Commentary & Suggestions For:
"Issues, Identified Data Gaps, and Possible Studies to Address Areas of Uncertainty Associated with Human Cancer Risks from the Inhalation of Naphthalene"

Background

As a result of an 7 October 2005 meeting among OMB, DoD, EPA, and other federal agencies regarding the potential inhalation carcinogenicity of naphthalene, my office offered to develop a report that discusses the science issues, identifies data gaps and suggests possible studies to address areas of uncertainty. The draft report is provided for your review and comment (Tab A). The report attempts to address more generic issues as well as the specific data gaps and technical uncertainties. We would also like the report to identify the information that would augment the naphthalene database, better inform the human health risk assessment, and reduce reliance on generic uncertainty factors.

The final report will be submitted to the Naphthalene State of the Science Symposium, proposed for fall of 2006 (Tab B). The objective of the Symposium is to identify, vet, and rank the key uncertainty issues regarding naphthalene's human health risk assessment in order to determine the need for additional research and studies.

The themes reflected among the "unresolved science issues" included species relevance, species sensitivity, metabolism & injury, mode of action, saturation effects and protective mechanisms, absence of supporting epidemiology, administered vs. actual dose. (Tab C)

The attached "talker" summarizes two comparatively recent EPA briefs (2004 & 2006) on naphthalene highlighting the issues and research needs as that agency sees them. (Tab D)

Task

The draft paper (prepared in response to an DUSD(I&E) tasker) captures and repackages the themes highlighted on 07Oct05 while identifying data gaps and areas of uncertainty warranting further study. This paper will be submitted for consideration to the upcoming Naphthalene State of the Science Symposium (NS^3) and is to represent DOD's perspective on the questions attending naphthalene's reassessment as a potent inhalation carcinogen. However, analysis of the larger issues as well as the particular data gaps and technical uncertainties has evolved since the October meeting and ought to be captured in this report as part of DOD's submission.

Using the draft paper as a basis, the task is to identify *all* the issues that would augment naphthalene's data base and better inform its application to *human* risk assessment. As an independent body embracing a broad array of expertise, it is NS^3's mission, rather than ours as a stakeholder, to identify, vet and rank all of the key issues attending naphthalene's risk assessment, and to come to consensus on what studies and study designs are needed to more fully inform naphthalene's assessment pertinent to human risk.

Issues

The following discussion, which highlights some of fundamental issues that should be considered, is provided for your review during the development of your comments on the draft report. Among the fundamental issues are:

- Are the National Toxicology Program (NTP) studies unconfounded?
- Is the rodent model in this case pertinent to and predictive of human risk – is it the right model?
- Is the LMS model an appropriate tool for characterizing naphthalene's carcinogenic potential – or is this chemical a threshold carcinogen – or both?
- If naphthalene is as potent as the EPA argues it to be based on the rodent model, then "where are the *human* bodies"? What does epidemiology, and our experience, tell us?

Are the National Toxicology Program (NTP) studies unconfounded?

The extraordinary incidence of cytotoxicity across all exposure concentrations in the NTP studies may suggest the exposure concentrations were too high. The selection of exposure concentrations were linked expressly to two different estimates of the vapor saturation limit for naphthalene in air, not to dose ranging studies. Tumors emerge in the context of a 90-100% incidence of irritation, inflammation, injury and repair – even at the lowest exposure concentration of 10 ppm (see Tab E).

The increased sophistication and changes in the testing conditions between the early and later rat bioassays suggests that operationally something was learned and the test systems changed. The exposure data itself suggests concerns; however, the rationale for the changes is not explained. For example, the presence/absence of an inhalable condensation aerosol, which would exaggerate the irritation and inflammatory effects of naphthalene, is not discussed.

Is the rodent model in this case pertinent to and predictive of human risk – is it the right model?

The rat nose has a proportionately greater surface area susceptible to naphthalene's effects (~50% olfactory epithelium vs ~10% in humans). The rat nose has a high metabolic capacity . Rates of metabolic turnover in rodents are reportedly 10-100X higher than primates. Glutathione depletion and potentially different cytochrome profiles also are implicated.

