Comments on the Report on Carcinogens Draft Background Document for Formaldehyde, September 3, 2009, U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program: Biological Mechanisms for Formaldehyde-Induced Leukemia

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Introduction

The following comments are directed to the specific issue of whether there are sufficient mode of action (MOA) data in support of a reported association between exposure to formaldehyde and increased risk of developing of leukemia. This issue has important implications with respect to interpreting the biological plausibility of the epidemiological findings because the absence of a credible explanation for how this might occur, in the context of the well established MOA for chemical leukemogenesis, undermines the extent to which the relevance of the epidemiological findings can be judged.

In 2006, the International Agency for Research on Cancer (IARC 2006) classified formaldehyde as a known human carcinogen based largely, but not exclusively, on the results of a study conducted by the National Cancer Institute (NCI) that reported increased mortality from nasopharyngeal cancer (NPC) in formaldehyde-exposed workers (Hauptmann et al. 2004). In addition to the conclusion regarding NPC, IARC (2006) also concluded that there was some information to link formaldehyde inhalation exposure to leukemia although there was skepticism about this because a biological mechanism to explain how this might have occurred could not then be identified. Subsequently, as reviewed in the DRAFT Report on Carcinogens (ROC) background document on formaldehyde (hereafter "background document") several hypotheses have been postulated in an attempt to fill this key knowledge gap. This is a critical overarching issue because, based on abundant data, it does not appear that formaldehyde is capable of inducing leukemia similar to any other known leukemogenic chemical. The MOA of all known leukemogenic chemicals is well established, so the absence of a plausible explanation for how formaldehyde might cause leukemia substantially undermines the likelihood that the positive epidemiological results can reliably be attributed to formaldehyde exposure. As noted in the

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background document (p. *xxi*), "*However, some authors have questioned the biological plausibility of an association between formaldehyde exposure and leukemia, because formaldehyde is rapidly metabolized and would not enter the systemic circulation. They state that formaldehyde does not cause bone marrow toxicity or pancytopenia, which are common features of known leukemogens, and that the genotoxic and carcinogenic effects in animals and humans are limited to local effects.* It is important to emphasize that this statement is entirely correct and supported by an abundance of empirical data.

The following comments address key issues pertaining to the hypothesized MOA for formaldehyde-induced leukemia which essentially avoids the inconsistencies noted in the above-quoted statement, as detailed in sections 5.7.6 (Other tumors, p. 447) and 5.4.2.4 (Hematological and immunological effects, pp 350-355) of the background document. In order to avoid the necessity for reviewers to refer to the background document, key statements are quoted verbatim followed by a detailed comment.

Deficiencies of Soffritti et al. (1989 Drinking Water Study

P. 447; *Other potential tissue target sites include lymphohematopoietic tumors in humans (acute myelogenous leukemia and other lymphohematopoietic tumors, see Section 3) and experimental animals (hemolymphoreticular tumors, see Section 4...")*

Comment: It is beyond the scope of these brief comments to address either the biological plausibility or common sense implications of the idea that inhalation exposure to formaldehyde might be capable of inducing lymphohematopoietic tumors other than leukemia (i.e., all forms of acute and chronic myeloid and lymphoid leukemia as well as Hodgkin's and non-Hodgkin's lymphoma, and multiple myeloma. If true, this would be an unprecedented finding, as no chemical or physical agent at any exposure level (including high dose-ionizing radiation), has been shown to induce all forms of such malignancies. Rather this comment focuses only on the above reference to lymphohematopoietic tumors as reported in the drinking water study by Soffritti et al. (1989). While it is understood that NTP background documents do not typically make judgments on the quality of a particular study, in the case of Soffritti et al. (1989) there would appear to be grounds for making an exception. Other than the questionable relevance of the route of exposure (i.e., drinking water) when the issue is clearly one of inhalation, as summarized below, the recognized deficiencies of this study suggest that it should be afforded little, if any, consideration in a scholarly document of this kind. Of the many carcinogenicity studies on formaldehyde, the only one that has reported a carcinogenic effect at a site distant from the point of administration (i.e., nasal passages or gastric mucosa) was by Soffritti et al. (1989). While the substantial deficiencies of this study are well known space does not permit a full accounting of them other than the conclusions of several critiques which are briefly summarized. For example, as noted by Feron et al. (1990, 1991), none of the contradictory findings from other oral dosing studies that were available when Soffritti et al. (1989) published their results were discussed. In addition, historical untreated control data in Sprague-Dawley rats of the colony used show that the incidence of leukemia varies widely, with reported spontaneous incidence rates similar to those reported by Soffritti et al., suggesting that treatment-related effects may have been unrelated to formaldehyde exposure. In reviewing the results of Soffritti

