Peer Reviewer Comments on Draft

Toxicological Profile for Uranium

June 2011

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I. Peer Reviewer Summary Comments

Comments Received from

Peer Reviewer #1

Replies to Reviewer Charges and Associated Comments

1. Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?

Not to my knowledge.

2. Are there any general issues relevant to child health that have not been discussed in the profile and should be?

Not to my knowledge.

3. If you answer yes to either of the above questions, please provide any relevant references.

n/a

CHAPTER 1. PUBLIC HEALTH STATEMENT

4. The tone of the chapter should be factual rather than judgmental. Does the chapter present the important information in a non-technical style suitable for the average citizen?

Yes.

5. Major headings are stated as a question. In your opinion, do the answers to the questions adequately address the concerns of the lay public? Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text? Please note sections that are weak and suggest ways to improve them.

Yes, answers to questions seem to adequately address concerns of the lay public.
Yes, summary statements are consistent and are reasonably well supported.

6. Are scientific terms used that are too technical or that require additional explanation? Please note such terms and suggest alternate wording.

Consider adding explanations to the following terms:

· isotope (versions of the same chemical with different atomic structures)
· adsorb (collect and condense on a surface or material)
· hematological parameters (measurements of chemical constituents in the blood)
· alterations (damaging changes)
· genotoxicity (toxic damage to genes and chromosomes)
· "absence of maternal effects" (not sure what to suggest here...)
· some typos: "no conclusive evidence suggesting **that**"
"most uranium **leaves** the body"
" for a long time after **you**"

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

7. Do you agree with those effects known to occur in humans as reported in the text?

As far as I know, yes.

8. Are the effects only observed in animals likely to be of concern to humans? Why or why not?

Likely, yes. The effects reported appear to connect with similar sorts of human endpoints.

9. Have exposure conditions been adequately described?

Reasonably so, yes.

CHAPTER 3. HEALTH EFFECTS

Toxicity - Quality of Human Studies

10. Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)?

To the available degree, yes. Study limitations were clearly described.

11. Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?

Reasonable discussion/conclusions/limitations were presented.

12. Were all appropriate NOAELs and/or LOAELs identified for each study? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations?

NOAELs and LOAELs are listed throughout, to a reasonable degree. However, see Question #13.

13. Were the appropriate statistical tests used in the studies? Would other statistical tests have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

I am afraid I strongly disagree with the Agency's primary reporting of and reliance on (to whatever degree) the NOAEL and/or LOAEL for building points of departure (PODs) in their risk analyses/assessments. The NOAEL/LOAEL's limitations as a statistical measure have been revealed to such a degree, and by a wealth of reputable authors (selected references listed at end of report), that its continued use by any scientific entity borders on embarrassment (with apologies for the hyperbole). I encourage the ASTDR to begin de-emphasizing use of the NOAEL/LOAEL. Replacement can be made with more modern statistical technologies, such as the BMDL/BMCL approach already mentioned in the report. (If scientific and experimental data of sufficient quality are not available for construction of BMDLs/BMCLs, then call should be made to raise the scientific community's standards for data generation and production –

accumulation of substandard data is a poor reason to resort to a substandard statistic such as the NOAEL/LOAEL.) Also see Question #20.

14. Are you aware of other studies which may be important in evaluating the toxicity of the substance?

No.

Toxicity - Quality of Animal Studies

15. Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)?

To the available degree, yes.

16. Were the animal species appropriate for the most significant toxicological endpoint of the study?

Unable to judge.

17. Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the text? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?

Reasonable discussion/conclusions/limitations were presented.

18. Were all appropriate NOAELs and LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies?

NOAELs and LOAELs are listed throughout, to a reasonable degree. However, Question #20.

19. If appropriate, is there a discussion of the toxicities of the various forms of the substance?

Discussion seemed reasonable.

20. Were the appropriate statistical tests used in the interpretation of the studies? If not, which statistical tests would have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

As in Question #13, I am afraid I strongly disagree with the Agency's primary reporting of and reliance on (to whatever degree) the NOAEL and/or LOAEL for building points of departure (PODs) in their risk analyses/assessments. The NOAEL/LOAEL's limitations as a statistical measure have been revealed to such a degree, and by a wealth of reputable authors (references available upon request), that its continued use by any scientific entity borders on embarrassment (with apologies for the hyperbole). I encourage the ASTDR to begin de-emphasizing use of the NOAEL/LOAEL. Replacement can be made with more modern statistical technologies, such as the BMDL/BMCL approach already mentioned in the report. (If scientific and experimental data of sufficient quality are not available for construction of

BMDLs/BMCLs, then call should be made to raise the scientific community's standards for data generation and production – accumulation of substandard data is a poor reason to resort to a substandard statistic such as the NOAEL/LOAEL.) Unfortunately, where the BMDL approach was employed in the report a *serious statistical error* in (at least) presentation occurs: the MRL summary analyses in Table A-3 list multiple P-values larger than 1.0 (some as high as 18.44). Since every form of P-value must lie between 0.0 and 1.0, the Table displays a flaw in fundamental statistical presentation (and, it appears, interpretation). I assume this is some sort of typographical error and/or simple misunderstanding, and that a straightforward explanation for it can be found. As currently presented, however, these flawed statistical summaries are suspect: due to potential forward-propagation of the unknown error(s) into the consequent calculations, I must warn that the results as presented should not be relied upon for something as important as MRL determinations until the nature and extent of the discrepancies can be determined.

21. Are you aware of other studies that may be important in evaluating the toxicity of the substance?

No.

22. Are the LSE tables and figures complete and self-explanatory? Does the "Users Guide" explain clearly how to use them? Are exposure levels (units, dose) accurately presented for the route of exposure? Please offer suggestions to improve the effectiveness of the LSE tables and figures and the "User's Guide."

Tables and User Guide seem reasonable. See, however, Questions #13 and #20.

23. Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

Categorizations seemed reasonable.

24. If MRLs have been derived, are the values justifiable? If no MRLs have been derived, do you agree that the data do not support such a derivation?

See Question #20 regarding a questionable statistical error.

25. Have the major limitations of the studies been adequately and accurately discussed? How might discussions be changed to improve or more accurately reflect the proper interpretation of the studies?

Discussion seemed reasonable.

26. Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?

Discussion seemed reasonable.

27. Have "bottom-line" statements been made regarding the relevance of the endpoint for human health?

Discussion seemed reasonable.

28. Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

Conclusions seemed acceptable, given limitations of available data. See, however, Questions #13 and #20.

29. Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

To the best extent possible, apparently. (See, however, Question #20 regarding a questionable statistical error.)

30. Has the animal data been used to draw support for any known human effects? If so, critique the validity of the support.

To the best of my understanding, yes. Conclusions seemed acceptable, given limitations of available data. See, however, Questions #13 and #20.

31. Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance?

To the best of my understanding, yes.

32. Have the major organs, tissues, etc. in which the substance is stored been identified?

To the best of my understanding, yes.

33. Have all applicable metabolic parameters been presented? Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

I am concerned that in §3.4.5, the various compartmental, PBPK, etc., model equations are displayed with specific numerical parameters (see, e.g., pp. 136+), but without any indication of the variation/error/uncertainty in these numerical values. How are these numbers derived? If from data, what are the data, and what statistical methods are used to estimate the parameters? And most importantly, what level of statistical uncertainty/error can be assigned to the point estimates? (Admittedly, much of this information can be delegated to an Appendix.) Perhaps I missed where this was presented – but if so, better signposting or textual emphasis on these details is warranted.

34. Is there adequate discussion of the differences in toxicokinetics between humans and animals?

Discussion seemed reasonable.

35. Is there an adequate discussion of the relevance of animal toxicokinetic information for humans?

Discussion seemed reasonable.

36. If applicable, is there a discussion of the toxicokinetics of different forms of the substance (e.g., inorganic vs. organic mercury)?

Discussion seemed reasonable.

37. Are the biomarkers of exposure specific for the substance or are they for a class of substances?

Discussion appeared to highlight and focus on Uranium.

38. Are there valid tests to measure the biomarker of exposure? Is this consistent with statements made in other sections of the text?

Discussion seemed reasonable, given limitations of available data.

39. Are the biomarkers of effect specific for the substance or are they for a class of substances?

Discussion appeared to highlight and focus on Uranium.

40. Are there valid tests to measure the biomarker of effect? Is this consistent with statements made in other sections of the text?

Discussion seemed reasonable, given limitations of available data.

41. Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites?

Discussion seemed reasonable, given limitations of available data.

42. If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions?

Discussion seemed reasonable.

43. Is there a discussion of populations at higher risk because of biological differences which make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?

Discussion seemed reasonable. I am unable to judge quality or extent of population choice(s).

44. Is the management and treatment specific for the substance, or is it general for a class of substances?

Discussion appeared to highlight and focus on Uranium.

45. Is there any controversy associated with the treatment? Is it a "well accepted" treatment?

Unable to judge.

46. Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

Unable to judge.

47. Are treatments available to prevent the specific substance from reaching the target organ(s), or are the actions general for a class of substances?

Unable to judge.

48. Is there any controversy associated with the treatment? Is it a "well-accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?

Unable to judge.

49. Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

Unable to judge.

50. Are there treatments to prevent adverse effects as the substance is being eliminated from the major organs/tissues where it has been stored (e.g., as a substance is eliminated from adipose tissue, can we prevent adverse effects from occurring in the target organ[s])?

Unable to judge.

51. Are treatments available to prevent the specific substance from reaching the target organ(s), or are the treatment's actions general for a class of substances?

Unable to judge. (Discussion seemed reasonable.)

52. Is there any controversy associated with the treatment? Is it a "well accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?

Unable to judge. (Discussion seemed reasonable.)

53. Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

Unable to judge.

54. Do you know of other studies that may fill a data gap?

No.

55. Are the data needs presented in a neutral, non-judgmental fashion? Please note where the text

shows bias.

Generally, yes.

56. Do you agree with the identified data needs?

Yes. However, on p. 160, I would propose that data for construction of dose-response curves should be of sufficient quality to allow for calculation and use of modern BMD/BMC technologies. Calculation of NOAELs is an outdated and substandard approach. See Question #13.

57. Does the text indicate whether any information on the data need exists?

Discussion seems reasonable.

58. Does the text adequately justify why further development of the data need would be desirable; or, conversely, justify the "inappropriateness" of developing the data need at present? If not, how can this justification be improved.

Discussion seems reasonable. However, see Question #56.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

59. Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables? Please provide appropriate references for your additions or changes.

Unable to judge. (I did find this Chapter very well-written and informative.)

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

60. Are you aware of any information that is wrong or missing? If so, please provide copies of the references and indicate where (in the text) the references should be included.

Unable to judge issues of production, use, and disposal.

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

61. Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

Discussion seems reasonable. I am unable to comment on other relevant information.

62. Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

Discussion seems reasonable. I am unable to comment on other relevant information.

63. Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

Discussion seems generally reasonable. Note, however, on p. 194 that a "population mean [of] 0.8 pCi/L" is likely not what is intended. (Perhaps "sample mean"?)

64. Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

Discussion seems reasonable. I am unable to comment on population selection.

65. For Sections 6.8.1, Identification of Data Needs and 6.8.2, Ongoing Studies, answer the same questions presented in Section 3.12.2, Identification of Data Needs and 3.12.3, Ongoing Studies.

All components of §6.8.1 seemed reasonable. Only 1 sentence is given for §6.8.2, which seemed reasonable.

CHAPTER 7. ANALYTICAL METHODS

66. Are you aware of additional methods that can be added to the tables? If so, please provide copies of appropriate references.

Unable to judge.

67. Have methods been included for measuring key metabolites mentioned previously in the text?

Unable to judge.

68. If unique issues related to sampling for the substance exist, have they been adequately addressed in the text? What other discussion should be provided?

Unable to judge.

69. For Section 7.3.1, Identification of Data Needs, answer the same questions presented in Section 3.12.2, Identification of Data Needs.

All components seemed reasonable. I encourage the call (p. 217) to improve sensitivity to accurately measure low levels of radio-nucleotides (ostensibly, to overcome/avoid problems of limits of detection).

CHAPTER 8. REGULATIONS AND ADVISORIES

70. Are you aware of other regulations or guidelines that may be appropriate for the table? If so, please provide a copy of the reference.

No. But, I reiterate my recommendation to begin immediate migration from NOAEL/LOAELs towards more modern PODs such as the BMDL; see Question #13.

