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Special Report: Comments for the Update to the ATSDR Toxicological Profile for JP-5 and JP-8 Occurring in FY14

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Interim Report

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14. ABSTRACT
Toxicologists at 711 HPW/RHDJ have reviewed the 2013 document published by the Agency for Toxic Substances and Disease Registry (ATSDR) entitled "Addendum to the Toxicological Profile for Jet Fuels (JP-5 and JP-8)" (ATSDR, 2013). The full document "Toxicological Profile for JP-5 and JP-8", published in 1998 (ATSDR, 1998), is scheduled for update in FY14. A review of the Addendum identified concerns over the quality of a subset of studies summarized in the 2013 document and the potential inclusion of this subset in the 2014 update. The purpose of this special report is to provide information to supplement the FY14 update of the ATSDR Toxicological Profile for JP-5 and JP-8, and to present recommendations for data inclusion. The authors conclude that the exposure compounds and concentrations are unknown in studies published by the University of Arizona utilizing the DeVilbiss® Ultra-Neb nebulizer exposure system. Therefore these studies are unsuitable for assessment of JP-8 risk. ATSDR should not handle these studies as "key studies" in their FY14 update.

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DEPARTMENT OF THE AIR FORCE
AIR FORCE RESEARCH LABORATORY
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30 December 2013

MEMORANDUM FOR DIVISION OF TOXICOLOGY AND HUMAN HEALTH SCIENCES
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SUBJECT: Comments for the update to the ATSDR Toxicological Profile for JP-5 and JP-8 occurring in FY14.

1. Executive Summary

1.1 Purpose. To provide information to supplement the FY14 update of the ATSDR Toxicological Profile for JP-5 and JP-8, and to present recommendations for data inclusion.

1.2 Conclusions. The authors conclude that the exposure compounds and concentrations are unknown in studies published by the University of Arizona utilizing the DeVilbiss® Ultra-Neb nebulizer exposure system. Therefore these studies are unsuitable for assessment of JP-8 risk. ATSDR should not handle these studies as "key studies" in their FY14 update.

2. Toxicologists at 711 HPW/RHDJ have reviewed the 2013 document published by ATSDR entitled "Addendum to the Toxicological Profile for Jet Fuels (JP-5 and JP-8)" (ATSDR, 2013). The full document "Toxicological Profile for JP-5 and JP-8", published in 1998 (ATSDR, 1998), is scheduled for update in FY14. A review of the Addendum identified concerns over the quality of a subset of studies summarized in the 2013 document and the potential inclusion of this subset in the 2014 update.

2.1 References in this report are listed in Attachment A.

3. The primary purpose of this report is to provide further clarification on the scientific drawbacks of the study subset in question. In the mid-1990s, the University of Arizona (UA) Department of Pediatrics was awarded a Basic Research grant from the Air Force Office of Scientific Research (AFOSR). With these funds, the UA utilized an inhalation exposure system

at their laboratory for a series of basic research studies on the jet fuel JP-8. The UA published jet fuel studies from circa 1996 through circa 2008 using this system featuring a DeVilbiss® Ultra-Neb nebulizer for exposure atmosphere generation and a 7-stage IN-TOX® cascade impactor for exposure concentration characterization. A list of 20 UA studies utilizing this generation system that were included in the 2013 ATSDR Addendum can be found in Attachment B.

3.1. Please find attached the description of the UA exposure system authored by John Hinz (AFRL/RSRE) and Maj Robert B. Walton (RSHI) and dated 10 Jul 2002 as part of their trip report to the 9th Annual Meeting of the AFOSR JP-8 Jet Fuel Toxicology Workshop (15 - 17 May 2002). The description of the exposure system begins on the fourth page of Attachment C. The description provides information unavailable from published accounts of the UA exposure system.

4. The UA studies are lacking in scientific merit in three areas that were identified in the 2013 Addendum and that have the potential to impact the FY14 update. First, unique requirements of using the DeVilbiss® Ultra-Neb nebulizer generation system with JP-8 resulted in undesirable inhalation exposure conditions.

4.1 Attachment C notes that plastic cups containing JP-8 were positioned above the nebulizer's ultrasonic generator to produce the JP-8 aerosol:vapor mixture. One-hour exposures were interrupted every 15 minutes in order for the cups to be replaced.

4.1.1 There is potential for the JP-8 vapor and aerosol mixture to contain minute particles of plastic if the plastic cups were disintegrated due to sonication. Alternatively, the JP-8 atmosphere may contain plastic components dissolved by JP-8. It is unclear if plastic contamination was ever evaluated.

4.1.2 The responses observed in UA studies (Attachment B) are potentially attributable to the chemical decomposition of plastic containers and subsequent nebulization of plasticizing chemicals into the inhalation chambers. Plasticizers are well-known lung and immune toxicants, resulting in endpoints similar to those seen in UA studies. The UA study by Wang et al. (2001) noted an increase in macrophages (ATSDR, 2013). Similar increases are produced with exposure to the plasticizer mono-2-ethylhexyl phthalate (MEHP) (Larsen *et al.*, 2004). The Addendum summary of the UA study by Pfaff et al. (1996) reported thickening of bronchiole epithelium (ATSDR, 2013); similar results were found in rats following exposure to di-(2-ethylhexyl) phthalate (DEHP) aerosols by Klimisch et al. (1992).

4.1.3 Replacement of the plastic cup required the exposure chamber to equilibrate four times over a one-hour exposure. A visual representation of replacement impact on the exposure concentration curve over time is shown in Attachment A, Figure 2.

4.2 Therefore, considerable uncertainty exists regarding the content (potential co-exposure with plastic particles or plasticizer compounds) and consistency of the conditions to which

animals were exposed throughout the many studies published by the UA while using this system (Appendix B).

4.2.1 A full description of this system is presented in Attachment C. It appears that much of this information is unavailable in published studies.

5. Second, exposure concentration characterization in the UA studies found in Attachment B was inadequate.

5.1 Attachment C describes the use of a cascade impactor to quantify aerosol concentration as an "uncommon approach". Cascade impactors are designed to measure aerosol size, not concentration. Recent attempts to utilize such a system to quantify JP-8 exposure resulted in underestimation of the aerosol concentration by at least 50 percent due to evaporation (Tremblay *et al.*, 2011).