et al. (1989), ATSDR (1999) also expressed skepticism: "*Another limitation to the strength of the evidence for formaldehyde-induced leukemia is the lack of a consistent dose-response relationship in the Soffritti et al. study.*" The Cancer Assessment Committee of the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration (FDA), also reviewed the study of Soffritti et al. (1989), concluding that the data reported were "*unreliable*" due to "*a lack of critical detail . . . questionable histopathological conclusions, and the use of unusual nomenclature to describe the tumors*." Consequently, the FDA "*determined that there is no basis to conclude that formaldehyde is a carcinogen when ingested*" (U.S. FDA 1998).

Given the well documented unreliable nature of their findings, in conjunction with the fact that leukemia was not reported in any of seven inhalation bioassays of formaldehyde, or three other drinking-water studies in which rats were exposed to even larger doses, suggests that the results reported by Soffritti et al. (1989) should either be put into proper perspective or not cited in this document because these findings are inconsistent with the rest of the peer-reviewed literature. Finally, given the intense controversy generated by the results of studies from the laboratory in which the Soffritti et al. study was conducted (e.g., Schoeb et al. 2009) this issue should be acknowledged in the background document.

Lack of Mechanistic Studies on Formaldehyde-Induced Leukemia

P. 447; *In contrast, numerous mechanistic studies were identified discussing the association between lymphohematopoietic cancers and formaldehyde exposure.*

Comment: The basis for this statement is unknown because the remainder of this section does not cite a single mechanistic study addressing an association between formaldehyde exposure and lymphohematopoietic (LHP) cancers. Indeed, part of the reason for the controversy surrounding this issue is the complete absence of any relevant data on this key issue. All that can be stated with any "certainty" about this issue are several untested hypotheses.

Proposed Hypothetical Mechanisms for Formaldehyde-Induced Leukemia

P. 447; *Two groups of researchers have proposed potential mechanisms for formaldehyde induced leukemia: (1) Zhang et al. (2009a) and (2) the Environmental Protection Agency (EPA) [Note the EPA did not publish their proposed mechanism in the peer-reviewed literature, but the major points are discussed in a criticism published by Pyatt et al. 2008.] The basic concepts of these proposed mechanisms are similar.*

Comment: It is somewhat surprising that the only way that EPA's proposed potential mechanism for formaldehyde-induced leukemia can be "addressed" is as a result of a critique of it by Pyatt et al. (2008) in which it was concluded that there was no scientific support that the proposed MOA or any of its elements actually occurs and that there were no relevant supporting data. If EPA deemed this to be an important issue (which it clearly is), it seems that publication in the peer reviewed literature should have been undertaken and this should be noted. Instead, as discussed below, other than two "hypothesis" posters by DeVoney et al. (2006a, b) EPA has neither addressed this issue nor undertaken any research to confirm their hypotheses.

As previously noted, IARC (2006) was skeptical about whether the reported epidemiological findings concerning an association between exposure to formaldehyde and leukemia were real due to the inability to identify a mode of action. Indeed, as discussed by IARC (2006), the idea that formaldehyde may cause leukemia "*...raises a number of mechanistic questions, including the processes by which inhaled formaldehyde may reach a myeloid progenitor.*" IARC continues "*...a clastogenic product of FA could conceivably be formed in the blood and circulate to the bone marrow although this has not been suggested in the literature.*" And finally, "*...it is possible that circulating myeloid progenitor stem cells could be the source of leukemia....such cells are present in the blood and plausibly could be exposed to formaldehyde in the respiratory tract vasculature; however, there is no known prototype for such a mechanism of leukemogenesis.*" It would appear, therefore, that IARC (2006) had already addressed and ruled out many of the critical issues pertaining to the biological plausibility of the MOA proposed by DeVoney et al. (2006a, b). Because there are still no data on any of the specific points raised by IARC (2006) it is suggested that the background document reconcile this issue with the MOA speculations raised by Zhang et al. (2009a, b).

