside other dose-response models used in the assessment, so that USEPA conclusions regarding the best choice of model would be more transparent.

3. Integrating the formaldehyde science on LHP cancer and potential for causality

There also are divergent conclusions about LHP cancer causation by formaldehyde (Table 6). In 2004, IARC concluded that there was sufficient evidence of NPC causation by formaldehyde, but that the evidence for leukemia and occupational exposures, while strong, was not sufficient to designate formaldehyde as a known human leukemogen. In their discussion of supporting data, the committee emphasized that the mechanism by which myeloid leukemia might be induced was unknown and that it was not possible to identify a plausible mechanism for induction of myeloid leukemia by formaldehyde. By 2009, IARC regarded the epidemiological evidence as showing that formaldehyde caused both cancer of the nasopharynx and leukemia. However, the IARC working group at the time was split; a small majority judged the evidence to be sufficient and the remainder considered it limited. The toxicological evidence at that time remained inconsistent; neither bone marrow toxicity, pancytopenia, a precursor to leukemia, nor leukemias were observed in high dose, well-conducted, chronic exposure studies in animals. Some emphasis was placed on a study (Zhang et al., 2010), while accepted for publication was available only in draft form, for the review, in formaldehyde-exposed workers that showed evidence of aneuploidy in white blood cells (Zhang et al., 2010). The IARC Working Group felt that this study needed to be replicated.

USEPA's DRAFT IRIS assessment (USEPA, 2010) followed much the same rationale in arriving at their conclusion that the epidemiological evidence was sufficient to conclude a causal relationship between formaldehyde exposures and all leukemias, myeloid leukemias (ML) or

Table 5
Key issues within each stream of evidence.

Cancer	Epidemiological	Animal	Mode of Action
NPC	• Evidence of upper respiratory cancer vs. other sites of cancer • Define strengths, weakness and inconsistencies of key studies • Statistical power of studies, due to rarity of type of cancer • Selection of studies for dose-response modeling	• Comparison of cancer sites in animals vs. humans • Evidence from animal models in various regions of the nose	• Understanding role of endogenous formaldehyde • Role of mutagenicity and cytotoxicity • Potential for a threshold in the animal modlel
LHP	• Specific vs. grouped LHM cancers • Define strengths, weakness and inconsistencies of key studies • Selection of studies for dose-response modeling • Understanding non-traditional exposure metrics	• Paucity of evidence for LHP from animal models	• Understanding role of endogenous formaldehyde • Data regarding potential for aneuploidy/cytogenetic effects at distant sites • Reconciling divergent statements regarding systemic delivery • Reconciling varying conclusions regarding causality

Table 6
Background Information on LHP Cancer in relation to Formaldehyde Exposures.

Organization	Toxicological Evidence	Epidemiological Evidence	Supporting Data
IARC Monograph 88 (2006)	"The Working Group was not aware of any good rodent models that simulate the occurrence of acute myeloid leukemia in humans."	"Strong but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde."	The mechanism for inducing myeloid leukemia is not known. Possible mechanisms considered included clastogenic damage to circulatory stem cells. "… on the basis of the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukemia in humans."
IAC Monograph 100F (2012)	"Studies of bone marrow cells in formaldehyde-exposed animals have been inconsistent." "Pancytopenia has not been among the hematological findings in experiments with laboratory animals …" "Inconsistent genotoxic effects [seen] …"	Formaldehyde causes cancer of the nasopharynx and leukemia. "The Working Group was not in full agreement on the evaluation of formaldehyde causing leukemia in humans …"	"Particularly relevant … was a recent study accepted for publication which, for the first time, reported aneuploidy in blood of exposed …" "Three possible mechanisms, all focused around genotoxicity, are moderately supported as …"
US EPA Draft IRIS Toxicological Review (2010)	Limited evidence to support conclusion that formaldehyde exposure causes leukemia.	"Human epidemiological evidence is sufficient to conclude a causal association between formaldehyde exposure and nasopharyngeal cancer, nasal and paranasal cancer, all leukemias, ML and lymphohematopoietic cancers as a group."	"Chromosomal damage in blood-borne immune cells, relevant to agent-induced lymphohematopoietic cancers has been documented in formaldehyde exposed workers, including increased micronuclei and chromosomal aberrations, increased incidence and aneuploidy in hematopoietic stem cells."
National Toxicology Program 12thReport on Carcinogens (2013)	"Hemolymphorecticular tumor (combined types) in rats of both sexes also were significantly increased after long-term exposure of adults; however, it is unclear whether these tumors were exposure- related, because of limitations in the reporting of these tumors (Soffritti et al., 2002)."	"Epidemiological studies have demonstrated a causal relationship between exposure to formaldehyde and cancer in humans. Causality is indicated by consistent findings of increased risks for nasopharyngeal cancer, sinonasal cancer, and lymphohematopoietic cancer, specifically myeloid leukemia "	"Most agents known to cause leukemia are thought to do so by directly damaging stem cells in the bone marrow. In order for a stem cell to become malignant, it must acquire genetic mutations and genomic instability (Zhang et al., 2010a)." "… how it can reach the bone marrow or cause toxicity or genotoxicity at distal sites."
European Chemical Agency (ECHA), Committee for Risk Assessment (RAC) (2012)	"No indication of carcinogenic potential on organs/tissues distant from the site of contact (respiratory tract) including lymphohematopoietic tumors in inhalation study of rats and mice (Kerns et al., 1983)."	Carcinogen 1b – H50 f (May cause cancer) "In conclusion, while some studies have found increased rates of leukemia, the epidemiology data do not show consistent findings across studies for leukemia rates … "Results based on cohort and case-control studies do not suggest an association between formaldehyde exposure and leukemia."	"Physiologically, formaldehyde occurs in most organisms, tissues and cells at very low concentrations." "… it may be expected that carcinogenic effects are not found at anatomical sites distant from the port of entry."
Scientific Committee on Occupational Exposure Limits for Formaldehyde (2016)	"In essence, new experimental data, reported since 2008, clearly indicate that systemic genotoxic action of inhaled formaldehyde is not likely, even at exposure concentrations leading to nasal malignancies in the rat."	"A possible induction of myeloid leukemias by formaldehyde in humans is not so easy to explain … Such an action would not be possible within a range where the external dose does not change the physiological level of formaldehyde."	"A plethora of arguments suggests that formaldehyde concentrations below 1 or 2 ppm would not increase the risk of cancer in the nose or any other tissue, or affect formaldehyde homeostasis within epithelial cells (Swenberg et al., 2013)."

