ENVIRON

February 8, 2010

Dr. Ruth Lunn
Director
Report on Carcinogens Center
National Institute of Environmental Health Sciences
P.O. Box 12233
K2-14
Research Triangle Park, NC 27709

RE: Comments on the Recommendation from the Expert Panel Report (Part B) on Formaldehyde, 74 Fed. Reg. 67,883 (December 21, 2009)

Dear Dr. Lunn:

On behalf of Hexion Specialty Chemicals, Inc., ENVIRON International Corporation submits the attached comments on the Expert Panel Report (Part B) on Formaldehyde.

Our primary comments may be summarized as follows:

We respectfully submit that the Panel's conclusion regarding myeloid leukemia was not based on a rigorous strength-of-evidence assessment in which a balanced evaluation of all the data – epidemiological, toxicological and mechanistic – was conducted.

The Panel's interpretation of findings for leukemia did not consider the totality of results available and ignored the lack of statistical significance of the observations. The strength of the evidence for a causal association between formaldehyde exposure and myeloid leukemia is lacking, and there is, at most, a weak association that does not rise to the level necessary for the classification of "known" human carcinogen.

The animal data provide sufficient evidence that formaldehyde exposure is not linked with the production of either leukemia or of non-neoplastic indicators of the potential for leukemia.

Insufficient evidence is available to support a potential mechanism for the development of myeloid leukemia following inhalation exposure to formaldehyde. The toxicokinetic data indicate that any transport of formaldehyde from the point of contact will not affect endogenous levels in the peripheral blood, and no *in vivo* evidence is available that formaldehyde can be transported to and affect the bone marrow. While chromosomal aberrations have been reported in *in vivo* studies of peripheral blood cells in formaldehyde-exposed individuals, these findings do not support a causal association between formaldehyde and myeloid leukemia.

The strength of the evidence for formaldehyde as a potential causal agent for human myeloid leukemia neither supports a "known" classification, nor rises to the level of "reasonably anticipated," based on NTP's classification requirements in the Background Document for Formaldehyde.

We recommend that the NTP, including the Expert Panel and the Board of Scientific Counselors, apply a strength-of-evidence approach discussed above in separately evaluating all human, animal and mechanistic data relevant to each of the three identified tumor types -- i.e. nasopharyngeal carcinoma, sinonasal adenocarcinoma, and myeloid leukemia. The scientific evidence for these three cancers varies widely and warrants independent reviews and findings.

Consistent with these recommendations, we specifically request the NTP to reconvene the Expert Panel and direct it to (1) carry out and document a separate strength-of-evidence evaluation for each of the three cancers, and (2) consider and determine the applicability of the RoC classification criteria on a *type of cancer*-specific basis.

Thank you for your consideration of these comments.

Respectfully yours,

Signatures of Dr. J.V. Rodricks, Dr. A. Shipp, Dr. R. Gentry, & Dr. D.Turnbull

[Redacted]

Comments on the National Toxicology Program Expert Panel Report (Part B) on Recommendations for the Listing Status of Formaldehyde in the 12th Report on Carcinogens

In Response to Federal Register Notice of December 21, 2009 (74 FR 67883)

Prepared for:

Hexion Specialty Chemicals, Inc.
Columbus, OH 43215

Prepared by:

ENVIRON International Corporation
Arlington, Virginia
Amherst, Massachusetts
Monroe, Louisiana

February 8, 2010

1.0 Background

The National Toxicology Program (NTP) has prepared a Background Document (NTP 2010) on the toxicity of formaldehyde that was used by the external Expert Peer Review Panel (Panel) as the basis for its conclusion that formaldehyde is a "known" human carcinogen. While guidance providing specifics as to this categorization is limited, Wolfe[1] (2009) specified that the "Listing determination is based on the strength of the evidence" and that the "[c]onclusion [is] based on scientific judgment with consideration of **all** relevant information" (emphasis added). That is, the listing decision needs to be based on a thorough, balanced, scientific evaluation of **all** of the pertinent data, with full consideration given to the strengths and weaknesses of each piece. We respectfully submit that the Panel did not perform such a rigorous assessment of all of the evidence in reaching its conclusion that formaldehyde is a "known" human carcinogen.

It appears that Panel members merged their analyses of the epidemiological data for myeloid leukemia, nasopharyngeal carcinoma, and sinonasal adenocarcinoma in order to reach their general conclusion that formaldehyde is a "known" human carcinogen. Of most concern is the apparent conclusion by the Panel that there is sufficient evidence that formaldehyde is causally associated with myeloid leukemia, which according to the Panel, was part of the justification of the classification of formaldehyde as a "known human carcinogen".