Pp. 448-9; *Zhang et al. (2009a, b) identified three potential mechanisms for formaldehyde-induced leukemia: (1)* ***direct damage to stem cells in bone marrow****, (2) damage to circulating hematopoietic stem/progenitor cells in the blood, or (3) damage to pluripotent stem cells present within the nasal turbinates and/or olfactory mucosa. Although the biological plausibility of the first model has been questioned (discussed below), these authors suggested that absorbed formaldehyde would dissolve in the blood and be converted to its hydrated form (methanediol) and* ***could be transported to bone marrow*** *in this form. However, if formaldehyde is not able to reach bone marrow in sufficient quantities to damage stem cells, the two alternate mechanisms involving damage to circulating stem/progenitor cells* ***that travel to bone marrow and become initiated leukemic cells*** *are plausible. Thus, the critical DNA or macromolecular binding occurs in the blood, and when the affected cells proliferate, unrepaired lesions could lead to mutations and cellular toxicity. The initiated stem cell could be* ***re-incorporated into the bone marrow, and eventually lead to leukemia.*** *The authors cited the detection of DNA-protein crosslinks and cytogenetic damage in circulating lymphocytes of exposed workers as supporting evidence. The same type of damage would be expected to occur in circulating hematopoietic stem cells. The third mechanism is similar to the second but involves pre mutagenic or mutagenic damage to primitive pluripotent stem cells that reside in the oral or nasal passages.* ***Damaged stem cells*** *could be released from the nasal passages, perhaps enhanced by formaldehyde-induced cytotoxicity, circulate through the blood, and* ***eventually be incorporated into the bone marrow****.* [emphasis added]

Comment: This is the key paragraph that must be considered in the context of biological plausibility and an abundance of relevant data. With respect to the first hypothetical MOA, i.e., ***direct damage to stem cells in bone marrow,*** it is well established that because inhaled formaldehyde does not change the normal concentrations of formaldehyde present in the blood in rats, monkeys or humans even following prolonged exposure to almost 15 ppm (Heck et al. 1985; Casanova et al., 1988), direct damage to the bone marrow is essentially impossible. This is confirmed by the fact that none of the numerous chronic 2-year cancer bioassays conducted

with airborne formaldehyde concentrations up to 15 ppm has ever produced pancytopenia or bone marrow toxicity.

Further confirmation that formaldehyde is unlikely to damage the bone marrow is also provided in the background document (p. 390) which summarizes the results of a study by Dallas et al. (1992) in which inhalation exposure to formaldehyde at up to 15 ppm for 8 weeks produced no increase in chromosomal aberrations (CA) in the bone marrow. In another study by Dean et al. (1984), which was not cited in the background document, female B6C3F1 mice were exposed via inhalation to 15 ppm formaldehyde 6 h/day for 21 days (Dean et al. 1984). Bone marrow cellularity and clonogenic potential of bone marrow derived progenitor cells were not significantly different between exposed and controls thereby providing evidence that subchronic exposure to 15 ppm formaldehyde does not damage the bone marrow and is not likely a target organ for formaldehyde toxicity.

As also summarized in the background document, another study by Kitaeva *et al.* (1990) reported CA in the bone marrow following inhalation exposure to formaldehyde. However, this study, as reported, is difficult to interpret because key experimental procedures (e.g., dose levels, number of animals tested, actual number of metaphases analyzed per animal) and statistical methods were not sufficiently described. The results lack plausibility because an uncommon pattern of aberrations (chromatid type and chromosome type aberrations, increase in hypo- and hyperdiploid cells) was reported. Hypoploidies were much more frequent than hyperploidies which may indicate limits in the quality of chromosome preparation. Altogether, this study is of poor scientific quality and therefore not reliable. Furthermore, the overwhelming majority of studies have not corroborated this finding, including some with considerably higher exposures. For example, a recent comprehensive inhalation study with Fischer-344 rats unambiguously excluded systemic genotoxic effects of formaldehyde (Speit et al. 2009). Groups of six rats were exposed for 4 weeks (6h/day, 5 days/week) to formaldehyde target concentrations of 0, 0.5, 1, 2, 6, 10 and 15 ppm. Clearly negative results were obtained with the comet assay, the sister chromatid exchange (SCE) test and the micronucleus test (MNT) with peripheral blood. For micronuclei determination the highly sensitive flow cytometry method was used enabling investigation of 20,000 cells per animal. Because micronuclei are formed during cell division in the bone marrow, this study confirms that exposure of the bone marrow leading to genotoxicity/mutagenicity can be excluded. Therefore, the single positive study reported by Kitaeva and coworkers is not sufficient in demonstrating formaldehyde-induced bone marrow toxicity/genotoxicity.