LHP cancer as a group. Although neither data integration nor weight-of-evidence considerations influenced their conclusions, USEPA recognized that only limited toxicological evidence was available to support the conclusion that formaldehyde causes leukemia (USEPA, 2010 Draft Assessment). While the 2012 National Toxicology Program's 12th Report on Carcinogens (NTP, 2011) stated unambiguously that epidemiological studies pointed to a causal relationship between formaldehyde and human cancer, they noted that the evidence for NPC was stronger than that for myeloid leukemia and voiced concern over uncertainty in understanding how a highly reactive, rapidly metabolized compound such as formaldehyde could reach the bone marrow or cause toxicity at sites distant from the portal of entry.

Reliable decisions about carcinogen classification, for formaldehyde or any other chemical, should include both the strength and consistency of the epidemiological evidence and integration with MOA evidence from animal studies into their final conclusions. This balanced approach is necessary to generate a more integrated assessment, but also raises some questions. For instance, (1) where do differences of interpretations exist for causation of human cancer and why are they present; (2) are there still data gaps, and can we identify practical ways of filling them; and, (3) if formaldehyde were regarded to be a human leukemogen, what would be the best framework for risk assessment and for low dose extrapolation? To adequately consider these questions, we first need to have a good sense of the current epidemiological support for human LHP cancers in exposed populations. Session 2 provided an overview (1) on the epidemiological evaluations of LHP cancer in occupational populations, (2) on studies conducted to evaluate the formation of formaldehyde-DNA reaction products in tissues from rodents and non-human primates, and (3) on other data streams of animal toxicology of formaldehyde that need to be considered in an integrated formaldehyde assessment for leukemia. A summary of the information presented is provided in the following sections.

Epidemiological evaluations of possible LHP cancer in occupationally exposed populations: There have been four influential studies addressing LHP cancer risks in occupational cohorts - the NCI cohort of workers from 10 US producing/using facilities; a UK cohort of employees from 6 formaldehyde producing/using facilities; a NIOSH study of US garment workers; and an NCI study of funeral industry workers (Supplemental File 4B). Although a reported association between "peak" occupational formaldehyde exposure and risk for myeloid leukemia (ML) was highly influential in the International Agency for Research (IARC) classification of formaldehyde as a Category 1 ("confirmed") human carcinogen, the same NCI cohort study indicated no association with cumulative exposure, the standard dose metric. However, re-analysis of the data by

an independent group also found no association with cumulative exposure and noted that very few employees who died of ML had peak exposures (Checkoway et al., 2015). The relative risk for various LHP cancers was reported to be increased in three of the four studies (Hauptmann et al., 2009; Beane Freeman et al., 2009; Meyers et al., 2013), with the most consistent signal observed for myeloid leukemia (ML) and peak exposures.

In contrast, a UK study of 14,008 subjects from 6 industries (Coggon et al., 2014) showed no increase in relative risk (RR) for ML. The UK study was analyzed using different exposure metrics - highest exposure levels and highest exposure levels for different periods of time (>2.0 ppm for <1 year and >2 ppm for more than 1 year). In a nested case-control analysis of incident or fatal ML, the highest relative risk estimate (Odds Ratio, OR) was for >2.0 ppm for <1 yr – with no elevation of risk for longer exposures at that level - a dose-response pattern contrary to that which might be expected if the relationship to formaldehyde exposure were causal (Coggon et al., 2014).

As with discussions at the meeting about NPC in humans, there were several conclusions regarding the LHP cancers: (1) epidemiological evidence for an association between formaldehyde exposure and LHP cancer was weak; (2) there was more support for an association with ML than for other LHP cancers, but even this association was inconsistent; (3) population-based case-control studies showed no or very weak associations with any LHP cancer; (4) there was no consistent or strong evidence that formaldehyde causes any of the LHP cancers; and (5) improvements in disease classification and exposure assessment in future epidemiologic research, and incorporation of evidence of modes of action were essential to advancing understanding of any possible role of formaldehyde exposure in the development of LHP. Overall, there do not appear to be strong, consistent signals indicating that formaldehyde causes LHP cancer in workers.

