Environmental and Molecular Mutagenesis 51:181–191 (2010)

Review Article

Formaldehyde and Leukemia: Epidemiology, Potential Mechanisms, and Implications for Risk Assessment

Luoping Zhang,[1]* Laura E. Beane Freeman,[2] Jun Nakamura,[3] Stephen S. Hecht,[4] John J. Vandenberg,[5] Martyn T. Smith,[1] and Babasaheb R. Sonawane[6]

[1]*School of Public Health, University of California, Berkeley, California*
[2]*Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland*
[3]*Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina*
[4]*Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota*
[5]*National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina*
[6]*National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington DC*

Formaldehyde is widely used in the United States and other countries. Occupational and environmental exposures to formaldehyde may be associated with an increased risk of leukemia in exposed individuals. However, risk assessment of formaldehyde and leukemia has been challenging due to inconsistencies in human and animal studies and the lack of a known mechanism for leukemia induction. Here, we provide a summary of the symposium at the Environmental Mutagen Society Meeting in 2008, which focused on the epidemiology of formaldehyde and leukemia, potential mechanisms, and implication for risk assessment, with emphasis on future directions in multidisciplinary formaldehyde research. Updated results of two of the three largest industrial cohort studies of formaldehyde-exposed workers have shown positive associations with leukemia, particularly myeloid leukemia, and a recent meta-analysis of studies to date supports this association. Recent mechanistic studies have shown the formation of formaldehyde-induced DNA adducts and characterized the essential DNA repair pathways that mitigate formaldehyde toxicity. The implications of the updated findings for the design of future studies to more effectively assess the risk of leukemia arising from formaldehyde exposure were discussed and specific recommendations were made. A toxicogenomic approach in experimental models and human exposure studies, together with the measurement of biomarkers of internal exposure, such as formaldehyde-DNA and protein adducts, should prove fruitful. It was recognized that increased communication among scientists who perform epidemiology, toxicology, biology, and risk assessment could enhance the design of future studies, which could ultimately reduce uncertainty in the risk assessment of formaldehyde and leukemia. Environ. Mol. Mutagen. 51:181–191, 2010. Published 2009 Wiley-Liss, Inc.

Key words: formaldehyde; epidemiology; myeloid leukemia; DNA damage; DNA adducts; risk assessment

Abbreviations: CA, Chromosomal Aberrations; CI, Confidence Interval; DPCs, DNA-Protein Crosslinks; EMS, Environmental Mutagen Society; FANC, Fanconi Anemia Complementation Group; FA, Formaldehyde; GSH, Glutathione; HR, Homologous Recombination; IRIS, Integrated Risk Information System; IARC, International Agency for Research on Cancer; LHP, Lymphohematopoietic; MN, Micronuclei; NHEJ, Non-Homologous End Joining; OEL, Occupational Exposure Level; ppm, Parts Per Million; RR, Relative Risk; STEL, Short-Term Exposure Limit; SCE, Sister Chromatid Exchange; SMR, Standardized Mortality Ratio; TWA, Time-Weighted Average; NCI, National Cancer Institute; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; WHO, World Health Organization.

Sponsors: The symposium and this work were in part supported by the US EPA and the Intramural Research Program of the National Cancer Institute, respectively.

*Correspondence to: Luoping Zhang, School of Public Health, 50 University Hall, University of California, Berkeley, CA 94720-7356, USA. E-mail: luoping@berkeley.edu

Received 18 July 2009; provisionally accepted 13 August 2009; and in final form 17 August 2009

DOI 10.1002/em.20534

Published online 29 September 2009 in Wiley InterScience (www.interscience.wiley.com).

INTRODUCTION

The International Agency for Research on Cancer (IARC) recently reclassified formaldehyde as a human carcinogen that causes nasopharyngeal cancer [Cogliano et al., 2005; IARC, 2006] and also concluded that there is "*strong but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde*" based on evidence from epidemiological studies. Leukemia is a much more prevalent and lethal tumor (12.3 and 7.5 per 100,000, respectively) than is nasopharyngeal cancer (0.7 and 0.2 per 100,000, respectively) [SEER, 2006]. Therefore, determining whether there is an association between formaldehyde exposure and leukemia is of great public health significance. However, inconsistencies in human and animal studies have presented challenges for the evaluation of the risk of leukemia associated with formaldehyde exposure. At the 39th Annual Environmental Mutagen Society (EMS) Meeting in October 2008, we held a symposium at which an up-to-date review of the epidemiological studies of formaldehyde and leukemia and potential mechanisms were presented. We also discussed the implications for risk assessment and how to incorporate recent findings into the design of future human exposure and mechanistic studies. The proceedings of the symposium are reported here.

FORMALDEHYDE EXPOSURE AND LEUKEMIA

Formaldehyde Exposure

Formaldehyde is an economically important chemical produced in the United States at a rate of over 11 billion pounds annually. It is used in the manufacture of numerous products such as consumer appliances, porcelain-like dishware and insulation, and as a tissue preservative or bactericide in embalming fluid and medical laboratories [Zhang et al., 2009]. In 1995, more than 2 million workers in the United States were occupationally exposed [OSHA, 1995], while many more are exposed in developing countries [Tang et al., in press]. The current U.S. Occupational Safety and Health Administration (OSHA) standards were established in 1992, and it includes a permissible exposure limit (PEL) of 0.75 ppm (parts per million) in air as an 8-hr time-weighted average (8 hr TWA) and a short-term (15 min) exposure limit (STEL) of 2 ppm [OSHA, 1992]. Despite much lower exposure limits of 0.016 ppm (8 hr TWA) and 0.1 ppm (STEL) [NIOSH, 2005] recommended by the U.S. National Institute for Occupational Safety and Health (NIOSH), the U.S. continues to retain higher occupational exposure levels (OELs) than the current or proposed levels of many other countries or provinces including Australia, Germany, Japan, and British Columbia [NICNAS, 2006; NIOSH, 2006; CAREX-Canada, 2007; JSOH, 2007].

Although much lower formaldehyde levels are typically encountered through environmental than occupational exposure, a greater number of people are impacted. Outdoor environment air concentrations in large cities sometimes exceed the NIOSH recommended exposure level for the workplace of 0.016 ppm (=16 ppb) [Khoder et al., 2000; Báez et al., 1995, 2003; Chen et al., 2004; NIOSH, 2005], and ambient levels in homes containing large amounts of pressed wood products can exceed 0.3 ppm [USEPA, 2007], much higher than the World Health Organization's (WHO) recommended indoor limit of 0.08 ppm [WHO-ROE, 2006], which many other countries have adopted as well [Tang et al., 2009]. Despite this, the U.S. continues to allow the use of formaldehyde, while the Council of the European Union has already placed formaldehyde, along with many other biocidal products, under strict review with the intention of eventually removing them from the European market [EU, 2007].