5.1.1 UA researchers acknowledged the variability and inaccuracy of utilizing cascade impactors to measure jet fuel aerosol, due to the high volatility of the fuel and its ability to transition between aerosol and vapor states (Herrin *et al.*, 2006).

5.2 Vapor concentrations during the UA animal exposure studies were not measured. Instead, the body of work relied on gas chromatograph (GC) samples from impactor plate deposits taken during preliminary mock exposures (not during actual animal exposures). The average aerosol:vapor ratio was stated to be 1.5 (Hays *et al.*, 1995). This is the value also stated in 2002 (Attachment C) and again in 2011 (Hilgaertner *et al.*).

5.2.1 An average aerosol:vapor ratio corresponds to an aerosol content of approximately 60 percent of the total exposure.

5.2.2 The use of a single aerosol:vapor ratio from initial trial/mock exposures to estimate total concentration is not expected to accurately describe multiple inhalation studies and study concentrations. The portion (percentage) of aerosol in a combined vapor and aerosol jet fuel exposure typically increases when the overall exposure concentration increases. Fully characterized exposures show aerosols ranging from 4.2 to 19 percent for Jet A concentrations of 500, 1000 and 2000 mg/m^3 in two 14-day inhalation studies (Sweeney *et al.*, 2013). The Jet A used in these studies was essentially JP-8 without the military additives and was generated with a Sonimist® ultrasonic spray nozzle.

5.2.3 The principle of increasing aerosol with increasing concentration holds true across fuels and generation methods. Aerosol percentages of 0.6 to 33 were measured for synthetic paraffinic kerosene (SPK) exposures of 200 to 2000 mg/m^3 (Mattie *et al.*, 2011). For another alternative jet fuel, Hydrolized Fatty Acids and Esters (HEFA) from a feedstock of mixed fatty acids (HEFA-F), aerosols ranged from 7 to 28 percent for HEFA-F exposures of 200 to 2000 mg/m^3 (Mattie *et al.*, 2012). Exposure concentrations were generated using commercial atomizers and were measured in two different laboratories.

5.3 The lack of adequate quantitation in the UA studies (Appendix B) alone undermines the validity of these studies. Additional quantitation deficits in these studies are discussed in Appendix C.

6. The third issue of scientific merit concerning the UA studies (Appendix B) stems from a 2011 publication which suggested incorrectly that the exposure concentrations in studies using the DeVilbiss® generation system may be "corrected" by multiplying the published aerosol concentration by a factor of 8. Hilgaertner et al. (2011) was able to correlate lung compliance measurements in mice exposed on their new Lovelace® jet nebulizer system to 1000 mg/m^3 JP-8 (6 to 10 percent aerosol) to lung compliance measurements in mice exposed to 125 mg/m^3 JP-8 (approximately 60% aerosol) using the DeVilbiss® system. The authors suggested that, since there was an 8-fold difference in concentration for this similar measurement of effect, all UA studies using the DeVilbiss® system may then be "corrected" by multiplying the aerosol concentration by a factor of 8.

6.1 There is no evidence that a single point correlation of effect (lung compliance in mice) will hold true over multiple studies, species or endpoints.

7. The authors would like to make some recommendations to ATSDR regarding the use of these UA studies (Appendix B) in the FY14 full toxicological profile update.

7.1 The ATSDR should include the UA studies in the reference list and should thoroughly discuss all shortcomings of these studies. No further use of the UA studies using the DeVilbiss system should be made, as the actual exposure contents and concentrations in these studies are unknown.

7.1.1 The ATSDR report in section 2.2.1 Inhalation Exposure discusses the UA studies performed with the DeVilbiss® Ultra-Neb nebulizer system (ATSDR, 2013). However, this discussion did not include the generation system's plastic cup issue (section 4 of this report).

7.2 If further mention of any of the UA studies list in Appendix B occurs in the full revised Toxicological Profile, a definitive footnote should be included to remind the reader of the inadequacies of these studies.

7.2.1 Although the ATSDR addressed the UA studies in their 2013 Addendum report in section 2.2.1 Inhalation Exposure and "noted whether the reported concentrations were for the aerosol component only or aerosol and vapor components" throughout the inhalation section, a reader interested only in immune response, for example, may not read the introductory materials and might miss the significance of the words "(aerosol component only)" behind the exposure concentration stated.

7.3 The ATSDR should not suggest that the UA studies concentrations may be "corrected" by using a factor of 8. Instead, ATSDR should discuss why a single point correlation of effect may not be applied across studies, endpoints and species.

7.3.1 Text in the 2013 ATSDR Addendum (section 2.2.1) states, "Although, Hilgaertner *et al.* (2011) and Herrin *et al.* (2006) estimated that the aerosol only concentrations represented only one-eighth of the total JP-8 exposure, ATSDR has not corrected the reported exposure concentration".

7.4 The ATSDR should not consider as "key literature" the UA studies listed in Appendix B, when assessing the health effects of JP-8 in the FY14 full Toxicological Profile update.

7.4.1 Under section 2.2.1 Inhalation Exposure of the 2013 ATSDR Addendum, the UA studies listed in our Attachment B were summarized in their appropriate subsections (e.g., 2.2.1.2 Systemic Effects), alongside studies from other laboratories in which adequate and industry-accepted characterization of exposure conditions were performed.

7.4.2 The authors understand that the 2013 ATSDR Addendum followed the template and purpose of ATSDR Addenda. "The purpose of this addendum is to provide to the public and to federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1998" (ATSDR, 2013).

7.4.3 However, in effect, the ATSDR gave the listed UA studies, in which exposure is unknown, as much weight in their document as fully characterized studies in the 2013 Addendum.

7.4.4 Fortunately, the format and purpose of a full ATSDR Toxicological Profile is different. "Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented but is described in less detail than the key studies" (ATSDR, 2012). Therefore, ATSDR is allowed to make distinctions between key literature and additional studies.

8. Further comments and recommendations to ATSDR regarding the FY14 full Toxicological Profile update are found in Attachment D. These comments/recommendations are independent of the primary purpose of this report, but should assist the ATSDR in completing their update.