Formaldehyde-DNA reaction products in tissues from rodents and non-human primates: Originally, DNA-protein cross-links (DPX) formed by radiolabeled ^{14}C-formaldehyde were used to measure increases in these reaction products from formaldehyde exposure in rats (Casanova et al., 1989) and also to examine DPX formation in tissues from the nose in non-human primates (Casanova et al., 1991). Newer technologies based on LC-MS-MS (liquid chromatography with tandem mass spectrometry) methods used exposures to mass labelled ($^{13}CD_2$)-formaldehyde to determine both endogenous and exogenous reaction products between DNA bases and formaldehyde. Studies were also conducted to measure N^6-formyllysine (a measure of protein-protein cross-links) in the nasal epithelium and several other tissues (lung, liver and bone marrow) after exposures of rats to formaldehyde for 6 h at 2 ppm and higher. The results from these studies help evaluate any ability of inhaled formaldehyde to reach tissues beyond the portal of entry.

Endogenous DNA-adduct levels were present throughout all the tissues, whereas exogenous lysine adducts were only seen in the nasal epithelium (Lu et al., 2010, 2011; Moeller et al., 2011; Yu et al., 2015; Lai et al., 2016). DNA-protein crosslinks were indirectly measured by examining dG-Me-cysteine (Table 7). Again, endogenous levels were present in the nose and other tissues, including peripheral blood monocytes and bone marrow. In both rats and non-human primates, exogenous dG-Me-Cys was also only found in the nose (Supplemental File 4C). The conclusion of studies of DNA-formaldehyde reaction products was that exogenously delivered formaldehyde does not reach tissues beyond the front of the nose. When both endogenous $^{12}CD_2$-N(2)-hydroxymethyl dG and exogenous $^{13}CD_2$-N(2)-hydroxymethyl dG, a direct product of the reaction of DNA bases with formaldehyde, were measured in the rat nose after exposures to 2 ppm for 28 days, the exogenous adduct concentration was only about a third of that of the endogenous adduct (Yu et al., 2015).

These analytical chemistry studies demonstrate that endogenous formaldehyde-DNA reaction products are found throughout the body. The concentrations of these reaction products are expected to be influenced by various detoxifying enzymes. For example, transgenic mice lacking aldehyde dehydrogenase 5 (*Adh5* $-/-$), an enzyme that converts formaldehyde to formate, show a modest increase, i.e. less than two-fold, in N2-methyldG in bone marrow compared to controls (Pontel et al., 2015). Mice lacking both aldehyde dehydrogenase5 (*Adh5* $-/-$) and an important DNA-repair related protein, Fanconi Anemia complementation group D2 (*Fancd2* $-/-$), develop bone marrow failure and leukemia (Pontel et al., 2015). These mice live only to 1 or 2 months of age. While exposures to higher concentrations of tissue formaldehyde appear to play a role in leukemia in mice lacking both *Adh5* and *Fancd2*, wild-type mice had adduct levels very similar to those found in *Adh5* ($-/-$) mice and both wild-type and the *Adh5* ($-/-$) mice had survival curves and levels of bone marrow toxicity that were similar to those seen in wild-type mice.

In addition to measurements of DNA-formaldehyde reaction products in various tissues, Starr and Swenbwerg (2013) proposed an alternative risk assessment approach, referred to by these authors as a bottom-up approach, using these formaldehyde-DNA reaction products to estimate a conservative upper bound of the low dose risks of formaldehyde based on adducts in the nose in rats and on the absence of adducts in tissues remote from the nose in rats and monkeys. The method was refined and extended in a later publication (Starr and Swenbwerg, 2016). This procedure represents an alternative to the other approaches for risk assessment, i.e. either using the low dose, linear default (USEPA, 2010) or the BBDR model (Conolly et al., 2003, 2004).

Other data streams pertinent to an integrated risk assessment for LHP cancer: Available animal toxicology studies also provide information on possible responses of tissues remote from the nose and the results of these toxicological studies can be useful in assessing the validity of proposed MOAs for human NPC or LHP cancer. In the available, well-conducted animal bioassays, there was no evidence for leukemia or lymphoma either with the inhalation (Kerns et al., 1983) or the oral-dosing studies (Til et al., 1989) (Supplemental File 4D). Despite the lack of direct evidence for cancer in these tissues in the animal studies, three hypothesized MOAs have been proposed for human LHP to account for epidemiological observation and the DNA-damage biomarker increases in formaldehyde exposed manufacturing workers (Zhang et al., 2010). These MOAs are: (1) passage of formaldehyde from lung to bone marrow leading to toxicity and leukemia; (2) formaldehyde affecting hematopoietic stem cells present in blood as these cells move through more highly exposed tissues in the front of the nose; and, (3) formaldehyde affecting primitive stem cells (with potential to become hematopoietic cells) within nasal tissues themselves.