Because the evidence for formaldehyde-induced neoplasia for each of these three endpoints (tumor types) is of different strengths, not only must these endpoints be evaluated separately, the classification of formaldehyde as a carcinogen must be based on the strength of the evidence for each endpoint separately. When evaluated on the strength of the evidence and the merits of the studies, the data from all types of evidence, as noted below, do not support the conclusion that the production of myeloid leukemia is causally associated with formaldehyde exposure. As discussed in the following sections, we respectfully submit that the Panel's conclusion regarding myeloid leukemia was not based on a rigorous strength-of-evidence assessment in which a balanced evaluation of all the data - epidemiological, toxicological and mechanistic - was conducted. Additional comments relevant to the consideration of nasopharyngeal and sinonasal cancers as part of the classification of formaldehyde are provided in a separate submission by the Formaldehyde Council, Inc. (Natz, 2010).

2.0 Epidemiological Studies

The Panel appears to have relied on just four studies, deemed by the Panel to provide "informative information", in reaching its conclusion that formaldehyde is a "known" human carcinogen based on the production of myeloid leukemia. There are three studies in workers in various industries (Beane Freeman *et al.* 2009; Pinkerton *et al.* 2004; Coggon *et al.* 2003), and one study of funeral home employees (Hauptmann *et al.* 2009).

- **The evidence presented in these four papers does not rise to the level of the "strength" of the evidence necessary to support the conclusion that formaldehyde is a "known" human carcinogen based on myeloid leukemia.**

Beane Freeman et al. (2009) is the latest follow-up of an NCI-sponsored study of more than 25,000 workers in 10 plants involved in formaldehyde-related processes. The incidence of myeloid leukemia was not statistically significantly increased by any of the metrics selected, i.e., concentration of peak

[1] Dr Wolfe is the Executive Secretary of the NTP Board of Scientific Counselors

exposure, number of peak exposures, average exposure concentration, cumulative exposure concentration, and duration of exposure.

- In a previous evaluation of this cohort (Hauptmann *et al.* 2003), the incidence of myeloid leukemia was significantly increased only in the subcohort with peak exposures greater than 4 ppm and average intensity of exposure (AIE) based on internal mortality rate comparisons, but not cumulative exposure or duration of exposure.
- In the reanalysis of the NCI data by Marsh and Youk (2004), no significant increase in either endpoint was noted in any alternative exposure categories: average intensity, cumulative exposure, duration of exposure, duration of time worked in the highest peak category, or time since first highest peak exposure. Marsh and Youk (2004) did not find a statistically significant increase in leukemia or myeloid leukemia when compared to external mortality rates (SMRs), and suggested that the positive findings in Hauptmann *et al.* (2003) were due to statistically significant deficits in deaths in the baseline group. An observation that was confirmed by Beane Freeman et al (2009). More than 1000 deaths among cohort members were missed in the previous investigation (Hauptman *et al.* 2003) with proportionally more deaths missed among the unexposed and non-exposed groups.

Pinkerton et al. (2004) is a study sponsored by NIOSH of more than 11,000 workers in the garment industry. No statistically significant increases in leukemia or all types of myeloid leukemia, including acute, chronic and other myeloid leukemia, were reported in standard analyses, even when stratified by duration of exposure or time since first exposure. A "multiple cause of death" (MCOD) analysis, which typically is used to assess the prevalence of non-cancer, non-fatal diseases, did not find a significant increase in myeloid leukemia (all types) for the entire cohort. When stratified either by duration of employment (10+ years) or by years since first exposure (20+ years), and evaluated using the MCOD method, modest increases in mortality from myeloid leukemia were noted, i.e., the lower bound on the confidence intervals were 1.02 for years of exposure and 1.10 for years since last exposure. Acute myeloid leukemia was not significantly increased when evaluated using MCOD analysis.

Coggon et al. (2003) is a study of more than 14,000 workers employed in factories where formaldehyde was manufactured or produced. No significant increase in leukemia (type not specified) was found even when the cohort was stratified by duration of exposure or when limited to only those workers considered to be in the high-exposure group. Formaldehyde exposures were in general higher for these workers than those included in the NCI study, and a higher percentage of workers were considered to be in the "high" exposure category compared to the NCI cohort. Although the Panel acknowledged that this study did not show an association with leukemia, the Panel provided no discussion/justification why these results did not influence their conclusions.