Formaldehyde is also a naturally occurring compound that is present in human plasma at concentrations ranging from 13 to 97 μM [Szarvas et al., 1986; Heck and Casanova, 2004]. Endogenous formaldehyde and its oxidation product, formic acid, are intermediates in the "one-carbon pool" [Neuberger, 1981] used for the biosynthesis of purines, thymidine, and some amino acids. As a naturally occurring metabolite in many living things, formaldehyde is also found at high background levels in many types of food, such as shiitake mushrooms and many types of seafood. There have also been instances of formaldehyde found in fruits, vermicelli noodles, and even beer [Tang et al., 2009]. Thus, formaldehyde exposure via food consumption is also a possibility.

Formaldehyde's pervasiveness has resulted in a number of reported health effects, including nasopharyngeal cancer and potentially leukemia. As both the incidence and mortality rates are higher for leukemia than for nasopharyngeal cancer, the public health implications of leukemia are potentially more significant than that of nasopharyngeal cancer.

Epidemiology of Formaldehyde and Leukemia

Much of the evidence for an association between formaldehyde exposure and leukemia comes from epidemiology studies, of which there are primarily three types: case–control studies in the general population, proportionate mortality studies of professionals, (e.g., funeral industry workers and pathologists), and cohort studies of industrial workers. Understanding exposure evaluation is important for understanding and interpreting results from these studies. Population-based case–control studies generally rely upon job histories to assign the probability of whether exposure occurred and may provide a category

of low or high exposure, but rarely provide an estimate of the level of exposure. Similarly, the proportionate mortality studies conducted in professionals rely upon employment where formaldehyde exposure likely occurred, such as in the funeral industry or among pathologists. Cohort studies in industrial settings have provided the best opportunity, thus far, for evaluating exposure-response associations.

Case–control studies suggested associations between formaldehyde exposure and various lymphohematopoietic (LHP) malignancies, but the evidence for an association with leukemia is less certain. There are three case–control studies and one nested case-control study [Ott et al., 1989; Linos et al., 1990; Partanen et al., 1993; Blair et al., 2001] that have specifically examined associations with leukemia. Although they all provide some evidence of an association with formaldehyde exposure and leukemia, these studies have a small number of exposed cases, ranging from 1 to 61 exposed, making confidence intervals (CI) wide and exposure-response analyses difficult or impossible.

Early epidemiological studies of formaldehyde exposure in industrial manufacturing environments did not consistently show an association between formaldehyde and leukemia [IARC, 2006]. However, studies of professionals exposed to formaldehyde, such as embalmers, mortuary workers, medical examiners, pathologists, and anatomists, showed excesses of leukemia [Milham, 1983; Levine et al., 1984; Walrath and Fraumeni, 1983, 1984; Hayes et al., 1990; Hall et al., 1991; Matanoski, 1991]. Recently, three of the largest cohorts in industrial settings have been updated, and two of the three did find an association between formaldehyde exposure and leukemia [Coggon et al., 2003; Hauptmann et al., 2003; Pinkerton et al., 2004]. The first included 14,014 workers in British chemical factories where formaldehyde was used or produced [Coggon et al., 2003]. Exposure was assessed by creating a job exposure matrix and assigning each job to one of five categories of exposure. Leukemia rates were compared to a local group, and no association was observed between either employment in the factories (Standardized Mortality Ratio, SMR = 0.91; 95% CI: 0.62–1.29) or work in a high exposure group (SMR = 0.71; 95% CI: 0.31–1.39). Myeloid leukemia was not examined specifically.

The second industrial cohort was conducted by NIOSH and included 11,039 workers from the garment industry, where fabrics are treated with formaldehyde resins [Pinkerton et al., 2004]. In an exposure assessment based on 594 randomly selected employees, the mean exposure levels were 0.09–0.20 ppm and were relatively constant with no peaks or intermittent exposures. For leukemia, the highest SMR occurred with those who had been employed in the garment factory 10 or more years (SMR = 1.53) and more than 20 years since the first exposure (SMR = 1.31). Results were stronger for myeloid leukemia, with the SMR = 2.19 for employment of 10 or more years and SMR=1.91 for those who had first been exposed more than 20 years ago.

The third study, conducted by the U.S. National Cancer Institute (NCI), included 25,619 participants [Hauptmann et al., 2003]. Exposure assessment included a number of time-dependent exposure metrics, duration, cumulative exposure, average 8-hr time-weighted average intensity, and highest peak category of exposure. The exposure assessment ended in 1980, when only 11% of the workers were still employed in formaldehyde-exposed jobs. The study demonstrated associations with formaldehyde exposure and leukemia overall, but the stronger associations were seen for myeloid leukemia. For the highest level of average intensity of exposure, the relative risk (RR) for myeloid leukemia was 2.49 (95% CI: 1.03–6.03), and 3.46 (95% CI: 1.27–9.43) for the highest category of peak exposure compared to workers with lower exposures.

Since the EMS symposium in October 2008, a more recent update of the NCI cohort has been published [Beane Freeman et al., 2009], which includes 10 years of additional follow-up from the previous publication. The cohort study demonstrated statistically significant increased risks for all LHP malignancies (RR = 1.37; 95% CI: 1.03–1.81, P_{trend} = 0.02), as well as nonsignificant increase for all leukemia (RR = 1.42; 95% CI: 0.92–2.18, P_{trend} = 0.12) for peak exposure. Additionally, the study found that the overall risk of myeloid leukemia had decreased since the last publication, but remained somewhat elevated (RR = 1.61; 95% CI: 0.76–3.39, P_{trend} = 0.4 for average intensity exposure; RR = 1.78; 95% CI: 0.87–3.64, P_{trend} = 0.07 for peak exposure). Although the risks have diminished since the previous report from the cohort, the pattern of larger risks occurring closer in time to relevant exposure and diminishing as time progresses is not inconsistent with a causal association. Conversely, they may also indicate that the previous stronger association was due to chance.

The epidemiological literature on formaldehyde and leukemia is extensive. Many, but not all of the studies have suggested associations. Since these studies took place in varied settings with different co-exposures, it is unlikely that uncontrolled confounders explain these results. More studies are needed to explore potential mechanisms and to establish if there is heterogeneity in association between the leukemia subtypes.

