II. Highlights of the Review

ILMA highlights the following comments contained in the Review:

- Not all metal removal fluids cause symptoms associated with respiratory irritation and some occupational respiratory effects are likely caused by microbiological decay products often found in in-use fluids, but not in fresh fluids.

  On page 25, please change the second line of the main paragraph so that the text becomes "compelling evidence that occupational exposure to some metalworking fluid aerosols is associated with..." Otherwise the sentence implies that *all* MWF have similar *causative* effects. Also, it would be helpful to add at an appropriate point that a MWF might contain unintended substances, such as bacterial decay products, in addition to the components used to blend the MWF. These unintended substances are in addition to the bacteria and fungi that might also be found and which are mentioned further in the first comments under "Introduction" in the "Other Comments." In other words, a *new* MWF is not necessarily the same as a *used* MWF.

- There are large differences between laboratory exposures and occupational exposures; moreover, characterization of just what the laboratory animals were exposed to is unclear.

  The choices of concentrations in the two-year exposures seem reasonable based on the range-finding data. However, it is important to note that these aerosol concentrations are significantly higher than occupational exposures. Based on work done by NIOSH, 80% of the mist levels in small shops in the 1990's were 0.5 mg/m^3 or less (Piacitelli, G., et al. Metalworking fluid exposures in small machine shops, Am. Ind. Hyg. Assoc. J., 62, 356–370, 2001). A separate in-plant metalworking fluid mist study by CIMCOOL (A. Ball.A survey of metalworking fluid mist in manufacturing plants.*Lubrication Engineering* Sept., 1997) showed that the mean aerosol value was 0.85 mg/m^3 and almost all were less than 2 mg/m^3. Since these studies were done, most plants now enclose and vent almost all machines and operations such that aerosol levels are even further reduced. NTP exposed the animal to concentration of 10, 30 and 100 $mg/m^3$of CIMSTAR® 3800 concentrate. Therefore, the exposure levels utilized in this NTP study were from 100 to 2000 times greater than levels expected during occupational use. We feel that the differences between experimental doses and those encountered during typical occupational use should be mentioned in the Introduction and/or the Discussion and Conclusions.

  What was the composition of the aerosol? What was the composition of any measured vapor phase? How closely did these data agree with the composition of the starting MWF? These are basic questions for which data should exist. More specifically, methanol, ethanolamine, and 1-amino-2-propanol apparently had appreciable vapor phases (page H-6). Was vapor phase measured in addition to the aerosol phase? These components represent ~7.3% of the original MWF, which also had 60% water. It is reasonable to assume that the water vaporized during the generation of the aerosolized MWF. Perhaps, NTP can verify that

assumption. In addition, other volatile components likely partially (or completely) vaporized. It would help if NTP would further explain this in "Chamber Atmosphere Characterization" and again in "Discussion and Conclusions."

- Support for characterization of observed results of squamous cell papilloma and keratoacanthoma (combined) as *equivocal evidence of carcinogenic activity* is weak for female Wistar Han rats, particularly when an increase in skin tumors in male rats was not observed.

  A positive, though not statistically significant, trend in the incidences of squamous cell papilloma or keratoacanthoma (combined) of the skin of female rats was noted on page 65. These tumors were considered possibly related to deposition of the MWF on the skin (page 96). NTP stated (also on page 96) that it was unclear if this is a treatment-related effect because of the lack of statistical significance and the lack of historical control data from inhalation studies in the Wistar Han rat. NTP concluded on page 98 that there was *equivocal evidence of carcinogenic activity* in female Wistar Han rats based on the incidences of squamous cell papilloma and keratoacanthoma (combined) of the skin. It is unclear how results that are neither statistically significant nor dose related can be considered evidence, equivocal or otherwise, of carcinogenic activity.

  The conclusion of equivocal evidence is further compromised because an increase in skin tumors was not observed in male rats (page A-4). In other words, one might expect a similar trend in males because a lack of a sex difference seems reasonable. In fact, the control group in males had one (1) basal cell carcinoma and one (1) keratoacanthoma compared to none in the female controls. As a consequence, it was not entirely clear why the results in female rats were considered equivocal evidence of carcinogenicity rather than a random incidence of tumors. A discussion of the uncertainty associated with these data on tumor incidence would be very helpful.

- While the non-statistically significant incidence of prostate tumors in male rats at 100 mg/m^3 was slightly higher than controls, NTP concluded on page 98 that there was *equivocal evidence of carcinogenic activity* in male Wistar Han rats based on the incidences of prostate gland adenoma or carcinoma (combined). The criteria used to define equivocal evidence of carcinogenic activity relative to statistically non-significant findings would be useful.

  The incidence of prostate tumors in male rats at 100 mg/m^3 was slightly higher than controls (6% vs 2%, not statistically significant) and higher than historical controls (page 61). NTP concluded on page 98 that there was *equivocal evidence of carcinogenic activity* in male Wistar Han rats based on the incidences of prostate gland adenoma or carcinoma (combined). A discussion of the criteria used to define equivocal evidence of carcinogenicity would be helpful to the reader.

