External Peer Review for the IRIS Reassessment of the Inhalation Carcinogenicity of Naphthalene

FINAL REPORT

Prepared for
Integrated Risk Information System (IRIS) Program
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Prepared by
ORISE IRIS Technical Assistance Team
Oak Ridge Institute for Science and Education
Oak Ridge Associated Universities

August 2004

This document was prepared for the EPA by ORISE under interagency agreement No. DW-89939822-01-0 between EPA and the U.S. Department of Energy. ORISE is managed by Oak Ridge Associated Universities under a contract with DOE.

EXTERNAL PEER REVIEWERS

James Chen, Ph.D.
NCTR/FDA

Michael Dourson, Ph.D.
Toxicology Excellence for Risk Assessment

Michelle V. Fanucchi, Ph.D.
University of California at Davis

David Gaylor, Ph.D.
Gaylor and Associates, LLC

Mary Beth Genter, Ph.D.
University of Cincinnati

John B. Morris, Ph.D.
University of Connecticut

Laura S. Van Winkle, Ph.D.
University of California at Davis

PEER REVIEW PROJECT

Lynn Flowers, Ph.D., Project Manager
U. S. Environmental Protection Agency

Leslie Shapard, Peer Review Manager
Oak Ridge Institute for Science and Education

Frederick M. O'Hara, Jr., Reporter
Oak Ridge Institute for Science and Education

External Peer Review for the IRIS Reassessment of the Inhalation Carcinogenicity of Naphthalene

TABLE OF CONTENTS

Charge to External Reviewers and Reviewer Comments for the IRIS Reassessment of the Inhalation Carcinogenicity of Naphthalene
July 30, 2004

1. Metabolism of Naphthalene

Have the metabolic pathways for naphthalene been transparently described? Are there additional studies that have not been included that might be useful for the discussion of mode of action or human relevance of naphthalene carcinogenicity in animals?

James Chen:
The description of the metabolic pathways for naphthalene is very comprehensive and clear. It covers several recent publications and review papers on metabolic mechanisms of naphthalene.

I am not familiar with any additional studies that should be included for a discussion.

Michael Dourson:
EPA could do a better job here. Transparency is achieved but the important "So what?" question is not asked nor answered. Does rodent metabolism reflect what could be expected in humans? How is the comparison of rat to monkey metabolism affect a rat to human extrapolation? What proportion of the metabolism in rodents results in genotoxic end products? Is this proportion similar in humans?

EPA has to address these, and likely other, questions, since they directly relate to the extrapolation between rats and humans that might be used as a basis of any risk assessment. For example, if the difference between rat and monkey CYP2F metabolism relates to the expected difference in the target cells between rats and humans, then any dosimetric adjustment done on the rat data needs to further adjusted to reflect the CYP2F difference. In the choice of mode of action, if the proportion of genotoxic metabolites is a tiny fraction, for example, <0.1 percent of total metabolism, then a genotoxic mode of action will likely not play a prominent role in the dose response assessment for this chemical. If this proportion is greater than 10%, is most likely will play a role.

EPA's risk assessment scientists are up to the job of answering these kinds of questions. They just need more time devoted to task.

Michelle V. Fanucchi:
Although all of the information known about the metabolic pathways for naphthalene are included in section **3.3 Metabolism** (p 14, June 2004 draft revision of the Toxicological Review of Naphthalene), it is difficult to read. There are many potential pathways suggested by the literature. Moving the proposed metabolic diagram (p18) next to the paragraph that summarizes all of the options would be very helpful.

The second paragraph of section **3.3** indicates that **Van Winkle et al (1996)** demonstrated that CYP 2B4 can epoxidate naphthalene. Could this information actually be from **Van Bladeren**? I don't believe that Van Winkle performed activity studies.

Nhamburo et al 1990 did not study the expression of CYP2F in primates, just humans and mice. The reference to primates should be removed (top p15).

At the end of the second paragraph, **Boland et al (2004)** ["Site specific metabolism of naphthalene and 1-nitronaphthalene in dissected airways of rhesus macaques." *J Pharmacol Exp Ther*. Epub 4/13/2004, to be published 1 August 2004, 310(2)] should be included in the references to the ability of non-human primates to metabolize naphthalene.

It is interesting to note that **Baldwin et al 2004** indicate that only the ethmoid turbinates from the rhesus macaque contain detectable CYP2F protein, yet the ability to metabolism naphthalene has been demonstrated in both non-human primate and human lung **(Buckpitt and Bahnson 1986, Buckpitt et al, 1992, Boland et al 2004)**. Either the metabolism of naphthalene to a reactive intermediate is mediated by different subfamilies of CYP2F in different species (which may not be immunoreactive to the same antibody), or more information needs to be known about the other potential CYP families that have the ability to metabolize naphthalene.

Also, age-specific differences in naphthalene metabolism and cytotoxicity have been reported in the mouse. This should be addressed here. [**Fanucchi et al (1997) Naphthalene** cytotoxicity of differentiating Clara cells in neonatal mice. *Tox Appl Pharm* 144:96-104**; Fanucchi et al (1997) Pulmonary** cytochrome P450 monooxygenase and Clara cell differentiation in mice*. Am J Resp Cell Mol Biol* 17:302-314; **Fanucchi, et al (2000)** Development of phase II xenobiotic metabolizing enzymes in differentiating murine Clara cells. *Tox Appl Pharm* 168:253-267].

David Gaylor:
Metabolic pathways have been adequately described, but this is outside my area of expertise.

Mary Beth Genter:
The metabolism of naphthalene has been well documented in the literature cited in the EPA Toxicological Review of Naphthalene draft document. It might be worthwhile to clarify the species to which the CYPs listed on pp. 14-15 belong.

John Morris:
The pathways for naphthalene metabolism have been clearly described. Figure 1 is particularly useful. The text might be further clarified by consideration of the following issues.

Generally speaking there are two aspects in which the overall thrust is hard to follow. The metabolism section correctly places an emphasis on CYP2F pathways. However, the rationale for this is not clearly stated. A concise lucid statement explaining why CYP2F pathways are thought to be particularly important would improve the clarity of the text. The metabolism section includes much detail on the 1S,2R-1R,2S enantiomers, yet not much is made of this issue in the subsequent text.

In the subsequent portions of the text it is stated that either the 1,2- or the 1,4-quinones may be involved in the toxicity (e.g. p86-p87). Appropriate balance should be maintained in the metabolism section. Considerable text is provided on the 1,2 quinone pathways, but virtually no information is provided on the 1,4- pathways.

A general review of the overall importance of metabolic activation would also seem to be important. Indeed, the subsequent text states that naphthalene toxicity has been linked to CYP-mediated metabolism (p55), but this issue is not explicitly reviewed/justified. As currently written there is a tacit, but unwritten, assumption that the toxicity of naphthalene is due to metabolic activation. In essence, considerable text is included on identifying which specific metabolic pathways are important, but no text is provided on the general role of metabolism itself. In my view this is an important point. A wide database exists which provides strong evidence that activation is necessary for toxicity. PBPK models could easily be formulated, based currently on available data, which include overall CYP metabolism rates. These would likely provide a significant improvement over default inhalation dosimetric approaches. However, the conceptual basis for this approach would be rooted in the belief that metabolic activation is an essential step in naphthalene toxicity.

Certain aspects of the nasal metabolism descriptions could benefit from clarification. Since the nose represents the target site for the critical response, information on nasal metabolism is highly important. In the context of nasal toxicity it is appropriate to recognize that the respiratory mucosa and olfactory mucosa represent two entirely different tissues. Thus, it is important to clearly distinguish between metabolic pathways in these two tissue types. Quantitative information on metabolism rates in the two sites is also

important; specifically, an indication of specific activities (or Vmax values) is essential in formulating informed decisions regarding categorization of naphthalene as a category 1,2 or 3 gas. Such information

is available in the literature, but is not highlighted in the document. Since the proposed categorization of naphthalene is wrong in my view (see below), it is clear this issue did not receive adequate emphasis in the metabolism section of the text.

A paper at this year's Society of Toxicology meeting (Van Winkle et al) provides data that indicate estrous cycle influences pulmonary metabolism of naphthalene in rodents. Since there are gender differences in naphthalene toxicity it might be helpful to cite this abstract.

As written, the text does appear to be somewhat dogmatic in that it does not clearly and explicitly indicate that only the major metabolic pathways are being described. For example the legend for Figure 1 states, "Scheme for naphthalene metabolism...." A literal reading would suggest that the figure contains all metabolic routes. "Scheme for **major pathways** for naphthalene metabolism.." might avoid this impression. The text might be suitably modified as well.

Clarity might also be provided if the text and the figure relied on the same terminology. It is confusing when differing terms for the same compound and/or enzyme are used. Subsequent portions of the text (dealing with cataract formation) discuss aldose reductase, yet this is not listed in Figure 1.

The text on CYP2F expression levels should be clarified. Statements of enzyme levels without a denominator (per organ, per gram tissue, per mg DNA, etc.) are difficult to precisely interpret. An example is the statement, "In both mice and rats, the lung was found to contain higher levels of CYP2F transcript than the liver.." The lung contains over 40 cell types. Does this statement mean the overall lung expression is higher or the level of expression in only Clara cells? Similarly, statements such as "expression is 4-fold higher" need to be clarified to indicate the basis (per g tissue, per cell, per housekeeping gene, etc). Similar issues relate to comparisons of nasal metabolism/enzyme expression as well.

The data on glutathione depletion and enhanced naphthalene toxicity data are probably over interpreted. True, GSH depletion may enhance toxicity if a glutathione-adduct is an essential detoxification pathway. However, GSH depletion will also enhance toxicity if redox cycling represents an important pathway, irrespective of whether or not a glutathione-naphthalene detoxication pathway exists.

Laura Van Winkle:

Have the metabolic pathways for naphthalene been transparently described? No. The metabolism of naphthalene still contains too many metabolites whose importance is unknown to be "transparently described". This is reflected in the most recent diagram of potential NA metabolites in a paper by Boland et al that is in Press in JPET (Boland et al. 2004) which contains 4 potential interaction pathways following epoxide formation with question marks noting their unknown relevance to toxicity. The current diagram on page 18 of this draft EPA—IRIS toxicological review of naphthalene accurately reflects the current uncertainty regarding causative metabolites in NA cytotoxicity. This is well discussed, although the possible relevance of protein covalent binding could be added to both the diagram and the discussion of toxicity. The importance of protein covalent binding by naphthalene metabolites is well discussed in a review by Buckpitt et al (Buckpitt et al. 2002).