New Meta-Analysis of Formaldehyde and Leukemia

Zhang et al. [2009] recently performed a meta-analysis of epidemiological studies based on the hypothesis that high formaldehyde exposure can induce leukemia, particularly myeloid type. Unlike past analyses, this new meta-analysis' novel approach focused on both industrial and

TABLE I. Summary of Current and Previous Meta-Analyses All Blood Cancers and Leukemia Associated With Formaldehyde

	Zhang et al., 2009				Bosetti et al., 2008				Collins & Lineker, 2004				Blair et al., 1990			
Blood cancer type	*N*	*n*	RR	95% CI[a]	*N*	*n*	RR	95% CI	*N*	*n*	RR	95% CI	*N*	*n*	RR	95% CI[b]
All types	19	392	1.25	1.09–1.43					18	287	1.1	1.0–1.2				
Industrial					4	234	0.85	0.74–0.96								
Professional					9	263	1.31	1.16–1.47								
All Leukemia	15	109	1.90	1.18–2.00												
Myeloid leukemia	6	61	1.9	1.31–2.76												
Industrial					4	122	0.9	0.75–1.07	8	164	0.9	0.8–1.0	5	122	1.1	0.91–1.31
Professional					10	106	1.39	1.15–1.68	3	45	1.4	1.0–1.9	12	107	1.6	1.31–1.93
Embalmers									7	78	1.6	1.2–2.0				

N, Numbers of included studies; *n*, reported number of cases; RR, relative risk; CI, confidence interval.
[a]Shore adjusted CI is used due to heterogeneity which is defined as present when chi-square > degrees of freedom.
[b]Calculated 95% CI by Byars approximation since data were not originally provided in the reference.

professional subjects with the highest exposure and selected RR estimates for myeloid leukemia (the primary type of leukemia associated with formaldehyde) whenever possible, to prevent dilution of RR estimates toward the null that occur when examining ever versus never exposure. The results (Table I) showed a strong association between formaldehyde and leukemia, and suggested that formaldehyde exposure can increase risk of leukemia by 54% (RR = 1.54; 95% CI: 1.18–2.00), particularly myeloid leukemia (RR = 1.90; 95% CI: 1.31–2.76). Compared to the largest national cohort discussed in the previous section, risk estimates of the peak exposures are very similar for all types of blood cancers, all types of leukemia, and myeloid leukemia [Beane Freeman et al., 2009].

Table I summarizes and compares the four past and present meta-analyses. For all types of blood cancers and all professions, Zhang et al. [2009] and Collins and Lineker [2004] found similar positive risk estimates, while Bosetti et al. [2008] found a significant increase in RR only in professional workers but not industrial workers. The risk estimates for all leukemias are positive for professional workers, and across the three older meta-analyses, risk estimates for industrial workers are inconclusive. Discrepancies found between these meta-analyses are a result of study and exposure group selection, and inclusion and exclusion criteria, which have all been discussed at length in the recent [Zhang et al., 2009] meta-analysis. And although past meta-analyses also included similar studies and data, this meta-analysis serves as an example of how a focused hypothesis, strict selection criteria, and meticulous review of all existing studies can strengthen the assessment of leukemia risks from formaldehyde exposures.

BIOLOGICAL PLAUSIBILITY AND POTENTIAL MECHANISMS

As discussed earlier, numerous epidemiological studies and a recent meta-analysis support the association of formaldehyde exposure with leukemia. However, because of its reactive nature, it has been considered unlikely that inhaled formaldehyde could damage stem cells in the bone marrow directly and cause leukemia similar to classical leukemogens. It has been previously suggested that formaldehyde could potentially reach bone marrow directly in its hydrate methanediol form. Two alternative mechanisms have been proposed, by which formaldehyde could cause leukemia through damage to hematopoietic stem/progenitor cells circulating in the peripheral blood and through damage to the primitive pluripotent stem cells present within the nasal turbinates and/or olfactory mucosa [Zhang et al., 2009]. In the latter two models, damaged stem/progenitor cells would then travel to the bone marrow and become initiated leukemic stem cells, potentially developing into leukemia following a protracted latency. Given the likely dynamics of stem cell turnover between the nasal/oral passages, blood, and bone marrow [Barnett et al., 1999; Murrell et al., 2005; Bryder et al., 2006], sufficient stem cells could be targeted through these two alternative models to induce leukemia, under conditions of chronic high formaldehyde exposure (such as occupational exposure).

The "insufficient evidence" for an association with formaldehyde and leukemia concluded by IARC was based on the inability of the Working Group to identify a mechanism for leukemia induction [IARC, 2006]. Most leukemogens exert their effects on stem or progenitor cells in the bone marrow, causing hematotoxicity and/or genotoxicity. Although there is limited and inconsistent data on formaldehyde hematotoxicity [Kuo et al., 1997; Madison et al., 1991; Vargova et al., 1993; Ye et al., 2005], a recent review on formaldehyde in China summarized the available studies published in Chinese and reported a positive association between formaldehyde exposure and decreased blood cell counts [Tang et al., 2009]. However, future studies are needed to further clarify the hematotoxic effects of formaldehyde in exposed people.

Although endogenous formaldehyde is usually rapidly metabolized by reduction, oxidation, and reduced glutathione (GSH)-dependent pathways, excess levels such as those encountered occupationally and/or environmentally could saturate metabolic capacity and lead to genotoxicity in DNA and chromosomes.

Formaldehyde-Induced DNA and Chromosome Damage

Formaldehyde is genotoxic, inducing both DNA damage expressed as DNA adducts [Wang et al., 2007] and DNA-protein crosslinks (DPCs) and chromosome changes, expressed as chromosomal aberrations (CA), sister chromatid exchanges (SCEs), and micronuclei (MN). These alterations have been demonstrated by a large number of studies in vitro, in exposed animals, and to varying degrees in the circulating lymphocytes of exposed people [ATSDR, 1999; Ye et al., 2005; Yu et al., 2005; IARC, 2006; Orsiere et al., 2006; Iarmarcovai et al., 2007; Wang et al., 2007]. As discussed previously, more molecular epidemiological studies examining the genotoxic effects of formaldehyde are needed [Zhang et al., 2009]. For example, the only human studies performed to date showing elevated DPCs in the peripheral mononuclear cells of formaldehyde-exposed workers [Shaham et al., 1996, 2003] need to be replicated because of the excessively high levels of DPCs reported in the controls.

Studies showing increased CA in humans have a number of methodological weaknesses, including poor exposure assessment, non-current measurement of exposure and outcome, small sample size, and so forth, necessitating replication of the findings in better-designed studies [Bauchinger and Schmid, 1985; Chebotarev et al., 1986; Vozenilkova et al., 1991; Kitaeva et al., 1996; He et al., 1998; Lazutka et al., 1999]. Despite these limitations, many studies report positive results indicating that formaldehyde is able to cause a range of genotoxic effects in the DNA and chromosomes of lymphocytes, and possibly other bone marrow-derived cells. Recent studies have investigated the potential mechanisms underlying DNA damage [Wang et al., 2007] and the DNA repair pathways [Ridpath et al., 2007] induced by formaldehyde.