There are species- and site-specific differences in response among test species such as: rats – nasal toxicity by mouth or injection; tumors by inhalation at high exposures; mice – pulmonary toxicity by mouth or injection; tumors at high exposure concentrations, and primates – not defined, less CYP2F suggests primates refractory to effects

The International Agency for Research on Cancer (IARC) is more cautious about the NTP rodent assays; while cautious, IARC has not concluded that naphthalene a human carcinogen.

The key issue could revolve around whether a rodent or a primate is the more predictive model for human risk assessment. Rodent-based work on modes/mechanisms of action and metabolism may be of academic interest – if the primate is the pertinent and predictive model, then the research needs to be recast/refocused on that more human model. What is the evidence that the rat isn't the appropriate model and would future work in primates be more beneficial than those rodent-based studies?

The past and current debates associated with the TCE, perchlorate, and light hydrocarbon risk assessments suggest that no assumptions about the pertinence of the rodent model should be made. In the case of light hydrocarbon nephropathy, it took a decade of research and scientific debate (augmented with mechanistic and human epidemiology studies) before it was determined that the effect was specific to male rats and not pertinent to human risk assessment.

Is the Linear Multi-Stage (LMS) model an appropriate tool for characterizing naphthalene's carcinogenic potential – or is this chemical a threshold carcinogen – or both?

The LMS model presupposes a one-hit linear no threshold approach to cancer development. On the other hand, naphthalene did not induce positive results in 30 in vitro assays with mammalian as well as non-mammalian cell systems. There is scant evidence for naphthalene's potency in these assays without activation with rodent enzyme systems; a response that could be checked in primate-based systems.

If the LMS is not applicable, then protecting vulnerable tissues from naphthalene's cytotoxic effects should also protect them from cancer. After the processes of irritation, inflammation, injury and repair begin, their vulnerability to naphthalene's active metabolites would likely increase and susceptibility to cancer may then begin.

Besides mechanistic studies (rodent- or primate-based?), the answer to this question may require a second bioassay with exposure concentrations beginning at, and ranging below 10 ppm, spanning perhaps from 10 down to 1.0 or 0.1 ppm. Additional bioassays would assist in addressing the adequacy of the current TLVs/OELs – all set at 10 ppm; 10 ppm caused cancer in the NTP rat bioassay.

Are there a noticeable number of naphthalene related cancers in exposed human populations?

While there is little, if any, epidemiological data on exposures exclusively to naphthalene, there is a body of literature on exposures to mixtures containing naphthalene, petroleum and creosote workers. The results of these epidemiological studies, including a new (unpublished) Air Force study, are negative, caution against over-investing in the rat bioassay. (Tab F)

Issues, Identified Data Gaps, and Possible Studies to Address Areas of Uncertainty Associated with Human Cancer Risks from the Inhalation of Naphthalene

July 2006

Executive Summary

In updating its Integrated Risk Information System database, the US Environmental Protection Agency (EPA) proposed that naphthalene be classified as a likely human carcinogen (U.S. EPA, 2005). This change in naphthalene's human carcinogenic potential from "possible" to "likely" is based primarily on the results of inhalation studies in mice and rats, conducted by the National Toxicology Program (NTP, 1998, NTP, 2000). The final classification of naphthalene's carcinogenic potential in humans is the subject of ongoing scientific peer review. There is a current lack of understanding regarding the mode of action for naphthalene and possible relevance of findings to humans.

Several steps should be examined by EPA prior to a final determination. The report discusses which studies we suggest should be considered based on what their results will provide regarding a weight-of-evidence assessment of naphthalene's human carcinogenic potential. Four priority areas of study are identified in order of relative important to assessing human health risk. We understand that academics, industry, and EPA are pursuing a state of the science symposium to further explore risk, uncertainty and which studies might be done to further understand human health risks. As new information is revealed or developed, we may revise these recommendations.

Genotoxicity studies using S9 fractions derived from the target tissues of rats and mice are warranted at this time. Current genotoxicity data for naphthalene suggest that the chemical is not genotoxic. However, one of the concerns that has been raised recently is that a unique naphthalene metabolite, which cannot be produced using standard liver S9 fractions, may be formed in rat and mouse target tissues. This hypothesis is worth testing using standard Ames assays.