Hauptmann et al. (2009) is a case-control study of professionals employed in the funeral industry. Of the more than 6,800 embalmers and funeral directors, 168 cases listed as either the cause of death or a contributing cause of death from lymphohematopoietic cancers were identified, with 265 control subjects randomly selected from the funeral industry whose deaths were attributed to other causes. Subjects were matched as to sex and dates of birth and death. The authors reported a significant increase in myeloid leukemia among those who performed embalming for more than 34 years, performed more than 3,000 embalmings, or had a cumulative exposure of more than 9200 ppm-hours. These elevations were modest, with lower bounds on the confidence interval of 1.2, 1, and 1, respectively. Based on the typical definition of statistical significance, the second two categories would not be considered statistically significant because the confidence intervals do not exclude the null (a value of 1). Numerous methodological limitations have been noted for this study:

- Although some effort was made to assure comparability between the cases and controls overall, important differences are apparent specifically between myeloid leukemia cases and the control group. For example, myeloid leukemia cases were 50% more likely than controls to have begun employment in the funeral industry prior to 1942. This suggests that the myeloid leukemia cases were generated by an older and earlier source population than is represented by the controls, and may explain why this group performed more embalmings. This and other differences between cases and controls, including earlier year of death, all white race, longer time employed and age first employed, appear not to have been fully considered by the authors.
- As reported by the authors, the standard statistical analyses initially reported are unreliable due to the fact that there was only one unexposed myeloid leukemia case, leading to very large and unstable OR's. Adjusting the lowest exposure group to include cases and controls performing fewer than 500 estimated embalmings resulted in drastically reduced OR's that the authors consider more realistic. However, no clear rationale is provided for using less than 500 embalmings as an "unexposed" group, and most exposed groups produce roughly similar ORs regardless of exposure category.
- Contrary to most results presented, myeloid leukemia cases and the controls had nearly identical mean estimated average formaldehyde exposure, TWA-8 hour exposure and peak formaldehyde exposure. As expected, estimated number of embalmings and the correlated cumulative exposure were slightly higher for cases – likely due to their earlier first employment, younger age at hire and older age at death, leading to longer average employment in the industry.

- **The Panel did not consider the results of the published meta analyses in reaching their conclusion to classify formaldehyde as a "known" human carcinogen.**

As stated, the Panel apparently relied on just 4 studies in reaching their conclusion regarding the evidence for causation of myeloid leukemia. The Panel failed to consider 12 additional cohort studies with leukemia findings among formaldehyde-exposed workers. When a number of studies have been conducted that may have dissimilar designs and different results, Meta analysis is an accepted statistical procedure to aid in reaching a strength-of-evidence conclusion based on epidemiological data. The most recent and comprehensive Meta analysis, conducted by Bachand *et al.* (2010), evaluated 18 studies in which an association was investigated between formaldehyde exposure and leukemia, in particular myeloid leukemia, and concluded that formaldehyde was not causally associated with leukemia or myeloid leukemia. The other Meta analyses have been conducted since 2004 (Bosetti *et al.* 2008; Collins *et al.* 2004; Zhang *et al.* 2009). These studies did not include the most recent data from the NCI cohort published by Beane Freeman *et al.* (2009). None of these adequately investigated potential sources of heterogeneity other than the effect of job type, did not include sensitivity analyses that could have been conducted with the available data, and included proportional mortality rates (PMRs), which are not useful in determining causal associations (Bachand *et al.* 2010).

In summary, the Panel's interpretation of findings for leukemia did not consider the totality of results available and ignored the lack of statistical significance of the observations. The strength of the evidence for a causal association between formaldehyde exposure and myeloid leukemia is lacking, and there is, at most, a weak association that does not rise to the level necessary for the classification of "known" human carcinogen.

3.0 Toxicological Studies

We would agree with the Panel that the animal data do not provide sufficient evidence that formaldehyde is associated with leukemia in general or myeloid leukemia specifically. However, we disagree with the Panel's questioning of the appropriateness of the species of animal tested. The Panel implied that the species tested may not be the appropriate "animal model" to assess leukemia in general and myeloid leukemia in particular.

- **A review of all of the chemicals identified in the NTP 11th RoC (NTP 2009) identified 45 agents classified as "known" human carcinogens, with 38 of these having concordant animal data.**

Seven of these were identified as known human leukemogens: benzene, ionizing radiation, MeCCNu, 1,3-butadiene, chlorambucil, thiotepa, and cyclophosphamide. For all of these agents, the production of leukemia or other hematopoietic cancers (benzene and 1.3-butadiene) was reported in mice and/or rats, and, in the case of ionizing radiation, positive results for leukemia in dogs, monkeys and rabbits were also reported.

- **No evidence of an effect on hematological systems, e.g., pancytopenia, was noted in animal studies that evaluated such effects.**

No effects have been seen on hematological parameters measured in animal toxicology and carcinogenicity studies (Appleman *et al.* 1988; Dean *et al.* 1984; Johannsen *et al.* 1986; Kamata *et al.* 1997; Kearns *et al.* 1983a; Til *et al.* 1988, 1989; Tobe *et al.* 1989; Vargova *et al.* 1993; Woutersen *et al.* 1987). Among these studies, Vargova *et al.* (1993) reported increased red blood cell counts and increased proportions of lymphocytes and monocytes in rats exposed to formaldehyde by gavage at 80 mg/kg/day for 28 days.