The FANC-BRCA Pathway and DNA Damage Response

DPCs appear to be critical DNA lesions for formaldehyde-induced carcinogenesis. A recent study was able to locate vulnerable binding sites for formaldehyde on N-terminus of histone and lysine residues that may be involved in the formation of DPC [Lu et al., 2008]. As such, DPC level has been used as a biomarker of formaldehyde exposure in mammalian cells [Casanova et al., 1991, 1994], and it has also been correlated with formaldehyde-induced carcinogenesis in animals [Hubal et al., 1997; IRIS, 1998]. However, little is known about which DNA damage response pathways are essential for cells to counteract damage from formaldehyde exposure. Yamazoe et al. [2004] investigated the DNA damage response to formaldehyde using a reverse genetic approach in the chicken DT40 cell model system by first assessing targeted mutations in various DNA repair or cell cycle checkpoint response pathway genes. As measured by the survival of DT40-derived mutants, results revealed a requirement for the homologous recombination (HR) pathway, but not the nonhomologous end joining (NHEJ pathway), in processing DNA damage induced by formaldehyde, strongly suggesting that the HR pathway is involved in repair of DPCs. DT40 mutants deficient in REV1, REV3, and RAD18 but not genes involved in translesion synthesis, showed hypersensitivity to formaldehyde, in agreement with the findings from an earlier study of DNA–DNA intercrosslinking agents, including cisplatin and mitomycin C [Nojima et al., 2005]. In contrast, the ATM pathway showed no major contribution in the DNA damage response to formaldehyde-induced DNA damage.

Fanconi anemia, an inherited disorder associated with progressive bone marrow failure and predisposition to malignant leukemia and solid tumors, is characterized by sensitivity to DNA interstrand crosslinking agents and an associated increase in chromosomal breakage [Kennedy and D'Andrea, 2005]. Given this sensitivity, the role of genes in the FANC (Fanconi anemia complementation group) pathway in the response to formaldehyde-induced DNA damage was investigated by the Nakamura laboratory [Ridpath et al., 2007]. FANC proteins are closely related to the breast/ovarian cancer susceptibility gene products BRCA1 and BRCA2, and to their partner proteins, and the FANC pathway is also called the "FANC-BRCA pathway" [D'Andrea and Grompe, 2003] or "FANC-BRCA network" [Venkitaraman, 2004]. *FANCD1 (BRCA2)*-deficient and *FANCD2*-deficient chicken DT40 cells were most hypersensitive to formaldehyde at concentrations irrelevant to human exposure. Furthermore, the same study showed that human RKO cells deficient in *FANCC* and *FANCG* were also hypersensitive to plasma levels of formaldehyde at concentrations 20 μmol/L or higher (*FANCC*) or 38 μmol/L or higher (*FANCG*) [Ridpath et al., 2007]. In addition, *FANCD2*-deficient DT40 cells, the most sensitive cell line to formaldehyde, were hypersensitive to acetaldehyde, but not to acrolein, crotonaldehyde, glyoxal, and methylglyoxal. These results indicate that the FANC-BRCA pathway is essential to counteract DPCs caused by formaldehyde and other aliphatic monoaldehydes.

Figure 1, adapted from data by Jacquemont and Taniguchi, summarizes all of the genes known to be involved in the resistance to DPC induced by formaldehyde [Jacquemont and Taniguchi, 2007]. Eight FANC proteins (FANC-A, -B, -C, -E, -F, -G, -L, and -M), a FANCM-interacting protein called FAAP24, and an unidentified

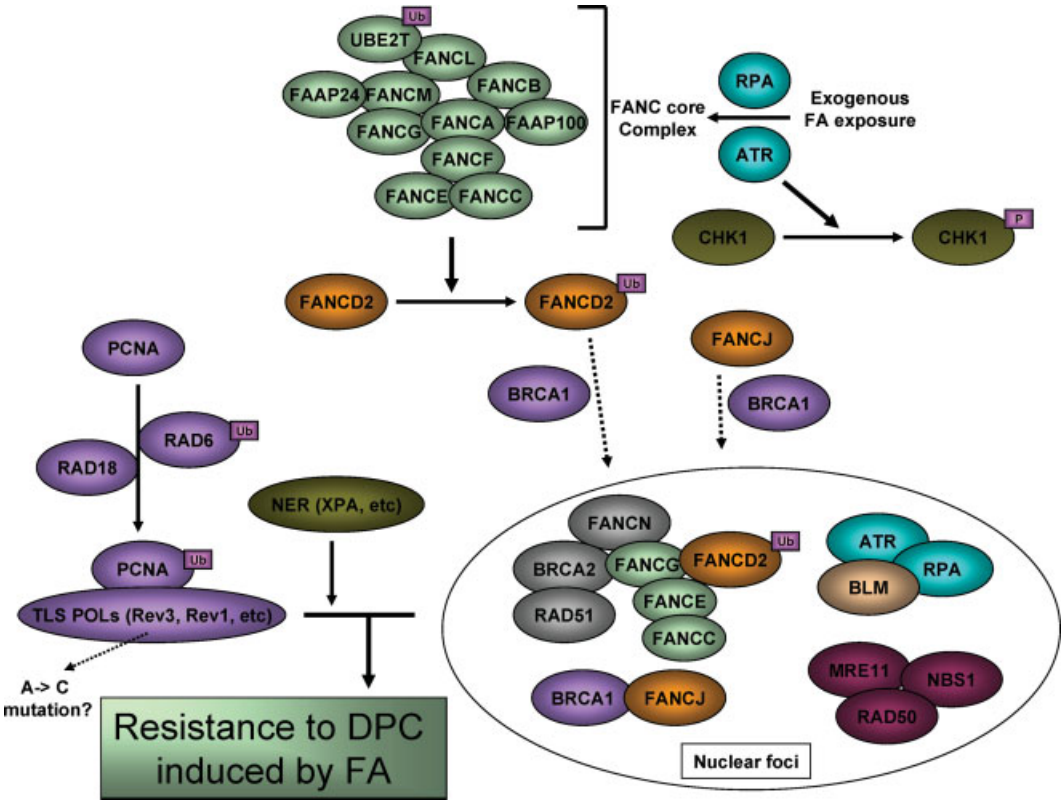

Fig. 1. Proposed model of DNA damage response pathways for DNA damage caused by formaldehyde (FA). Adapted with modifications from [Jacquemont and Taniguchi, 2007]. In response to DNA damage (e.g., DPC) caused by formaldehyde, the FANCD2 protein appears to be monoubiquitylated on lysine 561 (K561) in a Fanconi anemia core complex- and UBE2T-dependent manner that may also require ATR and RPA. Monoubiquitylation of FANCD2 is thought to target the protein into nuclear foci where it co-localizes with BRCA1, FANCD1/BRCA2, FANCN/PALB2, RAD51, FANCJ/BRIP1, and other proteins. Many of these factors appear to be required for cellular resistance to formaldehyde. Monoubiquitylation of PCNA on lysine 164 (K164) may require RAD6 as an E2 and RAD18 as an E3. In turn, modification of PCNA may cause recruitment of translesion synthesis (TLS) DNA polymerases at the site of stalled replication forks.

factor (FAAP100) form a nuclear complex (the FANC core complex) with E3 ubiquitin ligase activity. FANCL, the catalytic subunit of the Fanconi anemia core complex, directly interacts with the E2 ubiquitin conjugating enzyme UBE2T. In response to DNA damage (e.g., DPC) caused by formaldehyde, the FANCD2 protein is monoubiquitylated targeting it into nuclear foci where it co-localizes with BRCA1, FANCD1/BRCA2, FANCN/PALB2, RAD51, FANCJ/BRIP1, and other proteins. Many of these factors appear to be required for cellular resistance to formaldehyde. Reduced activity in one or more of these genes involved in the DNA damage response could render an individual hyper-susceptible to DPC induced by formaldehyde [Jacquemont and Taniguchi, 2007].