NA cytotoxicity has been found to be P450 dependent. However, it is not known which P450 activates NA in primates or humans or in the nasal compartments of the mouse and rat. In the airways of mice, CYP2F2 is clearly the dominant P450 due to the low Km and high Vmax of this enzyme for NA (Shultz et al. 1999; Shultz et al. 2001) and it's great abundance in very plentiful Clara cells of the mouse. The human protein, CYP2F1 had a rate of naphthalene turnover that was less than 0.1% the rate of metabolism observed with the mouse CYP2F2. Recent publications cast some doubt on CYP2F as the causative P450 in NA activation in distal airways of nonhuman primates (compare data from Boland et al and (Baldwin et al. 2004)) as CYP2F protein was not detected in rhesus airways, where there was some

NA metabolism. Other P450's could contribute. Further, little is known about NA metabolism in human lung so the metabolic pathways cannot be described, especially for the human. The rate of metabolism in humans and in nonhuman primates is 70-100 fold less than rodents if one compares the formation of water soluble metabolites in microsomes from whole lung. When specific lung subcompartments from the injury target regions in the airways are compared, the P450 activities were only 2-3 fold different between rodents and rhesus macaques (Lee et al. 1998). Regardless of this difference in P450 activity, adducted proteins from NA metabolism are found at similar levels in both rodents (Cho et al. 1994) and nonhuman primates (Boland et al. 2004). Toxicity in humans and non human primates may depend on which metabolite is more closely tied to the toxicity: the protein bound metabolites or the water soluble metabolites (that have the potential to be further metabolized to quinones and to redox cycle). This is unknown and both products likely contribute.

Are there additional studies that have not been included that might be useful for the discussion of mode of action or human relevance of naphthalene carcinogenicity in animals? The paper by Boland et al has interesting data regarding protein adduct formation in rhesus macaques, a species which is thought to more closely model human responses than rodents. Adducted proteins are found at similar levels (less than an order of magnitude different) in both rodents and nonhuman primates. However, very few of the proteins adducted in the primate (3) are identical to those identified as adducted in the mouse, a sensitive species (Buckpitt, personal communication). Baldwin et al describes detection of CYP2F in nasal tissue (ethomoturbinates; both transcript and protein) of rhesus macaque as well as mice and rats. However, neither protein nor transcript for CYP2F was found in the airways of rhesus. Given the role of GST in detoxifying the reactive NA metabolites, it would be helpful if we had additional human data on the effect of GST polymorphisms on NA metabolism. To my knowledge the information on GST polymorphisms is not available for naphthalene, although it is available for cancer outcomes especially associated with smoking (Miller et al. 2003; Wang et al. 2003).

Additional support for the importance of interaction of NA metabolites with glutathione can be found in the studies by West et al (West et al. 2001; West et al. 2002). These studies show that mice made tolerant to NA by repeated exposure have induction of gamma glutamylcysteine synthetase (GCS). Inhibition of GCS with buthionine sulfoximine eliminates tolerance. Depletion of GSH has been demonstrated in Clara cells exposed to NA in situ and this is an early change during acute cytotoxicity (Plopper et al. 2001). Tolerance can also be induced by inhalation so these findings are relevant to the discussion of inhalation effects (West et al. 2003).This paper also showed that the development of tolerance is due to factors intrinsic to the lung. If tolerance also occurs in humans then repeated exposures may be less of a concern than intermittent exposures to NA.

2. Physiologically Based Pharmacokinetic (PBPK) Modeling

Have the available PBPK models been transparently described? Are there additional PBPK models available that have not been included?

James Chen:

The review of the PBPK modeling is well presented. Primary sites for naphthalene metabolism to naphthalene oxide are the lung and the liver. Animal studies suggest that naphthalene is absorbed following oral or inhalation exposure. Exposure of rats by inhalation showed nasal respiratory epithelial adenomas in males and olfactory epithelial neuroblastomas in both males and females. In the halation in mice, there was an increase of alveolar/bronchiolar adenomas/carcinomas in female mice. A recent refined PBPK model indicated that tissue dosimetry of the parent compound alone does not explain why this compound was carcinogenic to the female mouse lung but not to the rat lung; this model cannot conclude regarding which metabolites may be responsible for the lung toxicity.

The current proposed PBPK models do not account for nasal deposition, epithelial absorption, and nasal metabolism of naphthalene because these data are not available. I am not aware of any additional PBPK models.

Interspecies adjustment of animal to human dose, and the conversion of from 1 ppm to the 5.2 mg/m^3 should be clearly described before the calculation of unit risk estimation.

Michael Dourson:
EPA could do a better job here also. Transparency is also achieved but the important "So what?" question is still not asked nor answered. Does the rodent PBPK model allow an extrapolation from higher to lower doses? Does the model allow for a choice in the dosimetric adjustment in extrapolation from rats to humans? One panel member suggested an exercise to add a nasal component to the existing PBPK model. This would allow the parsing of deposited dose between a category 1 and 3 gas. This is probably not a trivial task, but is one well within the capabilities of EPA and its colleagues. I encourage EPA to do this.

Michelle V. Fanucchi:
There are four PBPK models regarding the pharmacokinetics of naphthalene, three of the models refine the original model by **Sweeney (1996)**. The paragraph describing the cell culture reactor experiment (p22) by Ghanem and Schuler (2000) is very difficult to understand. There are an additional two publications (**Viravaidya et al (2004)** Development of a microscale cell culture analog to probe naphthalene toxicity. *Biotechnol. Pro*g 20:316-323; **Viravaidya and Shuler (2004)** Incorporation of 3T3-L1 Cells to mimic bioaccumulation in a microscale cell culture analog device for toxicity studies. *Biotechnol. Pro*g 20:590-597) that utilize naphthalene in PBPK modeling.

The PBPK models are a natural tool for interspecies scaling of pharmacokinetics, and none of these papers have addressed that issue. As written, I fail to see how the information on these models is helpful in this review. The models would be extremely useful to determine potential dose due to inhaled vs systemic naphthalene exposure if there were a nasal compartment added to the model. I think that this gap in knowledge should be addressed by this document.

David Gaylor:
PBPK modeling has been transparently and adequately described, but this is outside my area of expertise.

Mary Beth Genter:
An additional paper that might be cited as a potential model for understanding naphthalene toxicity is Viravaidya et al., 2004 [1].

John Morris:
The available PBPK models for naphthalene disposition have been well described. I think the facts that the estimated blood:air partition coefficient for naphthalene is 571 and that significant levels of naphthalene are found in the bloodstream after inhalation exposure should be clearly emphasized. These issues are of great importance relative to the assignment of naphthalene as a category 1,2 or 3 gas for inhalation dosimetry. (Since the proposed categorization of naphthalene is wrong in my view, see below, it is clear this issue did not receive adequate consideration.)

The interpretations of the current PBPK model limitations (e.g. lack of a nasal compartment) are well founded. The citation of Frederick et al 2001 (of which I am a co-author) is appropriate. The work of M.E. Anderson and M.S. Bogdanffy provide other state-of-the-art nasal modeling approaches. In my view, a significant advance towards scientifically-based risk assessment of naphthalene would be made by formulation of an appropriate inhalation dosimetry model. This would not represent a huge undertaking, because including the already published naphthalene data into the PBPK model structure of Frederick would be all that is required. Particularly with respect to nasal lesions, such would be fully consistent with the state of the art. Perhaps it is beyond the charge of EPA to actually formulate such a model. Initially such models might rely on total CYP metabolism rates, in the future the models could be easily updated to include specific CYP pathways once consensus is achieved on the precise role of the various CYP enzymes.

A less rigorous modeling approach could easily be applied to make a scientifically based decision regarding categorization of naphthalene for inhalation dosimetry. The likelihood of penetration of vapor through nasal tissues to the nasal bloodstream is the critical factor in assignment to category 1 vs. 2 vs. 3.

Solubility and metabolic data are all that are required in current nasal PBPK model structures to make a state-of-the-art evaluation of this issue. It would be unfortunate to fail to apply these widely available model structures for this purpose.

The statements in the text about the diffusion-limited models are confusing as written. The site of the diffusion limitation (I believe is was in the fat compartment) should be clearly indicated. The well-mixed assumption is the opposite of a diffusion-limited assumption. Thus statements such as "The diffusion-limited model.....which were assumed to be well-mixed and equilibrated..." appear to be oxymoronic.

Some clarifying text might be needed in the paragraph dealing with the possibility that a hepatic-derived metabolite of naphthalene may be involved in the pulmonary toxicity. Compounds that act through this mechanism typically cause endothelial, not bronchiolar or nasal epithelial injury. Thus the analogy is not clear. Moreover, numerous compounds produce olfactory injury due to *in situ* metabolism. Thus, I do not follow the logic that demonstration of olfactory injury after ip injection supports the concept of escape of a hepatic metabolite.

Laura Van Winkle:
Have the available PBPK models been transparently described? Yes, the models are well described and their inability to account for nasal deposition/nasal metabolism is appropriately noted.

Are there additional PBPK models available that have not been included? Should the recent paper by Viravaidya et al on the microscale cell culture model for NA toxicity (Viravaidya et al. 2004) be added to the discussion of Ghanem and Shuler 2000 cell culture model?

3. Mode of Action of Carcinogenesis

Has the available information on the mode of action for inhalation carcinogenesis been transparently and objectively described? Are there additional studies that should be included?

James Chen:
Nasal and pulmonary effects have been identified as critical effects from inhalation exposure to naphthalene. The mode of action for naphthalene is clearly described. Naphthalene affects mouse lung epithelial tissue and mouse and rat nasal tissues may involve the metabolic intermediates, naphthalene-1,2-oxide, 1,2-naphthoquinone, and 1-4-nathoquinone, which may damage tissue macromolecules either directly by their inherent electrophilicity or by the generation of reactive oxygen species. However, identification of which metabolites are responsible for naphthalene toxicity and carcinogenicity is unknown. Tissue sites of nonneoplastic cellular damage show some correlation with tissue sites of carcinogenicity, suggesting that naphthalene metabolites may act by nongenotoxic modes involving sustained cellular proliferation following cellular damage. Results from genotoxicity testing were mostly negative. But, a possible genotoxic model of carcinogenic action cannot be discounted as there are some data indicating genotoxicity of 1,2-naphthoquinone.

I am not aware of any additional studies should be included.

Michael Dourson:
I think that EPA could improve its efforts here by integrating the public comments on genotoxicity into its section and further discussing the potential role of cytotoxicity in the tumor response. The fact that no tumors were seen without extensive noncancer toxicity in the target organs is important. EPA needs to talk about this in its discussion of MOA, rather than mentioning that some organs with extensive toxicity do

not have tumors. I do not see why this later point has any impact on the MOA discussions, since some organs do not get cancer and yet have toxicity (e.g., lens of the eye).