- Support for characterization of observed results of brain tumors in male rats as *equivocal evidence of carcinogenic activity* is marginal since the higher incidence at 10 mg/m^3 was not statistically significant, not dose-related, close to the

incidence in female controls, and very close to historical controls. [1]. The incidence for males and females combined was at the upper end of historical controls. Further discussion would be useful.

Data on brain tumors in male rats are presented on pages 62-63 and in the table below. In the Discussion (page 95), NTP states that "the incidences of these tumors were not statistically significant or exposure concentration related; however, granular cell tumors of the brain are rare in rats" and the combined incidence of benign and malignant tumors in males at 10 mg/m^3 (6%) exceeded the historical control range of 0 to 4% in rats.

Although not mentioned on pages 62-63, the incidence of granular cell tumors in the brains of female rats was also 6% at 100 mg/m^3. (See following table.) However, the potential noteworthiness of these tumors in females apparently was lessened by 4% benign tumors in female controls. NTP concluded on page 98 that *equivocal evidence of carcinogenic activity* in male Wistar Han rats occurred based on the incidences of benign or malignant granular cell tumors (combined) of the brain. A discussion of the combined data for males and females in the Results and/or Discussion would be useful.

No. of rats with brain tumors	Historical Controls	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Males (page 63)					
Benign granular cell tumor	3/150	0	2	0	1
Malignant granular cell tumor	0/150	0	1	1	0
Benign and malignant combined	0 – 4%	0	3	1	1
Females					
Benign granular cell tumor		2	1	0	1
Malignant granular cell tumor		0	0	0	2
Oligodendroglioma		0	0	0	1
M & F benign & malignant granular cell tumors	0 – 4%	2%	4%	1%	4%

- While NTP concludes on page 98 that there was *some evidence of carcinogenic activity* in female B6C3F1/N mice based on the incidences of alveolar/bronchial adenoma or carcinoma (combined) of the lung and *no evidence of carcinogenic activity* in male B6C3F1/N mice, clarification of "overall rate" given for alveolar/bronchiolar carcinoma in female mice and of how adenomas and carcinomas were counted is needed.

The following table is a summary of lung tumors in both sexes of mice. NTP discussed these data on pages 93-94 and concluded on page 98 that there was *some evidence of carcinogenic activity* in female B6C3F1/N mice based on the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) of the lung and *no evidence of carcinogenic activity* in male B6C3F1/N mice exposed to 10, 30, or 100 mg/m^3.

	Historical inhalation	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Females					
Alveolar/bronchiolar adenoma (D-4)		1	3	2	4
Alveolar/bronchiolar adenoma (multiple, D-4)		0	1	0	0
Alveolar/bronchiolar adenoma (total, p. D-6)	2 – 12%	1	4 (P=0.181)	2 (P=0.506)	4 (P=0.168)
Alveolar/bronchiolar carcinoma (D-4)	0 – 10%	4	1 (P=0.176N)	4 (P=0.627N)	8 (P=0.163)
Overall alveolar/bronchiolar adenoma/carcinoma (p. D-6)	2 – 16%	4	5 (P=0.505)	6 (P=0.391)	12 (**P=0.021**)
Males					
Alveolar/bronchiolar adenoma	8 – 20%	5	2	8	9
Alveolar/bronchiolar carcinoma	16 – 24%	8	9	8	10
Alveolar/bronchiolar adenoma/carcinoma	26 – 40%	13	9 (P=0.288N)	14 (P=0.476)	17 (P=0.270)

However, it is not clear in Table 18 why the overall rate for alveolar/bronchiolar adenoma/carcinoma in the control group of female mice is given as 4/50 while there was 1 animal with an adenoma and 5 with carcinomas. Did one individual have both an adenoma and a carcinoma and, if so, was the total number reduced to show only the number of tumor-bearing individuals regardless of how many tumors any given individual might have? The definition of "overall rate" given for alveolar/bronchiolar carcinoma in female mice on pages 84-85 was the "number of animals with neoplasm per number of animals with lung examined microscopically". Should that be "number of animals with one or more neoplasm per…"? If this procedure was used, was it applied to both control and treated groups? Was consideration given to the "multiplicity in site-specific neoplasia"

(page 14) to both controls and treated groups equally? Clarification of these matters would be helpful.

- CIMSTAR® 3800 contains a formaldehyde-release biocide and that may be responsible for increases (but not doubling) in *E. coli*. We suggest that NTP revise the Report where appropriate to reflect this fact.

  Two oxazolidine compounds were tentatively identified in CIMSTAR® 3800 during the chemical analyses presented in the NTP report, but NTP states on pages 93 and 97 that there was no biocide in CIMSTAR® 3800. It should have been noted in the report that derivatives of 5-methyloxazolidine are often used as biocides in MWFs. The MSDS for CIMSTAR® 3800, dated 10-8-2012, indicates that a biocide, namely hexahydro-1,3,5-tris(2-hydroxyethyl)-S-triazine (CAS No. 4719-04-4), was in fact in the MWF at a concentration of 1-5%. This biocide has been commonly used in MWFs. A test of this formaldehyde condensate biocide in a micronucleus assay in rats at doses up to 410 mg/kg by three routes was negative (Urwin et al., 1976).