CHAPTER 9. REFERENCES

71. Are there additional references that provide new data or are there better studies than those already in the text? If so, please provide a copy of each additional reference.

n/a

UNPUBLISHED STUDIES (IF APPLICABLE TO REVIEW)

72. For each of the unpublished studies included with the profile, prepare a brief evaluation that includes your assessment of the:

72a. Adequacy of design, methodology, and reporting;
72b. Validity of results and author's conclusions; and
72c. Study inadequacies or confounding factors.
73. Provide a summary of your conclusions? Do you agree or disagree with those of the author? If not please explain why.

n/a (as far as I could tell)

Selected references on limitations of the NOAEL/LOAEL

Crump, K. S. (2008). Benchmark analysis. In *Encyclopedia of Quantitative Risk Analysis and Assessment*,***1***, Melnick, E. L. and Everitt, B. S. (eds.), 145-149. Chichester: John Wiley & Sons.

Davis, J. A., Gift, J. S., and Zhao, Q. J. (2011). Introduction to benchmark dose methods and U.S. EPA's Benchmark Dose Software (BMDS) version 2.1.1. *Toxicology and Applied Pharmacology*, in press. doi: 10.1016/j.taap.2010.10.016

Falk Filipsson, A., Sand, S., Nilsson, J., and Victorin, K. (2003). The benchmark dose method - Review of available models, and recommendations for application in health risk assessment. *Critical Reviews in Toxicology* **33**, 505-542.

Faustman, E. M., and Bartell, S. M. (1997). Review of noncancer risk assessment: Applications of benchmark dose methods. *Human and Ecological Risk Assessment* **3**, 893-920.

Foronda, N. M., Fowles, J., Smith, N., Taylor, M., and Temple, W. (2007). A benchmark dose analysis for sodium monofluoroacetate (1080) using dichotomous toxicity data. *Regulatory Toxicologyand Pharmacology* **47**, 84-89.

Gift, J. S., McGaughy, R., Singh, D. V., and Sonawane, B. (2008). Health assessment of phosgene:

Approaches for derivation of reference concentration. *Regulatory Toxicology and Pharmacology* **51**, 98-107.

Hansson, S. O. (2002). Replacing the no-effect level (NOEL) with bounded effect levels (OBEL and LEBEL). *Statistics in Medicine* **21**, 3071-3078.

Leisenring, W., and Ryan, L. (1992). Statistical properties of the NOAEL. *Regulatory Toxicology and Pharmacology* **15**, 161-171.

Kodell, R. L. (2009). Replace the NOAEL and LOAEL with the $BMDL_{01}$ and $BMDL_{10}$. *Environmental and Ecological Statistics* **16**, 3-12.

Öberg, M. (2010). Benchmark dose approaches in chemical health risk assessment in relation to number and distress of laboratory animals. *Regulatory Toxicology and Pharmacology* **58**, 451-454.

Sand, S., von Rosen, D., Victorin, K., and Falk Filipsson, A. (2006). Identification of a critical dose level for risk assessment: Developments in benchmark dose analysis of continuous endpoints. *Toxicological Sciences* **90**, 241-251.

Sand, S., Victorin, K., and Falk Filipsson, A. (2008). The current state of knowledge on the use of the benchmark dose concept in risk assessment. *Journal of Applied Toxicology* **28**, 405-421.

Suter, G. W. (1996). Abuse of hypothesis testing statistics in ecological risk assessment. *Human and Ecological Risk Assessment* **2**, 331-347.

West, R. W., and Kodell, R. L. (2005). Changepoint alternatives to the NOAEL. *Journal of Agricultural, Biological, and Environmental Statistics* **10**, 197-211.

Comments Received from

Peer Reviewer #2

Review of Draft 2 of Uranium Profile for ATSDR/CDC

by

Reviewer #2

1. *As implied, there is a significant need for studies on the toxic effects of U in children. Unfortunately, speculation and paralleling the effects of U in children from data in adult humans and animal studies serve currently as the main tool for estimating and predicting adverse effects of U in children. Since U does target bone and marrow spaces, especially during growth and remodeling phases, and since most of bone growth occurs prior to the age of 21, special considerations and careful allowances should be made in reference to this potential sensitive group of humans.*
2. *The opinion of this reviewer is that avoidance behaviors implemented by parents would serve well as a means to minimized exposure of a potential sensitive sector of society (i.e. children) to U. Some of these avoidance behaviors, and recommendation for alternate behaviors, are provided in the profile.*

CHAPTER 1: PUBLIC HEALTH STATEMENT:

Summary Statement (Sections 1.1 -1.10): The content of this section is readable by the lay public. It is on the whole informative and logically arranged. This section appears to be adequate and consistent with Public Health Statements for other Profiles. The sections of this chapter are presented in a logical and informative manner. Some sections are not as complete as one would want, implying future needs. Although questions may arise in the mind of some lay persons, there is not much more to add to this chapter, without data from more complete studies.

Some questions and issues in this section are outlined below.

In section 1.2 – Under SOURCES: What about uranium (U) found in wells drilled for drinking water, particularly in U-containing rock formations?...Deaths caused by co-exposure to U and arsenic (As) in wells for drinking water have been reported in the Halifax NS (Canada; personal communication with a clinical pathologist in Halifax). Although the precise agent cause death was difficult to determine, it would seem appropriate to include both surface and sub-surface water as potential sources of U since similar U (and As)can be co-localized in areas of significant rock-formation.

CHAPTER 2:

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO URANIUM IN THE UNITED STATES:

-This section reads well and seems appropriate in content.

2.2 SUMMARY OF HEALTH EFFECTS:

-Overall, this section is written well and provides the preponderance of currently available data. Concerns and suggestions are provided below:

-One concern about the lack of associated carcinogenicity is time. No mention of the potential effect of length of time of exposure on the potential for carcinogenic effects in bone and bone marrow. If the conclusion the author makes early in this section about U not being carcinogenic, I think some reference to the length of time subject were studied to come up with this conclusion. Cancer effects are sometimes delayed by decades following or during exposure to certain compounds. Some clarification of this issue would be helpful to the reader.

-Some of the biomarkers cited for use as indicators of toxic effects in the kidneys are not necessarily specific for U. Additional studies need to provided showing whether clearer relationships exist between the urinary excretion of transaminases such as ALT (alanine aminotransferase) and AST (aspartate aminotransferases, brush border enzymes γ-GT (gamma-glutamyltransferase) and AP (alkaline phosphatase) and the cytosolic enzyme LDH (lactate dehydrogenase) with proximal tubular injury. After all, the predominant manner by which these enter the urinary compartment is by cell death along the proximal portions of the nephron. In rodents exposed to a number nephrotoxicants tend to demonstrate correlative relationships between the urinary excretion of these enzymes and the level of proximal tubular injury. Dr. Paul Morrow at the U. of Rochester published some his urinary enyzymology findings in criteria documents published by DOE back in the early 1980s. However, biopsy samples for histopathology serve as the gold standard. Clearly, utilizing these measure may prove to be difficult in humans under most sampling conditions (i.e. spot urine sampling).

-It must be kept in mind that elevation in BUN and/or plasma creatinine are generally not going to occur until 75-80 of the functional renal mass has been acutely or chronically compromised. Care should be taken to separate out criteria indicating some level of acute or chronic proximal tubular necrosis vs. necrosis resulting in acute and/or chronic renal failure!

-An additional point relates to potential differences in sensitivity to various forms of U among different experimental animals. This is important in that dose-effect relationships for a number of nephrotoxicants vary greatly among a number of mammalian species.

-Although implied by the presented data, a very important issue not addressed directly is whether uranyl ions have the ability to cross the placental barrier and enter into fetuses. The fetus may prove to be one of the more sensitive to the toxic effects of U. Additional studies may link possible carcinogenic effects of U to the delicate hematopoietic stem-cells in the liver and marrow of developing bones.

2.3 MINIMAL RISK LEVELS (MRLs):

-This section appears to present a preponderance of relevant data on routes and types of exposure to U and the associated provided MRL(s).

CHAPTER 3: HEALTH EFFECTS:

- NOAELs and LOAELs reported appear to be appropriate and to date.

- In general, without reading the original manuscripts, evaluation of statistical analyses used is difficult to assess. Authors publishing data, especially from animals and in vitro studies, in the bio-medical-health journals, tend NOT to provide appropriate details on the nature of statistical evaluations performed. For example, very few provide information on selection of subjects, whether data fall within the realms of normal (or Gaussian) distributions and whether variances were statistically similar. Unlike statistical and epidemiological journals, values from statistical tests are not provided (such as t- or F-values, SS or sum of squares-values). Too many opinions are generated from manuscripts in which investigators used improper statistical analyses! This point applies more to investigative animal studies, particularly in older studies. In general, this important issue is impossible to assess for this profile due to the time allotted for review and nature of its presentation.

3.1 INTRODUCTION:

*-On page 33, line 22: The correct word to describe a toxic element or molecule not generated or made in/by a living organism is "**nephrotoxicant**".*

-One page 38, line 20: I would strongly suggest to state that the nephrotoxic effects of U occur mainly in the "proximal portions" of the renal tubule (nephron would be more precise).

-On Page 39. It is important to convey the fact that U tends to behave as an acute nephrotoxicant. This point and the fact that during chronic exposure additional variables may be involved, which may mask the deleterious effects of U.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE:

Toxicity- Quality of Human Studies:

-Better human studies are clearly needed. With this in mind, the human studies appear to have adequately discussed. Some of the section leaves the reader with significant questions about whether U does have toxic metal-based or radioactive effects. This may be inherent in the nature of the studies and the lack of important information lacking.

Toxicity- Quality of Animal Studies:

-As might be expected, the toxicological data from animals are more informative, but are also lacking. This again is likely due to the nature of the body of research done.

Level of Significant Exposure (LSA) Tables and Figures:

-The LSAs data in the text figures and tables appear appropriate.

3.3 GENOTOXICITY:

-Page 108, line 20: U does NOT have to localize in the gonads for it to be genotoxic! Genotoxicity could be associated with stem cells along the GI tract and stem cells in other body compartments, including bone marrow, which may be exposed to significant amounts of U.

-Overall this section seems to behave covered adequately.

3.4 TOXICOKINETICS:

-Page 114, line 20: "were" should be substituted for "was". Data is the plural form of datum, thus requiring the past-tense of the verb "were".

-Very little is mentioned about uranyl acetate. Are there experimental or human data pertaining to the inhalation and toxic effects of uranyl acetate associated with individuals using transmission electron microscopy? The acetate form of U has been utilized since the introduction of electron microscopy for allowing one to make cytological features more visible (electron dense).

-The remainder of the discussion of the toxicokinetics of U, i.e. depth and completeness, is consistent with that of other profiles.

3.5 MECHANISMS OF ACTION:

-Page 140, Line 7: Nephrotoxicity is NOT a measure of toxicity. It is an inherent entity. Moreover, nephrotoxicity is not induced. A nephropathy is induced. This should be corrected.

-On the whole, very little is understood about the mechanisms of action of U. Most of the data available provide only a implication of mechanisms. This notion is inferred in this section. As indicate by the section of Animal-to-Human extrapolation, the renal and pulmonary systems are significant targets for the adverse (toxic) effects of U as a metal. However, as stated, the toxic effects of U on the kidney are variable among species. This is not that surprising. The nephrotoxic effects of other heavy metals are also variable among various species, even among rodents (rabbit, rat and mouse).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS:

-As written, only limited data are available regarding the possible estrogen mimicking effects of U.

3.7 CHILDREN'S SUSCEPTIBILITY:

-The bolded section is appropriate and is worded well. My only additional comments about susceptibility of children to U were made above in section 2.2. Some of the data presented in this section tend to support the potential of U to cross the placental barrier and affect adversely the developing fetus.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT:

-Biomarker for the detection of the presence of U within an individual are covered and are discussed appropriately. However, some of the biomarkers used to assess organ-specific injury are not specific for

U. Many of the biomarkers discussed are also markers of injury induced by other heavy metals and various organic chemicals. Numerous measures of organ-specific injury are in many situations impractical, if not impossible, to obtain. This point is covered marginally.

3.8 INTERACTIONS WITH OTHER CHEMICALS:

-Very limited information appears to be available.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE:

-Although the author is correct that a sensitive group of individuals that may be particularly susceptibility to the toxic effects of U are individuals with impaired renal function, impairment of renal function is generally not detectable before one loses about 75-80% of their functioning renal mass. Individuals with low levels of reduced renal mass may also be at risk due to compensatory metabolic changes occurring in the remaining functional renal mass. Current statistics from various private and Federal agencies indicate that about 17% of the US population suffers with various forms chronic renal disease (CRD). This percentage covers renal diseases induced by hypertension, diabetes and autoimmune diseases. Consequently, a large subpopulation of US residents may be at greater risk of intoxication by U, assuming the potential for exposure. The author may want to stress this point.

3.11 METHODS FOR REDUCING TOXIC EFFECTS:

-As implied, limited efficacious treatments are available for reducing the burden and toxic effects of U.