The panel had other ideas in this area, some of which might lend themselves to desk study; others might necessitate effort in the lab. These are:

- Study the genotoxicity and cytotoxicity of other chemicals with similar tumors (desk),
- Determine the proportion of metabolites expected to be genotoxic in rodents and primates (desk and/or lab),
- Add a nasal component to existing PBTK models (desk),
- Investigate the metabolism, genotoxicity and cytotoxicity of naphthalene in target tissues in humans and primates (desk and/or lab),
- Conduct Ames assays with S9 fractions of target-tissue homogenates (lab).

Michelle V. Fanucchi:

Much of the information available regarding mode of action is the same information that is presented in section **3.3 Metabolism**. This information does not completely explain the site-specific and species-specific and age-specific differences in cellular toxicity and is also inadequate for use as carcinogenic mode of action information. Most of the mutagenicity and studies are negative, and although naphthalene metabolites are electrophilic, there is no data suggesting that they interact with DNA.

Since the important lesion in the rat is found in the respiratory epithelium in the septum in the nose, perhaps mutagenicity studies could be performed using S-9 fractions from this tissue. This tissue may be generating DNA-reactive metabolites not found in liver. Again, it would be very helpful if this document discussed the gaps in knowledge regarding naphthalene exposure.

On page 66 (middle of first paragraph), there is a statement "…neoplasms … occur in a limited number of chemical exposure studies". Which chemicals? Are they similar/different than naphthalene? This should be clarified.

In section **4.1.4, Ajao**'s description (**1988**) of 11 cases of colorectal carcinoma, 'half' of the patients indicated use of 'kafura'. Kafura is described by the author as a naphthalene compound, but there is no data to confirm this, nor has this been pursued by others. This data should be given very little weight, if any.

David Gaylor:

The mode of action has been transparently described and I am not aware of additional studies.

Mary Beth Genter:

a) There is considerable discussion in the document about the species-related differences in the formation of the naphthalene-1S, 2R-oxide vs. the 1R, 2S-oxide, and it was noted in the Lanza 1999 reference that human CYP2F1 produces more of the 1S, 2R-oxide (similar to the rat). This is a potentially important observation, particularly if a study could be performed to determine the relative mutagenicity of the 2 stereoisomers.

b) I would like to reiterate a point made on page 23 of the draft document, in that while abundant data are being generated about bioactivation enzymes, we need to know more about the relative detoxification enzyme expression across species.

c) Given the current research environment, in which different strains of mice are used to attempt to identify susceptibility genes, in all cases where mouse experiments are summarized, the strain of the mice used should be specified (e.g. O'Brien 1989 study, mentioned on p. 42).

d) The Lakritz 1996 reference cited, showing that tolerance imparted by prior naphthalene exposure is just the tip of the iceberg. For example, this same group also showed that glutathione S-transferase is induced in liver [2], and others have showed that enzymes involved in glutathione synthesis are also

induced by prior naphthalene exposure and may be involved in the development of tolerance to cytotoxicity associated with subsequent exposures [3]. Further, given that one or more quinone metabolites of naphthalene have been implicated in its toxicity, and the association between quinines and oxidative stress, some of the key studies showing that antioxidants confer protection against naphthalene-induced oxidative stress should be cited [e.g. 4-5]. Finally, basal levels of tissue antioxidant levels might confer protection/susceptibility to naphthalene toxicity—e.g. comparative studies of rat vs. mouse vs. primate lung and olfactory mucosal glutathione and ascorbate contents might be informative.

e) Comments on genotoxicity studies: What appears to be necessary are mutagenicity assays with the species-specific forms of CYP2F and target tissue (i.e. olfactory mucosa and lung) S9 bioactivation studies. The studies with the expressed 2Fs might be informative as to the risk of each species. The tissue S9 experiments would likely be even more informative, as it may be factors specific to the tissue microenvironment that determines susceptibility. For example, many olfactory P450s appear to be more active (on a per mg tissue basis) than the corresponding liver enzymes from the same species because of the higher ratio of P450 reductase to cytochrome P450 in olfactory mucosa compared to other tissues. For example, the ratio of reductase to cytochrome P450 is 1:2 in hamster, rat and mouse, compared to ratios of 1:11 – 1:15 in the liver. Similarly, rabbit reductase to cytochrome P450 ratio is ~1:8 in the rabbit olfactory mucosa and 1:32 in the liver [6-7]. (The optimal ratio for maximal catalytic activity would be 1:1).

f) Additional comments re genotoxicity section: The paragraph beginning on the lower one-third of p. 46 needs some work. The sentence 'Polycyclic aromatic hydrocarbons, including naphthalene, are generally planar ……..' should either be an introductory sentence to a new paragraph or omitted altogether (I would favor the latter) . The very next sentence 'In addition, benzo[a]pyrene-7,8-dione…….' has absolutely nothing to do with naphthalene and should be omitted.

g) On page 63, the possibility that naphthalene acts via a non-genotoxic mechanism of action, such as sustained induction of cell proliferation, is proposed. A simple series of BrdU incorporation studies could be conducted over a range of doses of naphthalene to determine whether there is a correlation between proliferation and ultimate carcinogenicity. A more definitive answer to the genotoxic vs. non-genotoxic issue could be ascertained using an *in vivo* mutagenesis assay such as the Stratagene Big Blue model; both Big Blue rats and mice are available.

John Morris:

Since my expertise does not extend to chemical carcinogenesis I will only provide limited comments on this area. My comments will focus primarily on the inhalation (particularly nasal) toxicity of naphthalene. From my perspective the text appears, for the most part, to be clearly written. The necessary groundwork to support the subsequent recommendations appears, for the most part, to be clearly laid out.

The section on inhalation toxicity of naphthalene is clearly written and appears to be appropriately interpreted with respect to nasal toxicological aspects. From a nasal toxicological viewpoint there are two issues that might benefit from further detail. First, mapping of lesion of lesion distribution within the nose is extraordinarily useful with respect to toxicological interpretation (see any review by KT Morgan). Mapping data should be described if available, or alternatively explicit statements should be provided indicating that data are lacking on the precise localization of lesions. Second, a common response of the olfactory epithelium is respiratory metaplasia (replacement of olfactory epithelium by what appears to be respiratory epithelium, see any review by JR Harkema or KT Morgan). It would be useful if statements regarding the presence of "olfactory metaplasia" would be more explicit relative the precise lesions that occurred.

The general issue of the potential role of cytotoxicity versus genotoxicity in the carcinogenesis of naphthalene could be presented more clearly. For example, the text should indicate whether it is essential that a perfect correlation between tumor incidence and cytotoxicity exist or whether the critical factor is the presence (or absence) of tumors in tissues that did not exhibit cytotoxic injury. As currently written, the exact basis for the judgments regarding cytotoxicity vis a vis carcinogenicity are not explicitly clear.

As highlighted above it is most appropriate to view the nose as consisting of two entirely different tissues. More rigorous application of this perspective would enhance clarity in the document. The issue of nasal toxicity following parenteral or oral administration of naphthalene is important relative to mechanism of action. An explicit summary of these data is needed. Importantly, this summary needs to clearly document whether respiratory or olfactory mucosal (or both) injury occurs following non-inhalation routes of administration. My understanding, based on Plopper's work, is that parenteral administration of naphthalene induced olfactory but not respiratory mucosal injury. If this is the case it might suggest that system (blood) delivery of naphthalene may play a role in the olfactory but not respiratory injury and consequently, that the inhalation dosimetric categorization of naphthalene relative to the two target sites might differ (e.g. category 2 vs. category 3).

The text correctly indicates that olfactory epithelial neuroblastomas are rare tumors. It is my view that respiratory adenomas are also rare tumors. "Equal time" might be provided to this point. Since subsequent text indicates that both are rare, it is confusing for the reader when the early text only highlights the low incidence of one tumor type.

The mode of action section (p55) states that "Animal studies have linked naphthalene toxicity in the lung to CYP-mediated metabolism." Are these studies adequately described in the earlier metabolism section? (See my previous comments.) The cross-species metabolism-nasal toxicity comparisons (p56-57) may be over interpreted. A metabolic rate of 87.1 vs. 43.5 in two rodent species may not be enormously significant from a toxicological view. Similarly a threshold dose of 200 vs. 400 mg/kg may not represent an enormous difference either.

Laura Van Winkle:

Has the available information on the mode of action for inhalation carcinogenesis been transparently and objectively described? There is no mechanistic understanding of the link between NA exposure and tumor formation. This has received very little study other than the NTP toxicology studies in rats and mice. These studies primarily look at the end stage lesions and so there is little known about the mechanism(s) of NA carcinogenesis particularly for the inhalation route of exposure. There is a possibility that there are susceptible populations to NA carcinogenesis based on elevated susceptibility to acute NA toxicity in the following groups: females (Van Winkle et al) and the young (Fanucchi et al) and the fact that tumors have been identified in sites that are susceptible to acute toxicity. It is possible that there are individuals with certain polymorphisms that may affect the metabolic activation or detoxification of NA. Some recent work suggests elevated susceptibility in female mice by stage of estrous cycle (Van Winkle et al. 2004). However, there is very little known about carcinogenesis in these potentially sensitive groups in relation to inhaled NA exposure with the exception of the sensitivity of the female mice to inhaled NA in the NTP 1992a study. The possibility of susceptible subpopulations is not fully discussed.

Another P450 activated cytotoxicant to lung (4-ipomeanol) in young postnatal rabbits causes a failure of repair that is due to inhibition of cellular proliferation and a lack of cellular re-differentiation (Smiley-Jewell et al. 1998; Smiley-Jewell et al. 1999; Smiley-Jewell et al. 2000). A similar failure of repair has been shown to occur in young postnatal mice exposed to naphthalene intraperitoneally (Fanucchi et al. 1997). The postnatal mice are more sensitive (Fanucchi et al. 1997) despite having significantly less NA metabolism (Fanucchi et al. 1997) . NA carcinogenesis may be due to faulty repair mechanisms in the adult but this has not been proven, nor has it been shown in humans. Although, impaired epithelial repair of NA injury has been shown in adult mice previously exposed to tobacco smoke (Van Winkle et al. 2001). It is not known if children are more susceptible to NA toxicity or carcinogenesis by either inhalation or ingestion, although a recent study has found that substantial exposure to NA occurs in children (Chuang et al. 1999). Clearly NA injury causes proliferation throughout the airway tree (Van Winkle et al. 1995; Lawson et al. 2002). Repeated episodes of cellular proliferation could lead to an event that precipitates formation of a tumor. But this has not been shown for NA. Further, a genotoxic mode of action of a metabolite downstream from the epoxide (perhaps one of the quinones) has still not been completely eliminated as a possibility. However, this reviewer feels that a genotoxic event (i.e. DNA reactive) likely contributes very little to the mode of action of NA in rodents and the primary mode of action is likely to be cytotoxicity.