Because substantial evidence suggests that naphthalene produces tumors in rats and mice via a cytotoxic mechanism of action, studies to fully characterize the cytotoxic response of target tissues upon repeat naphthalene exposure should be conducted. Such studies will likely require preliminary research be conducted first to ensure that the final experimental design is appropriate to address the questions at hand. Such studies will likely be highly complex, assessing the time-course, dose-response, and regional distribution of cytotoxic responses, as well as changes in cell proliferation and apoptosis rates. Because further information regarding the cytotoxic response to naphthalene treatment is still needed, specific experiments to address threshold response are not appropriate at this time; however, this issue may be examined in part via incorporation of suitable endpoints into the repeat naphthalene exposure studies.

Considerable basic research regarding the target tissue-specific metabolism of naphthalene in rats and mice is still needed. The information ultimately derived from such studies will likely prove important for assessing the human relevance of findings from naphthalene rodent inhalation studies. At this time, however, this area of research is still too basic to provide much practical data that can be immediately applied to assessing the human health risk of naphthalene. **Studies that have significant potential for providing information for immediate use should be given preference, include those to identify cytochrome P450s expressed in rat and mouse target tissues, followed by experiments using isoform-specific inhibitors to demonstrate**

which specific P450 isoforms are involved in naphthalene cytotoxic responses. The results of such studies can be used to compare with P450 expression patterns in human target tissues (as discussed in next paragraph).

With regard to assessing the relevance of findings from naphthalene rodent inhalation studies, an examination of naphthalene deposition patterns in human lungs will likely be of little value. Rather, the key to understanding the human relevance of these findings lies with understanding the likely naphthalene metabolic pathways of human target tissues. **Cytochrome P450-specific immunohistochemistry and/or *in situ* hybridization experiments on human nasal and respiratory epithelial tissues, as well as experiments using isoform-specific P450 inhibitors on human target tissues in explant culture treated with naphthalene will likely be helpful in addressing the question of human relevance.** Only after the results of rodent naphthalene inhalation studies are shown to be not relevant to human health risk assessment, should studies to identify a more appropriate animal model be conducted.

Although epidemiological data is extremely useful in assessing human relevance, such studies are not recommended as a high priority at this time. Epidemiology studies are extremely expensive and time-consuming. Even if (1) the challenges associated with identifying appropriate exposure cohorts can be overcome, (2) the complications of multiple chemical exposures can be convincingly reconciled, and (3) the outcome is negative in one or several such studies, these will likely have little impact on the human health risk assessment for naphthalene. Rather, only after a wealth of negative studies is published, will the epidemiological data likely carry substantial weight. Thus, while these studies are certainly needed to fill the void of epidemiological data available for naphthalene, other studies will likely have a larger immediate impact on naphthalene's human health risk assessment.

Concerns have been raised that the NTP study was conducted at vapor concentrations that were inappropriately high and that another bioassay should be done using naphthalene concentrations that are more environmentally relevant (at 10 ppm and below). Although such a study likely would be negative for tumors (the size of the treatment groups would probably need to be increased substantially to provide statistical robustness), the results would not erase or negate the findings of the previous NTP study. Furthermore, such a study provides no information regarding the mechanism by which tumors are produced upon chronic, high concentration naphthalene exposure. In order to address the human relevance of the NTP tumor findings, efforts should be spent addressing the mechanism of action for naphthalene carcinogenicity.

Introduction

In updating its Integrated Risk Information System (IRIS) database, the U.S. Environmental Protection Agency (EPA) has proposed that naphthalene be classified as a likely human carcinogen (U.S. EPA, 2005). This change in naphthalene's human carcinogenic potential from '*possible*' (based on inadequate human and limited animal data; U.S. EPA, 1998) to '*likely*' is based primarily on the results of inhalation studies in mice and rats, conducted by the National Toxicology Program (NTP, 1992; NTP, 2000). The final classification of naphthalene's carcinogenic potential in humans is the subject of ongoing scientific peer review. There is a current lack of understanding regarding the mode of action for naphthalene and possible relevance of findings to humans.

Critical Studies

The two NTP long-term toxicology and carcinogenicity studies for naphthalene used by EPA to support the change in the human carcinogenic potential are as follows.

Mouse Inhalation Study

In the mouse inhalation study (NTP, 1998), groups of male and female B6C3F1 mice were exposed to 0, 10, or 30 ppm naphthalene vapors for 6 hours/day, 5 days/week for two years. Additional animals per sex were also included in each exposure group for hematological evaluations at 14 days, and 3, 6, 12, and 18 months; however, because of decreased survival of control male mice due to fighting, only the 14-day evaluations were conducted and the remaining mice were incorporated into the two-year carcinogenicity study. Findings after two year's exposure to naphthalene vapors are as tabulated in Table 1.