It is hypothesized that the induction of DPCs by endogenous formaldehyde plays a critical role in the initiation of progressive bone marrow failure or predisposition to malignant tumors in patients with Fanconi anemia. Exposure to exogenous sources of formaldehyde could push susceptible individuals into a dangerous zone in which genotoxic levels of DPC are induced. One of the big limitations to this hypothesis is the uncertainty over whether exogenous formaldehyde can reach the bone marrow. A mouse model, such as a FANC deficient mouse could be a useful tool to better understand whether formaldehyde causes bone morrow toxicity by inhalation. Such a model would also allow us to investigate the potential role of endogenous formaldehyde on the etiology of acute myeloid leukemia in patients with Fanconi anemia.

Detection of Formaldehyde-DNA Adducts

DNA adducts are critical in the process of carcinogenesis because, if unrepaired, they lead to mutations in important growth control genes, ultimately resulting in genomic instability and cancer. While several formaldehyde DNA adducts have been identified in vitro, notably the dAdo cross-link, di-N^6-deoxyadenosylmethane (dAdo-CH_2-dAdo), and its precursor, N^6-hydroxymethyl-dAdo (N^6-$HOCH_2$-dAdo) [Cheng et al., 2003], they had not been previously analyzed in laboratory animals exposed to formaldehyde or its precursors. The Hecht laboratory developed a highly sensitive, accurate, and precise liquid

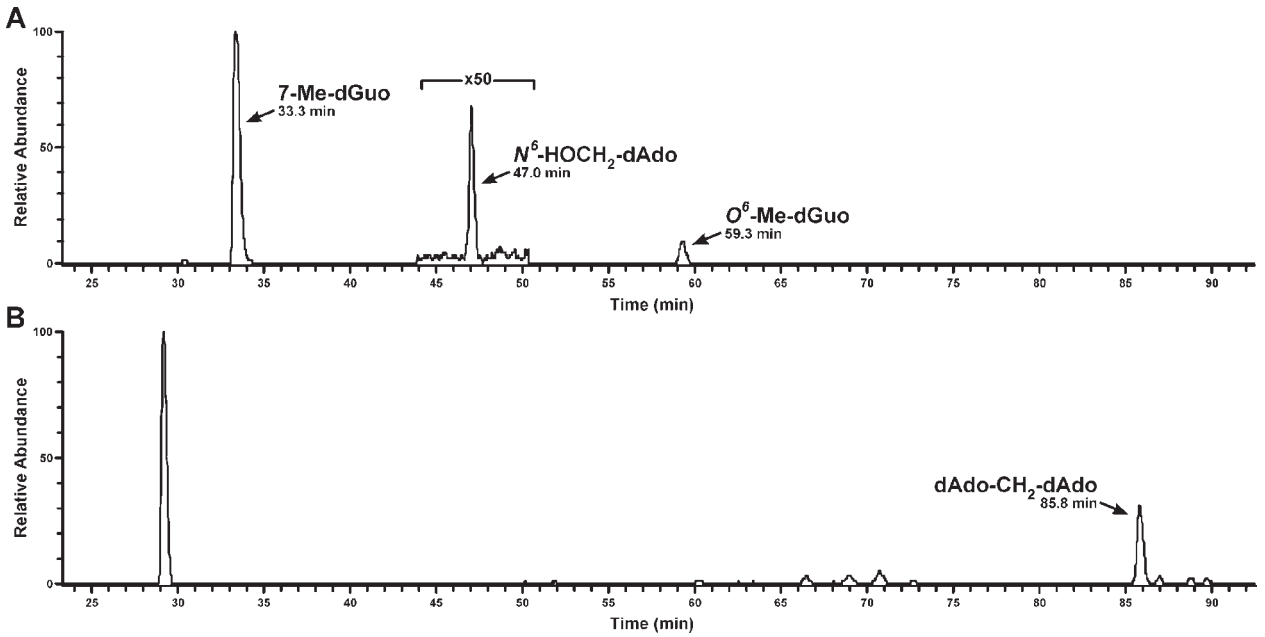

Fig. 2. Liquid chromatography-electrospray ionization-tandem mass spectrometry-selected ion monitoring (LC-ESI-MS-SIM) analysis of an enzymatic hydrolysate of hepatic DNA from a rat treated with NDMA [Wang et al., 2007]. Peaks corresponding to the retention times of standard 7-Me-dGuo, N^6-HOCH$_2$-dAdo, and O^6-Me-dGuo were observed. (**A**) The peak eluting at 47.0 min had a base peak at *m/z* 282 $[M + H]^+$, similar to standard N^6-HOCH$_2$-dAdo. (**B**) LC-ESI-MS-SIM analysis (*m/z* 515) showed a peak at 85.8 min, corresponding to the retention time of dAdo-CH$_2$-dAdo.

chromatography-electrospray ionization-tandem mass spectrometry-selected reaction monitoring (LC-ESI-MS/MS-SRM) method to quantify these adducts [Wang et al., 2007]. They applied the method to analyze hepatic and pulmonary DNA from rats treated with the strongly carcinogenic nitrosamines *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which release formaldehyde upon cytochrome P450 catalyzed metabolic activation [Preussmann and Stewart, 1984; Hecht, 1998]. Results such as those shown in Figure 2 clearly demonstrated the dose-dependent presence of N^6-HOCH$_2$-dAdo in all DNA samples, along with well-established nitrosamine-DNA adducts such as 7-methylguanine and O^6-methylguanine [Wang et al., 2007]. The cross-link adduct dAdo-CH$_2$-dAdo was observed in hepatic DNA of NNK-treated rats, with lower amounts in pulmonary DNA, and in hepatic DNA of NDMA-treated rats. Levels of these adducts were generally lower than those of DNA adducts formed by the classical diazohydroxide pathway of nitrosamine metabolism. This was the first demonstration of formaldehyde-DNA adducts in tissues of laboratory animals. The biological significance of these adducts requires further investigation.

The Hecht laboratory is presently analyzing N^6-HOMe-dAdo in human leukocyte DNA. Once standardized, this assay should be useful in directly assessing DNA damage as formaldehyde-DNA adducts in humans, and could be applied in molecular epidemiology studies of formaldehyde and cancer.