3.12 ADEQUACY OF THE DATABASE:

Existing Information on Health Effects of URANIUM:

-This section adequately conveys the body of existing information of the health effects of U.

Identification of Data Needs:

-This section is fairly well developed and presented. The section implies needs for study rather than discussing them directly, which is appropriate.

CHAPTER 4: CHEMICAL AND PHYSICAL INFORMATION:

-This section is covered appropriately.

CHAPTER 5: PRODUCTION, IMPORT/EXPORT, USE AND DISPOSAL OF URANIUM:

-This section covers the relevant information regarding production, import/export, use and disposal succinctly and appropriately.

CHAPTER 6: POTENTIAL FOR HUMAN EXPOSURE:

-This section appears to thoroughly cover available data. The only question this reviewer has pertains to available data on the content of U in the water of private wells drilled in areas of higher underground quantities of U.

CHAPTER 7: ANALYTICAL METHODS:

-Methods used (and their limitations) to detect U in vivo and in vitro are covered adequately. The section also provides an appropriate recommendation for improved methods for determining the effects of U in tissues and organs affected by U. No ongoing studies were identified.

CHAPTER 8: REGULATIONS AND ADVISORIES:

-As discussed, national and international regulations, advisories and guidelines for U in air, water and other media are provided and summarized in Table 8-1.

CHAPTER 9: REFERENCES:

-References appear to be quite comprehensive and up to date.

CHAPTERS 10 (GLOSSARY) and APPENDICES:

-The glossary and appendices for this profile appear complete and informative.

SUMMARY STATEMENT:

Overall, the 2nd draft of the profile for U is written clearly and is quite comprehensive. Although there are a few suggestions for change, the document presents a rather comprehensive summary of the preponderance of relevant data on U.

Comments Received from

Peer Reviewer #3

PEER REVIEW OF ATSDR
TOXICOLOGICAL PROFILE – URANIUM
Reviewer #3

SUMMARY REVIEW

In addition to these summary comments, editorial changes have made in the copy of the Profile.

CHAPTER 1. PUBLIC HEALTH STATEMENT

Track changes have been made to suggest a more readable document for the lay public.

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

Section 2.1 Background **and Environmental Exposures to Uranium in the United States.**
This section devotes many lines to the physical aspects of uranium and the deposition and clearance of particles from the lung. Few lines are devoted to where the US or what environments have relatively higher concentrations of uranium and where the uranium goes once it is in the body. I would like to see some of that information come in from the chapters on Potential for Human Exposure and Toxicokinetics. At the very least, those chapters could be referenced in Chapter 2.

Section 2.2 Summary of Health Effects
I agree with the effects in humans and animals discusses in the chapter. However, the summary gets off to a rocky start. The first paragraph states that there are no cancerous effects of uranium because it is not very radioactive but also suggests that the lungs and cardiovascular system might be affected (by radiation?). The discussion of cancer should come at the end, not lead off. The second brief paragraph talks about chemical toxicities and finishes with mentioning sensitive targets of toxicity. This paragraph should give more detail and spell out in more detail what the target organs are so we will know what is coming.

CHAPTER 3. HEALTH EFFECTS

Section 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

The Summary section of Chapter 3 is well prepared and written. The mention of linear, non-threshold assumptions and risk benefit analysis vs., cancer death predictions are helpful. Many people are unaware of these issues.

Toxicity - Quality of Human Studies

The review of both the human and animal studies of the carcinogenic effects of uranium are generally well presented, but certain points in the text and corresponding LSE figure need consideration. One glaring omission is the lack of mention in the text of the

Cookfair publication on lung cancer incidence in U processing workers (although it was presented in the Summary Toxicity Tables). The paper is used to establish a CEL for lung cancer in humans. It needs to be explained why a CEL is needed for cancer effects in humans when none has been identified to date, as noted in the text and by BEIR and UNSCEAR publications, to name just two. It also needs to be discussed why this epidemiologic study was chosen for the LSE figure. I question the quality of the Cookfair data. The work was published in the proceedings of a Health Physics Society meeting in 1983. In the text of the paper a new study was mentioned that would increase the size of the cohort by about two thirds. I could not find more recent publications from this group reporting on the results using the enlarged cohort that might determine whether the lung cancer risk of the younger hire group approaches that of the older group. This epidemiologic study is the only one that illustrates an increase risk of lung cancer in U workers. It is curious that the data has not been used by any of the regulatory groups. The authors should be queried on the status if this effort published nearly 30 years ago and if they still stand on the results.

-

Levels of Significant Exposure (LSE) Tables and Figures

With the help of the "User's Guide", the LSE tables and figures do stand alone.
Two points in the figures need to be examined. The NOAEL for renal effects after intermediate duration inhalation exposure to U is higher than the LOAEL. This can be explained by the fact that the NOAEL is based on insoluble UO2 and the LOAE is based on the soluble UF. However, this is not obvious looking at Figure 3-1 and is not emphasized in the text that discusses the renal effects after intermediate duration exposure.

The second point is the characterization of the "dose" used for the CEL in the Cookfair study and plotted in Figure 3-1. Apparently it is 20 rad, the lower boundary in the High Exposure group that ranged from 20 to 75 rads. Plotting rads on a graph that used mg/m3 for all other endpoints is misleading and unacceptable. It should be possible to calculate the mass of U in the lung and estimate the exposure concentration of U.

I agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables. The derived MRLs appear appropriate.

Evaluation of Text

There is a massive amount of data on the toxicity of uranium that has been collected over a period of nearly 75 years. Presenting the data in an understandable way is a challenge. The format of these Toxicological Profiles, categorization of effects by route of exposure, encourages repeated presentation of the effects using the same or similar references. Another approach is to discuss routes of exposure, toxicokinetics and distribution in the body followed by discussion of the effects related to the affected organ systems. This should reduce, for example, the repetition of the isotopes of U, the radiation vs. chemical

toxicity of U, the explanation of the deposition of particles in the lung and the discussion of the reason HF is so acutely toxic.

One of these repeated comments, which implies that U dust may cause emphysema, is misleading because occupational exposures to metal dusts do not show that. On Page 14 line 25 and P 47 line 2: "In acute exposures pulmonary damage may be limited to interstitial inflammation of the alveolar epithelium leading eventually to emphysema and pulmonary fibrosis." The text continues noting that the respiratory diseases in uranium miners may be aggravated by mine dusts. A list of references are presented, however, half are not relevant. A key reference here should be the book *Pathology of Occupational Lung Disease*, 2nd edition, by A.Churg and F,H.Y. Green, Williams and Wilkins, Baltimore, 1998. They point out that emphysema is not a feature of inhaled metals and metal compounds. The one inhaled dust that is associated with a form of emphysema is coal dust, but high concentrations in the lung are required for this to happen. For references, I recommend Churg (for lung diseases), Samet 1994 (for silicosis) in addition to Dungworth, Saccomanno and Waxweiler already in the text. The other references in the text do not really deal with the issue of uranium in the lung.

Section 3.4 TOXICOKINETICS

This section is well presented. Some of this material should be brought forward to Chapter 2.

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

This chapter is well done and parts should be mentioned in Chapter 2.

II. Annotated Pages from the Draft Profile Document

Annotations Received from

Peer Reviewer #3

DRAFT
TOXICOLOGICAL PROFILE FOR URANIUM

Reviewer #3

Formatted: Centered

Prepared by:

SRC, Inc.
Under Contract No. 200-2004-09793

Prepared for:

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

January 2011

1.1 WHAT IS URANIUM?

Description	Uranium is a naturally occurring radioactive element. Natural uranium is a mixture of three isotopes: ^{234}U, ^{235}U, and ^{238}U. The most common isotope is ^{238}U; it makes up about 99% of natural uranium. All three isotopes behave the same chemically, but they have different radioactive properties. The half-lives of uranium isotopes (the amount of time needed for half of the isotope to give off its radiation and change into a different element) is very long (4.5 billion years for ^{238}U). The industrial process called enrichment is used to increase the amount of ^{234}U and ^{235}U and decrease the amount of ^{238}U in natural uranium. The product of this process is enriched uranium and the leftover is depleted uranium.
Uses	Main civilian use of uranium is for fuel in nuclear power plants and as ballast on helicopters and airplanes. Depleted uranium is also used by the armed forces as shielding to protect Army tanks and parts of bullets and missiles to help them go through enemy armored vehicles.

For more information about the properties and uses of uranium, see Chapters 4, 5, and 6.

1.2 WHAT HAPPENS TO URANIUM WHEN IT ENTERS THE ENVIRONMENT?

Sources	Uranium can be released into the environment through natural processes including wind and water erosion and volcanic eruptions. Industries involved in mining, milling, and processing of uranium can also release it into the environment.

1.4 HOW CAN URANIUM ENTER AND LEAVE MY BODY?

Enter your body • Inhalation • Ingestion • Dermal contact	About 0.76–5% of the uranium you breathe will enter the bloodstream through the lungs. With time, the more poorly dissolved uranium is remove up the airways, swallowed, and excreted through the gastrointestinal tract Less than 0.1–6% of the uranium you ingest will enter the bloodstream through the gastrointestinal tract. Uranium compounds that dissolve in water enter the bloodstream more easily than uranium compounds poorly soluble in water. Unabsorbed uranium is excreted in the feces. Uranium can be absorbed through the skin; water-soluble uranium compounds are the most easily absorbed.
Leave the body	Most of the uranium that enters your body goes to bone, liver, and kidney. It takes 11 days to reduce the amount of uranium in bone by half. It takes 2–6 days for the same to happen in the kidney. Uranium that reaches the bloodstream leaves your body in the urine. Most ingested uranium leaves the body in the feces.

For more information about how uranium can leave your body, see Chapter 3.

1.5 HOW CAN URANIUM AFFECT MY HEALTH?

This section looks at studies concerning potential health effects in animal and human studies.

Humans	The main target for inhaled uranium in humans is the kidneys. Workers exposed to uranium hexafluoride in the air have also experienced respiratory irritation and accumulation of fluid in the lungs. However, these effects were attributed to the irritant hydrofluoric acid. Oral exposure to elevated amounts of uranium has also produced alterations in the kidneys. Evaluations of Gulf War veterans who retained depleted uranium shrapnel fragments have shown no consistent alterations in renal and liver function, hematological parameters, sex hormone levels, sperm parameters, bone function, neurocognitive tests, and genotoxicity.
Animals	The kidney is also the main target for uranium toxicity following inhalation, oral, or dermal exposure. In addition, inhalation exposure also produced alterations in the respiratory tract. Oral exposure studies in animals have shown that water-soluble uranium compounds will result in kidney effects at lower doses than following exposure to insoluble uranium compounds. Prolonged oral administration of uranium to rats has induced neurobehavioral changes as well as changes in the levels of certain chemicals in the brain. Uranium affected fertility in male rats and mice. Treating male rats or mice with uranium for a few weeks before mating with untreated females resulted in a reduced number of babies being born. Application of uranium compounds to the skin of animals has produced skin irritation and mild skin ~~lesions~~ damage.
Cancer	There is no conclusive evidence suggesting the

2. RELEVANCE TO PUBLIC HEALTH

Field Code Changed
Field Code Changed

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO URANIUM IN THE UNITED STATES

Uranium is an alpha-emitting, radioactive, heavy metal that occurs naturally in the earth's crust at an average concentration of about 2 ppm (approximately 1 pCi/g). Uranium exists in several isotopic forms. The most toxicologically important forms are ~~anthropogenic~~ man-made ^{232}U and ^{233}U and naturally occurring ^{234}U, ^{235}U, and ^{238}U. Uranium isotopes decay by alpha emission. ^{238}U decays through 16 radioactive progeny, including ^{234}U, to reach stable lead-206 (^{206}Pb), while ^{235}U decays through 13 radioactive progeny to reach stable ^{207}Pb. This profile discusses the chemical and radiological health effects of isotopes of uranium (natural, enriched, and depleted) and the various compounds in which uranium is usually found. The health effects of daughter radioactive elements (radium and radon) are addressed in other toxicological profiles (consult the ATSDR toxicological profiles for radium and radon for more information regarding these radioactive elements).

Naturally occurring uranium is an isotopic mixture containing a large percentage of ^{238}U and very small percentages of ^{234}U and ^{235}U, by mass. The industrial process called enrichment is used to increase the percentage of ^{235}U and decrease the percentage of ^{238}U in natural uranium; enrichment also increases the percentage of ^{234}U. This results in a continuum of additional isotope mixtures in which the percentage of ^{235}U is either larger (enriched uranium) or smaller (depleted uranium) than that of natural uranium. Natural uranium consists of 99.284% ^{238}U, 0.711% ^{235}U, and 0.005% ^{234}U by weight (49% ^{238}U, 2% ^{235}U, and 49% ^{234}U by radioactivity) and has a very low specific activity (0.68 µCi/g). Uranium enrichment for commercial nuclear energy produces uranium that contains about 3% ^{235}U; this is called 3% enriched uranium. Uranium enrichment for other purposes, including nuclear weapons production, can produce uranium containing as much as 97.3% ^{235}U and having a higher specific activity (~50 µCi/g). Depleted uranium is the byproduct of the enrichment process. Depleted uranium has even less specific activity (0.33 µCi/g) than natural uranium.