  It is stated on page 97 of the NTP report that "Three types of metalworking fluids (two soluble oils and one semisynthetic) did demonstrate clear mutagenicity in the E. coli strain in the presence of S9 mix, and all three contained biocides that release formaldehyde, a known bacterial mutagen." We suggest that NTP revise its report on this page and elsewhere to reflect the presence of the formaldehyde condensate biocide in CIMSTAR 3800.

  Urwin C, Richardson JC, Palmer AK. An evaluation of the mutagenicity of the cutting oil preservative Grotan BK. Mutat Res. 40(1):43-6, 1976.

H. The report states that CIMSTAR® 3800 was mutagenic in *E. coli*, but the data were less conclusive.

  Regarding genetic toxicology, the data on mutagenicity in Table E1 are not as definite as the summary of genetic toxicity on page 88 implies. At doses up to 10 mg/plate (twice the recommended maximum dose in Health Effects Test Guidelines, OPPTS 870.5100, Bacterial Reverse Mutation Test), a doubling of the number of revertants was not seen in either of the apparent duplicate tests in *E. coli*. The conclusion in the table was that the test material was "weakly positive" even at these doses. As stated on page E-2, there "is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background." Because that benchmark was not reached here, please reconsider the decision to call the results weakly positive or provide a clear rationale for retaining that categorization in light of the test guidelines.

The Review contains other important comments and observations, and ILMA requests that NTP consider all of them as it finalizes its draft Report on CIMSTAR® 3800.

ILMA appreciates the opportunity to provide these comments to NTP and, if there are any questions related to interpretation of our comments, we would be pleased to engage in further dialog before the draft Report is finalized.

Sincerely,

Celeste Powers

Celeste M. Powers, CAE
Executive Director

cc: ILMA Board of Directors
ILMA Metalworking Fluids Committee
Dr. John Howell, Ph.D.
Jeffrey L. Leiter, Esq.

Review of
NTP Technical Report on the Toxicology Studies of CIMSTAR 3800 in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of CIMSTAR 3800 in Wistar HAN [Crl:WI (Han)] Rats and B6C3F1/N Mice (Inhalation Studies)

Performed by DalbeyTox, LLC

Walden Dalbey. MA, PhD, DABT

May 5, 2014

The following pages contain comments made by DalbeyTox, LLC during a review of the NTP report on toxicology and carcinogenicity studies with CIMSTAR® 3800, a metalworking fluid (MWF). DalbeyTox, LLC performed this work under an agreement with the Independent Lubricant Manufacturers Association (ILMA). The comments are arranged in two groups: (1) comments provided to improve interpretation of the report and (2) other comments, many of which are related to technical aspects of NTP's report. The comments within these two groups follow the sections of NTP's report.

Comments Provided to Improve Interpretation of the Report

Introduction

1) On page 21, the MWF referred to as a "high production compound". The MWF is a mixture and not a compound. Please correct this wording; it is not an insignificant point. As a more minor point, CIMSTAR 3800 was not part of the US EPA's High Production Volume program, as this wording seems to imply. Can "high production" be changed to other wording?

2) On page 25, please change the second line of the main paragraph so that the text becomes "compelling evidence that occupational exposure to some metalworking fluid aerosols is associated with...". Otherwise the sentence implies that all MWF have similar causative effects. Also, it would be helpful to add at an appropriate point that MWF might contain unintended substances, such as bacterial decay products, in addition to the components used to blend the MWF. These unintended substances are in addition to the bacteria and fungi that might also be found and which are mentioned further in the first comments under "Introduction" in the "Other Comments". In other words, a new MWF is not necessarily the same as a used MWF.

3) In the discussion of carcinogenicity of TEA and DEA on page 27, it would be helpful to mention whether liver tumors with TEA or DEA were considered to be due to an epigenetic mechanism. DEA is structurally similar to ethanolamine and choline and studies have demonstrated that DEA treatment caused a "spectrum of biochemical changes consistent with choline deficiency in mice and demonstrate a clear dose concordance between DEA-induced choline deficiency and hepatocarcinogenic outcome" (Lehman-McKeeman et al, 2002). The authors also concluded that the "hepatocarcinogenic effects of DEA in mice are not predictive of similar susceptibility in other laboratory animals or humans." Newberne (2002) commented on these results by saying that "the induction of hepatocellular carcinomas in mice, associated with exposure to DEA, was likely a result of disruption of hepatocellular choline homeostasis." Please note that DEA is not part of the CIMSTAR 3800 formulation.

   Similar work was performed with TEA in B6C3F1 mice with the conclusion that "TEA might cause liver tumors in mice via a choline-depletion mode of action and that this effect is likely caused by inhibition of choline uptake be cells" (Stott et al, 2004). The authors also stated that "this nongenotoxic mode of tumorigenesis displays thresholds and differences in interspecies sensitivity, with higher primates being much more resistant than rodent species."