Are there additional studies that should be included? Yes, Lawson et al should be added because it describes the widespread cell proliferation response to focal naphthalene injury in several strains of mice as well as adds to and supports the findings of Van Winkle et al 1995. Stripp et al 1995 should also be added because it describes similar cellular responses at the molecular level to Van Winkle et al 1995 (including proliferative responses) but in another strain of mice that have a more severe injury pattern to the same dose (this is discussed in the Van Winkle 1995 paper) (Stripp et al. 1995).

4. *Inhalation Carcinogenicity of Naphthalene*

4.a An assumption has been made that the nasal tumors in rats and lung tumors in mice are relevant to human carcinogenesis. Has this assumption been transparently and objectively described?

James Chen:
The available human data on cancer incidences are restricted to a few case series reports. No inference on the carcinogenicity of naphthalene can be drawn from these reports. All tumors from the NTP (1992, 2000) studies are considered relevant to human carcinogenesis. The respiratory epithelial adenomas were observed in rats. These are rare tumors. The mechanism of tumor formation in rats is unknown.

The assumptions that the nasal tumors in rats and lung tumors in mice are relevant to human carcinogenesis are transparently and objectively described.

Michael Dourson:
I am comfortable with this assumption.

Michelle V. Fanucchi:
Yes.

David Gaylor:
The relevance to humans of nasal tumors in rats and lung tumors in mice has been transparently and objectively described.

Mary Beth Genter:
Yes; I would add that in both mice and humans, pulmonary adenomas have been shown to progress to carcinomas [8].

John Morris:
Again, as my expertise does not extend to chemical carcinogenesis I will only provide limited input on carcinogenesis aspects of this charge question.

From my perspective, the text clearly describes the assumption that the nasal tumors in rats and lung tumors in mice are relevant from a human risk assessment perspective. Part of the basis for this conclusion is that the nasal tumors (neuroblastomas and respiratory adenomas) are quite rare in rodents and do not appear to be a common sequelae of typical irritant-induced non-neoplastic nasal lesions in rodents. In my view, these are accurate statements.

Laura Van Winkle:
This is partially discussed on page 66 last paragraph. However, there are key anatomical/cell population differences in the two target zones in mice (distal airways) and in rats (nasal olfactory epithelium) compared to humans and nonhuman primates. In both cases the rodents have an abundance of cells targeted by NA. In both cases these cells are found in great excess of what is seen in humans/non human primates. For example in mice, Clara cells, the target of NA in distal airways, comprise 75% or greater of the epithelium and are the most abundant cell type in both proximal and distal airways. In humans Clara cells comprise a much smaller percentage of the total cells because they have a much more restricted

distribution, being found in respiratory bronchioles, the most distal airways, only (Plopper and Hyde 1992). In the rat, olfactory epithelium is much more abundant than in the human nasal cavity. In the rat, olfactory epithelium occupies as much as 50% of the nasal cavity surface. In humans it is limited to a very small area (Harkema 1992). That being said, the guidelines for cancer risk do state that site concordance between human and animals is not required to find a compound a likely carcinogen. However, it would be nice to have some human data that shows an increase in tumors after NA exposure, or barring that, data in nonhuman primates on NA metabolism and cytotoxicity.

4. b Naphthalene is described as likely to be carcinogenic to humans via the inhalation route of exposure based on the U.S. EPA 1999 Draft Revised Cancer Guidelines (www.epa.gov/ncea). Do the available data support this statement?

James Chen:
Naphthalene is described as likely to be carcinogenic to humus via inhalation route based on the three inhalation studies: (1) NTP 2002 rat study; (2) NTP (1992) mouse study; (3) Adkins et al. (1986) mouse study. Evidence from human studies is limited to a few case series reports of laryngeal cancer in naphthalene purification workers and colorectal cancer in several patients who reported taking an indigenous medication containing naphthalene.

The NTP rat study (2002) provided clear evidence of carcinogenic activity of both male and female F344/N rats. The study showed increased incidences of nasal respiratory epithelial adenomas in males (0/49, 6/49, 8/48, 15/48) and increased incidences of olfactory epithelial neuroblastomas in both males (0/49, 0/49, 4/48, 3/48) and females (0/49, 2/49, 4/48, 12/49). The NTP mouse study (1992) provided some evidence of carcinogenicity in female mice, but not in male mice (discussed below). In both sexes, nearly all mice in the exposure groups showed nonneoplastic nasal chronic inflammation, olfactory epithelial metaplasia, and respiratory epithelial heperplasia. Olfactory epithelial neuroblastomas and respiratory epithelial adenomas are considered rare tumors in the NTP tumor database. The Adkins et al. (1982) study provided support to the weight of evidence that naphthalene induced lung tumors in female mice. The study showed increased number of lung tumors per tumor-bearing animal. These experiments provide adequate evidence of carcinogenicity in two animal species; thus, the results show that naphthalene can have carcinogenic potential in humans.

The reviews of the NTP rat study (2002), NTP mouse study (1992) and Adkins et al. mouse study (1986) are well presented. Evidence from human studies is limited to a few case series reports of laryngeal cancer and colorectal cancer. Based on the available data, the characterization that naphthalene is likely to be carcinogenic to humans via the inhalation route of exposure is appropriate.

I suggest adding a statement that the overall evidence for naphthalene in the group of agents designed as likely human carcinogens is at the low end.

Michael Dourson:
I am comfortable with this statement and agree with several panel members that the supporting data fall at the animal end of the range of data in this definition. I may or may not agree with adding a statement that "naphthalene is likely to be carcinogenic to humans *at doses that exceed a threshold for cytotoxicity*", depending on further suggested work as shown in the answer to question 3.

Michelle V. Fanucchi:
Yes, the data support this statement based on the guidelines.

David Gaylor:
The data support the likelihood that naphthalene is carcinogenic to humans via inhalation.

Mary Beth Genter:
I believe, yes, that naphthalene is accurately categorized as "likely to be carcinogenic to humans via the inhalation route of exposure" based on the USEPA 1999 Draft Revised Cancer Guideline description, with the adequate data in this case represented by a positive 2 species bioassay, with the weight of experimental evidence showing that the animal carcinogenicity could occur by a mode or modes of action that are relevant or assumed to be relevant to humans.

John Morris:
From my perspective, the text clearly describes the basis for concluding that naphthalene is likely to be carcinogenic to humans. The text clearly describes the literature supporting the conclusion that naphthalene is carcinogenic by the inhalation route in two species of laboratory animals, and also, indicates that the tumors induced in the nose represented rare types of tumors. The mutagenicity data are clearly described. However, the current document should cite published comprehensive genotoxicity reviews, if any, of other agencies (IARC) and/or individuals. In my view, the absence of in vitro genotoxicity tests that include a target organ (e.g. nasal S9) activating system represents a critical data gap that might be highlighted.

Laura Van Winkle:
Yes, under the new, 1999, guidelines the available data meet the minimum required to support this statement with carcinogenic potential in humans based on the increased incidence of a rare tumor type in rats (caveats noted above) and the tumors in an additional species (female mice). In the guidelines it states that data that fulfill this requirement are a spectrum. The current data appear to be at the minimal end of fulfilling the requirement for NA to be considered as likely to be carcinogenic to humans because there is no convincing human data. Further information, not available at this time, regarding NA toxicity or carcinogenicity in humans and nonhuman primates might well justify reclassification of NA to "suggestive", particularly if it is shown that the rodent carcinogenicity and MOA are not relevant to humans and/or nonhuman primates. In the absence of this information we must assume that the animal carcinogenicity and mode of action are relevant to humans, but this is not known.

4.c An inhalation unit risk has been derived utilizing benchmark dose modeling to define the point of departure of 10% extra risk followed by linear low-dose extrapolation below the point of departure.

4.c.1 The inhalation dosimetry equations used in the calculation of the human equivalent concentrations are for a category 1 gas (U.S. EPA, 1994). Is the explanation for the dosimetry choice in the derivation of the inhalation unit risk scientifically justified and transparently described?

James Chen:
In the EPA (1994b) inhalation dosimetry document, a category 1 gas is highly water soluble and/or rapidly irreversibly reactive in the surface liquid and tissue of the respiratory tract, and a category 3 gas is relatively water insoluble and un-reactive in the ET and TB surface and tissue. In this document, a sentence (page 73 line -16) states as ``The nasal effects from inhalation exposure to naphthalene were considered to be extra respiratory effects of a category 3 gas, ..''. Another sentence states as ``The category 1 gas dosimetric equation was selected as the appropriate method for dose conversion because naphthalene is reactive in nasal cavity.'' More comments on the choice of category 1 over category 3 as the appropriate method should be given.

The calculation in pages 81-82 (below) seems incorrect or something is missing.

Category 1 unit risk = (11.1 x10^{-3} per ppm) x 7/5 (days) x 24/6 (hours) x 1/0.132 RGDR

- o = (11.1 x10^{-3} per ppm) / 0.1236
- = 9 x 10^{-2} per mg/m^3,

where $RGDR_{ET}$ = regional gas dose ratio in the extra thoracic (ET) region

- = $(DoseET)_A/(DoseET)_H$
- = [minute volume/ETsurface area]$_A$ [minute volume/ETsurface area]$_H$;

Reference minute volumes (L/min): 13.8 human, 0.137 rat;

Reference ET surface area (cm^2): 200 human, 15 rat;

$RGDR_{ET}$(Rat to Human) = [0.137/15] [13.8/200] = 0.132.

Using the numbers in the first line, I am not able to obtain the value 0.1236 shown in the second line. My calculation for $RGDR_{ET}$(Rat to Human) = [0.137/15] [13.8/200] is 6.302 x 10^{-4}.

This sentence ``The corresponding maximum likelihood estimate of risk for a category 1 gas is 8.45 x $10^{-3}$ per ppm/0.1236, or 7 x $10^{-2}$ per mg/$m^3$ `` (page 82, lines 7-8) appears abruptly. The maximum likelihood estimate (MLE) represnts the sum $q1_{combined,male}$. The 95% confidence limit is computed from the MLE (Equation 1, page 77). It needs to mention the maximum likelihood estimate of the unit risk is 8.45 x 10^{-3} before calculating the confidence limit.

The notation is used to denote the standard deviation, it is a bad notation. It should be a or simply uses s.d. as used in Section 5.3.2.2 (page 84). The section 5.3.2. Quantal Analysis and the section 5.3.2.2 Time-to-Tumor Modeling appear to be written by different authors. The section 5.3.2.2 gives a more clear description of the procedure to calculate the 95% UCL. This part needs to be revised; the notation should be consistent.