Table 1. Incidences of Survival, Neoplastic, and Non-neoplastic Lesions in Mice Exposed to Naphthalene via Inhalation.

	0 ppm		10 ppm		30 ppm	
	M	**F**	**M**	**F**	**M**	**F**
Total # mice	70	69	69	65	133	135
Survival	37% (26/70)	86% (59/69)	75% (52/69)	88% (57/65)	87% (118/135)	76% (102/135)
Chronic nasal inflammation	0/70	1/69	67/69	65/65	133/135	135/135
Hyperplasia of respir-atory epithelium of nose	0/70	0/69	66/69	65/65	134/135	135/135
Metaplasia of olfactory epithelium	0/70	0/69	66/69	65/65	134/135	135/135
Chronic lung inflammation	0/70	3/69	21/69	13/65	56/135	52/135
Alveolar/bronchiolar adenomas	7/70	5/69	15/69	2/65	27/135	28/135
Alveolar/bronchiolar carcinomas	0/70	0/69	3/69	0/65	7/135	1/135

Body weights of treated mice were slightly decreased, but still within 10% of control values. Females in the high dose group displayed a significantly increased combined incidence of

alveolar/bronchiolar adenomas and carcinomas. This incidence was above the historical control range from all NTP feed, drinking water, and inhalation studies to date (7.8%, range = 0-16%). In comparison, treated male mice exhibited only a marginally increased incidence of alveolar/bronchiolar adenomas and carcinomas, which was within the historical control range from previous NTP studies (19.5%, range = 6-42%). Histologically, the adenomas (which are considered benign tumors) and carcinomas were considered to represent a "morphologic continuum," and occurred within a background of other, non-neoplastic lesions generally considered to represent an overall inflammatory response of the lung to naphthalene exposure.

In the nose, several non-neoplastic lesions were observed in treated mice of both sexes. These lesions were localized to the posterior nasal cavity and classified as either chronic inflammation of the nasal tissues, hyperplasia of the respiratory epithelium of the nasal cavity, or metaplasia of the olfactory tissues. The almost universal incidence of these lesions in treated mice of both sexes was thought to represent a general inflammatory and regenerative process in response to naphthalene exposure. Also, nasal adenomas were observed in two females from the 10 ppm treatment group; however, these findings were not considered to be treatment-related because the incidence was within historical control ranges and no nasal adenomas were identified in females treated with 30 ppm naphthalene.

Rat Inhalation Study

In the rat inhalation study (NTP, 2000; Abdo et al., 2001; Long et al., 2003), groups of 49 male and 49 female F344 rats were exposed to 0, 10, 30, or 60 ppm naphthalene vapors for 6 hours/day, 5 days/week for two years. Findings from this study are found in Table 2.

Survival was similar across all treatment groups. Body weights of males from exposed groups were less than those of controls throughout study; body weights of females were similar across all treatment groups. Neuroblastomas of the olfactory epithelium were observed in male rats exposed to 30 and 60 ppm naphthalene and in female rats from all exposed groups, with positive trends in both sexes. Furthermore, the incidence of neuroblastomas in the 60 ppm females was significantly elevated over that of controls. Because neuroblastomas have not been observed historically in control rats from NTP inhalation studies, these tumors were concluded to be treatment-related. The tumors arose in the olfactory region of the nose, but often extended posteriorly. Larger tumors blocked the nasal passages and obliterated the normal nasal architecture. In a few cases, the neuroblastomas invaded the brain. Additionally, one male in each of the 30 and 60 ppm groups exhibited metastases in the lungs. These tumors occurred in the presence of extensive non-neoplastic lesions of the olfactory epithelium, including atypical hyperplasia, atrophy, chronic inflammation, and hyaline degeneration. The lesions occurred with almost 100% incidences in both sexes and at all exposure concentrations.