L.D. Lehman-McKeeman, et al. Diethanolamine induces hepatic choline deficiency in mice. Toxicol. Sci. 67:38-45. 2002.

P.M. Newberne. Choline deficiency associated with Diethanolamine carcinogenicity. Toxicol. Sci. 67:1-3. 2002.
W.T. Stott, et al. Evaluation of the potential of triethanolamine to alter hepatic choline levels in female B6C3F1 mice. Toxicol. Sci. 79:242-247. 2004.

Materials and Methods

1) A concise summary of the components analyzed in the starting MWF was not found in the report. The following table was derived from the text of the report in order to understand the composition of the MWF more clearly. Is this table correct? If not, the text needs to be modified to make the composition more clear. A table such as the one below would be helpful in this regard.

Percent of specific components in CIMSTAR 3800 in draft NTP report			
Component	**60224BBN**	**71205BN**	**90317JN**
Water	60	60	60
Methanol	0.3	0.3	0.35
Ethanolamine	4.7	5.7	5.6
Triethanolamine	3.4	3.3	3.2
1-amino-2-propanol	1.8	1.4	1.6
5-methyloxazolidine (tentative)			
Oxazolidine compound (tentative)			
Methyl palmitate	0.24	0.21	0.20
Methyl stearate	0.71	0.77	0.78
Methyl oleate	2.6	2.26	2.66
Methyl linoleate	1.68	1.54	1.77
Hexane-extractable material	26	25	26
Total	101.43	100.48	102.16

2) At the bottom of page 31, the text states that "Mineral oils (fatty acid methyl esters) was assessed using GC/FID." Methyl esters of fatty acids are separate from and unrelated to mineral oils. The methyl esters are esters of tall oil fatty acids. These methyl esters and mineral oil are likely to be soluble in hexane along with other non-polar compounds. However, the interpretation of data from the hexane extract is not clearly stated in the report. Does the total hexane-extractable material represent the remainder of the MWF that is not identified in the report as specific compounds, as was assumed (based on information in the report) in the table shown above?

Identification of compounds in the hexane extract is important since it represents ~63% of the non-aqueous portion of the MWF. Was the hexane extract analyzed and, if so, what was found? Note that the supplier of CIMSTAR 3800 stated on the MSDS (dated 10-8-2012) that CIMSTAR 3800 contained 1-5% hydrotreated heavy naphthenic petroleum distillate, a lubricating oil basestock (mineral oil). Overall, a more definitive and understandable presentation of the analytical data in the report would help in interpretation of the results.

3) Given that boron was the basis for monitoring the aerosol of MWF, in what form was the boron present? Boron should be accounted for in a listing of components in the MWF.

4) The description of aerosol generation on pages 32-33 indicates that undiluted MWF was used to generate the aerosol (i.e., it was not diluted to its normal use concentration before it was aerosolized). While the rationale behind the use of this methodology is more clearly stated on pages 96-97 where it is indicated that the aerosol concentrations used in the testing likely exceed levels encountered by those working with the more typically diluted MWF, the difference between laboratory exposures (10, 30 or 100 mg/m^3) and occupational exposures (generally, around 0.5 mg/m^3) should also be explained in this section of the report. It would also be useful to explain any potential effects on particle size more fully in pp 96-97.

5) How was mixing achieved with the aerosol stream and the dilution air? No mixing device is shown in Figure H2. This is a significant point given the lack of information on mixing prior to entry into the chambers. The uniformity of aerosol concentration in the inhalation exposure chambers with animals present was mentioned on page H-5. However, it is not clear what methods were used, whether the measurements were conducted in all chambers (all aerosol concentrations), or what the criteria for acceptability were.

6) The method for monitoring the aerosol was changed after July 24, 2008 to a gravimetric procedure for unstated reasons. The steps and rationale in that gravimetric procedure are not clear. More specifically, some of the components in the MWF would be soluble in hexane and others would not be. Because a basic step in the gravimetric analysis is extraction with n-hexane (3x), the procedures for quantification of the aerosol need to be specified. It was not clear if the extract, the unextracted residue, or both were weighed. Clarification is needed in order to relate the gravimetric data to the reported composition of the aerosol.

7) What was the composition of the aerosol? What was the composition of any measured vapor phase? How closely did these data agree with the composition of the starting MWF? These are basic questions for which data should exist. More specifically, methanol, ethanolamine, and 1-amino-2-propanol apparently had appreciable vapor phases (page H-6). Was vapor phase measured in addition to the aerosol phase? These components represent ~7.3% of the original MWF, which also had 60% water. It is reasonable to assume that the water vaporized during the generation of the aerosolized MWF; perhaps NTP can verify that assumption. In addition, other volatile components also likely partially (or completely) vaporized; it would help if NTP would further explain this in "Chamber Atmosphere Characterization" and again in "Discussion and Conclusions."