Michael Dourson:

I do not agree with EPA's use of a category 1 RGDR, nor with observer comments on the use of a category 3 RGDR. Naphthalene is a category 2 gas. EPA needs to do its homework here and parse out the proportion of dose expected to arrive at the target tissues by way of systemic circulation and direct absorption. If the data are truly not helpful in making a reasonable guess, then I would be comfortable with a 50/50 split. EPA and the rest of us use such a split all the time with uncertainty factors for noncancer risk assessment. It is ok to do this here as well.

Michelle V. Fanucchi:

The explanation is clearly described. However, I could not understand why equations were used for a category 1 gas. The definition of Category 1, 2 and 3 gases should be clearly stated. Then, the reason for only calculating with Category 1 and 3 equations should be explained.

David Gaylor:

It is not always clear what conversion factors from animals to humans are being used to calculate the human equivalent concentrations (HEC). There appear to be calculation errors resulting in discrepancies between values reported in tables and the text. **See specific comments below.**

Mary Beth Genter:

Based on the discussions and definitions of Category 1, 2, and 3 gases, naphthalene best fits the description of a Category 2 gas, not Category 1.

John Morris:
The selection of the dosimetric model is described with clarity. However, the inhalation dosimetric aspects of the document need to be rethought. Assignment of toxicants to category 1 2 or 3 can be difficult. This is the case for naphthalene. The current document recommends a different categorization (category 1) from that used previously (category 3). My view is that both are wrong. **A careful application of the RfC methodology would suggest that naphthalene is in fact best modeled as a category 2 gas.**

The categorization of gases for the Inhalation RfC dosimetric evaluation is based on the disposition of the gas/vapor in respiratory tissues. The fundamental issue is whether or not the gas/vapor penetrates to the blood. Category 1 gases are those that do not penetrate to the blood (and exert no backpressure). This is definitely not the case for naphthalene. Significant blood accumulation of naphthalene occurs during inhalation exposure. Indeed, the literature cited in this review (e.g. Willems et al, 2001) provides naphthalene blood data. Moreover, naphthalene possesses neither the high solubility nor rapid reactivity required for category 1 gases.

Naphthalene is not a category 3 gas. These gases are "relatively water insoluble and unreactive in the ET and TB surface liquid and tissue and thus result in relatively small dose to these regions" (EPA RfC Methodology document). The current naphthalene document cites numerous references indicating that naphthalene is metabolized (e.g. reactive) in nasal and tracheobronchial tissues, data that is contrary to assignment to category 3. Moreover, the cited Willems et al (2001) manuscript estimates a blood:air partition coefficient of 571. Vapors with partition coefficients of that magnitude deposit with some efficiency in the nose and tracheobronchial tree, which further indicates the inappropriateness of a category 3 assignment.

Naphthalene is moderately soluble as indicated by a partition coefficient of 571, and metabolized in nasal and tracheobronchial tissues. Therefore, it would be anticipated that some degree of deposition and metabolism would occur in the nasal and tracheobronchial tissues and that some fraction of inspired naphthalene would penetrate to the alveoli and be absorbed into the bloodstream in that site. "Gases in Category 2 are moderately water soluble and rapidly reversibly reactive or moderately to slowly irreversibly metabolized in respiratory tract tissue" (Inhalation RfC Methods document). In my view the data cited in the naphthalene document clearly indicates it is most appropriately treated as a category 2 gas.

Policy issues may dictate that category 2 gases be handled as either a category 1 or category 3. If so, this should be clearly stated. Although possible, it seems unlikely that nasal metabolism rates of naphthalene are sufficiently high to metabolize a large fraction of the deposited naphthalene within the nose (and thus prevent penetration to the nasal bloodstream as is required for category 1 gases). This conjecture is based upon two issues: 1) CYP pathways are not generally high-capacity pathways and 2) even high capacity pathways (such as esterases) do not cause total first pass metabolism within the nose. From this perspective, it seems very unlikely that naphthalene will behave like a category 1 gas as assumed in the document. As noted above PBPK model structures currently exist and should be applied to formulate a scientifically justified assignment of categorization for inhalation dosimetry. As is documented for other compounds (e.g. acetaldehyde) the relative quantitative importance of metabolism may differ dramatically at high compared to low exposure concentrations. This represents another factor that should be considered relative to the inhalation dosimetry.

Laura Van Winkle:
The rationale for the choice is explained on page 82 as being because NA reacts with the nasal cavity. This could be better explained in relation to regionally deposited dose being more relevant than blood gas partition coefficients for the nasal effects. It is logical to assume that local deposition is more important than the contribution of NA via the circulation for respiratory effects by inhalation particularly in light of the ability of the nose and the lung to metabolize NA.

4.c.2 Has support for the use of linear low dose extrapolation been objectively and transparently presented? Are there other modeling approaches that should have been considered instead of or in addition to the low dose linear extrapolation approach?

James Chen:

The use of a one-degree polynomial (multistage) model to fit tumor incedence data is very appropriate and clearly presented. For each data set, a one-degree polynomial provided an adequate fit (P > 0.10) as assessed with the chi-square goodness of fit test. Higher degree models did not show improved fit to data. However, the estimate of the unit risk is based on the q1 and $q1^*$ rather than the default benchmark dose (BMC) and the 95% lower bound ($BMCL_{10}$). These differences should be addressed.

In the EPA Draft Revised Cancer Guidelines (1999), in the absence of data supporting a biologically based model, the basic default for extrapolation outside of the observed range is to assume linearity when the mode of action is not understood. In the linear extrapolation, a point of departure (POD) is estimated to mark the beginning of extrapolation. The LED_{10} (or $BMCL_{10}$) is a default for the POD.

The document uses $q1^*$ as the POD (page 77); in Equation 1, $q1^* = q1 + 1.645$ s.d., where q1 is the MLE estimate of the linear term of the multistage model and s.d. is the standard deviation of the square root of the variance estimate of the MLE (q1). This approach is different from the standard (default) approach of using the BMC and BMCL estimates.

Procedures to compute the unit risk for a single tumor site and summing risks from multiple tumor sites are described as follows.

Unit risk can be calculated by dividing the estimated corresponding tumor rate by the POD. For example, unit risk = 0.10/POD (as shown in page 83, line –9). For male rats, the MLE of unit risk of respiratory epithelial adenoma (REA) is $p1_{REA} = 0.1/15.16 = 6.60 \times 10^{-3}$ per ppm and upper risk estimate is $p1^*_{REA} = 0.1/11.32 = 8.83 \times 10^{-3}$ per ppm. The estimates of unit risk for olfactory epithelial neuroblastoma are $p1_{OEN} = 0.1/70.11 = 1.43 \times 10^{-3}$ and $p1^*_{OEN} = 0.1/39.91 = 2.51 \times 10.^{-3}$ These estimated values are slightly less than the corresponding estimates from the q1 and $q1^*$: $q1_{REA} = 6.95 \times 10^{-3}$ and $q1^*_{REA} = 9.30 \times 10^{-3}$ and $q1_{OEN} = 1.50 \times 10^{-3}$ and $q1^*_{OEN} = 2.64 \times 10.^{-3}$

An estimate of an upper limit on the combined risk based on the p1 and $p1^*$ can be computed using a procedure proposed by Gaylor and Chen (Risk Analysis, 395-398, 1996). The Gaylor and Chen (1996) approach is similar to the method (Equations 1-3) described, but it bypasses using the standard normal percentile 1.645. For simplicity, consider two tumor sites. Let P1 and P2 be the MLE estimates with the corresponding upper confidence limits L1 and L2, respectively. The MLE for the total risk is (P1+P2). An upper limit for the total risk is

$$L = (P1+P2) + [(L1-P1)^2 + (L2-P2)^2]^{1/2}$$

Applying this formula, the 95% upper limit of the unit risk for developing respiratory epithelial adenomas or olfactory epithelial neuroblastomas in male rats is

$$L_m = (6.60+1.43) \times 10^{-3} + [(8.83-6.60)^2 + (2.51-1.43)^2]^{1/2} \times 10^{-3}$$
$$= 8.03 \times 10^{-3} + [4.9729 + 1.1664]^{1/2} \times 10^{-3}$$
$$= 10.51 \times 10^{-3}$$

The 95% upper limit on the combined risk for the female rats is

$$L_f = (1.19+2.66) \times 10^{-3} + [(2.18-1.19)^2 + (4.78-2.66)^2]^{1/2} \times 10^{-3}$$
$$= 3.85 \times 10^{-3} + [0.9801 + 4.4944]^{1/2} \times 10^{-3}$$
$$= 6.19 \times 10^{-3}$$

These two values are smaller than the estimates using q1 and $q1^*$. When q1 and $q1^*$ are the MLE and Wald confidence limit, respectively, this procedure is equivalent to the procedure described in 5.3.2.2.

Michael Dourson:
I am not comfortable with the support for the linear case being made by EPA, especially in light of the public comments, which show little support for naphthalene's supposed mutagenicity. Even EPA acknowledges this. EPA needs to define what data would cause it to move from this linear default, and not just state that an understanding of naphthalene's MOA is not sufficient. Although modeling a noncancer cytotoxicity precursor is unlikely to yield credible results, since the incidence of these noncancer lesions is so high at low dose and the dose response behavior is so flat, it would be enlightening to model both a linear and nonlinear curve at the same time---like a hockey stick, but with no threshold. Consistent with this duel approach is the fact that tumors are only found with extensive noncancer toxicity, and that no genotoxicity is found except with some metabolites that make up an unknown, but suspected to be small, fraction of the overall metabolites.

Michelle V. Fanucchi:
The support is clearly presented. This appears to be the appropriate modeling approach.

David Gaylor:
Low dose linear extrapolation is justified, but likely to be overly conservative. It is not always clear whether cancer potency is obtained from the multistage model estimate of q_1 or from $0.1/BMC_{10}$. **See specific comments below.**

Mary Beth Genter:
No comment.

John Morris:
The rationale for the linear low dose extrapolation was clearly and objectively described in my view. It is my belief that a significant advance would be made by reliance on a dosimetrically-based PBPK model for low dose extrapolation. The state-of-the-art is sufficiently advanced to allow inclusion of inhalation pharmacokinetic considerations in the risk assessment process. Greater clarity and objectivity would be provided by text that lays out the alternative risk assessment approaches (cytotoxicity driven non-linear, or mixed mode of action), and also explicitly indicates the basis for selection of the approach that was utilized.