The first two hypothesized MOAs appear unlikely because studies in rats and non-human primates provide no evidence of increased concentrations of formaldehyde in the blood after inhalation exposures (Heck et al., 1985; Casanova et al., 1989; Heck and Casanova, 2004). More recently no increases in formaldehyde-DNA reaction products found at sites beyond the nose. The absence of formaldehyde-DNA reaction products in peripheral blood monocytes also suggests that the third MOA is unlikely (Lu et al., 2010, 2011; Moeller et al., 2011; Yu et al., 2015; Lai et al., 2016). The plausibility of this MOA can also be tested based on results from a recent NTP (2017) study conducted in transgenic mice (B6.129-Trp53^{tm1Brd} and C3B6.129F1- Trp53^{tm1Brd}) exposed by inhalation to formaldehyde (NTP, 2017). These mice had squamous metaplasia in the nasal passages but showed no evidence for blood cancers or changes in hematology, suggesting that any direct genotoxic action on primitive pluripotent cells in the nose was unlikely. In addition, *in vivo* genotoxicity studies showed no evidence of mutagenicity or clastogenicity in nasal epithelial cells (Meng et al., 2010; Speit et al., 2011), observations that argue against mutations occurring in primitive stem cells within nasal tissues themselves.

Participants found it particularly valuable to take the hypothesized human MOAs for formaldehyde and subject them to the same MOA verification process used in assessing the validity of MOAs proposed for cancer in animals. In at least a tentative manner, these same

Table 7
Formaldehyde-DNA Adducts in the rat Nose and at distant sites.

Rats Exposed to 15 ppm				Rats Exposed to 2 ppm			
Formaldehyde induced dG-Me-Cys in nose, PBMC and bone marrow of rats exposed to 15 ppm of formaldehyde (6 h per day)				Formation of formaldehyde-induced DPCs in rats nose exposed to 2-ppm labelled formaldehyde for 28 days			
Tissue	Exposure period (day)	dG-Me-Cys (crosslink/108 dG)		Tissue	Exposure period (day)	dG-Me-Cys (crosslink/108 dG)	
		Endogenous	Exogenous			Endogenous	Exogenous
Nose	0	6.50 ± 0.30 (n = 5)	ND*	Nose	7 days	4.78 ± 0.64 (n = 4)	0.96 ± 0.17
	1	4.42 ± 1.10 (n = 6)	5.52 ± 0.80		28 days	4.51 ± 1.48 (n = 3)	2.12 ± 1.00
	2	4.28 ± 2.34 (n = 6)	4.69 ± 1.76		28 days + 24h post expo.	3.78 ± 0.69 (n = 4)	2.12 ± 1.00
	4	3.67 ± 0.80 (n = 6)	18.18 ± 7.23		28 days + 168h post expo.	3.51 ± 0.16 (n = 3)	2.14 ± 1.02
PBMC	0	4.98 ± 0.61 (n = 5)	ND				
	1	3.26 ± 0.73 (n = 4)	ND				
	2	3.00 ± 0.98 (n = 5)	ND				
	4	7.19 ± 1.73 (n = 5)	ND				
Bone Marrow	0	1.49 ± 0.43 (n = 3)	ND				
	1	1.67 ± 0.18 (n = 3)	ND				
	2	1.66 ± 0.57 (n = 3)	ND				
	4	1.41 ± 0.21 (n = 3)	ND				

* ND, Not detected.

considerations had arisen in risk assessments and hazard classifications by IARC, NTP and USEPA. When designating formaldehyde as a human carcinogen, these groups noted the lack of toxicological support for the proposed MOAs in humans and the difficulty in understanding how formaldehyde could be delivered to tissues remote from the portal of entry. However, none of these organizations took steps to integrate these discrepant observations into their risk assessments or in decisions regarding classification as a human carcinogen. The workshop participants emphasized that considering only one line of evidence, such as only the epidemiology, when drawing conclusions should not be considered preferable to assessments considering all available data.

Conclusions from looking to both the animal toxicology and proposed human MOA evaluations were (1) the animal data, cited in USEPA (2010) to support a hazard of leukemia, are weak; (2) inhalation of formaldehyde does not increase blood formaldehyde concentrations (3) biomarkers of formaldehyde exposure (e.g. formaldehyde-DNA adducts); are not detected beyond the portal of entry in exposed animals; (4) *in vivo* genotoxicity assays following formaldehyde inhalation are negative both systemically and at the portal of entry; and, overall, that (5) animal data have yet to be generated that support a hazard for leukemia or hematotoxicity following formaldehyde exposure.

Discussion of Charge Questions/Possible Follow-up Studies:

Following the presentations and discussions of the human and animal studies on epidemiology, toxicity, MOAs and dose-response modeling, workshop attendees attempted to address charge questions more directly (Table 2), including consideration of the value of conducting targeted research/analysis that might clarify issues for the risk assessment.