9. The first author of this report is Teresa R. Sterner of the Henry M. Jackson Foundation for the Advancement of Military Medicine, assigned to 711 HPW/RHDJ. Co-authors include David R. Mattie, 711HPW/RHDJ, and Shanna L. Clark, AFCEC/CZTE (Lackland AFB TX). Inhalation exposure expertise was provided by Brian. Wong, PhD, Naval Medical Research Unit-Dayton (NAMRU-D, Wright-Patterson AFB OH). For further information please contact David R. Mattie, PhD, Aerospace Toxicology Program Manager for 711 HPW/RHDJ, by phone (937-904-9569, DSN 674-9569), fax (937-255-1474, DSN 785-1474) or e-mail (david.mattie@us.a£mil).

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1st Ind, 711 HPWIRHDJ 30 December 2013

MEMORANDUM FOR DIVISION OF TOXICOLOGY AND HUMAN HEALTH SCIENCES
AT1N: HENRY G. ABADIN

This memorandum has been coordinated at the branch level and is approved for release.

1& J. CHLAGER, PhD
Chief, Molecular Bioeffects Branch
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Attachment A: Reference List
Attachment B: University of Arizona Studies Cited in the 2013 ATSDR Addendum that Utilized the DeVilbiss® Ultra-Neb Nebulizer Generation System
Attachment C: Trip Report (AFOSR JP-8 Jet Fuel Toxicology Workshop, UA, Tucson, AZ, 15-17May02)
Attachment D: Additional Comments/Recommendations

ATTACHMENT A: REFERENCE LIST

ATSDR. 1998. Toxicological Profile for JP-5 and JP-8. Atlanta, GA: Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/toxprofiles/index.asp.

ATSDR. 2012. Toxicological Profile for 1,4-Dioxane. Atlanta, GA: Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine/Applied Toxicology Branch. http://www.atsdr.cdc.gov/toxprofiles/.

ATSDR. 2013. Addendum to the Toxicological Profile for Jet Fuels (JP-5 and JP-8). Atlanta, GA: Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Sciences. http://www.atsdr.cdc.gov/toxprofiles/profilesaddenda.asp.

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Mattie, D.R., Sterner, T.R., Wong, B.A., Dodd, D.E., Layko, D.K., Ross, P.W., Gross, E.A., Willson, G.A., Hinz, J.P. and Wagner, D.J. 2011. 90-Day inhalation toxicity study of FT fuel. Wright-Patterson AFB, OH: Air Force Research Laboratory, 711 Human Performance Wing, Human Effectiveness Directorate, Molecular Bioeffects Branch. AFRL-RH-FS-2011-0014, ADA557260.

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Sweeney, L.M., Prues, S.L. and Reboulet, J.E. 2013. Subacute effects of inhaled Jet Fuel-A (Jet A) on airway and immune function in female rats. Inhal Toxicol 25(5): 257-271.

Tremblay, R.T., Martin, S.A. and Fisher, J.W. 2011. Evaluation of methods used to generate and characterize jet fuel vapor and aerosol for inhalation toxicology studies. Jet Fuel Toxicology. M. L. Witten, E. Zeiger and G. D. Ritchie. New York: CRC Press: 220-238.

Wang, S., Young, R.S. and Witten, M.L. 2001. Age-related differences in pulmonary inflammatory responses to JP-8 jet fuel aerosol inhalation. Toxicol.Ind.Health 17(1): 23-29.

ATTACHMENT B: UNIVERSITY OF ARIZONA STUDIES CITED IN THE 2013 ATSDR ADDENDUM THAT UTILIZED THE DEVILBISS® ULTRA-NEB NEBULIZER GENERATION SYSTEM

The following citations were copied verbatim from the 2013 ATSDR Addendum to the Toxicological Profile for Jet Fuels (JP-5 and JP-8). They include only the University of Arizona studies which utilized the DeVilbiss® Ultra-Neb Nebulizer Generation System.

Baldwin CM, Figueredo AJ, Wright LS, *et al.* 2007. Repeated aerosol-vapor JP-8 jet fuel exposure affects neurobehavior and neurotransmitter levels in a rat model. J Toxicol Environ Health A 70(14):1203-1213.

Baldwin CM, Houston FP, Podgornik MN, *et al.* 2001. Effects of aerosol-vapor JP-8 jet fuel on the functional observational battery, and learning and memory in the rat. Arch Environ Health 56(3):216226.

Harris DT, Sakiestewa D, He X, *et al.* 2007b. Effects of in utero JP-8 jet fuel exposure on the immune systems of pregnant and newborn mice. Toxicol Ind Health 23(9):545-552.

Harris DT, Sakiestewa D, Robledo RF, *et al.* 1997a. Immunotoxicological effects of JP-8 jet fuel exposure. Toxicol Ind Health 13(1):43-55.

Harris DT, Sakiestewa D, Robledo RF, *et al.* 1997b. Protection from JP-8 jet fuel induced immunotoxicity by administration of aerosolized substance P. Toxicol Ind Health 13(5):571-588.

Harris DT, Sakiestewa D, Robledo RF, *et al.* 1997c. Short-term exposure to JP-8 jet fuel results in long-term immunotoxicity. Toxicol Ind Health 13(5):559-570.

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Harris DT, Sakiestewa D, Titone D, *et al.* 2000b. Jet fuel-induced immunotoxicity. Toxicol Ind Health 16:261-265.

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Robledo RF and Witten ML. 1999. NK1-receptor activation prevents hydrocarbon-induced lung injury in mice. Am J Physiol 276:L229-L238.
Robledo RF, Young RS, Lantz RC, *et al.* 2000. Short-term pulmonary response to inhaled JP-8 jet fuel aerosol in mice. Toxicol Pathol 28:656-663.
Wang S, Young RS, Witten ML. 2001. Age-related differences in pulmonary inflammatory responses to JP8 jet fuel aerosol inhalation. Toxicol Ind Health 17:23-29.
Wong SS, Hyde J, Sun NN, *et al.* Inflammatory responses in mice sequentially exposed to JP-8 jet fuel and influenza virus. Toxicol 197:139-147.