Laura Van Winkle:
Has support for the use of linear low dose extrapolation been objectively and transparently presented? Yes. This reviewer agrees that in the absence of additional mode of action information, a linear low dose extrapolation is appropriate, although it may overestimate risk.

Are there other modeling approaches that should have been considered instead of or in addition to the low dose linear extrapolation approach? This reviewer does not feel qualified to answer this question.

4.c.3 The inhalation unit risk is based upon the summed risks of developing olfactory neuroblastomas and respiratory epithelial adenomas in male rats derived from a time-to-tumor analysis. Is this approach scientifically justified? Are there other modeling approaches that should have been considered instead of or in addition to the approach taken? Has the best data set been chosen for derivation of the inhalation unit risk? Has the modeling been accurately and transparently described?

James Chen:
The time-to-tumor analysis has been a commonly used statistical method to adjust for competing risk mortality and time of tumor onset. The use of the multistage-Weibull model to fit the time-to-response is appropriate. When multiple tumor sites are present, there are several options to derive a combined risk estimate. It commonly performs an estimate for each experiment and chooses an estimate that can be

justified, for example, the most conservative estimate or most representative of the overall response. The description of the rationale for summing of the two risks that is superior to the EPA's previous practice should be elaborated.

Specifically, in page 76, line -15, ``The summation of risks from multiple sites … superior to the calculation of risks from individual tumor sites alone.'' Here it does not discuss how to obtain the combined risk from individual unit risk estimates. In page 84, line 8, ``Summing the cancer risks in this manner … is superior to EPA's previous practice of carrying out one dose-response analysis of tumor-bearing animals.'' Here the EPA's practice appears to combine all tumors into one (total) response and carries out the analysis. In the EPA Draft Revised Cancer Guidelines (1999) page F-3, an example illustrates that the combined risk is computed as the sum of two individual risks minus their product under the independence assumption. This needs to be clarified.

The calculation of the combined risk is inadequately described. The estimated values of human equivalent unit risk given in the last column of Table 7 are erroneous.

My calculation of the combined risk for male rats is as follows.

The MLE and UCL unit risks for developing olfactory epithelial neuroblastoma are $p1_{OEN} = 1.75 \times 10^{-3}$ ppm and $p1^{*}_{OEN} = 2.92 \times 10^{3}$ per ppm. The estimates of unit risk for respiratory epithelial adenomas are $p1_{REA} = 9.17 \times 10^{-3}$ per ppm and $p1^{*}_{REA} = 12.50 \times 10^{-3}$ per ppm. The summed MLE (central tendency) risk is $(1.75+9.17) \times 10^{-3} = 10.92 \times 10.^{-3}$ Using the Gaylor and Chen (1996), the 95% UCL is

$$\text{Unit risk} = (1.75+9.17) \times 10^{-3} + [(2.92-1.75)^2 + (12.50-9.17)^2]^{1/2} \times 10^{-3}$$
$$= 10.92 \times 10^{-3} + [1.3689 + 11.0889]^{1/2} \times 10^{-3}$$
$$= 14.45 \times 10^{-3}$$

There are many unclear sentences and typos in Section 5.3.2.2

a) page 83, line -8. The highest unit risk for individual tumors. Delete the word highest.
b) page 84. The first paragraph is puzzling. Where do 2.5 ppm and 13 mg/m^3 come from?
c) page 84, line -11. 1.645 should be the 95% percentile of the standard normal distribution.
d) Typos: 1. page 82, line -4. The symbole should be $\geq$.
2. page 84, line -12. The symbol A in the equation should be a multiple x.
3. page 84, line –8. MLE should be MLEs

Needed Research on Low Dose Extrapolation for Nonlinear Dose Response Model

The purpose of low-dose extrapolation is to provide as much information as possible about risk in the range of doses below the observed data. The most versatile form of low-dose extrapolation is a dose-response model that characterizes risk as a probability over a range of environmental exposure levels. Two approaches can be used for low dose extrapolation: linear extrapolation and nonlinear extrapolation.

Linear extrapolation has been used as a default approach (Final Guidelines for Carcinogen Risk Assessment, 2002) when the available data fall short of establishing the mode of action at a tumor site. In the linear extrapolation, a point of departure (POD) is estimated to mark the beginning of extrapolation, where POD is a dose (expressed in human-equivalent terms) near the lower end of the observed data range without significant extrapolation to lower doses. The 95% lower confidence limit on a dose associated with 10% extra risk adjusted for background (LED_{10}) is a default for the POD. The ratio of the extra risk to POD, known as the *slope factor*, is an upper-bound estimate of risk per increment of dose that is the estimate of *unit risk*.

A *nonlinear approach* is used when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses. Currently, nonlinear approaches do not estimate risk probabilities or

provide a dose-response curve at low doses, because there is considerable model uncertainty inherent in the extrapolation of nonlinear models. A re*ference dose* or *reference concentration* is estimated using safety assessment. This approach may not be adequate for purposes that require a quantitative characterization of risks across a range of doses. The risk probabilities provide needed information for benefit/cost analyses of different decision options. One major issue in dose-response modeling is that different nonlinear models that fit the observed data can lead to a wide range of results at lower doses. This issue is more serious for a nonlinear dose response function. Research on methods for quantifying dose-response relationships for tumors that arise through a nonlinear mode of action and methods for low dose extrapolation for nonlinear dose response model are needed.

Risk assessment involves many steps, and each step has potential variation and uncertainty. In the dose-response assessment, ideally a biologically-based model should be developed to describe biological processes involved in a response. Nonlinearity observed in a dose-response curve often can be attributed to toxicokinetics, and nonlinearity can also occur between applied dose and tumor response. When there are insufficient data or scientific knowledge to ascertain an appropriate dose response model for a nonlinear mode of action, researches on characterization of model uncertainty assessment and development of methods to account for model uncertainty are needed.

Michael Dourson:
EPA's summation procedure was not clear. After I think that I understood it, my first question was why not add up all the nonsignificant tumors in males? Why just stop with neuroblastomas for males and epithelial adenomas for females? Why not take the approach adding both the male and female responses for individual tumor types? This would more accurately anticipate the situation in humans, correct?

It appears that the answers to these questions might be that EPA is trying to maximize the cancer potency factor. I have no conceptual problem with this, but if this is the case, EPA needs to clearly state it. Afterwards, EPA needs to estimate cancer potency with other combinations and then discuss the strengths and weaknesses of all of these procedures in its risk characterization.

Michelle V. Fanucchi:
This approach is very conservative, but given the lack of complete information, it appears to be justified. The modeling approach should be described in simple English, followed by the mathematical equations used. A non-mathematician should be able to follow the logic of this section, if not the equations.

David Gaylor:
It is appropriate to sum the risks of olfactory neuroblastomas and respiratory epithelial adenomas in male rats from a time-to-tumor analysis to arrive at the most sensitive (largest) estimate of cancer risk. It is not always clear how the upper limits of the summed risks were
calculated (**see specific comments below**). Consideration should be given to estimating the summed risks for female rats and the risk of alveolar/bronchiolar adenomas and carcinomas in female mice. A weighted mean of these three estimates may provide a better estimate for human risk.

The summed risks correctly estimate the risk of an olfactory neuroblastoma **or** a respiratory epithelial adenoma. This is the most conservative approach. However, if it is desired to estimate the risk that an **individual** develops either type of tumor (the more usual calculation), then the risk of an individual producing both types of tumors must be subtracted from the sum to avoid double counting of individuals with both types. For rare tumors, as is the case here, this is a minor adjustment. A simple approach is to calculate a single benchmark dose based on the incidence of animals that have either or both types of tumors. In this way, animals with both tumor types are not double counted.

To reiterate, if the risk of either tumor type is desired, the summed risks are appropriate. If the tumor risk per individual is desired, then the joint risk of an individual having both tumor types must be subtracted from the sum of the risks.

Mary Beth Genter:
I believe that the approach is valid, although this tumor type is very unusual (i.e. this reviewer knows of no other compound causing adenomas of the nasal respiratory mucosa in rodents). The olfactory neuroblastomas in female rats do have a human counterpart (the esthesioneuroblastoma).

John Morris:
My expertise precludes detailed comments on the appropriateness of the time to tumor modeling approach and its application. I offer two comments, however. First, it is not clear to me from a nasal toxicological viewpoint why the neuroblastomas were considered to be fatal tumors. Did they grow to such a degree that they occluded the nasal air passages? If so, this should be clearly described. If not, they may not have represented fatal lesions. If the term "fatal" has some specific meaning from a regulatory, policy, or modeling perspective, it should be clearly indicated.

There may have been mathematical/typographical errors in the data presented in Table 7. I cannot determine how the human equivalent risk numbers were calculated using the formula provided in footnote c (by dividing the unit risk of 0.0029 by 5/7*6/24 and 0.132 does not give 0.0033.) Moreover the unit risk value cited on p86 (0.1) differs by about 10-fold from the numbers provided in Table 7.

Laura Van Winkle:
The inhalation unit risk is based upon the summed risks of developing olfactory neuroblastomas and respiratory epithelial adenomas in male rats derived from a time-to-tumor analysis. ***Is this approach scientifically justified?*** Yes from a "choice of models" point of view. It is a strength of this modeling approach that it allows different tumor types to contribute to the estimate of risk. However, it should be pointed out that all the tumors used for this analysis were nasal tumors, which are extremely rare in humans. Further, there is a lack of epidemiologic data showing nasal tumors in people following NA exposure.

Are there other modeling approaches that should have been considered instead of or in addition to the approach taken? This is outside the area of this reviewer's expertise.

Has the best data set been chosen for derivation of the inhalation unit risk? The rat data is the most appropriate for derivation of human risk because rats are a better model for human responses to NA than the mouse. The mouse is an unusually sensitive species due to the presence of a P450 isozyme with extreme affinity for NA (CYP2F2). Humans and rats each have a different isoform. The human CYP2F1 has a NA turnover rate that is less than 0.1% the rate observed with mouse CYP2F2 (Shultz et al 1999). The rat isozyme is CYP2F4 (Baldwin et al).

Has the modeling been accurately and transparently described? Yes, the modeling has been well described. Reviewer feels determination of accuracy, outside of scientific concerns noted above, is beyond her area of expertise.