Evidence regarding NPC: With respect to nasal carcinogenicity, the key animal data are consistent with a non-linear dose-response curve for the tumors, observed only in the very front of the rat nose. There was general agreement that the MOA for rat nasal tumors was primarily cytotoxicity or some combination of cytotoxicity and mutagenicity, with mutagenicity only contributing at exposure concentrations that cause cell proliferation. The uncertainty in establishing unequivocally that the cytotoxicity alone is the MOA is likely the reason that the SCOEL decision places formaldehyde in Category 4C as a carcinogen likely to have a threshold rather than Category 4D (carcinogens with a threshold). The BBDR model for formaldehyde, though, included both enhanced proliferation and the possibility of increases in mutation rates. Importantly, the BBDR model described the tumor-incidence data without requiring any increase in the mutation rate (Conolly et al., 2003, 2004). Overall, there was general agreement that there is significant non-linearity in the low-dose region with behavior consistent with a threshold response in the front of the rat nose. In addition, while there could be value in updating the BBDR model to refine estimates of contribution of increased mutation rates in the ranges of exposure causing tumors, further work with the BBDR model is not expected to alter the essential conclusions regarding the MOA - cancer in the proximal regions of the rat nose is caused by cytotoxicity of formaldehyde at high exposures leading first to compensatory cell.

The epidemiological evidence of an association between occupational formaldehyde exposure and NPC is limited. Neither is there evidence from the toxicology or MOA studies indicating the development of cancer in the nasopharynx region would be likely. For instance, no tumors were seen in this region in the rat (even at 15 ppm, an exposure that produced a 50% incidence of tumors in the front of the nose); CFD modeling indicated much lesser delivery of formaldehyde into the back of the nose; and DNA-formaldehyde reaction products are not found in the trachea (a region just distal to the nasopharynx) in rats (Yu et al., 2015). The discrepancy between the MOA for tissues in the proximal region of the rat nose and the inconsistent epidemiological association with NPC in a distal part of the human nose that is expected to have very much lower exposures could also be explained if the tumors in Plant 1 were related to confounding exposures or to the occurrence of a chance, random cluster of cases.

Two other questions were raised that could influence decisions about plausibility of a causal relationship between formaldehyde exposure and NPC. The first was whether there might be regional differences in pharmacodynamics (i.e. differential sensitivity of rat and human NP tissues to irritant exposures) and the second whether there might be differential delivery of formaldehyde to regions of the human nose and nasopharynx. To address the first question, a review of experience with other nasal carcinogens and nasal cancer in humans in general could be useful in determining whether there are any reasons to expect differential sensitivity for cancer arising in particular portions of the human nose compared to similar regions in the rat nose. With regard to the second question, CFD modeling throughout the respiratory tract can be used to determine if the NP-region of the human nose is expected to have an unusually high formaldehyde uptake at lower inhaled concentrations. Through this modeling exercise expected uptake into the human nasopharynx region at workplace exposures can be

compared with uptake in the target sites in the rat nose for the high concentration (i.e. ≥ 6 ppm) that caused cancer. Some work of this kind already has been reported (Kimbell, 2006), showing the nasopharynx receives much lower exposures than regions in the front of the nose. Updating the CFD models for NP dosimetry in rats and humans would permit a more quantitative assessment of species differences in formaldehyde dose in the NP region.

With respect to the epidemiological cohorts, there may be opportunities to continue evaluation of the US NCI study population to see if results with increasing length of observation strengthen the evidence for increased risk of NPC across the entire group of work-sites or lend further support to the conclusion that the association in the single Plant was likely due to chance or the presence of some confounder. Lastly, the expectation from participants was that the MOA seen in rats - high dose cytotoxicity and cancer associated with cell proliferation and increased tissue formaldehyde - should be applicable for toxicity and cancer formation in deeper regions of the nasal tissue where tumors should only occur if exposure became sufficiently great to cause toxicity. Any risk assessment for formaldehyde for portal of entry effects, including the human nasopharynx, would be pursued based on expectations of a non-linear dose response.

Evidence regarding LHP cancers: A key question with LHP tumor-types relates to integrating evidence available from the totality of animal and human studies to determine the potential for formaldehyde exposures to cause these cancers. The toxicology studies provide no convincing evidence for leukemia in any animal study - either by inhalation or ingestion. The associations in the epidemiological studies are weak, at best, and not clearly related to the intensity or duration of exposure to formaldehyde. A challenge in interpreting the epidemiological evidence for LHP malignancies is that the evidence for myeloid leukemias – the type most commonly linked with chemical exposure – is largely negative and the animal studies that identified nasal cancer in the front of the nose did not indicate significant increases in any type of leukemia. Discussions among the entire group indicated that combining all types of leukemia into a single category is not considered best practice because these various cancers have very different etiologies.

In assessing the mechanistic data relating to a possible hazard of LHP cancer, a key question was whether inhaled formaldehyde reaches target tissues/cells. The research on formaldehyde-DNA adducts in the past 10 years shows no evidence of exogenous formaldehyde reaction products in white blood cells, bone marrow or any tissue beyond the nose. Nonetheless, there are the reports that formaldehyde exposure in workers in China was associated with lower blood counts, effects on hematopoietic and myeloid progenitor cells and aneuploidy (Zhang et al., 2010) and that exposures of mice at concentrations up to 3 mg/m^3 (~2.4 ppm) caused a variety of blood and tissue responses (Ye et al., 2013; Zhang et al., 2013; Wei et al., 2016). These observations contrast to multiple studies that have examined the *in vivo* mutagenicity of inhaled formaldehyde in rats (Speit et al. 2009, 2011; Meng et al., 2010). In arriving at a weight of evidence on LHP cancer, the entirety of these biomarker studies of blood effects of formaldehyde need to be fully evaluated to consider possible reasons for these discrepancies.