8) The descriptions in the two paragraphs on page H-6 starting with "Samples were collected" and "Methanol concentrations were" are not clearly presented. Was vapor phase actually measured routinely in the chambers and the distribution lines? What percent of the MWF was in aerosol phase and what percent in vapor phase? Do the conclusions presented with respect to analyzed concentrations relative to the bulk concentrations account for the mixed distributions between aerosol and vapor phases?

9) How often was the MWF changed in the generators during the course of the study? Was there any indication of bacterial or fungal growth? Were any measurements of bacterial endotoxin performed

on the MWF during the study? It would be very helpful to address these questions in the report in order to present an indication of the condition of the MWF over the course of the studies.

Results

1) Data on brain tumors in male rats are presented on pages 62-63 and in the table below. In the Discussion (page 95), NTP states that "the incidences of these tumors were not statistically significant or exposure concentration related; however, granular cell tumors of the brain are rare in rats" and the combined incidence of benign and malignant tumors in males at 10 mg/m3 (6%) exceeded the historical control range of 0 to 4% in rats.

Although not mentioned on pages 62-63, the incidence of granular cell tumors in the brains of female rats was also 6% at 100 mg/m^3. (See following table.) However, the potential noteworthiness of these tumors in females apparently was lessened by 4% benign tumors in female controls. NTP concluded on page 98 that *equivocal evidence of carcinogenic activity* in male Wistar Han rats occurred based on the incidences of benign or malignant granular cell tumors (combined) of the brain. A discussion of the combined data for males and females in the Results and/or Discussion would be useful.

No. of rats with brain tumors	Historical Controls	0 mg/m3	10 mg/m3	30 mg/m3	100 mg/m3
Males (page 63)					
Benign granular cell tumor	3/150	0	2	0	1
Malignant granular cell tumor	0/150	0	1	1	0
Benign and malignant combined	0 – 4%	0	3	1	1
Females					
Benign granular cell tumor		2	1	0	1
Malignant granular cell tumor		0	0	0	2
Oligodendroglioma		0	0	0	1
M & F benign & malignant granular cell tumors	0 – 4%	2%	4%	1%	4%

2) A positive, though not statistically significant, trend in the incidences of squamous cell papilloma or keratoacanthoma (combined) of the skin of female rats was noted on page 65. These tumors were considered possibly related to deposition of the MWF on the skin (page 96). NTP stated (also on page 96) that it was unclear if this is a treatment-related effect because of the lack of statistical significance and the lack of historical control data from inhalation studies in the Wistar Han rat. NTP concluded on page 98 that there was *equivocal evidence of carcinogenic activity* in female Wistar Han rats based on the incidences of squamous cell papilloma and keratoacanthoma (combined) of the skin. It is unclear how results that are neither statistically significant nor dose related can be considered evidence, equivocal or otherwise, of carcinogenic activity.

The conclusion of equivocal evidence is further compromised because an increase in skin tumors was not observed in male rats (page A-4). In other words, one might expect a similar trend in males since a lack of a sex difference seems reasonable. In fact, the control group in males had 1 basal cell

carcinoma and 1 keratoacanthoma compared to none in the female controls. As a consequence, it was not entirely clear why the results in female rats were considered equivocal evidence of carcinogenicity rather than a random incidence of tumors. A discussion of the uncertainty associated with these data on tumor incidence would be very helpful.

In addition, the report states on page 96 that animals "received significant dermal exposure to CIMSTAR 3800 during the 2-year whole body inhalation study due to condensation of the liquid aerosols on the fur and skin". Was that statement made on the basis of actual observations of the animals or measurement of deposition (not condensation) on both "the fur and skin", or is it a means of explaining the dermal tumors? Can observations of deposition during the study be included in the report to support this statement? Such deposition can lead to other questions. Has similar deposition happened in other inhalation studies with particles in this size range and what was the effect in those studies? Did the MWF aerosol have an electrostatic charge that led to deposition? How much of the deposited aerosol did the animals ingest and what might have been the effect of that ingestion? Is the word "condensation" possibly correct in that a vapor phase (if present) might have condensed on the fur? In short, if this statement on page 96 was proposed without supporting evidence, we suggest deleting it. Otherwise, a description and discussion of the occurrence and implications of dermal deposition is needed for this and possibly other similar inhalation studies.

3) The following table is a summary of lung tumors in both sexes of mice. NTP discussed these data on pages 93-94 and concluded on page 98 that there was *some evidence of carcinogenic activity* in female B6C3F1/N mice based on the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) of the lung and *no evidence of carcinogenic activity* in male B6C3F1/N mice exposed to 10, 30, or 100 mg/m3.