Additional Comments

James Chen:

Comments regarding the NTP (1992) mouse study

The incidences of lung lesions in male and female mice are

		0 ppm	10 pom	30 ppm
M	Alveolar/bronchiolar adenoma/carcinoma	7/70(10%),	17/69(25%),	31/135(23%)
	Chronic inflammation	0/70(0%)[t],	21/69(30%)*,	56/135(41%)*
F	Alveolar/bronchiolar adenoma/carcinoma	5/69(7%)*,	2/65(3%),	29/135(22%)*
	Chronic inflammation	3/69(4%)[t],	13/65(20%)*,	52/135(39%)*

- * statististical significance ($p < 0.05$)
- t significant trend ($p < 0 .05$)

In female mice, a statistically significant increase of alveolar/bronchiolar adenomas/carcinomas was found in 30-ppm group ($p = 0.010$) and in dose-response trend analysis ($p < 0.001$). Incidences of chronic inflammation were significant in both 10- and 30-ppm groups and dose response trend. Male mice did not show a statistically significant increase of alveolar/bronchiolar adenomas/carcinoma. But, incidences of chronic inflammation were significant in both 10-ppm group ($p <= 0.001$) and 30-ppm group ($p <= 0.001$), and dose response trend group ($p <= 0.001$).

There are no significant chemical-related decreases in terminal body weights in either sex.
Statistical significant decreases in survival were observed in the control male mice due to fighting compared with the exposed groups. The survival percentages at the end of the study in the male mice were 37% (26/70), 75% (52/69), and 89% (118/133) for the three groups. The treatment had no apparent effect on the rate of mortality and mean body weight at the 30-ppm group. The 30-ppm group may not be adequate as the MTD in accordance with criterion of the MTD, although there are the sentences: ``In addition, because nasal hyperplasia and metaplasia were seen in virtually all exposure animals, but not in the controls, the dose chosen likely offered a sufficient challenge for the evaluation of carcinogenic potential'' (page 32, line -18). I suggest adding some discussion about the MTD issue.

Michelle V. Fanucchi:

This is, for the most part, a very complete review of the literature. However, it is difficult to read due to the fact that several sections cover the same information (for example, metabolism of naphthalene) and are not cross referenced well. It would be easier to read if the full review regarding an aspect of naphthalene were placed in one spot and referenced. It appears to have been put together by multiple authors and it would benefit from a rigorous editorial effort.

Also, some of the sections read as though the information from the literature is being catalogued without an attempt to synthesize and make sense out of it. This is difficult to read. Every attempt should be made to define which information is relevant and important. In areas that discuss multiple sites, multiple species or multiple expressions (i.e. gene vs protein vs activity), the information must be clearly laid out so that the reader knows exactly what is being described/compared.

Overall, this document needs to be edited for clarity and simplicity. Each section should summarize the literature, delineate the gaps in knowledge, and explain why certain studies are given more weight in the overall determination of naphthalene carcinogenicity.

David Gaylor:
Specific Comments on the "Toxicological Review of Naphthalene", (External Review Draft), NCEA-S-1707, June 2004

Page 33, first full paragraph, line 14, should read: ---marginally increased tumor incidences in the "dosed" groups.

Page 75, second paragraph, line1. Olfactory epithelial neuroblastomas in male rats show a significant dose response trend (Table 5 and I also verified the significance with a statistical test). Drop statement from sentence.

Page 76, Section 5.3.2.1. This Section is titled Quantal Analyses, which is correct but not descriptive and initially confusing. The time-to-tumor modeling also is conducted on quantal data. The single word "quantal" does not adequately distinguish the two approaches. I would suggest that the Section title should be changed to "Quantal Analyses Unadjusted for Survival", along with the same changes throughout the text.

Section 5.3.2.1 vs section 5.3.2.2
For the Quantal Analyses Unadjusted for Survival (Sec. 5.3.2.1), the added risks are based on adding the q_1 for REA and OEN. The upper confidence is properly described by equations 1-3 on page 77.

For the time-to-tumor analyses, the risk estimates are based on the linear extrapolation cancer slope factors ($0.1/BMC_{10}$ and upper limit of $0.1/BMCL_{10}$) as described in the current EPA Carcinogen Risk Assessment Guidelines. It is not clear how the upper limit for the added risks based on slope factors was done (see later comments).

Although risk estimates based on q_1 and slope factors are similar (not equal), this inconsistency in the Report initially leads to some confusion. It is recommended that low dose linear extrapolation from the benchmark dose be used for cancer risk estimation in the Quantal Analyses Unadjusted for Survival.

Page 77, Equation 1. Replace the first = sign with a colon.

Page 81, line 7. After unit risk it would be helpful to insert (upper limit) as a reminder.

Page 81, line 3 from bottom. The conversion factor for ppm to mg/m^3 of (1/5.2) is missing. The numerical calculations are correct.

Page 82, line 3 and 6. Insert a division sign between the animal and human ratios.

Page 82, last sentence of Sec. 5.3.2.1. For clarity change (unit risk) to
(unit risk upper limit).

Page 82, Sec. 5.3.2.2, first sentence. Change to read: In addition to a quantal analysis unadjusted for survival-----

Page 83, 3rd full paragraph, last line should read: ---were approximately 20% lower {not higher} than those calculated using the quantal unadjusted for survival approach.

Page 83, last paragraph. The highest unit risk for individual tumors was for male rat REA, not female rat OEN as incorrectly indicated in this paragraph.

Page 84, line 1. Nowhere is there a value of BMC_{10} = 2.5 ppm?

Page 84, 95% UCL formula: change A to x.

Page 84, 2nd full paragraph. Rather than just stating in words how the upper limit on the summed unit risk was calculated, the actual numerical calculations should be displayed.

Page 84, line 3 from bottom. 35% should be 30%.

Page 84, last paragraph. In these summary statements it is useful to again indicate that the summed unit risk is an upper limit and the earlier quantal dose-response modeling was unadjusted for survival.

Page 85, next to last column. To emphasize male rat REA as the largest unit risk, change $1.3x10^{-2}$ to $12.5x10^{-3}$ for an easier comparison with the other tumor types.

Page 85, Table 7, last column. The calculations for Human Equivalent Unit

Risks $(ppm)^{-1}$ are not correct. For example, for male rats OEN,

Human Equiv Unit Risk = 2.9×10^{-3} per ppm x (7days/5days) x (24hrs/6hrs)

x (1/0.132 RGDR) = 0.123 per ppm.

Similarly, Human Equiv Unit Risk = 0.551 per ppm for male REA, 0.280 per ppm for female OEN (in agreement with the calculation in the last paragraph on page 83), and 0.123 per ppm for female rat REA. Further division by 5.2 converts risk per ppm to risk per mg/m^3.

Page 86, Sec. 5.3.4, first paragraph. Change 0.1 to 0.12.

Page 90, next to last paragraph, first sentence. Change to: An inhalation unit risk (upper limit) of 0.12 per mg/m^3 (rounded to 0.1 per mg/m^3) , based on -------

Page 90, next to last paragraph, second sentence. Change to: ----modeling of the same data unadjusted for survival.

Specific Comments on June 2004 IRIS Summary

2 Page 19, Sec. II.C.1.1. The value 0.1 per mg/m^3 is not the 95% upper bound and is so close to the central estimate of 0.09 per mg/m^3 that it incorrectly appears that there is little uncertainty in the estimate. Change to read:

Inhalation Unit Risk, 95% upper bound – 0.12 per mg/m^3 (rounded to 0.1 per mg/m^3

or $1x10^{-4}$ per $\mu g/m^3$)

Central tendency estimate – 0.09 per mg/m^3 ($9x10^{-5}$ per $\mu g/m^3$)

Page 19, Sec. II.C.1.2. The values listed are not 95% lower bounds. Change to read:

Extrapolation Method – An inhalation unit risk (upper bound) of 0.12 per mg/m^3

(rounded to 0.1 per mg/m^3), based on time-to-tumor ----- of the same data unadjusted

for survival.

Air Concentrations at Specified Risk Levels:

Risk Level	95% lower bound on Concentration Estimate	Rounded Conc. Estimate
1 in 10,000 ($1x10^{-4}$)	0.8 $\mu g/m^3$	1 $\mu g/m^3$
1 in 100,000 ($1x10^{-5}$)	0.08 $\mu g/m^3$	0.1 $\mu g/m^3$
1 in 1,000,000 ($1x10^{-6}$)	0.008 $\mu g/m^3$	0.01 $\mu g/m^3$

Page 21, 2nd full paragraph, last sentence. Add: ----using the quantal approach unadjusted for survival.

Page 21, next to last paragraph. Change to read: For the male rats, the summed unit risk (upper 95% confidence limit) was $1.5x10^{-2}$ per ppm,-----approximately 30% higher than the summed risks resulting from the quantal dose-response modeling unadjusted for survival. Results ------

Page 21, last full paragraph. Change to: An inhalation unit risk (upper limit) of 0.12 per mg/m^3 (rounded to 0.1 per mg/m^3), based on ------

Mary Beth Genter:

p. 37, first line of section 4.2.3. “noninhalation” should be changed to ‘inhalation’.

p. 38: I assume that the Boyland paper is discussed only for completeness. It is hard to imagine how that paper could contribute to a human risk assessment.

p. 38. 2nd paragraph should be broken into 2 paragraphs, one on the subcutaneous injection study and one on the skin painting study. Is the strain of the ‘white rats’ in the Knake study also unspecified?

p. 62. last paragraph. The following sentence should be reworded as it is unnecessarily vague as written: ‘However, identification of which metabolites are responsible for naphthalene toxicity and carcinogenicity is unknown.’

p. 62. The next sentence is not much better; it refers to tissue sites on nonneoplastic cellular damage in mice and refers to the bronchoalveolar region of mice, which in fact is a tumor site.

Throughout the text: 1994a vs 1994b USEPA documents should be distinguished from one another in the text.

Literature cited:

1. Viravaidya K, Sin A, Shuler ML (2004) Development of a microscale cell culture analog to probe naphthalene toxicity. Biotechnol Prog 20:316-23.

2. Mitchell AE, Lakritz J, Jones AD. (2000) Quantification of individual glutathione S-transferase isozymes in hepatic and pulmonary tissues of naphthalene-tolerant mice. Arch Toxicol 2000 74:215-21.

3. West JA, Williams KJ, Toskala E, Nishio SJ, Fleschner CA, Forman HJ, Buckpitt AR, Plopper CG (2002) Induction of tolerance to naphthalene in Clara cells is dependent on a stable phenotypic adaptation favoring maintenance of the glutathione pool. Am J Pathol 160:1115-27.

4. Vuchetich PJ, Bagchi D, Bagchi M, Hassoun EA, Tang L, Stohs SJ (1996) Naphthalene-induced oxidative stress in rats and the protective effects of vitamin E succinate. Free Radic Biol Med 21:577-90.

5. Stohs SJ, Ohia S, Bagchi D (2002) Naphthalene toxicity and antioxidant nutrients. Toxicology 180:97-105.

6. Ding XX, Koop DR, Crump BL, Coon MJ (1986) Immunochemical identification of cytochrome P-450 isozyme 3a (P-450ALC) in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. Mol Pharmacol 30:370-8.