Some efforts have been made to reconcile these disparate results (Albertini and Kaden, 2017) by conducting a critical review of the peer-reviewed, published literature addressing the genotoxicity of formaldehyde. Their review noted that chromosome changes in the form of aberrations or micronuclei in blood cells have been studied in formaldehyde exposed animals and humans, with most of the animal studies being negative. Human occupational studies had mixed results for such changes in peripheral blood lymphocytes (PBLs) - a group of cells that circulate widely but do not reflect recent bone marrow (BM) events. In addition, studies reporting changes in human BM or hematopoietic precursor cells (HPCs) either have had potentially confounding exposures or reported changes that may have been related to the *in vitro* treatments of the harvested cells rather than the *in vivo* exposures (Zhang et al., 2010; Albertini and Kaden, 2017).

The more detailed analysis of the data from the Zhang et al., (2010) study indicated that the reported changes could not validly be derived from a single cross-sectional assessment and were not dependent on formaldehyde exposure, most likely reflecting inherent differences between the exposed and unexposed groups (Mundt et al., 2017). The prevailing opinion from workshop participants was that the reported genetic changes in circulating blood cells do not provide convincing support for classifying formaldehyde as a probable or known human leukemogen. While attendees agreed that replication of the Zhang et al., (2010) findings would be helpful, it was acknowledged that finding a study group with comparably high formaldehyde exposures would be very difficult. Nonetheless, the reconciliation of the human and the animal studies on these blood changes remains an issue that will need to be addressed in any integrated assessment for formaldehyde.

Among the proposed MOAs in humans for LHP cancer was a suggestion that circulating stem cells in the blood compartment could be transformed by exposure to formaldehyde reaching the venous circulation in the nose. Based on the MOA in the rat nose and both the kinetics and rapid tissue clearance of formaldehyde, and highly sensitive analytical evidence that inhaled formaldehyde protein and/or DNA adducts are not detected in tissues beyond the portal of entry, it is difficult to see how significant amounts of formaldehyde would be delivered to either the bone marrow or circulating stem cells. Suggested follow-up in this area involved (1) further evaluation of any consistency between the LHP tumors in the epidemiological studies and those known to occur from other toxicants, such as benzene and certain chemotherapeutic compounds, that induce ML, and (2) extension of current pharmacokinetic models to assess likely delivery of formaldehyde to the venous blood exiting the front of the nose after exposure. Overall, the evidence for an association of formaldehyde exposure and LHP cancer is equivocal at best and even less plausible due to the absence of any association with cumulative exposures. Assessing the overall body of animal and human studies on leukemia within a construct such as the Bradford Hill criteria in the context of a MOA framework (Boobis et al., 2006) could help to organize the various, sometime conflicting lines of evidence and provide a more integrated, objective approach to drawing inferences about the possible associations of formaldehyde and LHP cancer.

Consideration of other compounds that are metabolized to formaldehyde: At several points during the meeting, there was discussion of compounds that are metabolized to formaldehyde, leading to systemic formaldehyde exposures to tissues throughout the body. These compounds include methyl chloride, methanol, caffeine and aspartame (EFSA, 2014). Dosing rats with one of these, methanol (as a 15% solution in drinking water), increased formaldehyde DNA-reaction products in bone marrow, kidney and liver by 2–3-fold (Pontel et al., 2015). In another study, in which rats were dosed with 500 or 2000 mg methanol/kg/day for 5 days with mass-labelled $^{13}CD_4$-methanol (Lu et al., 2012), there were increases in exogenous formaldehyde-DNA reaction products in multiple tissues, including white blood cells. Thus, it is clear that following methanol administration, formaldehyde is produced in tissues throughout the body at sufficient concentrations to increase DNA-reaction products; yet there is no evidence that methanol causes leukemia or other cancers (Cruzan, 2009).

Another commonly consumed compound - aspartame, an artificial sweetener - is metabolized to methanol and then to formaldehyde but is not carcinogenic in rats following high-dose (4 g/kg) dietary treatment (Shibui et al., 2019). With caffeine, demethylation produces formaldehyde directly in various tissues with 1 mol of caffeine producing up to 3 mol of formaldehyde. Methyl chloride is also extensively metabolized to formaldehyde in rats (Kornbrust and Bus, 1983) and significantly increases formaldehyde concentrations in rat liver testes and brain but not in nasal mucosa (Heck et al., 1982), yet it was carcinogenic only to the male mouse kidney and not in rats (ATSDR, 1998). Reviewing the animal toxicology and human studies for formaldehyde-producing compounds (and perhaps determining whether they lead to

increased formaldehyde-DNA reaction products in various tissues) could help clarify whether compounds that are delivered to tissues throughout the body and increase formaldehyde exposures in tissues distant from the portal of entry show any evidence for increased cancer in tissues either in the LHP system or in any other tissue beyond the portal of entry.