Clearly, another study demonstrating whether exogenous formaldehyde, particularly FA-DNA adducts can be detected in the bone marrow would provide an answer to this key question. In this regard, the paper by Zhang et al. (2009b) hypothesizes the involvement of endogenous formaldehyde in the induction of DNA protein crosslinks (DPCs) and subsequent bone marrow failure with a predisposition to tumors in patients with Fanconi anemia. When extended to exogenous formaldehyde with the hypothetical that such sources "*...could push susceptible individuals into a dangerous zone in which genotoxic levels of DPC are induced*" the authors note that *"One of the **big limitations** to this hypothesis is the uncertainty over whether exogenous formaldehyde can reach the bone marrow."* [emphasis added] Given the abundance of

available data, confirming the inability of exogenous formaldehyde to reach the bone marrow it would appear that this hypothesis is untenable. Finally, with respect to the paper by Zhang et al. (2009b) it is surprising that none of the directly relevant studies involving formaldehyde-induced mutations and repair both *in vitro* and *in vivo* by Speit et al. 2000, 2002, 2006a,b, 2007a,b, 2008, 2009, Merk and Speit 1998, 1999, Neuss and Speit 2008, and Schmid and Speit 2007 were cited.

As for the other two hypothetical mechanisms, i.e., *damage to circulating hematopoietic stem/progenitor cells in the blood, or damage to pluripotent stem cells present within the nasal turbinates and/or olfactory mucosa,* the unifying commonality for each is the eventual necessity and absolute requirement for bone marrow involvement as emphasized in the bolded passages in the above quoted paragraph. It is beyond dispute that other than the bone marrow, there is no other anatomic site in mammals where leukemia cells can reproduce in order to eventually display the clinical pathohematological picture of frank leukemia. Consequently, the statement that the *initiated stem cell could be re-incorporated into the bone marrow, and eventually lead to leukemia,* necessarily implies that the bone marrow would show evidence of hyperplastic growth characteristic of leukemia which has never been observed. As noted in ATSDR (1999) *"No exposure-related effects on hematological variables were found in rats exposed to up to 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks (Woutersen et al. 1987), in rats exposed to up to 10 ppm, 6 hours/day, 5 days/week for up to 52 weeks (Appelman et al. 1988), in rats or mice exposed to up to 14.3 ppm, 6 hours/day, 5 days/week for up to 24 months (Kerns et al. 1983b), or rats exposed to up to 15 ppm for 28 months (Kamata et al. 1997). Dean et al. (1984) reported that female mice exposed to up to 15 ppm for 6 hours/day, 5 days/week for 3 weeks showed a statistically significant decrease in absolute number of monocytes compared with control values, but no other hematological variable was affected by exposure in this study."* Given the striking lack of hematological effects in rodents chronically exposed to high concentrations of formaldehyde, the equivocal epidemiological findings in human populations, even if correct, suggest that exposure to something other than formaldehyde likely influenced the findings.

Finally, with respect to the Zhang et al. (2009a) hypotheses linking *"the detection of DNA-protein crosslinks and cytogenetic damage in circulating lymphocytes of exposed workers as supporting evidence"* and the unfounded speculation that *"the same type of damage would be expected to occur in circulating hematopoietic stem cells"* there are substantial uncertainties with this hypothetical scenario. The genetic endpoints measured in peripheral blood of exposed workers are considered to be either biomarkers of exposure (DPX, SCE) or biomarkers of early genetic effects (micronuclei, chromosomal aberrations). There is some evidence that increased frequencies in micronuclei (MN) or chromosomal aberrations (CA) may predict the risk of cancer in humans (Hagmar et al. 1998; Bonassi et al. 2007). However, these results were obtained from subjects not specifically exposed to potential mutagens and the frequencies of MN and CA measured are not related to external exposure but more likely to the intrinsic genomic instability (e.g., differences in DNA repair capacity) of the subjects studied.