7. Reed CJ, Lock EA, De Matteis F (1986) NADPH: cytochrome P-450 reductase in olfactory epithelium. Relevance to cytochrome P-450-dependent reactions. Biochem J 240:585-92.

8. Yao R, Wang Y, Lubet RA, You M (2003) Differential gene expression in chemically induced mouse lung adenomas. Neoplasia 5:41-52.

John Morris:

The external review draft of the Toxicological Review of Naphthalene is a concise well-written document. Overall, the text clearly describes the literature that is cited and clearly explains the basis for the conclusions that are drawn both with respect to the cited literature and to EPA guidelines. As all reviews, this review focuses on areas for improvement. This focus should not be construed to detract from my perception of the overall high quality of the document.

As a general comment, I would advise that additional clarity in all sections would be achieved by inclusion of summary paragraphs that highlight the most critical aspects of the available database, and perhaps more importantly, explicitly highlight the critical data gaps. This latter information would aid in the objectivity of the document and also might serve to appropriately direct future research efforts. Specific comments to the charge questions follow.

Laura Van Winkle:

Overall Comments on the EPA's draft revision of the Toxicological Review of Naphthalene. In general I felt that this was a well-written and extremely well-researched document. It could benefit from editing and cross referencing for clarity. Some of the studies cited (Adkins for example) could be given less weight based on the quality of their data and the small increases shown. It was difficult to discern from the text, as currently written, which studies should be given more weight. In several sections, particularly the modeling and calculation sections, the pros and cons (rationale) of the methods chosen could be better explained.

***Suggested Editing*:**

1. first sentence of section 4.2.3 "noninhalation" should read inhalation.
2. The table on page 16 would be easier to read if males and females were separate, as in the table on page 75.
3. p14, last line: Van Winkle 1996 is not the appropriate reference for NA metabolism by CYP2B4. Van Winkle 1996 shows protein expression. Suggest Van Bladeren et al.
4. p15, second paragraph, discussion of Baldwin et al. CYP2F "expression" is used repeatedly but it is not clear whether this is protein or gene expression. While CYP2F protein was found in rodent airways and the highest level was in distal airways, CYP2F was not detected in the rhesus airways (see the results section of Baldwin et al). Also, suggest throughout that the amount of metabolite/protein/activity be references to the compartment and tissue sampled as this can make a large difference in the result.
5. Clarify the phrase "administration of NA" by stating it was given intraperitoneally on page 56. This is important because target site susceptibility has been found to vary by route of administration of NA. By inhalation the proximal airways are more susceptible in mice see (West et al. 2001).

6. Move Figure 1 on pg 18 up a page or 2.
7. p.88 add the word “female” to the tumor bearing A/J mouse statement in #3.
8. p.65 change “know” to “known” in first sentence 3rd para
9. p.65 should make clear that Plopper 92 is an i.p. exposure
10. First para under gender differences (shouldn’t this be sex differences?) seems out of place on page 69.
11. p.69 2nd to last sentence top para- little to no developmental information on CYP expression in humans. Pattern of CYP2F expression has been defined for mouse postnatal development. (Fanucchi et al. 1997).

Literature Cited:

Baldwin, R. M., W. T. Jewell, M. V. Fanucchi, C. G. Plopper and A. R. Buckpitt (2004). "Comparison of pulmonary/nasal CYP2F expression levels in rodents and rhesus macaque." J Pharmacol Exp Ther 309(1): 127-36.

Boland, B., C. Y. Lin, D. Morin, L. Miller, C. G. Plopper and A. Buckpitt (2004). "Site specific metabolism of naphthalene and 1-nitronaphthalene in dissected airways of rhesus macaques." J Pharmacol Exp Ther.

Buckpitt, A., B. Boland, M. Isbell, D. Morin, M. Shultz, R. Baldwin, K. Chan, A. Karlsson, C. Lin, A. Taff, J. West, M. Fanucchi, L. Van Winkle and C. Plopper (2002). "Naphthalene-induced respiratory tract toxicity: metabolic mechanisms of toxicity." Drug Metab Rev 34(4): 791-820.

Cho, M., C. Chichester, D. Morin, C. Plopper and A. Buckpitt (1994). "Covalent interactions of reactive naphthalene metabolites with proteins." J Pharmacol Exp Ther 269(2): 881-9.

Chuang, J. C., P. J. Callahan, C. W. Lyu and N. K. Wilson (1999). "Polycyclic aromatic hydrocarbon exposures of children in low-income families." J Expo Anal Environ Epidemiol 9(2): 85-98.

Fanucchi, M., M. Murphy and C. Plopper (1997). "Long-term effects of acute naphthalene injury during postnatal development." Am J Resp Crit Care Med 155(4): A45.

Fanucchi, M. V., A. R. Buckpitt, M. E. Murphy and C. G. Plopper (1997). "Naphthalene cytotoxicity of differentiating Clara cells in neonatal mice." Toxicol Appl Pharmacol(144): 96-104.

Fanucchi, M. V., M. E. Murphy, A. R. Buckpitt, R. M. Philpot and C. G. Plopper (1997). "Pulmonary cytochrome P450 monooxygenase and Clara cell differentiation in mice." American Journal of Respiratory Cell and Molecular Biology 17(3): 302-14.

Harkema, J. R. (1992). Epithelial Cells of the Nasal Passages. Comparative Biology of the Normal Lung. R. A. Parent. Boca Raton, CRC Press**:** 27-36.

Lawson, G. W., L. S. Van Winkle, E. Toskala, R. M. Senior, W. C. Parks and C. G. Plopper (2002). "Mouse strain modulates the role of the ciliated cell in acute tracheobronchial airway injury-distal airways." Am J Pathol 160(1): 315-327.

Lee, C., K. C. Watt, A. M. Chang, C. G. Plopper, A. R. Buckpitt and K. E. Pinkerton (1998). "Site-selective differences in cytochrome P450 isoform activities. Comparison of expression in rat and rhesus monkey lung and induction in rats." Drug Metab Dispos 26(5): 396-400.

Miller, D. P., D. Neuberg, I. de Vivo, J. C. Wain, T. J. Lynch, L. Su and D. C. Christiani (2003). "Smoking and the risk of lung cancer: susceptibility with GSTP1 polymorphisms." Epidemiology 14(5): 545-51.

Plopper, C., L. V. Winkle, M. Fanucchi, S. Malburg, S. Nishio, A. Chang and A. Buckpitt. (2001). "Early events in naphthalene-induced acute Clara cell toxicity: II. Comparison of glutathione depletion and histopathology by airway location." Am. J. Resp. Cell Mol. Biol 24(3): 272-81.

Plopper, C. G. and D. M. Hyde (1992). Epithelial cells of bronchioles. Comparative biology of the normal lung. R. A. Parent. Boca Raton, CRC Press. 1**:** 85-92.

Shultz, M. A., P. V. Choudary and A. R. Buckpitt (1999). "Role of murine cytochrome P-450 2F2 in metabolic activation of naphthalene and metabolism of other xenobiotics." J Pharmacol Exp Ther 290(1): 281-8.

Shultz, M. A., D. Morin, A. M. Chang and A. Buckpitt (2001). "Metabolic capabilities of CYP2F2 with various pulmonary toxicants and its relative abundance in mouse lung subcompartments." J Pharmacol Exp Ther 296(2): 510-9.

Smiley-Jewell, S. M., F. J. Liu and C. G. Plopper (1999). "Age-related differences in airway cell proliferation following acute injury to neonatal rabbit Clara cells." Am J Resp Cell Mol Biol submitted.

Smiley-Jewell, S. M., F. J. Liu, A. J. Weir and C. G. Plopper (2000). "Acute injury to differentiating Clara cells in neonatal rabbits results in age-related failure of bronchiolar epithelial repair." Toxicol Pathol 28(2): 267-76.

Smiley-Jewell, S. M., S. J. Nishio, A. J. Weir and C. G. Plopper (1998). "Neonatal Clara cell toxicity by 4-ipomeanol alters bronchiolar organization in adult rabbits." Am J Physiol 274(4 Pt 1): L485-98.

Stripp, B. R., K. Maxson, R. Mera and G. Singh (1995). "Plasticity of airway cell proliferation and gene expression after acute naphthalene injury." Am. J. Physiol. (Lung Cell. Mol. Physiol.) 269(13): L791-L799.

Van Winkle, L., G. Baker, R. Stelck, C. Brown, C. Plopper and A. Buckpitt (2004). "Estrous Cycle alters Pulmonary Metabolism of Naphthalene (NA)." The Toxicologist. Vol 78((S-1)): p 145 A#705.

Van Winkle, L. S., A. R. Buckpitt, S. J. Nishio, J. M. Isaac and C. G. Plopper (1995). "Cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice." Am. J. Physiol. (Lung Cell. Mol. Physiol.) 269(13): L800-L818.

Van Winkle, L. S., M. J. Evans, C. D. Brown, N. H. Willits, K. E. Pinkerton and C. G. Plopper (2001). "Prior exposure to aged and diluted sidestream cigarette smoke impairs bronchiolar injury and repair." Toxicol Sci 60(1): 152-64.

Viravaidya, K., A. Sin and M. L. Shuler (2004). "Development of a microscale cell culture analog to probe naphthalene toxicity." Biotechnol Prog 20(1): 316-23.

Wang, Y., M. R. Spitz, M. B. Schabath, F. Ali-Osman, H. Mata and X. Wu (2003). "Association between glutathione S-transferase p1 polymorphisms and lung cancer risk in Caucasians: a case-control study." Lung Cancer 40(1): 25-32.

West, J. A., G. Pakehham, D. Morin, C. A. Fleschner, A. R. Buckpitt and C. G. Plopper (2001). "Inhaled naphthalene causes dose dependent Clara cell cytotoxicity in mice but not in rats." Toxicol Appl Pharmacol 173(2): 114-9.

West, J. A., L. S. Van Winkle, D. Morin, C. A. Fleschner, H. J. Forman, A. R. Buckpitt and C. G. Plopper (2003). "Repeated Inhalation Exposures of the Bioactivated Cytotoxicant Naphthalene (NA) Produce Airway Specific Clara Cell Tolerance in Mice." Toxicol Sci 190(3): 286-293.

West, J. A., K. J. Williams, E. Toskala, S. J. Nishio, C. A. Fleschner, H. J. Forman, A. R. Buckpitt and C. G. Plopper (2002). "Induction of tolerance to naphthalene in Clara cells is dependent on a stable phenotypic adaptation favoring maintenance of the glutathione pool." Am J Pathol 160(3): 1115-27.