Formaldehyde – Data Rich Chemical Ripe for Risk Assessment:

This final session and discussion period were intended to more fully flesh out possible approaches for completing a comprehensive risk assessment that considered (1) the diversity of studies on formaldehyde, (2) the new directions arising from evolving approaches for data integration and (3) the challenges that are likely to be encountered in conducting a comprehensive risk assessment for formaldehyde. The NAS review of USEPA's Integrated Risk Information System (NRC, 2014) used a flowchart to illustrate the organization of systematic review and data integration that would support hazard identification and dose response modeling (Supplemental File 4E). This depiction showed the contribution of animal, human and mechanistic data streams. Little guidance was provided on methods to apply when there were significant differences in conclusions reached across these lines of evidence. The issues with formaldehyde largely revolve around the need to reconcile or explain in some way differences pertinent to (1) the formaldehyde MOA derived from toxicology and mechanistic studies; (2) the relevance and consistency of the possible human NPC in relation to the detailed understanding of responses in the rat nose; and, (3) the relevance and plausibility of human LHP cancer in context of the larger understanding of the MOA of formaldehyde with respect to cytotoxicity and tissue reactivity.

Emphasizing Mode of Action: A clear area of consensus was that any systematic approach to organizing the available evidence on NPC into an integrated risk assessment should be structured around a MOA framework based on the extensive understanding of cancer causation in the front of the rat nose, i.e. the one unequivocal site of carcinogenicity associated with exposure of an obligate nasal breathing rodent to high concentrations of formaldehyde over their entire lifetime. This tissue receives the bulk of the dose of inhaled formaldehyde and has been extensively examined for dose-dependent induction of tumors, cell proliferation, DNA-formaldehyde reaction products, pathology, and gene expression. There were no other cancer sites identified in the inhalation or oral bioassays with formaldehyde that would indicate other MOAs are operable at sites distant from the portal of entry such as the nasopharynx, circulating blood cells or bone marrow. With respect to evaluation of likely effects at distant sites, the state-of-the-art chemical analysis of various DNA-reaction products following formaldehyde or methanol exposures should be central considerations in evaluating the ability of inhaled formaldehyde to reach sites distant from the front of the nose and of the possible role of any DNA-reaction products in cancer causation in these tissues in humans.

Among the suggestions for proceeding forward was to begin using a framework similar to that employed by the International Programme on Chemical Safety (IPCS) for evaluating animal carcinogens (Sonich-Mullin et al., 2001), including listing of various possible MOAs and evaluating the evidence for each using concordance tables to support conclusions about whether the MOA in the rat nose cytotoxicity, mutagenicity or some combination of is the two. The original ICPS framework for evaluating MOAs for cancer was updated to include a component for evaluating the human relevance of a carcinogenic MOA (Boobis et al., 2006). In an earlier evaluation, formaldehyde-induced rat nasal tumors were chosen as an example to demonstrate a preferred format for presenting MOA evidence and evaluating the human relevance of the MOA (McGregor et al., 2006). The key events (KEs) in the threshold MOA included cytotoxicity, cell proliferation, and DPX formation. These threshold events were determined to be relevant for humans (McGregor et al., 2006), recognizing that tissue cytotoxicity was necessary as a key event leading to tumors. At this meeting, participants emphasized the need to update the MOA to include research performed over the last decade on multiple key events. It was recognized that additional key and associated events, such as the overwhelming of intracellular detoxification mechanisms, the formation of DNA adducts, and metaplasia may be helpful to include in the updated framework for formaldehyde-induced rat nasal tumors. This extension of the earlier MOA analysis (McGregor et al., 2006) would fit the intended purpose of the ICPS framework, i.e. to present a harmonized and flexible framework, for systematically evaluating available data in a transparent manner that could be continually updated to include new data and scientific thinking. We are at an appropriate point with new information to revisit this MOA assessment for nasal tumors from formaldehyde exposures in rats. With more progress in organizing evidence for a rat MOA or MOAs and implementation of a dose-response model for nasal tumors, the conclusions about the rat MOA for nasal tumors could be used as part of the process of organizing MOA frameworks for human NPC and LHP.

Coordination across Groups for Systematic Review: Moving beyond considerations of the MOA in the rat nose, four areas – best available science, weight of evidence, systematic review and data quality – deserve attention for conducting a more integrated risk assessment that looks to consider and reconcile the different conclusions reached in various risk assessments. The same questions related to MOA also need to be asked in reaching conclusions about the support for particular methods for low dose extrapolation.

Regarding data integration, the discussions considered methods to operationalize a structured framework that would allow systematic review of the full suite of studies. As an example, the Methodological Standards for the conduct of new Cochrane Intervention Reviews (MECIR) was discussed as a resource for methods of grading the quality of evidence and strength of recommendation. This resource was developed by the Grading of Recommendations, Assessment, Development and Training (GRADE) working group at US Cochrane Center (http://us.cochrane.org/about-us). Other points discussed were intended to ensure that adequate steps are taken to understand the quality and consistency of various lines of evidence. The Bradford Hill criteria could be useful here in considering the consistency of results across the broader suite of available research and knowledge. While it was emphasized that MOA determination - arising primarily from the detailed mechanistic studies in rats - needs to be a central activity for the integrated assessment, workshop participants indicated that there had been little experience in systematic reviews from a MOA perspective: i.e. systematically selecting key studies, identifying key events, assessing the quality of individual studies, and using the information to draw MOA-related conclusions. This activity will likely be necessary in any new risk assessment with formaldehyde.