Numerous studies have investigated the potential *in vivo* genotoxicity (i.e., DPX, SCE, MN or CA) in the peripheral lymphocytes of occupationally exposed workers compared to unexposed controls (Bauchinger and Schmid, 1985; He et al., 1998; Yager et al., 1986; Ying et al., 1997,

1999; Vasudeva and Anand, 1996; Thompson et al., 1984). These studies led to inconsistent and conflicting results and a critical assessment of the majority of these studies (positive or negative) is difficult because of shortcomings in the study design and/or the evaluation of the data.

On the basis of comprehensive *ex vivo* experiments with cultures of human lymphocytes it has been questioned whether *in vivo* exposure to formaldehyde can actually lead to positive effects in genotoxicity tests with lymphocytes of exposed subjects. SCE and MN measured in blood cultures of exposed humans are formed *ex vivo* during the proliferation of lymphocytes. The formation of these cytogenetic effects as a consequence of *in vivo* exposure requires that the cells sampled retain the increased levels of DNA damage. This damage has to persist up to replication and cell division. It is known that lymphocytes start replication about 24 h after stimulation. Due to the rapid repair of DPX the probability that DPX will persist and effects will occur is extremely unlikely. Furthermore it is highly improbable that DPX may accumulate in lymphocytes after inhalation exposure in sufficient amounts. The conditions that are necessary to induce measurable effects (i.e., high DPX levels and persistence of DPX until S-phase) are simply not achieved (Schmid and Speit, 2007). Most likely, the positive effects reported are chance findings or due to other kind of exposures of the populations studied. Therefore, human biomonitoring studies should be interpreted with great caution as a supporting argument for systemic genotoxic/mutagenic effects of formaldehyde. Most importantly these markers are for circulating lymphocytes, and it has not been shown that these effects occur in stem cells or HPC that can then somehow transition to leukemia. With respect to this latter issue, there is no evidence cited by Zhang et al. (2009b) that any of the proposed events actually occur other than that *"...**one can imagine** the targeting of sufficient stem cells through these two alternative models to induce leukemia..."* [emphasis added] The inability of formaldehyde to induce systemic genotoxic/mutagenic effects (i.e., damage bone marrow or circulating lymphocytes directly) has recently been demonstrated in comprehensive *in vivo* animal experiments (Speit et al. 2009). Inhalation of formaldehyde in a 28-day study at concentrations up to 15 ppm did not lead to effects in the comet assay (DNA strand breaks and DPX), the SCE test and the MNT with peripheral blood.

In attempting to demonstrate that exposure to formaldehyde can induce adverse hematological effects the background document cites a number of studies. For example, Tang et al. (2009) briefly reviews a number of studies from China published mainly in Chinese journals. While several of these studies are summarized, the inability to review most of them limits the scope of potential comments. However, as summarized in Table 9 of Tang et al. (2009) from the various studies cited, while some formaldehyde levels are listed it is essentially impossible to know what additional exposure conditions were present in order to evaluate whether the reported results on white blood counts, platelet counts or hemoglobin levels were due to formaldehyde or another exposure. For example, the only study specifically cited in support of hematological effects in Section 5.4.2.4 (Hematological and immunological effects, p. 350) by Kuo et al. (1997), was conducted on 50 hemodialysis nurses and controls from four hospitals in Taiwan and concluded that the white blood cell counts were significantly lower in the exposed group compared to controls. However, this study is not credible given that the formaldehyde analytical data are not only suspect (e.g., one area sampling concentrations reported as 0.231 ppm, 0.054 ppm and 0.082 ppm for mean, lowest and highest, respectively), but also that overall formaldehyde levels

were implausibly low (e.g., mean personal sampling concentrations of 0.015 ppm, 0.017 ppm, 0.033 ppm and 0.054 ppm) in the 4 hospitals. This is the only study cited in this section suggesting that exposure to formaldehyde can cause hematological effects.