- **The significance of the changes in blood counts reported by Zhang *et al.* (2010) is of questionable clinical relevance.**

Zhang *et al.* (2010) reported results on blood cell counts in formaldehyde-exposed workers, compared to counts in workers not exposed to formaldehyde. The authors collected individual exposure information for the exposed workers; however results in the paper appear to be in the form of "pooled" analyses, with wide error bars in the exposed workers. The authors indicate in the text that ***unadjusted summary measures*** are presented for all endpoints; therefore, it is unclear whether the results presented in tables and figures are adjusted for relevant covariates. Although the changes in the exposed workers were reported to be statistically significant, the values all appear to be well within the normal range of variability for the hematological parameters considered. Also, the authors did not attempt to demonstrate that the changes in blood cell counts within the group of exposed workers correlated with formaldehyde exposure; rather, only pooled results were provided.

In summary, the animal data provide sufficient evidence that formaldehyde exposure is not linked with the production of either leukemia or of non-neoplastic indicators of the potential for leukemia.

4.0 Mechanistic Data

Since the first epidemiology studies suggesting a possible association between formaldehyde exposure and leukemia were published, a major area of debate has been how mechanistically formaldehyde could cause a disease that develops distant from the point of contact. Formaldehyde is rapidly metabolized and highly reactive, and, because it is an endogenous compound, a detectable change in the natural

background levels would need to occur to result in the potential for adverse effects. Although, the Background Document and the Expert Panel Report cite hypotheses proposed by Zhang *et al.* (2010) regarding the theoretical development of leukemia following inhalation of formaldehyde, there is no documented evidence to support the applicability of these hypotheses. In fact, Zhang *et al.* (2010) note that their hypotheses related to mechanisms of leukemia clearly require additional testing. The existing mechanistic data for formaldehyde, which consider the toxicokinetics and genotoxicity of a compound, provide no evidence that exogenous formaldehyde will be transported from the point of contact to distant sites, but do provide evidence that formaldehyde does not affect the relevant target cells (bone marrow) for leukemia.

4.1 Toxicokinetic Data

- **Exogenous formaldehyde does not result in significant changes in normally present endogenous formaldehyde concentrations in the blood of humans, nonhuman primates and rats.**

Heck and colleagues (Heck *et al.* 1985; Casanova *et al.* 1988; Heck and Casanova 2004) have conducted studies in rats, monkeys, and humans to determine whether inhalation exposure will result in a significant increase in blood concentrations of formaldehyde, thereby suggesting transfer from the point of contact. The Background Document notes that endogenous concentrations of formaldehyde in human blood are about 2 to 3 µg/g of blood and are similar to concentrations measured in the blood of monkeys and rats (Casanova *et al.* 1988, Heck *et al.* 1985). In rats, following inhalation exposure to approximately 14 ppm formaldehyde for 2 hours, blood concentrations in exposed animals (2.25±0.07 µg/g; mean ± SE) were not significantly different than those in control animals (2.24±0.07 µg/g) (Heck *et al.* 1985). No significant difference in blood concentrations of formaldehyde were observed in monkeys at 7 minutes (1.84±0.15 µg/g) or 45 hours (2.24±0.07 µg/g) following exposure to 6 ppm formaldehyde for 6 hours/day, 5 days/week for 4 weeks In humans, average blood concentrations of formaldehyde prior to exposure to 1.9 ppm for 40 minutes (2.61±0.14 µg/g) were not significantly different from those measured immediately following exposure (2.77±0.28 µg/g) The results of these studies provide evidence that concentrations as high as 14 ppm have no significant impact on endogenous levels of formaldehyde in the blood of multiple species.

- **If the chemistry and biochemistry of formaldehyde are considered, it is highly unlikely that the small amounts of formaldehyde or formaldehyde conjugates resulting from exogenous exposure can impact endogenous levels.**

As noted by Dr. Melvin Andersen in a separate submission to NTP (Andersen 2010), whether in the extracellular spaces or within cells, free formaldehyde will be present at extremely low concentrations. Formaldehyde readily reacts reversibly with water to form an acetal (methanediol), which can further interact with glutathione (GSH) to form a thioacetal (S-hydroxymethylglutathione). At all times and in all tissues, there is a high concentration of both the acetal and thioacetal; however, mammalian cells have robust systems to tightly control the endogenous forms of formaldehyde. Therefore, the small amounts of these forms of formaldehyde (i.e., the methanediol and S-hydroxymethyglutathioine) that would be expected to move from the contact site to distant tissue following inhalation or oral exposure will have no appreciable influence on total levels of formaldehyde in distant tissues. Neither will they serve as a delivery mechanism for unreacted formaldehyde to these tissues.