ATTACHMENT C: TRIP REPORT (AFOSR JP-8 JET FUEL TOXICOLOGY WORKSHOP, UA, TUCSON, AZ, 15-17MAY02)

10 Jul 02

MEMORAMDUM FOR AFIERA/RSR & RSH DIVISION CHIEFS
ATTENTION: LtCol K L COX
LtCol K A FOX
MR G L LONG

FROM: AFIERA/RSRE & RSHI
2513 Kennedy Circle, Bld #180
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SUBJECT: Trip Report (AFOSR JP-8 Jet Fuel Toxicology Workshop, UA, Tucson, AZ, 15-17 May02)

1. PURPOSE: Participate in, and provide presentations to, the 9th annual meeting of the AFOSR JP-8 Jet Fuel Toxicology Workshop.

2. DRIVER & CUSTOMER: Formal representation of AFIERA interests in the AFOSR Workshop.

3. TRAVELER(S): Mr. John P. Hinz (RSRE), Maj Robert B. Walton (RSHI)

4. PERSON(S)/OFFICES CONTACTED:
Dr. Walter Kozumbo (AFOSR)
Dr. Mark Witten (Un AZ)
The Workshop

5. SIGNIFICANT ACTIVITIES: The annual JP-8 Toxicology Workshop, hosted by Dr. Mark L. Witten and the University of Arizona (Un AZ), provides all researchers whose work is sponsored by the Air Force Office of Scientific Research (AFOSR) the opportunity to share their latest findings together and chart new directions for their research. Fifteen out of the 25 workshop participants came from academic establishments; many of their projects, usually investigations into rodent JP-8-mediated proteomics and genomics, reflected a mechanistic and academic bent. On the other hand, the AF's presentations were more applied than most. As Dr. Kozumbo reminded us all, AFOSR's research effort aims at disclosing the potentially toxic interactions between JP-8 and the biological tissues exposed to it as well as the mechanisms that mediate these interactions – an effort intended to contribute to and improve upon an integrated health risk assessment of this fuel. Attachments 1-3 present in order the workshop's agenda, list of attendees and executive summary of the meeting. Bound abstracts from the presentations are on file with RSRE.

AFIERA's Presentations. Maj Walton briefed the Workshop on the results of the AF's "acute epidemiology study" that lead to the development of a new, tri-layered, fuel-resistant uniform for better protection of tank entry personnel (see Attachment 4).

Mr. Hinz described RSRE's project to characterize JP-8's potential for respiratory irritation and its successful application to the development of acute exposure guidelines by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (see Attachment 5).

Industry's Participation. In addition to DoD's participation, building on concerns over JP-8's purported link to immunotoxic health effects voiced at AFIERA's second international conference on jet fuel, this workshop has begun to draw interest from industry. Dr. Cynthia Mann, an immunotoxicologist from ExxonMobil Biomedical Sciences, Inc., who has been brushing up on the literature in this area, attended the workshop. Dr. George Woodall (American Petroleum Institute) outlined (see his abstract) for the meeting's participants industry plans for its own examination of jet fuel's immunotoxicity. Unlike the apparent flexibility allowed in the academic research described at the workshop, industry's study design is obliged to follow more consistent protocols outlined in the U. S. EPA's "Health Effects Test Guidelines, OPPTS 870.7800, Immunotoxicity; EPA 712-C-98-351, Aug98". It was not clear whether the academic community understood these constraints. Most of the work at the University of Arizona has used mice, and tissues obtained from them, as the principle test system. Industry's protocol, a 28-day, EPA-defined dermal immunotox bioassay with Jet-A, will use rats (to add a second species to the data base) and include positive, negative and vehicle controls. The contract for this study has been awarded to ExxonMobil's laboratory; the study should start sometime in June'02.

Book Proposal. Dr. Witten urged the workshop to consider writing a book on JP-8's toxicology; he already has a publisher in mind. The workshop's participants would author chapters of this book, with Witten serving as the book's senior editor. There was support for the idea, although no final commitments or decisions were made. If this proposal develops legs, both industry and the military might consider preparing their own chapters for it.

Laboratory Tour. Dr. Witten graciously gave Mann, Walton and Hinz a guided tour of his laboratory at the Un. AZ's Health Science Center. A number of the studies described at this workshop used animals or tissues dosed and obtained from Witten's lab. Most real world exposure to JP-8 comes through the dermal or inhalation routes. Witten's laboratory chose the latter approach, exposing mice to atmospheres laden with a mixture of JP-8 aerosol and vapor. To date, the laboratory appears to have focused most of its attention on the aerosol fraction. Dr. Witten described the exposure system (it was on display in a laboratory fume hood) and some of the studies that have been done with it. The laboratory appears to be conducting inhalation studies with a sophisticated chamber system and conscientious personnel supported by limited analytical or technical resources. A summary based on what we heard and saw is presented in Attachment 6. We also plan to obtain copies of the published descriptions of the design and operation of the Un AZ exposure system.

6. ACTION ITEM(S) & RECOMMENDATIONS:
   a) AFIERA should continue to monitor, participate in and support as warranted future AFOSR JP-8 workshops.
   b) Maintain professional liaison with industry and AFOSR, lending technical support or know-how where needed and as warranted.

7. For additional information, please contact Mr. John P. Hinz at 4-6136, or Maj Robert B. Walton at 4-6049.

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cc: AFIERA/RS, CD

Attachments:
1. AFOSR JP-8 Jet Fuel Toxicology Workshop – agenda
2. AFOSR JP-8 Jet Fuel Toxicology Workshop – list of attendees
3. AFOSR JP-8 Jet Fuel Toxicology Workshop – executive summary
4. Maj R. B. Walton – presentation
5. Mr. J. P. Hinz – presentation
6. Un AZ / Witten Exposure System - discussion
   Fig 1 – Representation of Exposure System
   Fig 2 – Representation of Exposure Profiles

UNIVERSITY of ARIZONA / WITTEN EXPOSURE SYSTEM.

- **Main Elements (see Figure 1)**

DeVilbiss Ultrasonic Nebulizer. This medical device, an ultrasonic humidifier used to introduce moisture into breathing air, has been adapted to generate a fuel aerosol that is gently purged from the device by fan-forced fresh air. A plastic cup, charged with ~15 mls of fuel, has been inserted over the ultrasonic generator. There is no direct connection between the generator and the exposure chamber.