Adenomas of the respiratory epithelium were observed in the noses of male rats from all exposure groups with a dose-related trend of increased incidence with increased exposure concentrations. Similarly, adenomas of the respiratory epithelium of the nose occurred in the 30 and 60 ppm females, although the increased incidences were not statistically significant. Like the aforementioned neuroblastomas, these adenomas occurred in the presence of high incidences of a variety of non-neoplastic lesions, including, hyperplasia, squamous metaplasia, hyaline degeneration, and goblet cell hyperplasia. Because adenomas of the respiratory epithelium of the

nose are not seen in the historical database of control rats from NTP inhalation studies, these tumors were concluded to be related to exposure.

Table 2. Incidences of Survival, Neoplastic, and Non-neoplastic Lesions in Rats Exposed to Naphthalene via Inhalation.

	0 ppm		10 ppm		30 ppm		60 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Total # rats	49	49	49	49	49*	49	49*	49
Survival	49% (24/49)	57% (28/49)	45% (22/49)	43% (21/49)	48% (23/48)	57% (28/49)	43% (21/49)	49% (24/49)
Lesions of the Olfactory Epithelium								
Atypical hyperplasia	0/49	0/49	48/49	48/49	45/48	48/49	46/48	43/49
Atrophy	3/49	0/49	49/49	49/49	48/48	49/49	47/48	47/49
Chronic inflammation	0/49	0/49	49/49	47/49	48/48	47/49	48/48	45/49
Hyaline degeneration	3/49	13/49	46/49	46/49	40/48	49/49	38/48	45/49
Neuroblastoma	0/49	0/49	0/49	2/49	4/48	3/49	3/48	12/49
Lesions of the Nasal Respiratory Epithelium								
Hyperplasia	3/49	0/49	21/49	18/49	29/48	22/49	29/48	23/49
Squamous metaplasia	0/49	0/49	15/49	21/49	23/48	17/49	18/48	15/49
Hyaline degeneration	0/49	8/49	20/49	33/49	19/48	34/49	19/48	28/49
Goblet cell hyperplasia	0/49	0/49	25/49	16/49	29/48	29/49	26/48	20/49
Adenoma	0/49	0/49	6/49	0/49	8/48	4/49	15/48	2/49
Lesions of the Nasal Glands								
Hyperplasia	1/49	0/49	49/49	48/49	48/48	48/49	48/48	42/49
Squamous metaplasia	0/49	0/49	3/49	2/49	14/48	20/49	26/48	20/49

*One male was missexed at 30 ppm; thus, lesions incidences are calculated based on a total of 48 males. The reason for calculating incidences based on 48 males at 60 ppm is not known.

Draft EPA Reassessment

The original IRIS toxicology assessment for naphthalene, which was published in 1998, deemed that the human carcinogenic potential of naphthalene via the oral and inhalation routes could not be determined based upon the inadequate human and limited animal data available at the time (U.S. EPA, 1998). Furthermore, the EPA suggested that the issue of human carcinogenic potential would be revisited upon availability of data from the NTP's rat naphthalene inhalation study (in progress at the time the original IRIS assessment was published).

Since 2000, the EPA has been in the process of revising its naphthalene toxicology assessment, taking into consideration new data developed since the first toxicology review, including those from the NTP rat study. The EPA has proposed that naphthalene's carcinogenic potential be changed from 'possible' to 'likely' (U.S. EPA, 2005), based on evidence of neuroblastomas in

male and female rats and respiratory adenomas of the nose in male rats following inhalation exposures (NTP, 2000; Abdo et al., 2001). It should be noted that no additional human data demonstrating possible carcinogenicity associated with naphthalene exposures have been published between 1998 and 2000. The EPA also derived an inhalation unit risk value based on the findings of the NTP rat inhalation study using a linear low dose extrapolation below the point of departure (U.S. EPA, 2004; U.S. EPA, 2005), although data supporting a genotoxic mode of action are weak.

These changes to the EPA's toxicology assessment for naphthalene raise questions related to (1) the lack of human epidemiological evidence demonstrating an association of naphthalene exposure with cancer; (2) the limited evidence indicating a genotoxic mode of action (and therefore, lack of support for using a linear low dose extrapolation to derive an inhalation unit risk value); and (3) the relevance of findings from inhalation studies conducted in rats and mice to human health risk assessment.

(1) If naphthalene 'likely' causes cancer in humans (and warrants the unit risk value calculated by the EPA), why are there no reports in the epidemiological literature indicating such an association?