4.2 Genotoxicity Data

- **Following inhalation exposure, DNA adducts resulting from exogenous formaldehyde were not detected in the bone marrow or in points distant from the point of contact.**

Recent work by Swenberg and colleagues (Lu *et al.* 2010a, b) evaluated the formation of DNA-adducts resulting from endogenous formaldehyde, versus those formed from exogenous formaldehyde in nasal tissues, liver, lung, thymus, spleen and bone marrow in rats following inhalation exposure to 10 ppm radiolabeled formaldehyde for 1 or 5 days. No exogenous formaldehyde-induced DNA adducts were detected in any distant tissue, while the DNA adducts from endogenous formaldehyde were present in all tissues examined in similar amounts. Lu *et al.* (2010a, b) concluded that the results do "not support the biological plausibility for the causation of leukemia". Other studies of formaldehyde-DNA adducts were in the blood and did not distinguish the contribution from exogenous versus endogenous formaldehyde.

- **DNA-protein crosslinks (DPX) were not detected in the bone marrow of rats or monkeys following inhalation exposure to formaldehyde.**

As noted in the Background Document, DPX formation is an important indicator of tissue and DNA exposure (Casanova-Schmitz *et al.* 1984a, Casanova *et al.* 1989, Casanova *et al.* 1994). Casanova-Schmitz *et al.* (1984b) studied formation of DPX and protein adducts in bone marrow of rats exposed via inhalation to [H^3]- and [^{14}C]-formaldehyde. Exposure was to concentrations of formaldehyde ranging from 0.3 to 15 ppm, with tissue samples collected from the nasal respiratory mucosa and the femoral marrow. Although DPX were noted in the nasal mucosa, the results indicated no delivery of radiolabelled formaldehyde to the bone marrow and no formation of DPX within the bone marrow. Similar results were observed in monkeys, with no DPX found in the bone marrow following exposure to concentrations of up to 6 ppm formaldehyde for 6 hours (Casanova *et al.* 1991).

Although DPX have been measured in blood and respiratory tissues in several studies, evidence indicates that these cross links do not persist for long periods of time, further limiting the possibility of transfer from tissue to tissue. Speit *et al.* (2000) demonstrated the complete removal of DPX from human cell lines within 24 hours, including both normal and repair-deficient cells. Similar results were reported in cultured human cells, with formaldehyde-induced DPX removed within a few hours (Fornace *et al.* 1982; Grafstrom *et al.* 1984). In the nasal respiratory tissue of rats, there was a lack of persistence of DPX following 12 weeks of exposure (Casanova *et al.* 1994).

- **Indicators of genotoxicity in tissues distal to the point of contact were not detected following inhalation exposure of formaldehyde.**

Exogenous formaldehyde did not produce significant increases in 1) DNA strand breaks, or chromosomal aberrations in bone marrow or olfactory mucosa, in rats following exposure to 15 ppm (Casanova-Schmitz *et al.* 1984; Dallas *et al.* 1992); 2) DNA cross-links in the maxillary sinuses of Rhesus monkeys exposed to 6 ppm (Casanova *et al.* 1991); or 3) sister chromatid exchange or other chromosomal aberrations in lymphocytes of rats exposed to 15 ppm (Kligerman *et al.* 1984).

- **Results reported by Pala *et al.* (2008) provide evidence of exposure to formaldehyde, but no evidence of effect.**

The Panel incorrectly considered the only direct evidence of transfer of formaldehyde from the nasal and pharyngeal passages to the blood to be the study by Pala *et al.* (2008). Pala *et al.* (2008) evaluated potential relationships between formaldehyde exposure in the workplace and biomarkers of exposure

(formaldehyde human serum albumin conjugate) and biomarkers of effect (chromosomal aberrations, sister chromatid exchanges, and micronucleated cells) in 36 workers exposed to formaldehyde in a cancer research facility. A statistically significant relationship between formaldehyde exposure, measured with personal samplers, and biomarkers of exposure was noted. However, the authors reported no statistically significant relationship between formaldehyde exposure and biomarkers of effect.

- **Presence and /or frequency of chromosomal aberrations in the peripheral blood are not a validated marker of specific types of cancer.**

The Panel relied heavily on the observation of chromosomal aberrations in the peripheral blood as evidence of genotoxic effects that are relevant to the mechanism of myeloid leukemia. Bonassi *et al.* (2008), which includes the genetic screening in 22,358 ***cancer-free*** individuals with follow-up for an average of 10 years, is cited to suggest that chromosomal aberrations are the only validated biomarker of human cancer. However, the only cancer site significantly associated with the frequency of chromosomal aberrations was stomach cancer (Bonassi *et al.* 2008). In particular, no significant association between cancers of the lymphohematopoietic system and the frequency of chromosomal aberrations was reported by Bonassi *et al.* (2008).