Uranium is present in the body at very low or trace concentrations and is not known to be an essential element. Human intakes are constant through very small amounts of natural uranium in food and water, and even smaller amounts in air. The following anthropogenic activities increase the potential for human exposure to uranium: mining, milling, and handling uranium; processing uranium ore end products (uranium dioxide, uranium hexafluoride); producing nuclear energy and nuclear weapons; producing phosphate fertilizers from phosphate rocks that contain much higher-than-average levels of uranium; and

improperly disposing of wastes. Occupational exposure to airborne uranium ore dust occurs in uranium mines and mills and in processing plants. Typically, uranium represents only 0.2–5% by weight of the ore.

The deposition of inhaled dust particles in the lungs depends on the particle size and the absorption depends on the solubility of the compound. Particle size determines the point of deposition site of pulmonary-inhaled aerosols. The pulmonary deposition site is an important factor in determining the toxicity of an aerosol. Small particles (≤2 µm activity median aerodynamic diameter [AMAD]) are deposited in the deep respiratory tract. Larger particles are deposited in the tracheobronchial region, where they are transported by mucociliary action to the throat and swallowed into the gastrointestinal tract where absorption is minimal. The less soluble compounds are more likely to remain in the lung tissue and associated lymph ~~glands~~ nodes either for weeks (uranium trioxide, uranium tetrafluoride) or years (uranium dioxide, triuranium octaoxide), resulting in significant pulmonary retention in inhalation-exposure toxicity and a greater dose of alpha radiation. Long-term retention of inhaled particles of insoluble compounds can cause pulmonary ailments if the dose is sufficiently high.

Dust particles that have deposited are rapidly transported out of the tracheobronchial region by mucociliary action and swallowed. The more soluble compounds are more likely to be absorbed into the blood at the alveolar level within days. Regardless of solubility, a portion of uranium quickly reaches the systemic circulation and the kidney, where it is cleared from the body in the urine. Ingested uranium that has been cleared from the lungs by mucocilliary action and swallowed is only partly absorbed into the blood. This is true even for the more common soluble salts (uranium hexafluoride, uranyl fluoride, uranium tetrachloride, uranyl nitrate hexahydrate). Uranium is usually found in compounds that can break down and recomplex to form other compounds. In body fluids, tetravalent uranium is likely to oxidize to the hexavalent form, followed by formation of the uranyl ion. Uranium generally complexes with citrate, bicarbonates, or protein in plasma.

According to the International Commission on Radiological Protection (ICRP 1995), the more soluble compounds (uranium hexafluoride, uranyl fluoride, uranium tetrachloride, uranyl nitrate hexahydrate) are more likely to be absorbed into the blood from the alveoli within days and are assigned to inhalation Type F (fast dissolution). The less soluble compounds (uranium tetrafluoride, uranium dioxide, uranium trioxide, triuranium octaoxide) are more likely to remain in the lung tissue and associated lymph ~~glands~~ nodes for weeks and are designated Type M (medium dissolution). The relatively insoluble compounds (uranium dioxide, triuranium octaoxide) may remain in the lungs for years and are designated Type S

(slow dissolution). ~~The ICRP (1995) recommends the following absorption factors for humans for inhaled compounds that subsequently enter the gastrointestinal tract: 2% for soluble compounds and 0.2% for less soluble compounds.~~

~~The main site of long-term retention for inhaled soluble uranium compounds (uranyl nitrate, uranium tetrachloride, uranium dioxide) and ingested uranium compounds is the bone, while the inhaled insoluble compounds (uranium tetrafluoride, uranium dioxide) that are deposited in the deep respiratory tract tend to accumulate in the lungs and pulmonary lymph nodes.~~

Ingested uranium is excreted mostly in the feces. The absorption factors for uranium compounds in the gastrointestinal tract of humans is 2% for soluble compounds and 0.2% for less soluble compounds (ICRP 1995). Uranium that enters the blood stream is excreted in the urine by the kidney. ~~urinary excretion is generally low.~~ The biological half-times of soluble uranium compounds (uranium hexafluoride, uranyl fluoride, uranium tetrachloride, uranyl nitrate hexahydrate) are estimated in days or weeks; those of the less soluble compounds (uranium tetrafluoride, uranium dioxide, triuranium octaoxide) are estimated in years. No information is currently available on the excretion of dermally absorbed uranium. Transdermally absorbed uranium and uranium released from embedded fragments is expected to behave identically to uranium compounds absorbed through the lungs and the gastrointestinal tract.

The main site of long-term retention for inhaled soluble uranium compounds (uranyl nitrate, uranium tetrachloride, uranium dioxide) and ingested uranium compounds is the bone, while the inhaled insoluble compounds (uranium tetrafluoride, uranium dioxide) that are deposited in the deep respiratory tract tend to accumulate in the lungs and pulmonary lymph nodes.

2.2 SUMMARY OF HEALTH EFFECTS

Because the specific activities of radiation ~~of~~ in natural and depleted uranium are low, no remarkable noncancerous radiological health hazard is expected (and none has been observed) from exposure to natural and depleted uranium. The results of the available studies in humans and animals are consistent with this conclusion. According to the BEIR IV report, if uranium's radiation were carcinogenic in humans, the most likely carcinogenic effect in humans would be bone sarcoma. However, even highly-enriched uranium has not been found to produce cancer, including that of the bone, in exposed humans. Evidence from animal studies suggests adverse effects reported from such exposures include damage to the epithelium of the lungs (fibrosis) and cardiovascular abnormalities (friable vessels).

The chemical action of all isotopes and isotopic mixtures of uranium are identical, regardless of the specific activity, because chemical action depends only on chemical properties. Thus, the chemical toxicities of natural, depleted, and enriched uranium are identical. Current evidence from animal studies suggests that the toxicity of uranium is mainly due to its chemical damage to kidney tubular cells, leading to nephritis. Other sensitive targets of toxicity include the respiratory tract (inhalation only), neurological system, reproductive system, and the developing organism.

There are limited data on the renal toxicity of uranium following inhalation exposure in humans. A number of studies found no alterations in mortality due to renal disease in uranium workers (Archer et al. 1973a, 1973b; Checkoway et al. 1988; NIOSH 1987; Polednak and Frome 1981). However, a study of uranium mill workers exposed to uranium found evidence of renal dysfunction (β-2-microglobinuria, aminoaciduria) (Thun et al. 1985); the severity and incidence of the effects appeared to be related to exposure duration. Several epidemiology studies have found associations between parameters of renal dysfunction (e.g., urine levels of albumin, β_2-microglobulin, glucose, and protein HC) and elevated uranium levels in drinking water (Kurttio et al. 2002; Limson Zamora et al. 1998, 2009; Mao et al. 1995; Seldén et al. 2009). These studies did not find overt signs of toxicity and in many cases, the biomarkers of renal dysfunction were within the normal range. Although most of the epidemiology studies provided information on uranium levels in the drinking water, there was often a large range of exposure levels; thus, the human oral exposure studies do not provide reliable dose-response data.

Renal effects have been observed in a number of animal species exposed to various uranium compounds at sufficiently high doses. The observed effects have primarily involved damage to the proximal tubules and have been observed following inhalation (Dygert 1949a, 1949b, 1949d; Roberts 1949; Rothemel 1949; Rothstein 1949a, 1949b, 1949c; Spiegl 1949; Stokinger et al. 1953;), oral (Domingo et al. 1987; Gilman et al. 1998a, 1998b, 1998c; Martinez et al. 2003; Ozmen and Yurekli 1998), dermal (DeRey et al. 1983; Lopez et al. 2000; Orcut 1949) exposures; and from embedded fragments (Zhu et al. 2009). The preponderance of the data on the renal toxicity of uranium come from a collection of experiments conducted in 1949. The results of these studies demonstrate compound- and species-related differences in toxicity. Soluble uranium compounds (e.g., uranyl nitrate, uranyl fluoride, uranium hexafluoride, and uranium tetrachloride) are more toxic than insoluble uranium compounds (e.g., uranium dioxide, uranium peroxide, uranium trioxide, and triuranium oxtaoxide). Renal effects have been observed in animals exposed to aerosols of soluble uranium compounds at concentrations of ≥0.13 mg U/m^3 for intermediate durations (Roberts 1949; Rothstein 1949a; Spiegl 1949; Stokinger et al. 1953). However, no renal effects

were observed in animals exposed to 1.0 mg U/m^3 as insoluble compounds (Rothstein 1949b; Stokinger et al. 1953); the lowest LOAEL was 8.2 mg U/m^3. These data suggest that soluble compounds are at least 5 times more toxic than insoluble compounds. The difference in toxicity is likely due to the more efficient absorption of soluble uranium compounds. Of the animals tested in intermediate-duration inhalation studies, dogs and rabbits are the most sensitive followed by rats, mice, and guinea pigs. The severity of renal lesions increases with increasing exposure concentrations; very slight renal tubular damage is observed at low concentrations and marked degeneration and necrosis ~~and degeneration~~ are observed at higher concentrations. Little differences have been found between the adverse effect levels following 30 days of exposure and 1 year of exposure, suggesting that in animals ~~models~~, the toxicity is not strongly influenced by the duration of exposure. The oral and dermal databases are more limited than the inhalation database. Acute- and intermediate-duration oral studies in laboratory animals (rats, mice, rabbits, and dogs) provide strong support for identifying the kidney as a sensitive target of uranium toxicity. Acute exposure to lethal doses of uranyl nitrate or uranyl acetate resulted in renal dysfunction in rats and mice as evidenced by increases in urine volume, plasma urea, blood urea nitrogen (BUN), and urinary total protein (Domingo et al. 1987; Martinez et al. 2003; Ozmen and Yurekli 1998). Minimal histological alterations in the glomerulus, proximal tubules, and/or interstitium have been observed in rats and rabbits exposed to intermediate-duration doses of soluble uranium compounds as low as 0.05 mg U/kg/day (Berradi et al. 2008; Gilman et al. 1998a, 1998b; Maynard and Hodge 1949; McDonald-Taylor et al. 1992, 1997); the severity of the renal lesions increased with dose. Additionally, a rabbit study demonstrated that the severity of the lesions increased after exposure termination (Gilman et al. 1998c). Due to the poor absorption of ingested insoluble uranium compounds, there are significant differences in the renal toxicity of various uranium compounds. No renal effects were observed in rats exposed to doses as high as 11,000–12,000 mg U/kg/day as uranium dioxide, uranium trioxide, uranyl octaoxide, or uranium tetrafluoride for 30 days (Maynard and Hodge 1949). In contrast, adverse renal effects were observed at doses of 140–270 mg U/kg/day as uranyl nitrate, uranium peroxide, or uranyl fluoride and doses of 440–790 mg U/kg/day as uranium tetrachloride or uranium acetate. As with other routes of exposure, proteinuria, renal failure, and renal lesions were observed in laboratory animals following acute dermal exposure to uranyl nitrate (De Rey et al. 1983; Lopez et al. 2000; Orcutt 1949) and in rats with depleted uranium fragments embedded in the gastrocnemius muscle (Zhu et al. 2009).

General damage to pulmonary structures, usually noncancerous alveolar epithelium damage o~~f type II cells~~, can occur upon inhalation of insoluble reactive chemicals such as some uranium compounds (uranium tetrafluoride, uranium dioxide, uranium trioxide, triuranium octaoxide). In acute exposures, pulmonary damage may be limited to interstitial inflammation of the alveolar epithelium leading

eventually to ~~emphysema or~~ pulmonary fibrosis (~~Clayton and Clayton 1981; Cooper et al. 1982~~; Dungworth 1989; Saccomanno et al. 1982; ~~Wedeen 1992~~). In studies of the pulmonary effects of airborne uranium dust in uranium miners (~~Dungworth~~ 1989; Waxweiler et al. 1983), the respiratory diseases reported were aggravated by the insoluble aerosol particles (mine dust) to which these miners were exposed because most of the noncancerous respiratory diseases reported in these studies were consistent with toxicity of inhalable dust particles other than uranium, such as silica (~~Dockery et al.~~ 1993).