As discussed in the original EPA toxicity assessment for naphthalene (U.S. EPA, 1998), almost no epidemiological data exist to suggest that naphthalene is carcinogenic to humans, although numerous studies show that exposure may be associated with hemolytic anemia and cataract formation (see U.S. EPA, 1998 for references). In 1998, the EPA identified only two epidemiology studies suggesting carcinogenicity (Wolf, 1976; Kup, 1978), both of which are confounded by factors that limit the conclusions that can be drawn from them. As noted in the EPA assessment (U.S. EPA, 1998), the Wolf study (1976) had no controls, a limited number of subjects, simultaneous exposures to other chemicals, and no calculation of exposure concentrations. The four cases of larynx cancer that were observed were diagnosed in tobacco smokers. Two non-respiratory cancers (of the stomach and cecum) were also identified. In the other study (Kup, 1978), 12 cases of larynx cancer, two cases of epipharyngeal cancer, and one case of nasal carcinoma were examined for work-related associations. Of these 15 cancers, 12 were diagnosed in smokers. Of the 12 larynx cancers, four were diagnosed in patients reporting occupational exposure to naphthalene. The study's author concluded that most of the cancers likely developed due to non-work-related causes.

Since the original EPA toxicity assessment for naphthalene, only one additional case study has been identified. In Ajao et al., (1988), 23 cases of colorectal cancer admitted to a Nigerian hospital were examined for association with ingestion of Kafura, a substance used in the treatment of anorectal problems, and according to the study authors, purported to contain naphthalene. Approximately half of the patients reported prior use of Kafura. Unfortunately, the naphthalene concentration of Kafura is unknown. Furthermore, no controls were examined, and the frequency of Kafura use in the normal population is not reported.

With such limited epidemiological evidence, an association between naphthalene exposure and cancer in humans is certainly equivocal.

(2) What evidence exists to indicate a genotoxic mode of action for naphthalene-induced lung tumors in mice and nasal tumors in rats (thereby supporting use of linear low dose extrapolation to develop a cancer potency factor)?

A recent review of the genetic toxicity data for naphthalene clearly found limited evidence to suggest genotoxicity (Schreiner, 2003). Of the 42 genetic toxicity studies analyzed (38 previously reported studies and four new studies; 18 bacterial assays, 10 cytogenic assays [seven *in vitro* and three *in vivo*], and 14 other assays [six cell transformation studies, four unscheduled DNA synthesis assays, two alkaline elution assays, one *Drosophila* assay, and one human cell mutation assay]), 38 assays were negative for genetic toxicity. These included 33 *in vitro* assays and five *in vivo* mammalian assays. The four studies that showed positive results included an NTP *in vitro* chromosome aberration assay, an *in vitro* micronucleus assay (conducted in a human lymphoblastoid cell line), an *in vitro* embryo chromosome assay, and the *Drosophila* assay. However, the results of the NTP study were considered negative by the U.K. Health and Safety Executive because positive results were seen only in the second of two trials and appeared to be due to lower control values in that trial (Scheiner, 2003). Overall, the 42 studies indicate that naphthalene is not mutagenic (i.e., does not cause changes to the genetic code), although limited data suggest a possible clastogenic effect (i.e., ability to cause chromosomal breaks), albeit one that requires naphthalene metabolism. Thus, the existing results from genotoxicity investigations appear sufficient to support a cytotoxic (cell-damaging) metabolite of naphthalene, which upon continued tissue injury, induces increased cell replication and subsequent chromosomal changes.

(3) Are the naphthalene carcinogenicity findings in rats and mice relevant to humans?

The human relevance of findings from rat and mouse naphthalene inhalation studies is not clear. Rats and mice are less than ideal models for extrapolating inhalation toxicity studies to humans (DeSesso, 1993; Reznik, 1990). The upper airways of rats and mice are laid out in a linear fashion while that of humans (and upper primates) exhibits an L-shaped arrangement. Rodents are obligate nose-breathers, while humans and some primates can breathe through both their noses and mouths. Finally, the overall morphology of the nasal cavity of rodents is directed towards olfaction (thereby requiring the presence of rather extensive olfactory epithelium in the nasal cavity), while that of humans is focused primarily on breathing. Interestingly, intraperitoneal naphthalene administration was shown to induce cellular damage in the Clara cells of mice and olfactory epithelium of rats (O'Brien et al., 1985; Buckpitt et al., 1995; Plopper et al., 1992; Lee et al., 2005), suggesting that differences in inhalation patterns alone may not be the seminal factor in species-specific expression of naphthalene cytotoxicity. However, these results do not completely rule out a role for nasal/respiratory airflow in determining region-specific injury patterns, as illustrated in the study by Lee et al., (2005), which compared results in the nasal cavity of rats treated with naphthalene via the inhalation and intraperitoneal routes of administration.