- **In the Zhang *et al.* (2010) study, no statistically significant changes in formaldehyde-exposed workers compared to workers not exposed to formaldehyde were found for the biological marker of effect on stem cells, i.e., the number of granulocyte-macrophage colony-forming units (CFU-GM).**

According to Zhang *et al.* (2010), human leukemogens lower the ability of progenitor cells to replicate in colony-forming cell culture assays. They further state that, "If formaldehyde were a human leukemogen, one would expect to see a lowering of peripheral blood counts in exposed workers and an effect on the ability of progenitor cells to form CFU-GM." However, the colony formation of CFU-GM hematopoietic progenitor cells was not statistically different between formaldehyde-exposed workers and workers not exposed to formaldehyde in the study (Figure 2 in Zhang *et al.* 2010). In addition, no conclusions can be drawn from the *in vitro* data reported (Figure 3 in Zhang *et al.* 2010) because the myeloid progenitor cells were from only one individual and formaldehyde was added directly to the culture medium, thereby, by-passing all normal metabolic and kinetic processes that prevent formaldehyde from reaching the target tissue, the bone marrow.

- **The clinical relevance of the changes in the levels of monosomy of chromosome 7 and trisomy of chromosome 8 reported by Zhang *et al.* (2010) is unclear.**

Zhang *et al.* (2010) recently reported an increase in the incidence of monosomy 7 and trisomy 8 in colonies of cultured granulocyte-macrophage colony-forming units from 10 "highly" (undefined) exposed healthy workers in two factories that produced formaldehyde-melamine resins. However, these changes are not predictive of the development of myeloid leukemia and are not commonly observed in individuals diagnosed with either acute (AML) or chronic myeloid leukemia (CML). In 200 patients with AML, only a limited number of patients had monosomy 7 (2.2%) and trisomy 8 (5.6%) (Ahmad *et al.* 2008). In addition, in 122 AML patients in China, monosomy 7 was not reported, and only approximately 3% had trisomy 8 (Zheng *et al.* 2007). For chronic myeloid leukemia (CML), translocation of chromosomes 9 and 22 of the Philadelphia chromosome is the chromosomal change most commonly found (Bonassi *et al.* 2008). The authors indicate in the text that ***unadjusted summary measures*** are presented for all endpoints; therefore, it is unclear whether the results presented in tables and figures are adjusted for relevant covariates. While the small cohort was said to have been matched by age, only 10 exposed and 12 nonexposed individuals were included in this analysis and the results reported were not adjusted for age of these subject. Because these chromosomal changes reported by Zhang *et al.* (2010) are not

commonly observed in myeloid leukemia patients, and because this is the first study to report such findings in a small number (n=10) of formaldehyde-exposed workers, this study must be replicated with larger numbers of individuals, and in combination with documented exposure to formaldehyde. It is our understanding that Zhang and colleagues have secured the funding to conduct their investigation with a larger number of workers.

In summary, insufficient evidence is available to support a potential mechanism for the development of myeloid leukemia following inhalation exposure to formaldehyde. The toxicokinetic data indicate that any transport of formaldehyde from the point of contact will not affect endogenous levels in the peripheral blood, and no *in vivo* evidence is available that formaldehyde can be transported to and affect the bone marrow. While chromosomal aberrations have been reported in *in vivo* studies with peripheral blood cells of formaldehyde-exposed individuals, these findings do not support a causal association between formaldehyde and myeloid leukemia.

5.0 Lack of Overall Strength of the Evidence Regarding an Association with Myeloid Leukemia

In evaluating the available epidemiological, toxicological, and mechanistic data for formaldehyde, the strength of the evidence does not justify classifying formaldehyde as a "known" human carcinogen based on myeloid leukemia. Overall, the epidemiological data reviewed by the Panel do not show an association between formaldehyde exposure and myeloid leukemia . At best, one study reports a weak association. Moreover, the toxicological data in animals provide further evidence that formaldehyde would not be expected to cause myeloid leukemia, as the available animal data are negative. While the Panel questioned the potential appropriateness of the rat as an animal model, numerous chronic studies in the rat provide no evidence of changes in blood cell count, an endpoint that would be anticipated if effects on the bone marrow were occurring following formaldehyde exposure. Further, according to the NTP 11th annual RoC, all "known" carcinogens (e.g., benzene, 1,3-butadiene) that cause leukemia in humans, also cause leukemia or other hematopoietic cancers in rats and/or mice. The mechanistic data support no significant transfer to or effects of exogenous formaldehyde in tissues distant from the point of contact, and there remain significant uncertainties in a mechanism of action for leukemogenic effects. Therefore, the strength of the evidence for formaldehyde as a potential causal agent for human myeloid leukemia neither supports a "known" classification, nor rises to the level of "reasonably anticipated", based on NTP's classification requirements in the Background Document for Formaldehyde.