Comment [LU1]: Not related to lung

Comment [LU2]: Not a great reference for this

Comment [LU3]: Not a good reference , no reference to silica

Respiratory effects reported in workers acutely exposed to uranium hexafluoride were caused by hydrogen fluoride, a potent lung irritant and a spontaneous byproduct of uranium hexafluoride (Kathren and Moore 1986; USNRC 1986). Similar to human studies, signs of respiratory irritation (rhinitis and lung edema, hemorrhage, and emphysema) have been observed in animals exposed to high concnerations of uranium hexafluoride, uranyl fluoride, and uranium tetrafluoride (Dygert 1949b; Rothstein 1949a; Speigl 1949). It is likely that these effects were due to co-exposure to hydrogen fluoride. Inhalation exposure to insoluble uranium compounds also results in pulmonary damage. Very slight pulmonary lesions were observed in rats and dogs exposed to uranium trioxide for 4 weeks (Dygert 1949c); mild to severe renal tubular necrosis was also observed at this concentration. In contrast, chronic exposure to uranium dioxide for at least 3.5 years resulted in lung fibrosis in monkey and dogs (Leach et al. 1970, 1973); renal effects were not observed in either species.

In general, studies of uranium workers have not provided evidence of adverse neurological effects, although tests to detect subtle neurological alterations were not conducted (Carpenter et al. 1988; Cragle et al. 1988; Hadjimichael et al. 1983; Kathren and Moore 1986; NIOSH 1987; Polednak and Frome 1981; Reyes et al. 1984; USNRC 1986). Although poorer performance on neurological tests were observed in Gulf War veterans exposed to depleted uranium from embedded shrapnel, the effects were not consistently observed, and were found to be strongly influenced by two subjects with extremely high uranium levels and severely complex co-morbid conditions (McDiarmid et al. 2000, 2001a, 2004b, 2006, 2007, 2009). Frank neurological effects have been observed in animals exposed to lethal inhalation concentrations or oral doses of several uranium compounds (Dygert 1949a; Rothstein 1949a). It is not known if these effects were directly related to uranium exposure. The effect of uranium compounds on neurobehavior and on neurotransmitter levels in various areas of the brain has been investigated in several studies. Uranium increased motor activity in rats exposed to 28 mg U/kg/day and mice exposed to 6 mg U/kg/day in acute-duration drinking water studies (Briner 2009; Briner and Murray 2005). Uranium also increased motor activity in rats dosed with 28 mg U/kg/day for 6 months (Briner and Murray 2005) and

decreased spatial working memory in rats dosed with 2.5 mg U/kg/day as enriched uranium for 9 months (Houpert et al. 2007b). However, these effects were not observed in rats similarly exposed to depleted uranium (Houpert et al. 2007b). It should be mentioned that a study in rats implanted with up to 20 depleted uranium pellets (approximately 760 mg depleted uranium) for 150 days reported no significant alterations in tests of spontaneous motor activity (Arfsten et al. 2007). Investigators have tried to identify biochemical and morphological substrates that, when altered, could explain the behavioral alterations. Increased motor activity showed a weak correlation with increased lipid oxidation in the brain of rats in a 2-week study (Briner and Murray 2005). Oxidative stress was also increased in the brain of rats exposed to ≥5.6 mg U/kg/day for 90 days, but this study did not conduct neurobehavioral tests (Linares et al. 2007). Uranium also altered the levels of neurotransmitters and their metabolites in brain areas from mice (Briner 2009) and rats (Bussy et al. 2006). Of various brain areas examined, the hippocampus from rats exposed to uranium in the drinking water for 90 days had the most uranium (Linares et al. 2007), suggesting that this area may play an important role in the neurobehavioral alterations caused by exposure to uranium. Implantation of depleted uranium pellets in rats resulted in measurable uranium in the brain at 6–18 months after implantation (Pellmar et al. 1999a) and was accompanied by electrophysiological changes in hippocampal slices from the treated animals at 6 and 12 months, but not at 18 months (Pellmar et al. 1999b). The mechanism(s) by which uranium induces neurological alterations is not known, but as is the case with other metals, it could be interfering with calcium by mimicking or blocking its actions. A recent study in rats also suggested that uranium may affect genes involved in cholinergic transmission (Bensoussan et al. 2009). These investigators reported that exposure to uranium seemed to induce transcriptional alterations in the hippocampus aimed at preserving acetylcholine levels, whereas in the cortex, exposure led mainly to translational alterations.

Limited data are available regarding reproductive effects of uranium in humans. Studies of uranium miners, millers, and processors found that male uranium miners had more first-born female children than expected, suggesting that uranium=s alpha radiation damaged the y-chromosomes of the miners (Muller et al. 1967; Waxweiler et al. 1981b; Wiese and Skipper 1986). However, the workers were also exposed to ^{222}Rn, chlorine, hydrofluoric acid, lead sulfate, nickel, nitric acid and nitrogen oxides, silicon dioxide, and sulfuric acid. Longitudinal assessments of relatively small numbers of males who were exposed to depleted uranium in the Gulf War via retained metal shrapnel fragments have not provided evidence of adverse reproductive effects (McDiarmid et al. 2000, 2001a, 2004b, 2007, 2009). Uranium reduced fertility, likely due to reductions in spermatozoa counts, was observed in male mice exposed to ≥5.6 mg U/kg/day in drinking water and mated with untreated females (Llobet et al. 1991). However, fertility was not significantly affected in another study in mice in which males and females were treated by gavage

Comment [LU4]: Did they smoke cigarettes?

with up to 14 mg U/kg/day (Paternain et al. 1989). These apparently discrepant results may be due to the different mode of dosing between the two studies (i.e., gavage vs. drinking water), which may have resulted in different rates of absorption. Uranium also reduced fertility in male rats dosed with 11.2 mg/kg/day and mated with untreated females; the NOAEL was 5.6 mg U/kg/day (Linares et al. 2005). Effects on female reproductive health have also been observed in mice orally exposed to uranium. Alterations in ovarian folliculogenesis and oocyte maturation were observed at ≥1.25 mg U/kg/day (Arnault et al. 2008; Feugier et al. 2008; Kundt et al. 2009). Another study (Raymond-Whish et al. 2007) reported slight alterations in ovarian folliculogenesis in mice at very low doses (≥0.00039 mg U/kg/day in the drinking water). A reduced number of small primary follicles were observed on postnatal day (PND) 5; however, all other follicle populations including primordial, secondary/growing, healthy, and atretic were unchanged. Since effects were reported at considerably lower doses than in other studies, it would be helpful to try to replicate these results. Uranium also showed estrogenic properties in mice at very low doses. Exposure of ovariectomized mice to 0.005 mg U/kg/day for 10 days beginning at 50 days of age significantly increased uterine weight and 0.009 mg U/kg/day significantly accelerated vaginal opening; both responses could be blocked by treatment with an antiestrogenic drug (Raymond-Whish et al. 2007); an increase in the presence of cornified vaginal cells, indicative of an estrogenic effect was also observed at 0.005 and 0.009 mg U/kg/day. Again, it would be helpful to try to replicate these results observed at such low doses. Enriched uranium, but not depleted uranium, increased serum testosterone and the expression of genes involved in steroidogenesis in male rats in a 9-month drinking water study (Grignard et al. 2008). In addition, enriched uranium significantly increased the expression of transcription factors involved in the regulation of steroidogenic genes. These results suggested that the observed effects were mainly due to the radiological activity of the compound.

Developmental effects have been observed in the offspring of mice; these effects have often been observed at maternally toxic doses. The observed effects included lethality, reductions in growth, increase in visceral and skeletal abnormalities, and reproductive effects. Lethality effects consisted of reductions in viability on PND 21 in offspring of mice dosed with 28 mg U/kg/day on gestation day (Gd) 13 to PND 21 (Domingo et al. 1989b), decreases neonatal viability in the offspring of mice exposed to 5.6 mg U/kg/day prior to mating and throughout gestation and lactation (Paternain et al. 1989), and increases in late resorptions and decreases in the number of live fetuses in the offspring of mice exposed to 14 mg U/kg/day prior to mating and during gestation (Paternain et al. 1989). Reductions in fetal body weight were observed in the offspring of mice dosed with ≥2.8 mg U/kg/day on Gds 6–15 (Domingo et al. 1989a). This study also reported significant increases in the total number of external malformations at ≥2.8 mg U/kg/day and the total number of skeletal defects at ≥14 mg U/kg/day. Maternal toxicity may

have played a role in this study since maternal weight gain during exposure was reduced by at least 43%. Alterations in ovarian folliculogenesis, similar to those described in the discussion of reproductive toxicity have been observed in the female pups of mice exposed to uranium prior to mating and/or during gestation (Arnault et al. 2008; Raymond-Whish et al. 2007). In rats, doses of 22.5 or 45 mg U/kg/day administered from before mating until Gd 14 were not fetotoxic; however, continued dosing during lactation resulted in a significant reduction in pups weight on PND 21 (Sánchez et al. 2006). Uranium did not affect developmental landmarks or neuromotor maturation in the pups, but the high dose altered learning and memory. Pups from rats exposed to a smaller dose of approximately 4.3 mg enriched U/kg/day during gestation showed delayed hyperactivity when tested at 5 and 9 months of age (Houpert et al. 2007a). Uranium was also shown to interfere with tooth eruption and development in young rats (Pujadas Bigi and Ubios 2007; Pujadas Bigi et al. 2003).

Uranium has not been implicated in the production of human lung cancer. Since uranium is weakly radioactive, it has been assumed to be potentially carcinogenic at occupational levels by NIOSH. EPA had classified uranium similarly, but has since withdrawn this classification for review. The International Agency for Research on Cancer (IARC) has no classification for uranium. Studies do not indicate any level of uranium carcinogenicity. No significant difference in cancer (of the lungs) was found between workers occupationally exposed to uranium and control populations. Other detailed studies conducted between 1950 and 1967 on the association between uranium mining and an increased incidence of cancer found lung cancer in the miners over 6 times the rate expected. However, the miners were concurrently exposed to other known or potential cancer-causing substances such as radon and its progeny, tobacco smoke, phosgene gas, mercury, and solvents (carbon tetrachloride and trichloroethylene). Radon progeny in the mines, and not the uranium, were clearly identified as the carcinogenic agents. (For further information on cancer risks from radon, refer to the ATSDR *Toxicological Profile for Radon* [Agency for Toxic Substances and Disease Registry 2008.])

Comment [LU5]: Provide some references here. Like BEIR or IARC

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for uranium. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic

Table 2-1 shows the mass equivalents for natural and depleted uranium for radiation levels that caused potential radiological effects in rats exposed once for 100 minutes to airborne 92.8% enriched uranium with an estimated specific activity of 51.6 µCi/g (Morris et al. 1989). These mass equivalent values for natural and depleted uranium for the minimal concentration of radioactivity that is expected to induce potential radiological effects are well above levels that would be expected to be inhaled or ingested. In addition, the mass equivalents for natural and depleted uranium for potential radiological effects are 3,600 and 76,500 times higher, respectively, than the occupational exposure limits (short-term exposure) recommended by NIOSH (1997). Therefore, MRLs for uranium based on studies that used enriched uranium are inappropriate.

Chemically, natural and depleted uranium are identical. Therefore, the MRLs calculated for chemical effects, based on studies that tested natural uranium, are applicable to the chemical actions of depleted uranium because the nature and extent of chemical toxicity are determined only by chemical properties. The health effects associated with exposure to natural and depleted uranium appear to be solely chemical in nature and not radiological; exposure to enriched uranium may also include a radiological component, but the data are limited. Although there are inadequate human data evaluating the toxicity of inhaled uranium, the toxicity of a variety of uranium compounds has been investigated in a number of animal species. Regardless of the exposure duration, the animal data provide strong evidence that kidney damage is the principal toxic effect of uranium and that the toxicity varies according to solubility of the uranium compound, regardless of how it enters the body. Other sensitive end points include the respiratory tract following chronic exposure to insoluble uranium compounds and developmental toxicity following acute oral exposure to soluble uranium compounds. The more soluble uranium compounds (uranium hexafluoride, uranium tetrachloride, uranyl fluoride, uranyl nitrate) have the highest renal toxicity, followed by the less soluble compounds (ammonium diuranate, sodium diuranate, uranium tetrafluoride) and the insoluble uranium compounds (uranium dioxide, uranium trioxide, uranium peroxide, triuranium octaoxide). The difference in toxicity is due to the easier absorption of soluble compounds from the lung or gastointestinal tract into the blood and distribution to other organs~~distal tissues~~ (Tannenbaum et al. 1951).

~~A~~TSDR has determined that the toxicity database for uranium justifies the derivation of separate MRLs for soluble and insoluble forms of uranium for certain durations and routes of exposure. This is based on toxicokinetic evidence that absorption of uranium (and concentration in target tissue) is significantly greater during exposure to the more water-soluble compounds. Where the database is not extensive

enough to allow separate MRLs, the MRL for the soluble form should be protective for health effects due to all forms of uranium.

Inhalation MRLs.