Glass Beaker. Suspended over the gap between the ultrasonic generator and the chamber inlet, the beaker was recruited as a dilution and mixing vessel for the test atmosphere before the atmosphere is aspirated into the exposure chamber.

Multi-port, Nose-only Inhalation Exposure Chamber. Made of stainless steel, about the size and shape of an attaché case, this IN-TOX product will hold up to 24 mice, each contained in its own restraining tube. The restraining tubes, each sealed air tight to the chamber by an O-ring, plug into one side of the chamber. Seen end-on, the chamber is divided sagittally by an internal baffle, which is nippled to receive the nose cones of the restraining tubes. The chamber on one side of the baffle supports the tubes, while the opposite side serves as a supply plenum for the test atmosphere. The chamber's volume was stated to be 3 L; however, on inspection this appeared to represent the supply plenum. The animal's side of the chamber [another 3 L?] is operated at an exhaust rate of ~0.225 LPM to expel exhaled air; the supply plenum is exhausted at ~2 LPM.

Restraining Tubes. Lucite and plastic tubes, bunged to prevent escape, with a conical nosepiece. The tubes serve to limit/direct exposure to the nose and respiratory tract while minimizing dermal and oral intake of the test agent.

2 LPM Personal Sampling Pump. The pump aspirates air (thereby ventilating the chamber) laden with the test atmosphere from the nebulizer and beaker into and through the plenum side of the exposure chamber. Operating on the exhaust side of the system, it draws the atmosphere from the inhalation chamber and through a cascade impactor before expelling the air.

Cascade Impactor. Normally used to help characterize by inertial impaction the size distribution of an aerosol, this particular 7-stage device (made by IN-TOX) was also recruited to determine exposure concentration.

- **System Operation**

As Dr. Witten described the system – we did not see it in operation – the ultrasonic humidifier/nebulizer creates a generous, turbulent cloud of aerosol that must be diluted before it is sucked into the exposure chamber. As explained to us, the inverted beaker and gaps in supply tubing (see Fig. 1) help to attenuate the atmosphere as it is drawn into the chamber. It is our supposition that while the output from an ultrasonic humidifier is apt to be turbulent, it is not evident how an open system such as theirs dampens the turbulence (it certainly dilutes the concentration), obtaining a more consistent exposure.

Each animal's restrainer penetrates the external port of the chamber, its nose cone applied to the small nipples in the chamber's internal baffle, the mouse receiving its breathing air from the plenum side of the system. It was not clear whether port-to-port variability in exposure concentration had been characterized. Except for the vent port on the animals' side of the baffle, the "3 L" chamber (actually just the supply plenum side of it) is ventilated at 2 LPM.

Theoretically, at this volume and flow rate, the supply plenum should reach equilibration (t99) in ~6.9 minutes, ~12% of the total exposure time. This would not be unacceptably long, except that equilibration must be repeated three more times during each 1 hour exposure (see Fig 2 and discussion of plastic cups below).

All air drawn through the supply plenum exits through the cascade impactor. All determinations of chamber aerosol concentration depend on results obtained from the cascade impactor and are based gravimetrically on changes in weight of the collection plates contained within the instrument. This uncommon approach to concentration measurement may yield a time weighted average approximation of the aerosol content, but cannot reflect chamber equilibration or the constancy of chamber concentration during each exposure. The plates with the two heaviest deposits may be subjected post-exposure to GC analysis, although such analysis (it's expensive) does not appear to be routine. The lab's quantitation methods appear to overlook the volatiles obtained from the jet fuel, relying instead on initial chamber trials conducted some years ago that suggested then a mean aerosol to vapor ratio of ~1.5x.

Dr. Witten stated that the nebulizer's plastic cups, charged with 15 mls of jet fuel, do not last very long – they begin to disintegrate and have to be replaced every 15 minutes (they retrieve about 10 mls from the used cup) during a 1-hour exposure. Cup replacement, no matter how prompt, shuts the generation system down repeatedly and subdivides each exposure into four 15-minute increments. From our perspective, the exposure chamber must re-equilibrate with fresh test atmosphere each time the generation system is restarted. Witten attributed the cup's failure to the ultrasonic generator; but, the loss of the cup's integrity might also be due to the chemical action of the fuel. From his description, we were not sure whether his lab has assayed, fingerprinted and compared the original fuel with the cup's residue and the content of the chamber's atmosphere to ascertain which (if any) of the fuel's or the atmosphere's constituents have gained from or been lost to the plastic in the generation system.

- **Assessments & Suggestions**

The research projects of many participants at the Workshop depend on Dr. Witten's lab for animals and tissues dosed with JP-8. Published reports state that the lab has exposed rodents to levels ranging from 5 to 2500 mg/m3. The responses obtained from the animals tell us that they have been dosed during these exposures. However, since much of this research depends on one lab's efforts, the record of these exposures might benefit from a more thorough characterization of the test atmosphere and the exposure process. How faithfully did the test atmospheres represent the constituents of the original fuel? What constituents were present? Since all of JP-8 is at least to some degree volatile, how did the fuel partition between aerosol and vapor phases? How repeatable and stable were the exposure atmospheres – and the dosages that the animals received from them? These exposures ought to be fully and regularly characterized to identify which constituents (ideally all) of JP-8 are present in these test atmospheres and to verify that these atmospheres are faithfully reproduced each time.

Dr. Witten's lab built its inhalation facility around the IN-TOX chamber. We believe that this sophisticated system dates back some years to a time when radioactive aerosols were being studied. With limited quantities of a potentially dangerous and expensive material to work with then, a low volume, directed exposure system (the mice inhale the test atmosphere directly from a "nipple") made sense. These constraints do not apply now to jet fuel, which is readily available and comparatively nontoxic. A simpler chamber, perhaps modeled after the IN-TOX

design to exploit the equipment at hand, but without the internal baffle and operated at higher flow rates, should be easier to work with. It would process enough test atmosphere to permit sufficient grab sampling to better characterize exposure concentration, stability and distribution within the chamber without compromising the dosing process. Grab samples should simultaneously capture both the aerosol and vapor phases of the test atmosphere, as both are present in these exposures. At least some samples should be fingerprinted to confirm whether all of JP-8's constituents are represented. Periodically, samples might be taken to characterize the size distribution, respirability and constituent composition vs size of any aerosol – a cascade impactor serves this assessment.