6.0 Recommendations to NTP Regarding Further Evaluation of, and RoC Decisions Concerning, Formaldehyde.

We recommend that the NTP, including the Expert Panel and the Board of Scientific Counselors, apply a strength-of-evidence approach discussed above in separately evaluating all human, animal and mechanistic data relevant to each of the three identified tumor types -- i.e. nasopharyngeal carcinoma, sinonasal adenocarcinoma, and myeloid leukemia. The scientific evidence for these three cancers varies widely and warrant independent reviews and findings. Moreover, although the NTP may generally classify formaldehyde based upon a single tumor type, we recommend that the NTP both apply the RoC classification criteria to each separate tumor type, and justify the listing classification with respect to each such endpoint. Thus, even if NTP were to conclude that formaldehyde is a known human carcinogen with respect to one (but not a second or third) endpoint, the NTP findings should clearly delineate both the scientific bases for, and the NTP RoC classification concerning, each separate cancer type. This is particularly important because, as our comments clearly delineate, the strength of the evidence for formaldehyde does not rise to the level necessary to qualify, according to the RoC classification scheme, formaldehyde as either a known or reasonably anticipated human myeloid leukemogen, when a comprehensive strength-of-evidence evaluation is performed on the full range of existing scientific data.

Consistent with these recommendations, we request the NTP to reconvene the Expert Panel and direct it to (1) carry out and document a separate strength-of evidence evaluation for each of the three cancers, and (2) consider and determine the applicability of the RoC classification criteria on a cancer-specific basis. We recommend that these further Expert Panel evaluations and classification decisions be spelled out in supporting documentation that addresses all available human, animal and mechanistic data for each cancer endpoint. We make this request because the current Expert Panel Report might be read to conclude that formaldehyde is a known human myeloid leukemogen, when the strength of the evidence does not support such a classification. This also will be very important in the examination of the Expert Panel's findings during the future peer review by the NTP's Board of Scientific Counselors.

REFERENCES

Ahmad F, Dalvi R, Das BR, Mandava S. 2008. Specific chromosomal aberrations in de novo acute myeloid leukemia: a comparative analysis of results with a report of three novel chromosomal rearrangements t(7;14)(q35;q13), t(8;18)(p11.2;q12), t(13;15) in Indian population. *Cancer Detect Prev* 32(2): 168-177.

Andersen, M. 2010. Letter submission to Dr. Ruth Lunn, Re: Comments on the Recommendation from the Expert Panel Report (Part B) on Formaldehyde, 74 Fed. Reg. 67,883 (December 21, 2009). Dated February 8, 2010.

Appelman LM, Woutersen RA, Zwart A, Falke HE, Feron VJ. 1988. One-year inhalation toxicity study of formaldehyde in male rats with a damaged or undamaged nasal mucosa. *J Appl Toxicol* 8(2): 85-90.

Bachand AM, Mundt KA, Mundt DJ, Montogomery RR. (2010). Epidemiological studies of formaldehyde exposure and risk of leukemia and nasopharyngeal cancer: A meta-analysis. *Crit Rev Toxicol* 40(2): 85-100.

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(10): 751-61.

Bonassi S, Norppa H, Ceppi M, Stromberg U, Vermeulen R, Znaor A, Cebulska-Wasilewska A, Fabianova E, Fucic A, Gundy S, Hansteen IL, Knudsen LE, Lazutka J, Rossner P, Sram, RJ, Boffetta P. 2008. Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. *Carcinogenesis* 29(6): 1178-1183.

Casanova-Schmitz M, Starr TB, Heck HD. 1984a. Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [14C]- and [3H]formaldehyde. *Toxicol Appl Pharmacol* 76(1): 26-44.

Casanova-Schmitz M, David RM, Heck HD. 1984b. Oxidation of formaldehyde and acetaldehyde by NAD+-dependent dehydrogenases in rat nasal mucosal homogenates. *Biochem Pharmacol* 33(7): 1137-1142.

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(4): 525-536.

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(2): 409-428.

Casanova M, Deyo DF, Heck HD. 1989. Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: analysis of formaldehyde and DNA by high-performance liquid chromatography and provisional pharmacokinetic interpretation. *Fundam Appl Toxicol* 12(3): 397-417.

Casanova M, Heck HD, Everitt JI, Harrington WW, Jr., Popp JA. 1988. Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. *Food Chem Toxicol* 26(8): 715-716.

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(21): 1608-1615.

Dean JH, Lauer LD, House RV, Murray MJ, Stillman WS, Irons RD, Steinhagen WH, Phelps MC, Adams DO. 1984. Studies of immune function and host resistance B6C3CLF1 mice exposed to formaldehyde. *Toxicol Appl Pharmacol* 72(3): 519-529.

Fornace AJ, Jr., Lechner JF, Grafstrom RC, Harris CC. 1982. DNA repair in human bronchial epithelial cells. *Carcinogenesis* 3(12): 1373-1377.

Grafström RC, Fornace A, Jr., Harris CC. 1984. Repair of DNA damage caused by formaldehyde in human cells. *Cancer Res* 44(10): 4323-4327.