IMPLICATIONS FOR THE HEALTH RISK EVALUATION OF FORMALDEHYDE

The U.S. EPA is evaluating potential human health risks from environmental exposures to formaldehyde as part of the Integrated Risk Information System (IRIS) [IRIS, 1998]. Both cancer and noncancer outcomes are being evaluated and the resulting assessment will provide qualitative (hazard identification) and, if possible, quantitative (dose–response) evaluations. The IRIS toxicological review may then be coupled with estimates of human exposures to formaldehyde to estimate potential risks to exposed populations, thereby supporting informed decision making under statutes such as the Clean Air Act, as amended in 1990 [USEPA, 1990]. Importantly, the qualitative and quantitative assessment of formaldehyde includes characterization of the nature and strength of the evidence as well as characterization of uncertainty in interpreting and using available data. The case of formaldehyde-induced LHP malignancies presents challenges in these areas resulting in uncertainties in the risk evaluation.

Uncertainties in hazard identification arise from interpretation and extrapolation of data from both experimental animal and human epidemiological studies. Data from in vivo and in vitro laboratory studies are evaluated in terms of study protocol, limitations of study design, selection of endpoints of toxicity and their relevance to humans, and coherence across levels of biological organi-

zation. Epidemiological studies may present uncertainties in exposure estimation, appropriate dosimetrics, misclassification of disease, confounding exposures, unidentified risk factors, and use of an appropriate referent population. Evidence related to mode of action, such as induction of formaldehyde-induced DNA adducts and chromosome damage, and the FANC-BRCA pathway, may help to support evaluation of the biological plausibility of the epidemiological findings. The EPA's 2005 Cancer Guidelines [USEPA, 2005a] and Supplemental Guidance [USEPA, 2005b] to protect children recommend the use of human data, where adequate and appropriate, for quantitative risk assessment. Recent developments, not only in hazard identification, but also mode and mechanism of action of formaldehyde on hematopoietic and immune cells provide an increasingly informed basis on which to evaluate the utility of human studies for human health risk assessment of formaldehyde exposure.

FUTURE DIRECTIONS

The updated findings discussed here suggest that future studies are warranted to more effectively assess the risk of leukemia arising from formaldehyde exposure. It was recognized that increased communication among risk assessment scientists, epidemiologists, toxicologists, and biologists would enhance the design of such studies, and reduce some of the uncertainty associated with risk assessment. Accordingly, the following specific recommendations have been made.

Several approaches were suggested to address gaps in knowledge regarding the association between formaldehyde and leukemia. Although leukemia arises from damaged blood stem cells, little is known about the sensitivity of blood stem cells to formaldehyde and whether formaldehyde produces mutations related to leukemia in these cells. As discussed earlier, some studies report that formaldehyde produces chromosome damage in circulating blood cells of exposed humans, but it is not known if it also does so in blood progenitor/stem cells or how consistent its effects are. Studies of $CD34^+$ cells exposed to formaldehyde in culture were suggested to address these issues.

Molecular epidemiology/biomarker studies of occupationally exposed populations should be designed to determine whether formaldehyde causes hematotoxicity, as this has not been definitively shown. A biomarker discovery approach should be applied in these studies using toxicogenomic, proteomic, metabolomic and epigenetic tools. Leukemia-specific markers, such as chromosome aneuploidy and translocations, should be examined in peripheral blood leukocytes and progenitor cells. Together, the study of leukemia-specific chromosome damage in cultured $CD34^+$ cells and of hematoxicity in human populations will strengthen the biological plausibility and help to elucidate a mode of action.

Members of the audience proposed that further studies in transgenic mice with DNA repair deficiencies are one possible future research direction. The determination of whether adducts are formed in the bone marrow of mice treated with formaldehyde-generating chemicals and whether the FANC-BRCA pathway is involved in the response to such damage in the bone marrow could help to determine if exogenous formaldehyde reaches the bone marrow. The potential application of the *Pig-A* mutation assay and/or a knockout mouse model to clarify the mechanisms of formaldehyde-induced leukemogenesis was also proposed. These various research approaches will provide lines of evidence that can be used to ascertain causality.

Finally, it was recognized that because few tools are available to measure formaldehyde exposure internally, chemical-specific methodologies to specifically detect adducts of formaldehyde to DNA and proteins in blood, bone marrow and other target tissues are urgently needed. The recently developed assay for the formaldehyde-DNA adduct N^6-HOMe-dAdo in leukocytes is one example. The ability to accurately measure formaldehyde exposure would address one of the key aspects of causality judgment in risk assessment, that of biologic gradient or exposure-response relationship. According to this relationship, increasing effects associated with greater exposure strongly suggest cause and effect. Swenberg and coworkers recently demonstrated that formaldehyde can cross-link GSH with DNA by forming *S*-[1-(N^2-deoxyguanosinyl)methyl]glutathione in the test tube, and proposed utilizing this adduct as a biomarker of formaldehyde exposure and toxicity [Lu et al., 2009]. Further, the authors proposed that this adduct, coupled with isotope-labeled formaldehyde, could differentiate between endogenous and exogenous origin of formaldehyde-derived adducts.

In conclusion, much of the uncertainty in the risk assessment of formaldehyde and leukemia could be limited through a concerted effort among all associated disciplines in the design of future studies. Risk assessment does not weigh one type of evidence against another, but rather weighs all of the evidence taken together. Research that strengthens the consistency, strength, specificity, exposure-response relationship, or biological plausibility of an observed association, or that provides experimental evidence in human populations, will aid in making supportable causality judgments.

ACKNOWLEDGMENTS

We thank Dr. Priscilla Cooper, current President of the Environmental Mutagen Society, for encouraging us to

organize this formaldehyde symposium. We thank all the symposium participants for their engaging discussion and insightful questions. We are grateful to Dr. Cliona McHale and Ms. Anh Duong for their discussion and critical review of this manuscript.

REFERENCES

ATSDR. 1999. Agency for Toxic Substances and Disease Registry: Toxicological Profile for Formaldehyde. Atlanta, GA:ATSDR.

Báez AP, Belmont R, Padilla H. 1995. Measurements of formaldehyde and acetaldehyde in the atmosphere of Mexico City. Environ Pollution 89:163–167.

Báez AP, Padilla H, Garcia R, Torres Mdel C, Rosas I, Belmont R. 2003. Carbonyl levels in indoor and outdoor air in Mexico City and Xalapa. Mexico Sci Total Environ 302:211–226.

Barnett D, Janossy G, Lubenko A, Matutes E, Newland A, Reilly JT. 1999. Guideline for the flow cytometric enumeration of CD34+ haematopoietic stem cells. Prepared by the CD34+ haematopoietic stem cell working party. General Haematology Task Force of the British Committee for Standards in Haematology. Clin Lab Haematol 21:301–308.

Bauchinger M, Schmid E. 1985. Cytogenetic effects in lymphocytes of formaldehyde workers of a paper factory. Mutat Res 158:195–199.

Beane Freeman LE, Blair A, Lubin JH, Stewart PA, Hayes RB, Hoover RN, Hauptmann M. 2009. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: The National Cancer Institute Cohort. J Natl Cancer Inst 101:751–761.

Blair A, Saracci R, Stewart PA, Hayes RB, Shy C. 1990. Epidemiologic evidence on the relationship between formaldehyde exposure and cancer. Scand J Work Environ Health 16(6):381–393.