	Historical inhalation	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Females					
Alveolar/bronchiolar adenoma (D-4)		1	3	2	4
Alveolar/bronchiolar adenoma (multiple, D-4)		0	1	0	0
Alveolar/bronchiolar adenoma (total, p. D-6)	2 – 12%	1	4 (P=0.181)	2 (P=0.506)	4 (P=0.168)
Alveolar/bronchiolar carcinoma (D-4)	0 – 10%	4	1 (P=0.176N)	4 (P=0.627N)	8 (P=0.163)
Overall alveolar/bronchiolar adenoma/carcinoma (p. D-6)	2 – 16%	4	5 (P=0.505)	6 (P=0.391)	12 (**P=0.021**)
Males					
Alveolar/bronchiolar adenoma	8 – 20%	5	2	8	9
Alveolar/bronchiolar carcinoma	16 – 24%	8	9	8	10
Alveolar/bronchiolar adenoma/carcinoma	26 – 40%	13	9 (P=0.288N)	14 (P=0.476)	17 (P=0.270)

However, it is not clear in Table 18 why the overall rate for alveolar/bronchiolar adenoma/carcinoma in the control group of female mice is given as 4/50 while there was 1 animal with an adenoma and 5 with carcinomas. Did one individual have both an adenoma and a carcinoma and, if so, was the total

number reduced to show only the number of tumor-bearing individuals regardless of how many tumors any given individual might have? The definition of "overall rate" given for alveolar/bronchiolar carcinoma in female mice on pages 84-85 was the "number of animals with neoplasm per number of animals with lung examined microscopically". Should that be "number of animals with one or more neoplasm per..."? If this procedure was used, was it applied to both control and treated groups? Was consideration given to the "multiplicity in site-specific neoplasia" (page 14) to both controls and treated groups equally? Clarification of these matters would be helpful.

4) Regarding genetic toxicology, the data on mutagenicity in Table E1 are not as definite as the summary of genetic toxicity on page 88 implies. At doses up to 10 mg/plate (twice the recommended maximum dose in Health Effects Test Guidelines, OPPTS 870.5100, Bacterial Reverse Mutation Test), a doubling of the number of revertants was not seen in either of the apparent duplicate tests in *E. coli*. The conclusion in the table was that the test material was "weakly positive" even at these doses. As stated on page E-2, there "is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background." Since that benchmark was not reached here, please reconsider the decision to call the results weakly positive or provide a clear rationale for retaining that categorization in light of the test guidelines.

Discussion and Conclusions

1) Two oxazolidine compounds were tentatively identified in CIMSTAR 3800 during the chemical analyses presented in the NTP report, but NTP states on pages 93 and 97 that there was no biocide in CIMSTAR 3800. It should have been noted in the report that derivatives of 5-methyloxazolidine are often used as biocides in MWF. The MSDS for CIMSTAR 3800 dated 10-8-2012 indicates that a biocide, namely hexahydro-1,3,5-tris(2-hydroxyethyl)-S-triazine (CAS No. 4719-04-4), was in fact in the MWF at a concentration of 1-5%. This biocide has been commonly used in MWFs. A test of this formaldehyde condensate biocide in a micronucleus assay in rats at doses up to 410 mg/kg by three routes was negative (Urwin et al, 1976).

It is stated on page 97 of the NTP report that "Three types of metalworking fluids (two soluble oils and one semisynthetic) did demonstrate clear mutagenicity in the *E. coli* strain in the presence of S9 mix, and all three contained biocides that release formaldehyde, a known bacterial mutagen." We suggest that NTP revise its report on this page and elsewhere to reflect the presence of the formaldehyde condensate biocide in CIMSTAR 3800.

Urwin C, Richardson JC, Palmer AK. An evaluation of the mutagenicity of the cutting oil preservative Grotan BK. Mutat Res. 40(1):43-6, 1976.

2) Among the other hazardous ingredients listed on the MSDS is synthetic sodium sulfonate (CAS No. 78330-12-8), present at 1-5% in the MWF and not found as ingredients in the NTP analyses.

3) As stated in the NTP report, metalworking fluids are mixtures that can contain many components. In this instance, a MWF has been associated with equivocal evidence of carcinogenic activity in the

skin, prostate, brain, and uterus of rats and with some evidence of carcinogenic activity in thyroid and lungs of mice. No mutagenic activity of the MWF was seen in two strains of *S. typhimurium* and only weakly positive results at very high doses were seen with one strain of *E. coli* and only without metabolic activation. Micronucleus tests *in vivo* were negative. With the lack of a definite indication of genotoxicity at this point, does NTP have any insight into which components in the MWF might be associated with the results observed in the 2-year study? At present, ILMA is not aware of any ingredients in CIMSTAR 3800 that might have led to the development of tumors. Therefore, it would be helpful if this question were addressed in the report even if there is no definite answer.

4) Is would be helpful to readers of this report to elucidate the criteria for "equivocal" findings more clearly than "a marginal increase of neoplasms that may be chemical related".

Other Comments

Introduction

1) Issues related to bacteria and fungi in MWF are mentioned on page 20. This is a significant point and perhaps the text can be expanded. Many references are available, such as (1) Gordon, T. Metalworking fluid - the toxicity of a complex mixture, J. Toxicol. Environ. Health, Part A, 67, 209–219, 2004 and (2) Lim, C-H. et al. Inflammatory and Immunological Responses to Subchronic Exposure to Endotoxin-contaminated Metalworking Fluid Aerosols in F344 Rats, Wiley Interscience, 2005, www.interscience.wiley.com, DOI 10.1002/tox.200097.