In contrast to the above, the vast majority of more credible data show essentially no reported hematological effects following exposure of either humans or animals to formaldehyde. While accidental ingestion of a large quantity of formaldehyde was reported to cause an intravascular coagulopathy (Burkhart et al., 1990), several reports of human ingestion of lower doses have not shown any effects on the blood or blood-forming organs (Eells et al. 1981, Freestone and Bentley 1989, Koppel et al. 1990). In animal studies, neither inhalation exposure (Appelman et al. 1988, Kamata et al. 1997, Kerns et al. 1983, Woutersen et al. 1987) nor oral exposure (Johannsen et al. 1986, Til et al. 1989, Tobe et al. 1989) to high doses of formaldehyde has produced any evidence of adverse hematological effects. A single study in rats exposed to massive oral doses of formaldehyde (e.g., 80 mg/kg for 4 weeks) reported minor alterations in erythrocyte count and hemoglobin values (Vargova et al. 1993). As noted in ATSDR (1999), the lack of hematopoietic toxicity in these studies is *"likely related to rapid metabolism prior to the formaldehyde reaching the blood and blood-forming components (bone marrow)."* Many of the above-cited studies are included in the background document and demonstrate that formaldehyde is unlikely to cause adverse hematological effects.

Lack of Experimental or Clinical Support for Hypothesized MOA for Formaldehyde-Induced Leukemia

P. 449; *Supporting evidence for this mechanism includes toxicity and DNA-protein crosslinks in the nasal passages of laboratory animals exposed to formaldehyde, reports of increased micronuclei in the nasal and oral mucosa of formaldehyde-exposed humans, and a study (Murrell et al. 2005) that showed that olfactory epithelial cells obtained from rat nasal passages contained hematopoietic stem/progenitor cells. These cells were shown to re-populate the hematopoietic tissues of irradiated rats and to form hematopoietic stem/progenitor cells of multiple lineages in vivo.*

Comment: The supporting evidence pertaining to DPX and MN in circulating lymphocytes has already been addressed above and as noted neither is relevant with respect to indirectly indicating that inhaled formaldehyde might be capable of mutating circulating stem or hematopoietic progenitor cells (HPCs). As for the idea that DPX or MN in nasal passages or in the nasal or oral mucosa is a relevant basis for assuming that similar effects would occur in circulating stem or HPCs there is a fundamental problem of assuming that a finding in a fixed tissue (e.g., nasal passages) with chronic exposure would translate to uncommon cells in the circulation. In general terms, the hypothesized MOA involves inhaled formaldehyde reaching susceptible cells (stem cells or HPC) in the nasal associated lymphoid tissue (NALT) and then, via an unknown mechanism, resulting in malignant transformation of these target cells that ultimately leads to the development of leukemia via the bone marrow. However, these potentially susceptible cells are not in direct contact with formaldehyde (i.e., they are not the first site of contact) and *in vitro* co-cultivation experiments indicate that formaldehyde (even at high concentrations) that has entered a cell is not released again and does not damage other cells

(Neuss and Speit 2008). Although it cannot be excluded that a circulating stem cell or HPC is somehow damaged in the first place, the likelihood that this cell is actually transformed with subsequent leukemogenesis can be dismissed particularly because it would still require bone marrow involvement.

Furthermore, in a very real sense, the hypothesized MOA has already been extensively tested as an unforeseen but now relevant consequence of the numerous long-term cancer bioassays conducted with formaldehyde in rodents. Like humans, both rats and mice have hematopoietic tissue at the portal of entry (i.e., NALT) as well as peripheral HPC with comparable functions across different species (Haley 2003, Kuper et al. 2003). Because both NALT and HPC are hypothesized as key targets in the MOA for formaldehyde-induced leukemia, these tissues should also be vulnerable to formaldehyde-induced toxicity and malignant transformation. While there are well documented and important differences in hematopoiesis and lymphopoiesis between humans and rodents, all chemicals known to produce leukemia in humans have also shown this capability in rodent studies with either rats or mice. In this regard, the various hematopoietic tissues (e.g., NALT) hypothesized by DeVoney et al. (2006a,b) or Zhang et al. (2009) to be potential targets of formaldehyde toxicity have, in fact, been chronically exposed to high concentrations of inhaled formaldehyde in numerous independent rodent cancer bioassays. As part of routine carcinogenicity testing protocols, numerous tissues (including lymph nodes, blood and bone marrow) are typically collected and assessed microscopically for pathological changes. While some peripheral tissues, such as the NALT are not usually collected, there is no evidence that chronic, high dose-exposure to formaldehyde (via either oral or inhalation dosing) causes hematopoietic toxicity or an increased incidence of any type of hematopoietic malignancy including leukemia. If formaldehyde were capable of inducing leukemia in humans, it seems likely that there would be some indication of a similar potential in rodents.