Periodic replacement of the nebulizer's cup during each exposure temporarily stops generation and delivery of the test atmosphere, leading to a variable, saw-tooth exposure profile (see Fig. 2). Analogue vapor and aerosol monitors can help characterize the degree and impact of the saw-tooth on the process of dosing as well as the chamber's return to a constant exposure.

Dr. Witten's lab may have answered these questions, typically asked of any inhalation study, already – they were simply not addressed at this workshop. We offer our suggestions and our help in support of this important research and to buttress its documentation – JP-8's immunotoxic potential remains an open question. The added information we propose should also support Dr. Kozumbo's goal of a well-developed and informed risk assessment for JP-8.

Figure 1 - Representation of UAz Exposure System

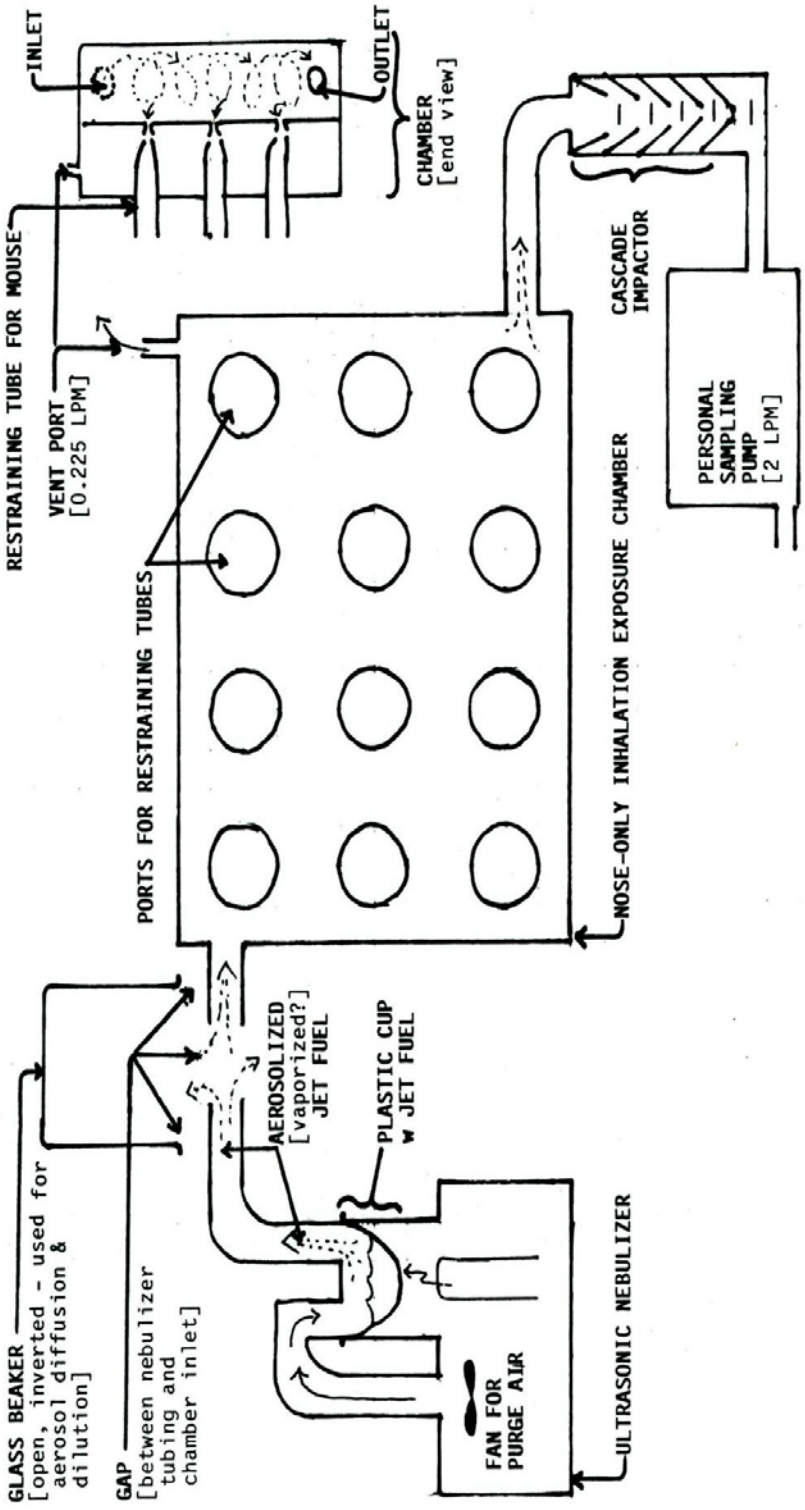
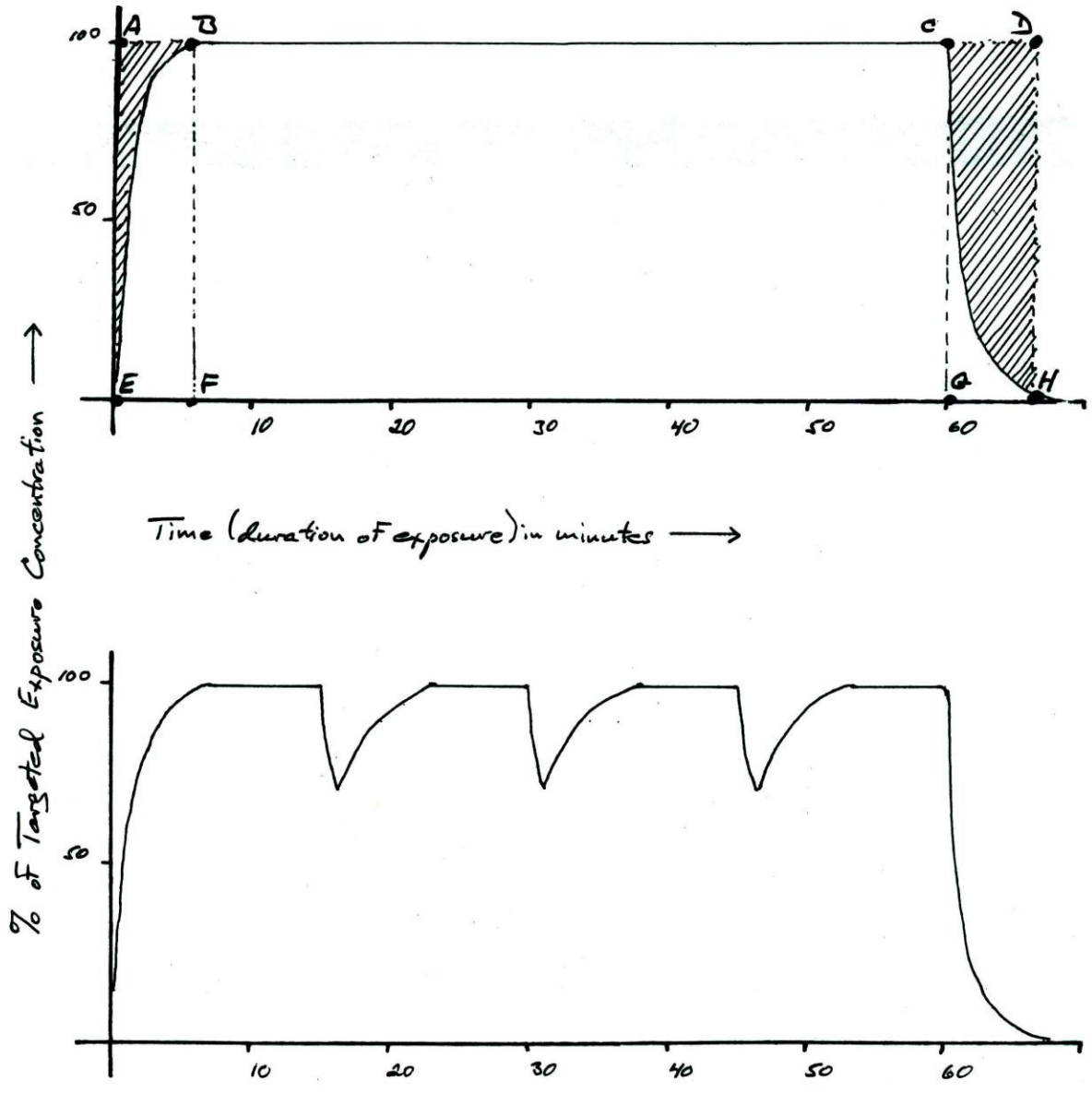