Blair A, Zheng T, Linos A, Stewart PA, Zhang YW, Cantor KP. 2001. Occupation and leukemia: A population-based case-control study in Iowa and Minnesota. Am J Ind Med 40:3–14.

Bosetti C, McLaughlin JK, Tarone RE, Pira E, La Vecchia C. 2008. Formaldehyde and cancer risk: A quantitative review of cohort studies through 2006. Ann Oncol 19:29–43.

Bryder D, Rossi DJ, Weissman IL. 2006. Hematopoietic stem cells: The paradigmatic tissue-specific stem cell. Am J Pathol 169:338–346.

CAREX-Canada. 2007. Information System on Occupational Exposure to Carcinogens: Formaldehyde. School of Occupational and Environmental Hygiene. University of British Columbia.

Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck HD. 1991. Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: Pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. Fundam Appl Toxicol 17:409–428.

Casanova M, Morgan KT, Gross EA, Moss OR, Heck HA. 1994. DNA-protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. Fundam Appl Toxicol 23:525–536.

Chebotarev AN, Titenko NV, Selezneva TG, Fomenko VN, Katosova LM. 1986. Comparison of chromosome aberrations, sister chromatid exchanges and unscheduled DNA synthesis in the evaluation of the mutagenicity of environmental factors. Tsitol Genet 20:109–115.

Chen J, So S, Lee H, Fraser MP, Curl RF, Harman T, Tittel FK. 2004. Atmospheric formaldehyde monitoring in the Greater Houston area in 2002. Appl Spectrosc 58:243–247.

Cheng G, Shi Y, Sturla SJ, Jalas JR, McIntee EJ, Villalta PW, Wang M, Hecht SS. 2003. Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: Formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. Chem Res Toxicol 16:145–152.

Coggon D, Harris EC, Poole J, Palmer KT. 2003. Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. J Natl Cancer Inst 95:1608–1615.

Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghissassi F. 2005. Meeting report: Summary of IARC monographs on formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2-propanol. Environ Health Perspect 113:1205–1208.

Collins JJ, Lineker GA. 2004. A review and meta-analysis of formaldehyde exposure and leukemia. Regul Toxicol Pharmacol 40:81–91.

D'Andrea AD, Grompe M. 2003. The Fanconi anaemia/BRCA pathway. Nat Rev Cancer 3:23–34.

EU. 2007. Commission Regulation (EC) number 1451/2007. Official Journal of the European Union.

Hall A, Harrington JM, Aw TC. 1991. Mortality study of British pathologists. Am J Ind Med 20:83–89.

Hauptmann M, Lubin JH, Stewart PA, Hayes RB, Blair A. 2003. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J Natl Cancer Inst 95:1615–1623.

Hayes RB, Blair A, Stewart PA, Herrick RF, Mahar H. 1990. Mortality of U.S. embalmers and funeral directors. Am J Ind Med 18:641–652.

He JL, Jin LF, Jin HY. 1998. Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. Biomed Environ Sci 11: 87–92.

Hecht SS. 1998. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem Res Toxicol 11:559–603.

Heck H, Casanova M. 2004. The implausibility of leukemia induction by formaldehyde: A critical review of the biological evidence on distant-site toxicity. Regul Toxicol Pharmacol 40:92–106.

Hubal EA, Schlosser PM, Conolly RB, Kimbell JS. 1997. Comparison of inhaled formaldehyde dosimetry predictions with DNA-protein cross-link measurements in the rat nasal passages. Toxicol Appl Pharmacol 143:47–55.

IARC. 2006. International Agency for Research on Cancer: Formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2propanol. Monogr Eval Carcinog Risks Hum 88:37–325.

Iarmarcovai G, Bonassi S, Sari-Minodier I, Baciuchka-Palmaro M, Botta A, Orsiere T. 2007. Exposure to genotoxic agents, host factors, and lifestyle influence the number of centromeric signals in micronuclei: A pooled re-analysis. Mutat Res 615:18–27.

IRIS. 1998. Integrated Risk Information System: Formaldehyde (CASRN 50–00-0). United States Environmental Protection Agency.

Jacquemont C, Taniguchi T. 2007. The Fanconi anemia pathway and ubiquitin. BMC Biochem 8 (Suppl 1):S10

JSOH. 2007. Japan Society for Occupational Health: Recommendation of occupational exposure limits (2007–2008). J Occup Health 49: 328–344.

Kennedy RD, D'Andrea AD. 2005. The Fanconi Anemia/BRCA pathway: New faces in the crowd. Genes Dev 19:2925–2940.

Khoder MI, Shakour AA, Farag SA, Abdel Hameed AA. 2000. Indoor and outdoor formaldehyde concentrations in homes in residential areas in Greater Cairo. J Environ Monit 2:123–126.

Kitaeva LV, Mikheeva EA, Shelomova LF, Shvartsman P. 1996. [Genotoxic effect of formaldehyde in somatic human cells in vivo]. Genetika 32:1287–1290.

Kuo H, Jian G, Chen C, Liu C, Lai J. 1997. White blood cell count as an indicator of formaldehyde exposure. Bull Environ Contam Toxicol 59:261–267.

Lazutka JR, Lekevicius R, Dedonyte V, Maciuleviciute-Gervers L, Mierauskiene J, Rudaitiene S, Slapsyte G. 1999. Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations:

Effects of occupational and environmental exposures. Mutat Res 445:225–239.

Levine RJ, Andjelkovich DA, Shaw LK. 1984. The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies. J Occup Med 26:740–746.

Linos A, Blair A, Cantor KP, Burmeister L, VanLier S, Gibson RW, Schuman L, Everett G. 1990. Leukemia and non-Hodgkin's lymphoma among embalmers and funeral directors. J Natl Cancer Inst 82:66

Lu K, Boysen G, Gao L, Collins LB, Swenberg JA. 2008. Formaldehyde-induced histone modifications in vitro. Chem Res Toxicol 21(8):1586–1593.

Lu K, Ye W, Gold A, Ball LM, Swenberg JA. 2009. Formation of S-[1-(N2-deoxyguanosinyl)methyl]glutathione between glutathione and DNA induced by formaldehyde. J Am Chem Soc 131:3414–3415.

Madison RE, Broughton A, Thrasher JD. 1991. Immunologic biomarkers associated with an acute exposure to exothermic byproducts of a ureaformaldehyde spill. Environ Health Perspect 94:219–223.

Matanoski GM. 1991. Risk of pathologists exposed to formaldehyde [NTIS/PB91-173682]. Springfield VA: National Technical Information Service.

Milham S Jr. 1983. Occupational Mortality in Washington State 1950–1979. DHHS (NIOSH) Publication (United States) (Department of Health and Human Services, National Institute (83–116).

Murrell W, Feron F, Wetzig A, Cameron N, Splatt K, Bellette B, Bianco J, Perry C, Lee G, Mackay-Sim A. 2005. Multipotent stem cells from adult olfactory mucosa. Dev Dyn 233:496–515.