2) First paragraph on page 25 describes three old dermal studies (1955, 1977, and 1989) in which mice developed skin tumors. NTP's report has the following statement regarding the most recent paper, which "did not specify how the cutting oil was refined, although the cutting oil was probably highly refined as the PAH content was only 5.22% (Gupta and Mehrotra, 1989)." However, if the PAH content was measured by a method comparable to IP346, a level of 5.22% PAH would indicate that the oil is not highly refined and would be expected to be dermally carcinogenic (IP, 2004). Clarification of the method used and the appropriateness of the specification of "highly refined" are needed.

IP (Institute of Petroleum). *Methods for Analysis and Testing, IP 346/92. Determination of polycyclic aromatics in unused lubricating base oils and asphaltene free petroleum fractions – Dimethyl sulphoxide extraction refractive index method.* London; 2004.

3) In the middle paragraph on page 27, the text states that "Specific components of metalworking fluids [e.g., diethanolamine (DEA), TEA, nitrosoamines, and formaldehyde] have been evaluated for carcinogenic potential on an individual basis." For accuracy, we suggest the addition of the following sentence: "Although these four substances have been measured in MWFs in use, only TEA is added to original formulations."

4) Again in the middle paragraph on page 27, the text states that the "carcinogenicity of TEA was investigated because of its potential conversion to the carcinogen *N*-nitrosodiethanolamine (NDELA)." More accurate alternate wording could be "The carcinogenicity of TEA was investigated primarily because of its use in cosmetics and a concern about conversion of TEA to DEA which, in turn, can form the carcinogen N-nitrosodiethanolamine (NDELA). It has

subsequently been shown that some DEA can be formed under conditions of heat and age of fluid (Kim et al, 2010)."

S. Kim, C. Yoon, and D. Park. Vaporization and conversion of ethanolamines used in metalworking operations. Safety Health work 1:175-182. 2010.

5) Regarding nomenclature, CIMSTAR 3800 is a semi-synthetic MWF, not a soluble oil that is sometimes referenced in the report. Consistency in the definition of CIMSTAR 3800 as a semi-synthetic would be helpful.

Materials and Methods

1) Fatty acid methyl esters are again equated with mineral oil on page 32 and the true amount of mineral oil was left unaddressed.

2) On pages 32-33, why were heated lines needed to supply the nebulizers? Was this a standard procedure at the laboratory? Why was it necessary to heat the air diluting the output of the nebulizers? Why were the conducting lines between the nebulizers and the chambers insulated? In essence, was the temperature in the rooms unstable such that a constant temperature could not be maintained without these measures? If so, how stable was the temperature within the chambers where the animals were?

3) Significant effort was made to maintain a controlled relative humidity (RH), but it's not clear how that was done from the methods described in the text and Appendix H. Where and how was RH measured? What was the RH in the air stream from the nebulizers? Was the RH in that air different from RH in air from the RH control system? What were the relative flows of air that contained the aerosol from the nebulizer and air from the RH control system with which it was mixed? That is, how much influence did the air from the nebulizers have on the RH in the inhalation chambers, particularly at the high concentration where the air from the nebulizers would be mixed with less dilution air?

4) The levels of boron in the MWF was reported at the nearest 0.1% and differed by 0.1% between lots. Was that difference due to expected variation near the limit of analytical precision? Since the calculation of aerosol concentrations was based on boron concentrations in the air (?), how much variation in the reported aerosol concentration might be due to extrapolation from data on a small portion of the total aerosol (boron) to the total aerosol?

5) At the time of changing to a gravimetric method (described on page H-6), was there a significant difference in the measured aerosol concentrations before this change in method compared to after the change? If so, were the settings on aerosol generation and dilution adjusted to compensate for any change?

6) Are there any data on the content of water in the aerosol that the animals inhaled?

7) Questions on "chamber atmosphere characterization" on pages 34-36 are similar to those in regard to measuring boron analytically and later some unstated portion of the aerosol gravimetrically.

8) Is the last sentence in 1st paragraph under Chamber Atmosphere Characterization on page H-5 meant to say that the "MMAD of all samples was within the 1 to 3 μm range required by protocol"?

9) What was the size of the chambers? It would be helpful to know given the emphasis on T90. Were these H-2000 chambers?

10) Second paragraph on page H-6 refers to generation of vapors rather than the aerosols that were actually generated.

11) According to the description in the 1st paragraph on page H-6, the aerosol was analyzed for methanol (MeOH), boron, fatty acid methyl esters, and alkanolamines. Samples from generator reservoir were analyzed for the same constituents. MeOH is not mentioned as having been analyzed in the generator reservoir, but MeOH in the liquid reservoirs is mentioned in the next paragraph. This should be clarified.