Figure 2 Representations of Exposure Profiles

Top – Representation of idealized equilibration and exposure profile
Bottom – Representation of an interrupted exposure regimen



Explanation for Figure 2

Referring to the diagrams in Figure 2, the top diagram represents an idealized profile for chamber equilibration and operation. The diagram, modeled after the precepts for the design and operation of inhalation exposure equipment elaborated by Dr. Harold MacFarland (see reference below), mimics how most standard inhalation chambers operate.

The test atmosphere is generated between times EG, spanning in the case of the U Az system a period of 1 hour, to achieve exposure level AC. ACEG represents the "exposure box" within which the animals are "dosed." Time EF represents the period during which the chamber is charged with the contaminant-laden test atmosphere; the curve E-B describes the rate and manner in which the chamber fills and equilibrates with the test atmosphere. At time G, the end of the exposure period, the test atmosphere generator is turned off; GH describes the post-exposure interval during which the chamber's atmosphere is replenished with fresh air. The equilibration curve EB is mirrored by the clearance curve CH. The piece of the box (EAB) seemingly "missing" during equilibration is made up by/compensated for by CGH as the chamber is replenished by, equilibrates with, clean air. The animals are usually removed at or after time H.

MacFarland recommends that equilibration take no more than $1/13^{th}$ of the exposure period (EF = EG/13; and, EF = GH). In my studies EF was never more than 10% of the total exposure time (EF = EG/10). Either way, you won't spend (nor should you) a disproportionate amount of time equilibrating the chamber before the animals get fully exposed or dosed. Of course, the chamber's atmosphere is sampled after equilibration to determine its concentration at intervals between times F and G during which it has reached and stabilized at level BC.

As MacFarland describes the process, equilibration, or T_{99} (defined as the time to reach 99% of the targeted test atmosphere exposure concentration), theoretically requires 4.6 changes of the chamber air. Thus, time EF/4.6 = the time allowed for 1 air change. We can back-calculate to the requisite flow rate for a short-term, 1-hour exposure:

1-hour exposure (60 minutes)
T_{99} </= 7.7% of EF = 4.6 min.
4.6 min/4.6 = T for 1 air change = 1 min
3L/1 min = ~3 LPM

The bottom figure represents an exposure regimen interrupted by the need to replace and replenish the plastic cups that are charged with jet fuel. The duration and magnitude of these interruptions should to be documented. These interruptions may be trivial, but they cannot be dismissed until they are more fully described.

MacFarland, HF. 1987. Designs and Operational Characteristics of Inhalation Exposure Equipment. Chapter 4 in *Inhalation Toxicology* (H. Salem, Ed.), pp. 93-120, Marcel Dekker, Inc., NY.

Attachment D: Additional Comments/Recommendations

*Section/Page refers to relevant place in the 2013 ATSDR Addendum to the Toxicological Profile for Jet Fuels (JP-5 and JP-8)

Comment Author: 711 HPW/RHDJ
Section/Page: 2.2.1 Inhalation Exposure/1
Comment: An unpublished report that was recommended for inclusion was omitted. It has since been published.

Unpublished citation: Sweeney, L.M., Prues, S.L., Wilfong, E.R., Reboulet, J.E. and Hess, K. 2012. Subacute Effects of Inhaled Jet Fuel-A (Jet A) on Airway and Immune Function in Rats. Wright-Patterson AFB, OH: Naval Medical Research Unit-Dayton. NAMRU-D Report Number 12-39, ADA564442.
Recommendation: We recommend inclusion of the published version in the updated Toxicological Profile.

Published citation: Sweeney, L.M., Prues, S.L. and Reboulet, J.E. 2013. Subacute effects of inhaled Jet Fuel-A (Jet A) on airway and immune function in female rats. Inhal Toxicol 25(5): 257-271.