Hauptmann M, Stewart PA, Lubin JH, Beane Freeman LE, Hornung RW, Herrick RF, Hoover RN, Fraumeni JF, Bair A, Hayes RB. 2009. Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. *J Natl Cancer Inst* 101(24): 1696-1708.

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(2): 92-106.

Heck HD, Casanova-Schmitz M, Dodd PB, Schachter EN, Witek TJ, Tosun T. 1985. Formaldehyde (CH2O) concentrations in the blood of humans and Fischer-344 rats exposed to CH2O under controlled conditions. *Am Ind Hyg Assoc J* 46(1): 1-3.

Johannsen FR, Levinskas GJ, Tegeris AS. 1986. Effects of formaldehyde in the rat and dog following oral exposure. *Toxicol Lett* 30(1): 1-6.

Kamata E, Nakadate M, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M, Kurokawa Y. 1997. Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. *J Toxicol Sci* 22(3): 239-54.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA. 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res* 43(9): 4382-92.

Lu K, Collins LB, Ru H, Bermudez E, Swenberg JA. 2010a. Distribution and Molecular Dose of Inhalation-derived and Endogenous Formaldehyde DNA Adducts Support Causation of Nasal Carcinoma, but not Leukemia. Need citation

Lu K, Collins LB, ru HY, Bermudez E, Swenberg JA. 2010b. DNA adducts caused by inhaled formaldehyde are found in nasal epithelium, but not bone marrow. Submitted to *Toxicological Sciences*.

National Toxicology Program (NTP). 2010. Report on Carcinogens Background Document on Formaldehyde. U.S. Department of Health and Human Services, Research Triangle Park, NC. http://ntp.niehs.nih.gov/ntp/roc/twelfth/2009/November/Formaldehyde_BD_Final.pdf.

Natz, B. 2010. Letter submission to Dr. Ruth Lunn, Re: Comments on the Recommendation from the Expert Panel Report (Part B) on Formaldehyde, 74 Fed. Reg. 67,883 (December 21, 2009). Dated February 8, 2010.

Pala M, Ugolini D, Ceppi M, Rizzo F, Maiorana L, Bolognesi C, Schiliro T, Gilli G, Bigatti P, Bono R, Vecchio D. 2008. Occupational exposure to formaldehyde and biological monitoring of Research Institute workers. *Cancer Detect Prev* 32(2): 121-6.

Pinkerton LE, Hein MJ, Stayner LT. 2004. Mortality among a cohort of garment workers exposed to formaldehyde: an update. *Occup Environ Med* 61(3): 193-200.

Smith MT, Zhang L, Jena M, Wang Y, Guo W, Duramad P, Hubbard AE, Hofstadler G, Holland NT. 2000. Hydroquinone, a benzene metabolite, increases the level of aneusomy of chromosomes 7 and 8 in human CD34-positive blood progenitor cells. *Carcinogenesis* 2198): 1485-1490.

Speit G, Schütz P, Merk O. 2000. Induction and repair of formaldehyde-induced DNA-protein crosslinks in repair-deficient human cell lines. *Mutagenesis* 15(1): 85-90.

Til HP, Woutersen RA, Feron VJ, Hollanders VH, Falke HE, Clary JJ. 1989. Two-year drinking-water study of formaldehyde in rats. *Food Chem Toxicol* 27(2): 77-87.

Til HP, Woutersen RA, Feron VJ, Clary JJ. 1988. Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking-water study in rats. *Food Chem Toxicol* 26(5):447-452.

Tobe M, Naito K, Kurokawa Y. 1989. Chronic toxicity study on formaldehyde administered orally to rats. *Toxicology* 56(1): 79-86.

Vargová 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(3): 255-75.

Wolfe MS. 2009. Peer review of draft substances profiles for the 12th report on carcinogens. NTP Board of Scientific Counselors Meeting. Research Triangle Park, NC. Available at http://ntp.niehs.nih.gov/files/Wolfe20090224.pdf. Accessed in January 2010.

Woutersen RA, Appelman LM, Wilmer JW, Falke HE, Feron VJ. 1987. Subchronic (13-week) inhalation toxicity study of formaldehyde in rats. *J Appl Toxicol* 7(1): 43-9.

Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, Qiu C, Guo W, Liu S, Reiss B, Freeman LB, Ge Y, Hubbard AE, Hua M, Bair A, Galvan N, Ruan X, Alter BP, Xin KX, Li S, Moore LE, Kim S, Xie Y, Hayes RB, Azuma M, Hauptmann M, Xiong J, Stewart P, Li L, Rappaport SM, Huang H, Fraumeni JF Jr., Smith MT, Lan Q. 2010. Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. *Cancer Epidemiol Biomarkers Prev* 19(1): 80-88.

Zheng J, Wang X, Hu Y, Yang J, Liu J, He Y, Gong Q, Yao J, Li X, Du W, Huang S. 2007. A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China . *Cytometry B Clin Cytom* 74(1): 25-29.