Getting Various Stakeholders and Discipline Experts to Work Together: The task of data integration for such a risk assessment needs to be multi-disciplinary; however, communication between and among the various risk assessment-oriented disciplines is often lacking. Moving forward with new approaches for integrated risk assessments could draw on work going on in the evidence-based toxicology consortium at Johns Hopkins (http://www.ebtox.org/) to create a flowchart for such reviews. One concern voiced was that, current approaches for risk assessment, as evident from those with formaldehyde by IARC, NTP and USEPA IRIS, focus primarily on strength of evidence from specific studies – in these cases on select epidemiological investigations - rather than on the overall weight-of-the evidence provided by a comprehensive assessment of all epidemiological studies or all relevant information. This concern is perhaps best exemplified by the selection of epidemiology studies for labelling formaldehyde as a likely or known human carcinogen, followed by use of linear low-dose extrapolation for estimating risk, despite the larger body of MOA studies from non-human primates, rats and mice indicating that these inconsistent epidemiological associations lack toxicological plausibility and that a linearized low-dose model for cancer responses to formaldehyde appears implausible.

Reviewing both Modes of Action and Extrapolation Models: Finally, systematic review for any formaldehyde risk assessment process needs to evaluate both the qualitative evidence for various modes of action and the manner in which they are brought together to support the choice of extrapolation models and other aspects of quantitative risk assessment, such as identifying a point-of-departure. This type of robust evaluation appears beyond the scope of the current USEPA approach for systematic review, which has traditionally focused only on guideline toxicity studies rather than on custom-designed MOA studies or evaluations of quantitative BBDR models. In looking at the flowchart from the NAS IRIS review (Supplemental File 4E), the differentiation of the three data streams – human, animal and mechanistic – might need revision. In the current version, the three data streams merge into an activity box, "integrate evidence with a single output – first moving on to "hazard identification" and then "dose response assessment and derivation of toxicity values". It may be necessary to explicitly show the decision-making process within each of the three different data streams and how, prior to full integration, each supports hazard identification and dose-response, providing clear reasoning for differences in hazard identification indicated by the three data streams and how weight of evidence should influence choosing particular models for quantifying perceived hazards.

Any integrated risk assessment approaches would also need to include consideration of dose to target tissues/cells at the portal-of-entry, sites deeper in the front of the nose, and in presumed targets for LHP cancers - bone marrow stem cells and circulating blood stem cells. The expected delivered dose at these more distant sites is important when considering the implausibility that inhaled formaldehyde reaches these distant sites. The potential for exogenously delivered systemic formaldehyde as a plausible contributor to systemic cancer is likely to be informed by integration of dosimetry and toxicity data both from formaldehyde and from a broad group of chemicals whose metabolism systemically generates formaldehyde, including caffeine, aspartame, methanol, methyl chloride and various high usage pharmaceuticals.

Establishing and Implementing New Approaches in Risk Assessment: USEPA's history of implementing the Guidelines for Cancer Risk Assessment has established an extremely high bar for relying on MOA information to depart from using the default linear-no-threshold model for risk assessment. With one exception, chloroform, this bar has never been met in historical EPA evaluations of human cancer risks for non-pesticide chemicals. With chloroform, (https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0025_summary.pdf), the risk assessment utilized a threshold dose-response argument and concluded that chloroform was not likely to be carcinogenic to humans by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration. This conclusion is equally valid for the tumors seen in rats inhaling formaldehyde. The availability of the particularly rich data streams on toxicology, MOA, epidemiology and PK/BBDR modeling with formaldehyde should now help forge new protocols and new integrated strategies for assessing the value of default models and the types of information needed to introduce approaches other than defaults and apply them to more than just a single chemical. The challenge though is not simply to determine which approach has the greatest support and use that to the exclusion of all others, but to develop decision analytic tools that permit consideration of all the available approaches for hazard identification and dose response analysis and to weigh the various approaches appropriately.

4. Summary

Producing a more integrated risk assessment with formaldehyde faces challenge in considering diverse and somewhat discordant information on MOA, toxicology and epidemiology. Despite the lack of consistent or solid evidence for association between formaldehyde exposures and human cancer, select epidemiology studies for NPC and LHP cancers have been interpreted in various assessments as conclusively showing that formaldehyde is a human carcinogen. In contrast, toxicology, MOA, formaldehyde-DNA adduct, CFD, BBDR and PK modeling studies are consistent in supporting a conclusion that formaldehyde does not reach sites distant from the front of the nose and would not be expected to cause cancer or any other endpoints in tissues other than at the immediate portal of entry. The integration of these various data streams will likely have to include formal, quantitative procedures to reconcile these differences by providing clear evaluation frameworks, such as the Bradford-Hill criteria, to gauge how the risk assessment process weighs confidence in various conclusions. Completing this type of comprehensive integration of MOA, toxicology and epidemiology represents a novel challenge for the field of risk assessment, and the development of these procedures with formaldehyde would likely serve as a prototype for application with similar integrated assessments that might be pursued with other well-studied compounds. While some specific studies were suggested as potentially useful follow-up evaluations, the overall body of information with formaldehyde already appears sufficient for moving forward with a more integrated risk assessment.

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Appendix A. Supplementary data

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