Acute-Duration Inhalation MRL

There are limited data on the toxicity of uranium compounds in humans and animals following acute-duration inhalation exposure. Several case reports of individuals briefly exposed to uranium hexafluoride (Kathren and Moore 1986; USNRC 1986) or uranium tetrafluoride (Lu and Zhao 1990) are available. The observed effects included eye irritation and respiratory irritation, chemical burns, renal toxicity, and gastrointestinal irritation; however, some of these effects may have been caused by hydrogen fluoride, which was released when the uranium compounds rapidly degrade in the atmosphere.

Respiratory and renal effects have been observed in acutely exposed laboratory animals. Severe alveolar septal fibrosis was observed in rats exposed to 5,051 mg U/m^3 as enriched uranium dioxide for 100 minutes (Morris et al. 1990) and gasping and nasal irritation were observed in mice exposed to 637 mg Um/m^3 as uranium hexafluoride for 10 minutes (Voegtin and Hodge 1949). The renal effects included proteinuria and glucosuria in rats exposed to 426 mg U/m^3 for 10 minutes or 1,430 mg U/m^3 for 2 minutes as uranium hexafluoride (Leach et al. 1984) or in guinea pigs exposed to 23,040 mg U/m^3 as uranium hexafluoride for 2 minutes (Leach et al. 1984).

The available data were not considered adequate for derivation of an acute-duration inhalation MRL for uranium; the human studies did not reliably report exposure levels and the animal studies involved very short (<2 hours) exposure durations. Using one of the short exposure animal studies to derive an MRL may not be protective for continuous exposure for 2 weeks; longer-term animal studies with serial sacrifices (Stokinger et al. 1953) reported renal lesions following 3 days of exposure to soluble uranium compounds. These data are poorly reported and involved very small number of animals and are not suitable for MRL derivation.

Intermediate-Duration Inhalation MRL

- An MRL of 0.002 mg U/m^3 has been derived for intermediate-duration inhalation exposure (15–364 days) to insoluble compounds of uranium.

Exposure to enriched uranium, used as uranium fuel in nuclear energy production, may present a radiological health hazard. Although uranium-associated cancers have not been identified in humans, even following exposure to highly enriched uranium, higher doses associated with highly enriched, high specific activity uranium may be able to produce bone sarcomas in humans. Evidence from animal studies suggests that high radiation doses associated with large intakes of ^{234}U and ^{235}U-enriched uranium compounds can be hazardous. Adverse effects reported from such exposures include damage to the interstitium of the lungs (fibrosis) and cardiovascular abnormalities (friable vessels). However, access to ^{235}U-enriched or other high specific-activity uranium is strictly regulated by the USNRC and the U.S. Department of Energy (DOE). Therefore, the potential for human exposure to this level of radioactivity is limited to rare accidental releases in the workplace.

The potential for adverse noncancerous radiological health effects from uranium is dependent on several factors, including physicochemical form and solubility, route of entry, distribution in the various body organs, biological retention time in the various tissues, and energy and ~~intensity~~ dose of the radiation. The potential for such effects is generally thought to be independent of the known chemical toxicity of uranium. While the chemical properties affect the distribution and biological half-life of a radionuclide, the damage from radiation is independent of the source of that radiation. In this profile, there is little, or equivocal, specific information regarding the influence of radiation from uranium on certain biological effect end points in humans, such as reproductive, developmental, or carcinogenic effects. There is evidence, however, from the large body of literature concerning radioactive substances that alpha radiation can affect these processes in humans (see Appendix D for additional information on the biological effects of radiation). However, because the specific activities of natural and depleted uranium are low, no radiological health hazard is expected from exposure to natural and depleted uranium. Since the radiological component of natural uranium has essentially been discounted as a significant source of health effects, this leaves only the chemical effects of uranium to contend with. The chemical (non-radiological) properties of natural uranium and depleted uranium are identical; therefore, the health effects exerted by each are expected to be the same. The results of the available studies in humans and animals are consistent with this conclusion.

Uranium is a heavy metal that forms compounds and complexes of different varieties and solubilities. The chemical action of all isotopes and isotopic mixtures of uranium is identical, regardless of the specific activity (i.e., enrichment), because chemical action depends only on chemical properties. Thus, the chemical toxicity of a given amount or weight of natural, depleted, and enriched uranium is identical.

uranium miners and mill workers exposed to dusts of both soluble and insoluble uranium compounds are particularly significant in view of the high levels of exposure.

A histological kidney study of chronically exposed workers found no pathological differences at the low kidney concentrations (~0.3 µg/g) when compared to unexposed workers (Russell et al. 1996). In animal studies, observations in acute- and intermediate-duration exposures to uranium compounds conclusively show that high doses of uranium are nephrotoxic. Histopathological examination of the kidneys of these animals following oral, inhalation, or parenteral exposure revealed a thickened glomerular capsular wall, shrinkage of the glomerular capillary network, and decreased glomerular filtration rates. The damage in animals is histologically manifested as glomerular and tubular wall pathology. A mechanism involving bicarbonate uptake in the kidneys and subsequent precipitation of uranium in the tubule was proposed for uranium-induced renal toxicity. An alternative mechanism involving the inhibition of both sodium transport-dependent and transport-independent adenosine triphosphate (ATP) utilization and of mitochondrial oxidative phosphorylation in the renal proximal tubule has also been proposed.

Respiratory diseases have been associated with human exposure to the atmosphere in uranium mines. Respiratory diseases in uranium miners (fatal in some cases) have been linked to exposure to silica dust, oxide dusts, diesel fumes, and radon and its daughters in conjunction with cigarette smoking. In several of these studies, the investigators concluded that, although uranium mining clearly elevates the risk for respiratory disease, uranium contributes minimally, if at all, to this risk. ~~The mine air also contained radon and its daughters, and cigarette smoke, which are proven carcinogens.~~ redundant As in human studies, several animal studies in which uranium-containing dusts, such as carnotite uranium dust, were used reported the occurrence of respiratory diseases.

Epidemiologic studies among workers who had been exposed to uranium aerosols in strip and underground mines, mills, and processing facilities found more than the expected number of lung cancers only among underground miners and especially among miners who were cigarette smokers. No significant difference in the incidence rate of lung cancer was found between other workers who had been occupationally exposed to uranium and control populations. In addition to uranium dust, the mine air contained many other noxious aerosols (including silica, oxides of nickel, cobalt, and vanadium), radon and its daughters, diesel fumes, and cigarette smoke. Excess cancers were found among those underground miners whose radon daughter exposure exceeded 120 WLM. The rate of cancer incidence increased with increasing exposure to radon daughters.

tr

No significant difference in cancer (of the lungs) was found between workers who are occupationally exposed to uranium and control populations. Other detailed studies conducted between 1950 and 1967 on the association between uranium mining and an increased incidence of cancer found lung cancer in the miners over 6 times the rate expected. However, some of the miners were exposed to other potentially cancer-causing substances such as radon and its progeny, tobacco smoke, diesel smoke, and solvents (carbon tetrachloride and trichloroethylene). These studies and a review of 11 uranium miner studies attributed the increased incidence of lung cancer to radon and its progeny and not to uranium.

The evidence for the cancer-inducing potential of uranium in animals is also inconclusive. Animals exposed to very high doses of uranyl nitrate hexahydrate, uranium tetrachloride, uranium dioxide, uranium trioxide, uranium tetroxide, uranyl fluoride, uranium tetrafluoride, or uranium acetate, through the inhalation or oral route in acute-, intermediate-, or chronic-duration exposures, failed to develop these respiratory cancers. The lack of significant pulmonary injury in oral animal studies indicates that other factors such as diverse inorganic dust or radon daughters may contribute to these effects. Because uranium is a predominantly alpha-emitting radionuclide, current theories on cellular necrosis by high linear energy transfer (LET) alpha radiation also imply a contributory role to the cellular degenerative pulmonary changes. In studies in which human subjects and animals were exposed to uranium hexafluoride, hydrogen fluoride was probably responsible for, or aggravated, the observed respiratory effects. Uranium hexafluoride is hydrolyzable to uranyl fluoride and hydrogen fluoride, and death occurred shortly after intake with signs and symptoms of acute acid-induced cellular damage.

Comment [LU6]: What about the Mitchel study with rats exposed to U ore dusts

Comment [LU7]: What about the Leach study in dogs?

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are

cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, metabolic, dermal, ocular, body weight, or other systemic effects of uranium following intermediate-duration inhalation exposure. No studies were found regarding the cardiovascular, gastrointestinal, musculoskeletal, renal, endocrine, metabolic, dermal, ocular, body weight, or other systemic effects in humans following chronic-duration inhalation exposure. The existing human data on the respiratory and hepatic effects of uranium are limited to acute- and chronic-duration inhalation exposures, hematological effects are limited to chronic-duration inhalation exposure, and gastrointestinal and renal effects are limited to acute-duration inhalation exposure.

No animal studies were located regarding the endocrine, metabolic, dermal, or ocular effects of uranium in animals following acute-duration inhalation exposures to uranium. Nor were any studies located regarding the metabolic, dermal, ocular, or other systemic effects in animals following intermediate-duration inhalation exposure to uranium. There are animal data for acute-, intermediate-, and chronic-duration inhalation exposures to uranium for respiratory, hematological, cardiovascular, gastrointestinal, renal, or body weight effects. However, animal data on hepatic effects are limited to acute- and chronic-duration inhalation exposures to uranium.

The highest NOAEL values and all reliable LOAEL values in each species and duration category for systemic effects from chemical exposure to uranium by the inhalation route are presented in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. The hazard from inhaled uranium aerosols, or from any noxious agent, is the likelihood that the agent will reach the site of its toxic action. Two of the main factors that influence the degree of hazard from toxic airborne particles are: (1) the site of deposition in the respiratory tract of the particles and (2) the fate of the particles within the lungs. The deposition site within the lungs depends mainly on the particle size of the inhaled aerosol, while the subsequent fate of the particle depends mainly on the physical and chemical properties of the inhaled particles and the physiological status of the lungs.

The respiratory tract is a system of air passages that starts at the nose and includes the larynx, trachea, and a branching series of bronchi and bronchioles that terminate in several thousand alveoli. Small particles (about 2 micrometers [µm] or smaller in diameter) tend to be deposited in the alveoli. ~~The alveoli, frequently called the "deep respiratory tract,"~~ that form the functional part of the lungs where gas exchange occurs. As the particle size increases, progressively fewer particles penetrate into the deep respiratory tract, and increasingly greater fractions of the inhaled particles are deposited in the upper

respiratory tract. The respiratory tract is a system of ducts that starts at the nares and includes the pharynx, larynx, trachea, and a complex series of bronchi and bronchioles that terminate in several thousand alveoli. Three different mechanisms are involved in the removal of particles from the respiratory tract. The first is mucociliary action in the ~~upper respiratory tract (~~ nose, trachea, bronchi and ~~,~~ bronchioles~~, and terminal bronchioles),~~ which sweeps particles deposited there into the throat, where they are either swallowed into the gastrointestinal tract or spat out. The two other clearance mechanisms, dissolution (which leads to absorption into the bloodstream) and phagocytosis (removal by specialized cells in the process), deal mainly with the particles deposited in the alveoli ~~deep respiratory tract (respiratory bronchioles, alveolar ducts, and alveolar sacs)~~ (ICRP 1994; NCRP 1997). The less soluble uranium particles may remain in the lungs and in the regional lymph nodes for weeks (uranium trioxide, uranium tetrafluoride, uranium tetrachloride) to years (uranium dioxide, triuranium octaoxide).

In acute exposures, respiratory disease may be limited to interstitial inflammation of the alveolar ~~epithelium,~~ septa leading eventually to ~~emphysema or~~ pulmonary fibrosis (Clayton and Clayton 1981; Cooper et al. 1982; Dungworth 1989; Wedeen 1992). In studies of the pulmonary effects of airborne uranium dust in uranium miners and in animals, the respiratory diseases reported are probably aggravated by the inhalable dust particles' (the form in which uranium is inhaled) toxicity because most of the respiratory diseases reported in these studies are consistent with the effects of inhaled dust (Dockery et al. 1993). In some of these instances, additional data from the studies show that the workers were exposed to even more potent respiratory tract irritants, such as silica and vanadium pentaoxide (Waxweiler et al. 1983).

The effects of massive acute exposures to uranium in humans, as well as epidemiologic or clinical studies of uranium mine workers chronically exposed to mine atmospheres (containing other noxious agents that include silica, diesel fumes, cigarette smoke, and radon and its daughters), have been investigated. Several epidemiologic studies have reported respiratory diseases in uranium mine and mill workers, who were also exposed to significant amounts of dust and other pulmonary irritants, but not in uranium-processing workers, who were not exposed to these potential aggravants.