Neuberger A. 1981. The metabolism of glycine and serine. Amsterdam: Elsevier.

NICNAS. 2006. National Industrial Chemicals Notification and Assessment Scheme: Formaldehyde, Priority Existing Chemical Assessment Report No. 28. Department of Health and Ageing, Australian Government. http://www.nicnas.gov.au/Publications/CAR/PEC/PEC28/PEC_28_Full_Report_PDF.pdf

NIOSH. 2005. Pocket Guide to Chemical Hazards. National Institute for Occupational Safety and Health. http://www.cdc.gov/NIOSH/npg/npgd0293.html

NIOSH. 2006. The Registry of Toxic Effects of Chemical Substances: Formaldehyde. National Institute for Occupational Safety and Health. http://www.cdc.gov/niosh/rtecs/lp882f48.html

Nojima K, Hochegger H, Saberi A, Fukushima T, Kikuchi K, Yoshimura M, Orelli BJ, Bishop DK, Hirano S, Ohzeki M, Ishiai M, Yamamoto K, Takata M, Arakawa H, Buerstedde JM, Yamazoe M, Kawamoto T, Araki K, Takahashi JA, Hashimoto N, Takeda S, Sonoda E. 2005. Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells. Cancer Res 65:11704–11711.

Orsiere T, Sari-Minodier I, Iarmarcovai G, Botta A. 2006. Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to formaldehyde by use of personal air sampling and analysis of DNA damage in peripheral lymphocytes. Mutat Res 605:30–41.

OSHA. 1992. Formaldehyde. Occupational Safety & Health Administration. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10075

OSHA. 1995. Occupational Safety and Health Administration: Occupational Exposure to Formaldehyde Fact Sheet. Department of Labor. http://www.medgasexperts.com/docs/01-01-1995%20-%20Occupational%20Exposure%20to%20Formaldehyde.htm

Ott MG, Teta MJ, Greenberg HL. 1989. Lymphatic and hematopoietic tissue cancer in a chemical manufacturing environment. Am J Ind Med 16:631–643.

Partanen T, Kauppinen T, Luukkonen R, Hakulinen T, Pukkala E. 1993. Malignant lymphomas and leukemias, and exposures in the wood industry: An industry-based case-referent study. Int Arch Occup Environ Health 64:593–596.

Pinkerton LE, Hein MJ, Stayner LT. 2004. Mortality among a cohort of garment workers exposed to formaldehyde: An update. Occup Environ Med 61:193–200.

Preussmann R,Stewart BW, editors. 1984. *N*-Nitroso carcinogens. Washington, D.C.: American Chemical Society. pp 643–828.

Ridpath JR, Nakamura A, Tano K, Luke AM, Sonoda E, Arakawa H, Buerstedde JM, Gillespie DA, Sale JE, Yamazoe M, Bishop DK, Takatam M, Takeda S, Watanabe M, Swenberg JA, Nakamura J. 2007. Cells deficient in the FANC/BRCA pathway are hypersensitive to plasma levels of formaldehyde. Cancer Res 67:11117–11122.

SEER. 2006. Cancer Statistics Review, 1975–2004: Overview. Bethesda, MD:National Cancer Institute. p 31.

Shaham J, Bomstein Y, Meltzer A, Kaufman Z, Palma E, Ribak J. 1996. DNA–protein crosslinks, a biomarker of exposure to formaldehyde—In vitro and in vivo studies. Carcinogenesis 17: 121–125.

Shaham J, Bomstein Y, Gurvich R, Rashkovsky M, Kaufman Z. 2003. DNA-protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. Occup Environ Med 60: 403–409.

Szarvas T, Szatlóczky E, Volford J, Trézl L, Tyihák E, Rusznák I. 1986. Determination of endogenous formaldehyde level in human blood and urine by dimedone-14C radiometric metho. J Radioanalytical Nucl Chem 106(6):357–367.

Tang X, Bai Y, Duong A, Smith MT, Li L, Zhang L. 2009. Formaldehyde in China: Production, consumption, exposure levels, and health effects. Environ Int doi:10.1016/j.envint.2009.06.002.

USEPA. 1990. Clean Air Act. 42 U.S.C. Section 7401 et seq. USA. http://www.epa.gov/air/caa/.

USEPA. 2005a. Guidelines for Carcinogen Risk Assessment. Washington, DC.:U.S. Environmental Protection Agency. EPA/630/P-03/001b.

USEPA. 2005b. Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. Washington, DC.:U.S. Environmental Protection Agency. EPA/630/R-03/003F.

USEPA. 2007. United States Environmental Protection Agency: Indoor Air Quality (IAQ). Environmental Protection Agency. http://www.epa.gov/iaq/formalde.html

Vargova M, Wagnerova J, Liskova A, Jakubovsky J, Gajdova M, Stolcova E, Kubova J, Tulinska J, Stenclova R. 1993. Subacute immunotoxicity study of formaldehyde in male rats. Drug Chem Toxicol 16:255–275.

Venkitaraman AR. 2004. Tracing the network connecting BRCA and Fanconi anaemia proteins. Nat Rev Cancer 4:266–276.

Vozenilkova H, Tmejova M, Srb V, Kubzova E, Rossner P, Pohlova H, Zudova Z, Polak J, Chylkova V, Turkova M, et al. 1991. [Environmental monitoring and biological monitoring of young people exposed to nonoccupational levels of formaldehyde, toluene and other hydrocarbons]. Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove Suppl 34:407–476.

Walrath J, Fraumeni JFJ. 1983. Mortality patterns among embalmers. Int J Cancer 31:407–411.

Walrath J, Fraumeni JFJ. 1984. Cancer and other causes of death among embalmers. Cancer Res 44:4638–4641.

Wang M, Cheng G, Villalta PW, Hecht SS. 2007. Development of liquid chromatography electrospray ionization tandem mass spectrometry methods for analysis of DNA adducts of formaldehyde and their application to rats treated with *N*-nitrosodimethylamine or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Chem Res Toxicol 20:1141–1148.

WHO-ROE. Regional Office of Europe: Development of WHO Guidelines for Indoor Air Quality 2006; Bonn, Germany. World Health

Organization. http://www.euro.who.int/Document/AIQ/IAQ_mtgrep_Bonn_Oct06.pdf

Yamazoe M, Sonoda E, Hochegger H, Takeda S. 2004. Reverse genetic studies of the DNA damage response in the chicken B lymphocyte line DT40. DNA Repair (Amst) 3:1175–1185.

Ye X, Yan W, Xie H, Zhao M, Ying C. 2005. Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters. Mutat Res 588:22–27.

Yu LQ, Jiang SF, Leng SG, He FS, Zheng YX. 2005. Early genetic effects on workers occupationally exposed to formaldehyde. Zhonghua Yu Fang Yi Xue Za Zhi 39:392–395.

Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT. 2009. Formaldehyde exposure and leukemia: A new meta-analysis and potential mechanisms. Mutat Res 681:150–168.

Accepted by—
B. Parsons