Results

1) The choices of concentrations in the 2-year exposures seem reasonable based on the range-finding data. However, it is important to note that these aerosol concentrations are significantly higher than occupational exposures. Based on work done by NIOSH, 80% of the mist levels in small shops in the 1990's were 0.5 mg/m^3 or less (Piacitelli, G., et al. Metalworking fluid exposures in small machine shops, Am. Ind. Hyg. Assoc. J., 62, 356–370, 2001). A separate in-plant metalworking fluid mist study by CIMCOOL (A. Ball. A survey of metalworking fluid mist in manufacturing plants. *Lubrication Engineering* Sept., 1997) showed that the mean aerosol value was 0.85 mg/m^3 and almost all were less than 2 mg/m^3. Since these studies were done, most plants now enclose and vent almost all machines and operations such that aerosol levels are even further reduced. NTP exposed the animal to concentration of 10, 30 and 100 mg/m^{3}of CIMSTAR 3800 concentrate. Therefore the exposure levels utilized in this NTP study were from 100 to 2000 times greater than levels expected during occupational use. We feel that the differences between experimental doses and those encountered during typical occupational use should be mentioned in the Introduction and/or the Discussion and Conclusions.

2) On page 57, what do torso/dorsal mass, torso/dorsal ulcer/abscess, and torso /lateral mass describe? Do these refer to superficial lesions on the skin, subcutaneous masses, or something else?

3) The incidence of prostate tumors in male rats at 100 mg/m3 was slightly higher than controls (6% vs 2%, not statistically significant) and higher than historical controls (page 61). NTP concluded on page 98 that there was *equivocal evidence of carcinogenic activity* in male Wistar Han rats based on the incidences of prostate gland adenoma or carcinoma (combined). Again, a discussion of the criteria used to define equivocal evidence of carcinogenicity would be helpful to the reader.

4) Based on data summarized on pages 63-65, NTP concluded on page 98 that there was *equivocal evidence of carcinogenic activity* in female Wistar Han rats based on the incidences of adenocarcinoma or mixed malignant Mullerian tumor (combined) of the uterus. We have no comment on this finding.

5) Nonneoplastic findings in nose, larynx, and lung of rats (page 68) were generally consistent with the 3-month study. Tumors did not occur in the respiratory tract where inflammation was noted. Histological findings in the lung-associated lymph nodes (pages 68-69) are consistent with other inhalation studies. We have no comments on these findings.

6) Some evidence of a possible weak effect on thyroid tumors in female rats is presented on page 69, but the non-statistically significant results for males reported on page A-3 are not discussed. The data in the following table were from page B-3. NTP concluded on page 94 that the C-cell neoplasms in female rats were not considered to be exposure-related. We have no comment on this conclusion.

No. of rats with thyroid tumors	Historical				
	Controls	0 mg/m3	10 mg/m3	30 mg/m3	100 mg/m3
Females (page 69)		(50)	(50)	(50)	(49)
C-cell, adenoma		3	2	4	4
C-cell, carcinoma		0	0	0	3
C-cell adenoma & carcinoma combined	6-14%	3	2	4	7
Follicular cell, adenoma		1	0	3	1
Follicular cell, carcinoma		1	1	0	0

7) Questions on the description of clinical signs for mice in the 2-year study (page 78) are the same as with the 2-year study in rats.

8) Regarding tumors in the thyroid gland of mice, the following table was compiled to include data for males (copied from Appendix C). Males had no carcinomas, but one animal did have follicular cell hyperplasia at 100 mg/m3. NTP stated that the 6% incidence of follicular cell carcinoma was not statistically significant relative to concurrent chamber controls, and these neoplasms were not present in females exposed to lower concentrations or in exposed male mice. Follicular cell carcinomas have been observed in only 0% to 2% of all female historical control mice exposed by inhalation or by all routes. The incidence of follicular cell hyperplasia, a potential precursor to neoplasia, was increased in female mice in an exposure concentration-dependent manner up to 6% in the 100 mg/m3 group. Because the neoplasms that were malignant are uncommon and there were exposure concentration-related increased incidences of follicular cell hyperplasia, the follicular cell carcinomas were considered to be treatment-related. NTP concluded on page 98 that there was *some evidence of carcinogenic activity* in female B6C3F1/N mice based on the incidences of follicular cell carcinoma of the thyroid gland. We have no comment on this finding.

No. of mice with follicular cell hyperplasia or carcinoma in thyroid	Historical Controls	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Females (page 83)					
Follicular cell hyperplasia		1	1	2	3
Follicular cell carcinoma	0 – 2%	0	0	0	3 (P=0.112)
Males					
Follicular cell hyperplasia (p. C-12)		0	0	0	1

Follicular cell carcinoma (p. C-3)		0	0	0	0

Discussion and Conclusions

1) NTP concludes on page 98 that exposure to CIMSTAR 3800 resulted in increased incidences of nonneoplastic lesions of the nose, larynx, and lung in male and female rats and mice, lymph nodes in male and female rats, and thyroid gland in female mice. We have no comment on these findings.