Accidental exposure of workers to estimated airborne concentrations of 20 mg uranium hexafluoride/m^3 for a 1-minute exposure and 120 mg uranium hexafluoride/m^3 for a 60-minute exposure (15.2 and 91 mg U/m^3, respectively) resulted in acute respiratory irritation, which is attributed to the hydrofluoric acid decomposition product. One worker died of pulmonary edema a few hours after the accident (USNRC 1986, 1990). In another report, 20 men who were seriously injured following accidental

morbidity. Histopathological evidence of toxicity was observed in several studies, including slight degenerative changes in rats and dogs exposed to 16 mg U/m^3 as uranium trioxide (Rothstein 1949c) and dogs exposed to 9.5 mg U/m^3 as uranyl nitrate (Roberts 1949). Uranium dioxide and triuranium octaoxide did not cause toxicity (Dygert 1949c; Rothstein 1949b). Carnotite uranium ore did not cause toxicity in mice or guinea pigs, but hemorrhagic lungs were observed in dogs (Pozzani 1949). The species differences may reflect deeper penetration of this material into the dog respiratory tract. Rabbits were more sensitive to respiratory effects of uranium compounds than other species. Severe respiratory effects (pulmonary edema, hemorrhage) were observed in this species with exposure to 6.8 mg U/m^3 as ammonium diuranate (Dygert 1949b), 15.4 mg U/m^3 as uranium peroxide (Dygert 1949d), 16 mg U/m^3 as uranium trioxide (Rothstein 1949c), and 22 mg U/m^3 as carnotite uranium ore (Pozzani 1949). Uranium dioxide at 19.4 mg U/m^3 did not cause respiratory effects in rabbits (Rothstein 1949b).

In chronic-duration exposure tests, a total of 3,100 test animals, including rats, rabbits, guinea pigs, and dogs were exposed to aerosols containing 0.05–10 mg U/m^3 of various uranium compounds for 7–13 months. ~~Histological examination of the lungs revealed no signs of injury attributable to uranium exposure.~~ No histological damage attributable to uranium exposure to the lungs was observed. There was an absence of any other type of histological damage outside the kidneys (Cross et al. 1981a, 1981b; Stokinger et al. 1985). Dogs exposed to 15 mg/m^3 of carnotite ore dust containing 0.6 mg U/m^3 with a particle size AMAD of 1.5–2.1 µm 4 hours/day, 5 days/week for 1–4 years showed very slightly increased pulmonary resistance, which may not have been statistically significant. Histological findings included vesicular emphysema, which was present to a lesser degree in control animals. Fibrosis was not noted at this concentration (Cross et al. 1981a, 1982).

Exposure of 200 rats, 110 dogs, and 25 monkeys to 5 mg U/m^3 as uranium dioxide dust 5.4 hours/day, 5 days/week for 1–5 years did not result in histological damage in the lungs of the dogs or rats. Minimal patchy hyaline fibrosis was occasionally seen in the tracheobronchial lymph nodes of dogs and monkeys exposed for >3 years. No atypical epithelial changes were noted (Leach et al. 1970).

Because particles containing insoluble uranium compounds can reside in the lung for years, it is likely that radiotoxicity as well as chemical toxicity can result from inhalation exposure to highly enriched uranium compounds. Radiation effects on tissues from the alveolar regions of the lungs were examined in Albino HMT (F344) male rats exposed, nose-only, for 100 minutes to an aerosol of to 92.8% ^{235}U-enriched uranium dioxide with a concentration ranging from 2,273 nCi/m^3 (84.1 kBq/m^3) to

Comment [LU8]: What organ or tissue

5,458 nCi/m^3 (202 kBq/m^3). Increases in the sizes and numbers of lung macrophages and type II[3] cells and the numbers of macrophages and type I cells, and a significant increase in the size of lysosomal granules within the macrophages were reported 8 days postexposure. At 7 days postexposure, 35 of the rats were further exposed to thermalized neutrons at a fluence of 1.0x10^{12} neutrons/cm^2 over 2.5 minutes in order to study the combined effects of radiation and chemical toxicity. The radiation dose due to the neutrons and the fission fragments was about 600 rads, which is about 300 times greater than the radiation dose from the uranium dioxide alpha particles. No significant difference was found between the uranium dioxide-only group and those that were subsequently irradiated with neutrons, indicating that the extra radiation exposure caused no immediate pulmonary cellular reaction above that produced by uranium dioxide alone. This finding implies that the observed acute pulmonary effects were due to the metallotoxicity of the uranium dioxide rather than to the alpha radiation from the uranium (Morris et al. 1989). ~~General damage to pulmonary structures, usually noncancerous alveolar epithelium damage of type II cells, can occur upon inhalation of insoluble reactive chemicals such as uranium salts and oxides. The main responses of epithelial cells to chronic injury are hyperplasia, hypertrophy, and trans-differentiation (metaplasia). These changes occur predominantly in proximal acinar regions where chronic injury often causes persistent lining of alveolar spaces by enlarged cuboidal cells that are derived from pre-existing type II cells, nonciliated epithelial cells from adjacent bronchioles, or a mixture of the two.~~

Comment [LU9]: Interpretation not based on observations in the studies. Adds little

There is evidence that exposure to highly enriched uranium through inhaled or intratracheally instilled enriched uranium compounds adversely affect the epithelium of the lungs. Severe alveolar fibrosis or metaplasia was found in 72% of the sampled lung tissues from F344 rats exposed for 100 minutes to an aerosol of 92.8% enriched uranium dioxide at a radioactivity concentration of 5 µCi/m^3 (137 kBq/m^3; ~150 mg U/m^3) to 10 µCi/m^3 (270 kBq/m^3; ~300 mg U/m^3). Extensive lung disease of an unspecified nature was observed only in animals sacrificed at 720 days postexposure. The radioactivity concentration of the mixture was estimated as 1.91 kBq/g (51.6 nCi/mg), and the AMAD of the particles ranged from 2.7 to 3.2 µm (Morris et al. 1990).

In other animal studies, changes suggestive of damage from either radiation or diverse inorganic dust (fibrosis) were reported in lungs and tracheobronchial lymph nodes in Rhesus monkeys exposed by

[3]Type I cells are alveolar lining cells that are involved with the transfer of oxygen and other substances ~~from~~ between the alveolus ~~through the wall to~~ and the blood. Type II cells are alveolar cells with two functions: oxidative enzymes for lung metabolism, and the production and secretion of the surfactant coating the alveolar surface.

uranyl nitrate hexahydrate for 26 weeks; rats exposed to 14.5 mg U/m^3 as triuranium octaoxide dust for 26 days; rats exposed to 16 mg U/m^3 as uranium trioxide for 4 weeks; mice and guinea pigs exposed to 3 mg U/m^3 as high-grade uranium ore dust for 30 days; and rabbits exposed for 30 days to 22 mg U/m^3 as high-grade uranium ore dust (contains uranium dioxide, triuranium octaoxide, and other potentially toxic contaminants) (Dygert 1949c; Pozzani 1949; Rothstein 1949c; Stokinger et al. 1953).

In chronic-duration exposure studies with animals, an unspecified strain of dogs exposed to ambient air concentrations of 0.05–0.2 mg U/m^3 as uranium hexafluoride for 1 year exhibited increased and persistent bromosulfalein retention, indicative of impaired biliary function, at the 0.2 mg U/m^3 concentration level (Stokinger et al. 1953).

Renal Effects. Uranium has been identified as a nephrotoxic metal, exerting its toxic effect by chemical action mostly in the proximal tubules in humans and animals. However, uranium is a less potent nephrotoxin than the classical nephrotoxic metals (cadmium, lead, mercury) (Goodman 1985). Many of the non-radioactive heavy metals such as lead, cadmium, arsenic, and mercury would produce very severe, perhaps fatal, injury at the levels of exposures reported for uranium in the literature (especially for miners and millers). ~~The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) has considered that limits for natural (and depleted) uranium in drinking water (the most important source of human exposure) should be based on the chemical toxicity rather than on a hypothetical radiological toxicity in skeletal tissues, which has not been observed in either people or animals (UNSCEAR 1993; Wrenn et al. 1985).~~ However, it has been suggested that the renal damage from exposure to high-LET alpha-emitting heavy metals, such as uranium, may be the complementary effect of both the chemical toxicity and the radiotoxicity of these metals (Wrenn et al. 1987).

Several epidemiologic studies found no increased mortality in uranium workers due to renal disease (Archer et al. 1973a, 1973b; Checkoway et al. 1988; NIOSH 1987; Polednak and Frome 1981). Also, case studies showed that workers accidentally exposed to high levels of uranium did not suffer renal damage, even up to 38 years postexposure (Eisenbud and Quigley 1956; Kathren and Moore 1986), although the tests for renal damage used in these studies were not very sensitive. A comparison of kidney tissue obtained at autopsy from seven uranium workers and six referents with no known exposure to uranium showed that the groups were indistinguishable by pathologists experienced in uranium-induced renal pathology (Russell et al. 1996). Three of seven workers and four of six referents were categorized as abnormal. Uranium levels in the workers kidney tissue (estimated by alpha particle emission) ranged from 0.4 to 249 µg/kg. As reviewed by Eisenbud and Quigley (1956), no evidence of renal toxicity was

Comment [LU10]: This has been mentioned before and adds nothing here

inflammatory lung responses than animals from other exposure groups. Specifically, one pheochromocytoma (zero in controls), one melanoma (zero in controls), one hemangioendothelioma (one in controls), two reticulum cell sarcomas (three in controls), and one adrenal cell carcinoma (zero in controls) were seen in animals exposed to uranium dust alone. Two osteosarcomas (zero in controls) were reported in animals exposed to the mixture of uranium ore dust and radon progeny. Four reticulum cell sarcomas (three in controls) and one adrenal cell sarcoma (zero in controls) were also seen in these animals. In animals exposed to radon progeny alone, one undifferentiated sarcoma (zero in controls), three reticulum cell sarcomas (three in controls), and one myelogenous leukemia (one in controls) were observed (Cross et al. 1981b).

In chronic animal studies, evaluation of Beagle dogs exposed to 3.4 nCi/m^3 (126 Bq/m^3 or 5 mg U/m^3) uranium dioxide for 5 years found ~~frank~~ benign and malignant pulmonary neoplasms ~~and atypical epithelial proliferation in 39–46%~~ in 4 of 9 (44%) of the ~~animals~~ dogs examined at 22-75 months after the exposure. The lung dose was estimated as 600–700 rads (6–7 Gy). Spontaneous tumors are rarely found in dogs under 10 years of age ~~were infrequent~~, and the incidence found in this study was 50–100 times higher than the expected rate of spontaneous tumors. The authors of the study recommended against the extrapolation of these findings to humans because these glandular neoplasms do not occur frequently in humans (Leach et al. 1973).

A study was conducted with uranium ore dust in male Sprague-Dawley rats (Mitchel et al. 1999). The rats were exposed nose-only to uranium ore dust that was delivered to the rats as an aerosol under positive pressure. The ore was without significant radon content. The rats were exposed to 0, 8.4, or 22 mg U/m^3 4.2 hours/day, 5 days/week for 65 weeks and were allowed to live for their natural lifetime. Exposure to uranium significantly increased the incidence of malignant and nonmalignant lung tumors. The frequency of primary malignant lung tumors was 0.016, 0.175, and 0.328 and the frequency of nonmalignant lung tumors was 0.016, 0.135, and 0.131 in the control, low- and high-dose groups, respectively. The main malignant tumor was bronchioalveolar carcinoma. No bronchial lymph node tumors were detected even though the lymph node specific burdens were considerably higher than in the lung in the same animal. The average absorbed doses for the low- and high-dose groups were 0.87 and 1.64 Gy, respectively, resulting in an average risk of malignant lung tumors of about 0.20 tumors per animal per Gy in both exposed groups. Lung tumor frequency appeared to be independent of dose, but exhibited a direct linear relationship with dose rate (as measured by the lung burden at the end of dust inhalation). Mitchel et al. (1999) noted that this suggested that rate may a more important determinant of lung cancer risk than dose.