Comment Author: 711 HPW/RHDJ and AFCEC/CZTE
Section/Page: Renal Effects/4
Comment: The damage described in these male rats is likely due to α-2-microglobulin nephropathy. The authors allude to this by stating that α-2-microglobulin concentrations increase with exposure concentration. Although the 1998 Toxicological Profile discusses that α-2-microglobulin nephropathy in rats does not have human relevance, that fact was omitted from the 2013 Addendum. This omission, in effect, **lends scientific validity to the reported renal effects** in this publicly available document.

There are references that discuss the lack of relevance of male rat hydrocarbon nephropathy on human health risk that were not included in the 1998 Toxicological Profile (e.g., Flamm and Lehman-McKeeman 1991; Hard et al 1993; Swenberg, 1993, U.S. EPA, 1991).
Recommendation: We recommend full discussion and interpretation of α-2-microglobulin nephropathy in male rats and its lack of relevance to human health in the updated Toxicological Profile. Possible references that the ATSDR may consider include:

Flamm and Lehman-McKeeman 1991 The human relevance of the renal tumor inducing potential of d-Limonene in male rats Implications for risk assessment. Regul.Toxicol.Pharmacol. 13:70-86.

Hard, G.C., Rodgers, I.S., Baetcke, K.P., Richards, W.L., McGaughy, R.E. and Valcovic, L.R. 1993. Hazard evaluation of chemicals that cause accumulation of alpha 2μ-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ.Health Perspect. 99: 313-349.

Swenberg, J.A. 1993. Alpha 2μ-globulin nephropathy: review of the cellular and molecular mechanisms involved and their implications for human risk assessment. Environ.Health Perspect. 101 Suppl 6: 39-44.

U.S. EPA. 1991. Alpha 2μ-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/625/3-91/019F, NTIS PB92143668.

Comment Author: 711 HPW/RHDJ
Section/Page: 2.2.1.3 Immunological and Lymphoreticular Effects/5-6
Comment: A new immunological study was just released that we request the ATSDR include in this section in the updated Toxicological Profile. Female mice and rats were exposed for 6 hours daily for 28 days at fully characterized concentrations of JP-8 vapor and aerosol up to 2000 mg/m^3. No immune effects were found.
Recommendation: Please include a summary of the following study in the updated Toxicological Profile.

White, K.L., Delorme, M.P., Beatty, P.W., Smith, M.J., Peachee, V.L. 2013. Jet Fuel Kerosene is not Immunosuppressive in Mice or Rats Following Inhalation for 28 Days. J Toxicol Environ Health A. 76(13):778-97.

Comment Author: 711 HPW/RHDJ
Section/Page: 2.2.1.4 Neurological Effects/8-9
Comment: The noise studies were not adequately summarized.

Fechter et al. (2007) performed three separate experiments, which was not clear from the summary. The noise exposures were not characterized (dB, kHz, whether the intensity is considered damaging to humans over the specified period of time). Aerosol exposure was characterized as "mostly vapor" instead of the reported 1-5%. GSH levels were measured only in liver, lung and brain, not in cochlea as was written in the summary.

Fechter et al. (2010) did more than replicate the results of the 2007 study. Three JP-8 exposure concentrations were used. The octave band noise used was stated to be 100-102 dB_{lin}, which is correct; this level correlates to 97-99 dB_A, on the human equivalent scale, which is relevant. The octave band was not characterized (centered at 8 kHz). The study indicated a dose-response effect with JP-8 concentration, which was not reported as only one dose was mentioned.

Fechter et al. (2012) performed two separate studies; the second study using intermittent noise was not summarized at all. This paper was different from previous studies in that Sprague-Dawley rats were utilized (not mentioned in the summary) and that the noise exposure was simultaneous, replicating human exposure (not emphasized in the summary). The octave band was not stated and the noise level should be characterized as 'non-damaging".
Recommendation: We recommend a more thorough summation of the noise studies in the updated Toxicological Profile.

Comment Author: 711 HPW/RHDJ

Section/Page: 2.2.3.8 Cancer/19
Comment: A study that was recommended for inclusion was omitted (Nessel et al., 1999). This study expands on the 1998 study that was summarized in the Addendum by concluding that dermal irritation is key to skin tumor promotion in mice. The omitted study has direct relevance to human exposure to JP-8.
Recommendation: We recommend including the following study in the updated Toxicological Profile:

Nessel, C.S., Freeman, J.J., Forgash, R.C. and McKee, R.H. 1999. The role of dermal irritation in the skin tumor promoting activity of petroleum middle distillates. Toxicol.Sci. 49(1): 48-55.

Comment Author: 711 HPW/RHDJ
Section/Page: 2.3.5.1 Summary of PBPK Models/21
Comment: The model description, "The model for jet fuel which examined aerosol and vapor exposure was developed using submodels for six aliphatic and aromatic hydrocarbon markers (n-octane, n-decane, n-tetradecane, toluene, ethylbenzene, and m-xylene)" is incomplete and therefore incorrect. The model, in addition to the six specific submodels, also contains submodels for three chemical 'lumps' which incorporate all other jet fuel components based on their physical properties.
Recommendation: Minimally, the sentence should be expanded in the updated Toxicological Profile (possible additional wording underlined below):

"The model for jet fuel which examined aerosol and vapor exposure was developed using submodels for six aliphatic and aromatic hydrocarbon markers (n-octane, n-decane, n-tetradecane, toluene, ethylbenzene, and m-xylene), plus three chemical lumped compartments based upon physical property similarities (aromatic hydrocarbons, 8 to 10-carbon hydrocarbon aliphatics, and heavier aliphatic hydrocarbons).

Comment Author: 711 HPW/RHDJ
Section/Page: 2.5 Relevance to Public Health: Genotoxicity/21
Comment: This description of in vitro genotoxicity studies is incomplete. An older unpublished study that was recommended for inclusion was omitted. This study was also not present in the original Toxicological Profile. The study contains an Ames test, a mouse lymphoma assay, an unscheduled DNA synthesis assay and a dominant lethal assay.
Recommendation: We recommend including the following study in the updated Toxicological Profile, for which the URL is also included:

Brusick, D.J. and Matheson, D.W. 1978. Mutagen and oncogen study on JP-8. Wright-Patterson AFB, OH: Aerospace Medical Research Laboratory. AMRL-TR-78-20. www.dtic.mil/dtic/tr/fulltext/u2/a064948.pdf