Due to the central importance of NALT in the MOA proposed by DeVoney et al. (2006a, b) a study was undertaken to investigate whether NALT and local lymph nodes might be affected by inhaled formaldehyde. This 28-day inhalation study was conducted in F344 rats and B6C3F1 mice at formaldehyde concentrations of 0, 0.5, 1, 2, 6, 10 and 15 ppm to determine if local lymphoid tissues are a target of formaldehyde. Both NALT and upper-respiratory tract-draining lymph nodes were stained either with H&E or immunohistochemically for cell proliferation (i.e., BrdU incorporation). Light microscopy revealed slight to moderate simple hyperplasia of NALT lymphoepithelium and an increased proliferation rate of the epithelial cells in rats exposed to 15 ppm indicating that the NALT had been reached by formaldehyde. Other effects on the local lymphoid tissues (NALT and lymph nodes) were not observed. Analysis of rat NALT and lymph nodes did not reveal any effects at lower exposure levels while similar tissues from mice were not affected at any dose. Consequently, the only distinct effect of formaldehyde vapor on local lymphoid tissues (NALT and lymph nodes) of Fischer 344 rats and B3C3F1 mice was hyperplasia of the lymphoepithelium in rats exposed to 15 ppm (Kuper et al. 2009). Given the lack of any observable responses of NALT or local lymph nodes at doses of <15 ppm other than what would be expected based on histopathology, there does not appear to be any evidence supporting the hypothesis that NALT tissues are involved in leukemogenesis.

The above noted study by Murrell et al. (2005) does not support the hypothesized MOA for formaldehyde-induced leukemia. This highly complex study focused on pluripotent cells found in the human olfactory mucosa that had the potential to differentiate mainly into neurons and glia. While the article stated that cells from rat olfactory tissues appeared to repopulate the bone marrow of irradiated hosts it also stressed that *"several experiments indicate that the olfactory cell preparations* ***did not contain hematopoietic stem cells*** *that could account for the observations."* [emphasis added] Because this study offers no support for the hypothesized formaldehyde-induced malignant transformation of hematopoietic stem cells in the nasal mucosa it should be deleted from the background document.

Finally, in a recent critique, Goldstein (2009) reviewed proposed mechanisms for formaldehyde-induced acute myelogenous leukemia (AML) that avoided the necessity for pancytopenia and myelotoxicity concluding that *"The lack of evidence that high-dose formaldehyde produces pancytopenia in humans or laboratory animals, and the longer latency period for its reported epidemiological association with AML, distinguish formaldehyde from the broad variety of known human leukemogens, and suggests that if formaldehyde is a myeloid leukemogen it does not follow known leukemogenic mechanisms of action. One inference raised by the proposal that myeloid precursors within the nasal mucosa may be the site for leukemogenesis is the expectation that chloromas, which are local collections of myeloid tumor cells, would be found in the nose. However, review of the literature suggests that nasal tissues are rarely a site for a chloroma."* Goldstein (2009) also notes that *"...all of the agents now known to cause AML will at high doses invariably cause pancytopenia or frank aplastic anemia. Any proposed mechanism for formaldehyde leukemogenesis must explain why pancytopenia has not thus far been observed with formaldehyde despite extensive animal and human studies."* The background document offers no explanation for why pancytopenia has not been observed with formaldehyde despite extensive animal and human studies. Because there are no empirical data supporting a mode of leukemogenic action that does not involve myelotoxicity the only biologically plausible conclusion that can be reached at this time is that this proposed mode of action does not exist.