Renal Effects. In general, no significant alterations in parameters of kidney function were observed among Gulf War veterans (Hooper et al. 1999; McDiarmid et al. 2000, 2001a, 2004b, 2006, 2007, 2009); a summary of these data are presented in Table 3-4. However, the differences in retinol binding protein approached statistical significance for several parameters including glomerular filtration rate, serum glucose, serum creatinine, and urine retinol binding protein and β-2 microglobulin levels. No alterations in *N*-acetyl-β-glucosaminidase levels (a biomarker of renal proximal tubule cell cytotoxicity) were found (McDiarmid et al. 2009). Of the biomarkers of renal function, only urine retinol binding protein and β-2 microglobulin levels were in the direction that would be indicative of renal damage (McDiarmid et al. 2009), and retinol binding protein levels were altered at several examination periods. During the period of 2001–2007, increases in retinol binding protein excretion were observed in the high exposure group at three of the four examinations. Urine retinol binding protein levels at the 2001, 2003, and 2007 examinations were 46.13, 27.33, and 31.00 µg/g creatinine in the low exposure group and 65.68, 80.51, and 48.11 µg/g creatinine in the high exposure group. The difference between the two groups did not reach statistical significance and the levels are within the normal range (<610 µg/g creatinine, McDiarmid et al. 2009). Although the difference was not statistically significant and values were within the normal range, there is concern (Squibb and McDiarmid 2006) because retinol binding protein may be a potential sentinel marker of proximal tubular effects from uranium.

Comment [LU11]: Check Table 3-4. The headings are mislabeled

Alterations in renal function and histopathology were observed in rats 90, 180, or 360 days after 0.1, 0.2, or 0.3 g depleted uranium fragments were embedded in the gastrocnemius muscle (Zhu et al. 2009b). The histological alterations included swollen glomeruli with infiltrated inflammatory cells, turgidity and epithelial necrosis in the tubules, and interstitial fibrosis (360 days after fragment implantation). Significant increases in urinary β_2-microglobulin levels were observed in the 0.3 g group 180 and 360 days after implantation, and an increase in urinary albumin levels was observed in the 0.3 g group after 90 days (but not after the longer exposure durations); however, there were large standard deviations for these measurements. Additionally, significant increases in serum creatinine levels were observed in all three exposed groups at 90 days, in the 0.3 g group at 180 days, and in the 0.2 and 0.3 g groups at 360 days; BUN levels were significantly increased in the 0.3 g group at 90 days and 0.2 and 0.3 g groups at 360 days (no significant alterations were observed at 180 days). Another study by this group also found significant decreases in 1-α-hydroxylase activity (responsible for the hydroxylation of $25(OH)D_3$ to $1\alpha,25(OH)_2D_3$ the active form of vitamin D) in the kidneys of rats exposed to 0.2 or 0.3 g depleted uranium embedded in the gastrocnemius muscle for 3 months (Yan et al. 2010); no significant alterations were observed at 6 or 12 months.

offspring of male mice exposed to ^{60}Co gamma radiation. To assess the role of radiation in the observed effects of depleted uranium, male mice were exposed to equal concentrations of depleted or enriched uranium in the drinking water (approximately 1 mg U/mouse) for 2 months. Exposure to either form of uranium significantly increased the frequency of mutations compared with controls and also suggested that the increase was specific-activity dependent. While this experiment showed that radiation can play a role in the observed effects of depleted uranium, the investigators noted that the mutation model used measures point mutations and cannot measure large deletions characteristic of radiation damage, the role of the chemical effects of depleted uranium may also be significant.

Table 3-5 presents results of genotoxicity tests conducted *in vivo*.

Comment [LU12]: Check this table. Should include resuts from Gulf War vets Section 3.2.4

With few exceptions, studies of genotoxicity of uranium in eukaryotic cells *in vitro* have yielded positive results (Table 3-6). For example, incubation of Chinese hamster ovary (CHO) cells with uranyl nitrate hexahydrate resulted in significant dose-dependent increases in micronuclei, SCEs, and chromosomal aberrations (Lin et al. 1993). Incubation of human osteosarcoma (HOS) cells with depleted uranium for 24 hours resulted in cell transformation into a tumorigenic phenotype and in significant increases in micronuclei, SCEs, and chromosomal aberrations in the form of dicentrics (Miller et al. 2002a). The increase in dicentrics suggested that the radiological component may play a role in depleted uranium's ability to induce both DNA damage and neoplastic transformation. To further study the role of the radiological component in the genotoxicity of depleted uranium, the same group of investigators incubated HOS cells with one of three uranyl nitrate compounds at the same concentration but varying in specific activity (Miller et al. 2002b). The results showed a statistically significant difference in transformation frequency between the three uranyl nitrates that was specific activity-dependent, indicating that radiation can play a role in the genotoxicity of depleted uranium. A subsequent report from these investigators showed that depleted uranium induced *de novo* genomic instability in HOS progeny cells (Miller et al. 2003). Delayed reproductive death was evident for many generations. A similar effect was induced by nickel or gamma radiation. Depleted uranium stimulated delayed production of micronuclei up to 26 days after exposure, longer than the 12 days it took the cells to return to normal after exposure to nickel or gamma radiation. Miller et al. (2003) noted that the precise mechanism by which depleted uranium induced genomic instability is unknown but it might be similar to that for radiation.

Comment [LU13]: Check this Table Should include all the cited drudies, not just a selected fes. Also should include dose. Which is alwa problematic in-vitro

Studies conducted with particulate (water-insoluble) and water-soluble depleted uranium showed that while both forms were cytotoxic to human bronchial cells *in vitro*, only the particulate form induced chromosome aberrations above background levels (LaCerte et al. 2010; Wise et al. 2007). Wise et al.

(2007) speculated that the different results may be related to different uptake mechanisms by the cell. Particulate depleted uranium would be able to enter the cell by phagocytosis, whereas soluble uranium would not.

In addition to causing DNA strand breaks in CHO cells, depleted uranium also produced uranium-DNA adducts. Incubation of CHO cells with depleted uranyl acetate showed the presence of DNA adducts on the order of a few uranium atoms per thousand nucleotides (Stearns et al. 2005). The formation of adducts was concentration- and time-dependent and suggested that uranium was acting through a chemical mechanism rather than a radiological mechanism. Characterization of the uranium-induced mutation in CHO cells showed the mutation spectrum to be different from the spectra generated spontaneously or by exposure to hydrogen peroxide or alpha and beta particles (Coryell and Stearns 2006). This suggested that depleted uranyl acetate had distinct effects on cells that result in a mutagenic response. A study that assessed DNA damage in rat kidney (NRK-52^{E}) proximal cells using several methods reported DNA damage and apoptosis occurring in a concentration-dependent manner (Thiébault et al. 2007). Apoptosis cell death was caspase-dependent and activated via the intrinsic pathway of the cells.

Comment [LU14]: -How does concentration and time dependence differentiate these two mechanisms?

Implantation of depleted uranium pellets in rats resulted in an increase in the mutagenic potential of urine towards the *Salmonella* tester strain TA98 (Miller et al. 1998a). Responses were dose- and time-dependent and strongly correlated with levels of uranium in the urine. In contrast to urine, tests conducted with rats' serum showed no significant increase in mutations, which was consistent with the low levels of uranium in blood. In support of the view that uranium in the urine and not other factor was responsible for the urine mutagenicity was the fact that the urine from controls, both non-surgical and implanted with an inert tantalum pellet, did not show an increase in mutagenic activity.

3.4 TOXICOKINETICS

Overview. Absorption of uranium is low by all exposure routes (inhalation, oral, and dermal). Absorption of inhaled uranium compounds takes place in the respiratory tract via transfer across cell membranes. The deposition of inhalable uranium dust particles in the lungs depends on the particle size, and its absorption depends on its solubility in biological fluids (ICRP 1994, 1996). Estimates of systemic absorption from inhaled uranium-containing dusts in occupational settings based on urinary excretion of uranium range from 0.76 to 5%. A comprehensive review of the available data for a pharmacokinetic model used lung absorption factors of 2–4% for 3-month-old children and 0.2–2% for adults, based on

compound absorbability (ICRP 1996). Gastrointestinal absorption of uranium can vary from <0.1 to 6%, depending on the solubility of the uranium compound. Studies in volunteers indicate that approximately 2% of the uranium from drinking water and dietary sources is absorbed in humans (Leggett and Harrison 1995; Spencer et al. 1990; Wrenn et al. 1989), while a comprehensive review indicates that the absorption is 0.2% for insoluble compounds and 2% for soluble hexavalent compounds (ICRP 1996). Dermal absorption has not been quantified, but toxicity experiments in animals indicate that water-soluble uranium compounds are the most easily absorbed. Once in the blood, uranium is distributed to the organs of the body. Uranium in body fluids generally exists as the uranyl ion $(UO_2)^{2+}$ complexed with anions such as citrate and bicarbonate. Approximately 67% of uranium in the blood is filtered in the kidneys and leaves the body in urine within 24 hours; the remainder distributes to tissues. Uranium preferentially distributes to bone, liver, and kidney. Half-times for retention of uranium are estimated to be 11 days in bone and 2–6 days in the kidney. The human body burden of uranium is approximately 90 µg; it is estimated that 66% of this total is in the skeleton, 16% is in the liver, 8% is in the kidneys, and 10% is in other tissues. The large majority of uranium (>95%) that enters the body is not absorbed and is eliminated from the body via the feces. Excretion of absorbed uranium is mainly via the kidney. The case of Gulf War veterans who were exposed to depleted uranium from inhalation, ingestion, and wounds, showed average urinary excretion, 7 years postexposure, of 0.08 µg U/g creatinine, with the highest rates around 30 µg/g (McDiarmid et al. 1999b).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

The deposition of inhalable uranium dust particles in the various regions of the lungs (extrathoracic, tracheobronchial, and deep pulmonary or alveolar) depends on the aerodynamic size of the particles. Particles >10 µm are likely to be transported out of the tracheobronchial region by mucocilliary action and swallowed. Particles that are sufficiently small to reach the alveolar region (≤10 µm AMAD) may transfer rapidly or slowly into the blood, depending on the solubility of the uranium compound. According to the ICRP (1996), a more soluble compound (uranium hexafluoride, uranyl fluoride, uranium tetrachloride, uranyl nitrate hexahydrate) is likely to be absorbed into the blood from the alveoli within days and is designated inhalation Type F (fast dissolution). A less soluble compound (uranium tetrafluoride, uranium dioxide, uranium trioxide, triuranium octaoxide) is likely to remain in the lung tissue and associated lymph glands for weeks and is designated Type M (medium dissolution). A relatively insoluble compound (uranium dioxide, triuranium octaoxide) may remain in the lungs for years and is designated Type S (slow dissolution).

target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figures 3-3 through 3-9 show models for radionuclides in general or specifically for uranium.

Comment [LU15]: Fig 3-3 is not discussed. Does not add anything as it now stands. Is it necessary?

in most inhalation animal studies indicates that other potentially toxic contaminants such as inhalable dust particles, radium, or radon may contribute to these effects.

Large doses of ionizing radiation have the actual or theoretical potential of being carcinogenic, teratogenic, and mutagenic. Since uranium has a low specific activity but emits high LET alpha particles that are densely ionizing along their track length, studies have been conducted to determine if uranium can produce these effects in humans and animals. The 4–8 MeV alpha particles from uranium travel through 40–70 µm in soft tissue, incrementally transferring their kinetic energy to the series of atoms and molecules with which they interact along their short, straight paths. Consequently, only structures within this range from the site of the deposition of uranium may be affected. If a DNA molecule is intersected and damaged without resulting in cell death, a range of theoretical effects can result. DNA has been found to be the most radiosensitive biological molecule, and ionizing radiation has been observed to damage individual chromosomes. The main result from low level ionizing radiation exposure is DNA damage or fragmentation. Viable cells repair the damage, but repair errors can result which produce gene mutations or chromosomal aberrations. Such events may result in such highly rare events as carcinogenesis or teratogenesis, but there is currently no evidence for radiation mutagenesis in humans. Chromosomal aberrations following large radiation doses have been demonstrated in humans and in research animals, showing that ionizing radiation can both initiate and promote carcinogenesis, and interfere with reproduction and development. Cancer is a well-known effect of ionizing radiation exposure, but it has never been associated with exposure to uranium. Likewise, no genetic changes due to radiation have ever been observed in any human population exposed at any dose (BEIR 1980, 1988, 1990; ~~Leach et al. 1970;~~ Morris et al. 1990; Muller et al. 1967; Otake and Schull 1984; ~~Sanders 1986; Stelzner et al. 1952~~; UNSCEAR 1982, 1986, 1988). For these reasons, UNSCEAR has stated that limits for natural (and depleted) uranium in drinking water (the most important source of human exposure) should be based on the chemical toxicity rather than on a hypothetical radiological toxicity in skeletal tissues, which has not been observed in either humans or animals (Wrenn et al. 1985). The EPA also used chemical toxicity as the basis for their 20 µg/L interim drinking water limit for uranium published in 1991 (currently withdrawn).

Comment [LU16]: I question whether these references measured or discussed genetic changes

3.5.3 Animal-to-Human Extrapolations

Kidney damage and respiratory disease are the most significant health effects in animals from the metallotoxicity of uranium. Because the biological systems through which these effects are mediated are common to both animals and humans (Brady et al. 1989; Clayton and Clayton 1981; Cooper et al. 1982;