Conclusions

Other than untested hypotheses purporting to explain how inhaled formaldehyde might be capable of inducing leukemia, there are no actual data documenting that any of the elements in the proposed MOA actually occur. All three proposed mechanisms in the MOA, i.e., (1) direct damage to stem cells in bone marrow, (2) damage to circulating stem or progenitor cells in the blood or (3) damage to stem cells in the nasal mucosa ultimately require the involvement of the bone marrow in order to complete the leukemogenic process. However, the striking lack of myelotoxicity in numerous chronic high dose animal bioassays or any indication of pancytopenia or myelotoxicity in humans would appear to undermine the likelihood that the proposed MOA actually occurs. The 28-day study showing no effects on NALT at formaldehyde concentrations <15 ppm would appear to eliminate nasal epithelial tissues from consideration as a target for formaldehyde-induced leukemogenesis. Consequently, until there is a credible biologically plausible explanation for the equivocal epidemiological findings concerning a possible association between formaldehyde and leukemia, the causal nature of that association remains suspect.

References Cited not in Bibliography of Background Document

Bonassi, S., Znaor, A., Ceppi, M., Lando, C., Chang, W.P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S., Barale, R., Bigatti, M.P., Bolognesi, C., Cebulska-Wasilewska, A., Fabianova, E., Fucic, A., Hagmar, L., Joksic, G., Martelli, A., Migliore, L., Mirkova, E., Scarfi, M.R., Zijno, A., Norppa, H., and Fenech, M. 2007. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis. 28(3):625-31.

Dean, J.H., Lauer, L.D., House, R.V., Murray, M.J., Stillman, W.S., Irons, R.D., Steinhagen, W.H., Phelps, M.C., and Adams, D.O. 1984. Studies of immune function and host resistance in B6C3F1 mice exposed to formaldehyde. Toxicol. Appl. Pharmacol. 72(3):519–529.

DeVoney, D., Vandenberg, J., Whalen, J., 2006a. Hypothesized mode of action for formaldehyde-induced lymphoid malignancies. Society of Toxicology Annual Meeting, San Diego, CA.

DeVoney, D., Jinot, J., Keshava, C., Hsu, C., Whalan, J., Vandenberg, J., 2006b. A hypothesized mode of action in support of the biological plausibility of formaldehyde-induced lymphohematopoietic malignancies. Society for Risk Analysis, Baltimore, MD.

Feron, V.J., Til, H.P., Woutersen, RA., 1990. Letter to the Editor. Toxicol. Ind. Health 6, 237–239.

Feron, V.J., Til, H.P., de Vrijer, F., 1991. Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. Mutat. Res. 259, 363–385.

Freestone, J., and Bentley, A. 1989. Case of formaldehyde poisoning. Br. J. Pharm. Pract. 11:20–21.

Hagmar, L., Bonassi, S., Stromberg, U., Brogger, A., Knudsen, L.E., Norppa, H., and Reuterwall, C. 1998. Chromosomal aberrations in lymphocytes predict human cancer: A report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer Res. 58(18):4117–4121.

Haley, P.J., 2003. Species differences in the structure and function of the immune system. Toxicology 188 (1): 49–71.

Johannsen, F.R., Levinskas, G.J., and Tegeris, A.S. 1986. Effects of formaldehyde in the rat and dog following oral exposure. Toxicol. Lett. 30:1–6.

Kuper, C.F., Arts, J.H., Feron, V.J., 2003. Toxicity to nasal-associated lymphoid tissue. Toxicol. Lett. 140–141, 281–285.

ED_014350_00000987-00011

C. Frieke Kuper, C.F, van Oostrum, L., Ma-Hock, L., Durrer, S. and Woutersen, R.A. 2009. Hyperplasia of the lymphoepithelium of NALT in rats but not in mice upon 28-day exposure to15 ppm formaldehyde vapor. Exper. Toxicol. Path. (in press)

Schoeb, T.R., McConnell, E.E., Juliana, M.M., Davis, J.K., Davidson, M.K., and Lind, J.R. 2009. Mycoplasma pulmonis and lymphoma in bioassays in rats. Environ. Pathobiol. 46:952-9.

U.S. Food and Drug Administration. 1998. Indirect food additives, adjuvants, production aids, and sanitizers. *Fed. Reg.* **63:**35134–35135.