In addition to functional and anatomical distinctions, differences in metabolic capacity of the olfactory and respiratory tissues between rodents and humans also exist. In fact, such differences between mice and rats are believed to account for the differences in regional-specific injury observed in these two species following naphthalene treatment. Cytotoxicity in each species is generally confined to those areas shown to have high metabolic activity towards naphthalene –

that is, the lung Clara cells in mice and the olfactory and nasal respiratory epitheliums in rats (Plopper et al., 1992; Buckpitt et al., 1995; Lee et al., 2005). Furthermore, research has indicated a role for cytochrome P450 isoform CYP2F in naphthalene metabolism and its subsequent cytotoxicity in both species (Baldwin et al., 2005; Shultz et al., 1999; Buckpitt et al., 1995). Other studies suggest that humans express CYP2F in the lungs and nasal cavity at substantially lower concentrations than mice and rats. Such differences suggest that more research is needed regarding the metabolic capacity of target tissues among species before the relevance of findings in rodents for assessing the human carcinogenic potential of naphthalene is fully understood.

Data Gaps

A number of data gaps regarding naphthalene's carcinogenic potential in humans have been identified.

Epidemiology

- To date, sound epidemiological studies have not been published investigating whether an association exists between naphthalene exposure and cancer (or more specifically, cancer of the nasal and/or respiratory tissues). Robust cohort studies (which follow a population with known exposure) and case-control studies (which retrospectively examine exposure histories of cancer cases and controls) should be conducted. Such studies are especially important for assessing the human carcinogenic potential of naphthalene because data demonstrating naphthalene's genotoxicity are weak at best, which suggests that this chemical may induce tumors in rodents via a mode of action not relevant to humans.

Mode of Action

- Data demonstrating genotoxic modes of action for naphthalene are limited (Schreiner, 2003). Additional studies looking at possible genotoxicity in the nasal cavities and lungs, specifically, are needed.
- Substantial evidence exists supporting a cytotoxic mechanism of action for naphthalene's carcinogenicity (i.e., tumors only develop in tissues exhibiting a high background incidence of chronic damage; NTP, 1998, 2000; Abdo et al., 2001; Long et al., 2003). Studies to further explore the role of cytotoxicity in naphthalene's carcinogenicity in rats and mice are required.
- A cytotoxic mode of action (especially one that likely involves metabolism of naphthalene to the critical cytotoxicant) often also translates to a possible threshold effect. That is, below a certain naphthalene exposure concentration (or threshold), the body can protect against the associated cytotoxicity – either by detoxification via metabolism and/or glutathione (GSH) conjugation and excretion (thereby preventing cytotoxicity from developing) and/or repair and regeneration of damaged tissues. Above the threshold concentration, however, detoxification/excretion and/or repair mechanisms are overwhelmed (i.e., become saturated) and cytotoxicity accumulates. Additional study is required to better understand the dose-response relationships among naphthalene concentrations, production of toxic metabolite(s), detoxification rates, cytotoxicity, and tumor development in rats and mice.

Metabolism

- Studies have suggested that naphthalene metabolism plays an important role in its subsequent carcinogenicity (Wilson et al., 1996; Buckpitt et al., 2002). Further research

is needed to determine (1) the relevant metabolic pathways, (2) the toxic metabolite(s) of concern for tumor development, (3) the rates of metabolism towards production and detoxification of toxic metabolite(s), and (4) the site-specificity of these metabolic pathways in the rat and mouse.

Species Relevance

- Data exploring how the innate differences between human and rodent respiratory systems affect their sensitivities to naphthalene toxicity are lacking. Studies are required to elucidate the differences and similarities between humans and rodents (rats and mice) in terms of (1) nasal/respiratory naphthalene vapor deposition, (2) naphthalene metabolism and detoxification in target tissues, and (3) sites of tissue toxicity/injury and associated time-courses for development.
- If data demonstrate that rodents are not an appropriate inhalation model for assessing naphthalene's human carcinogenic potential, then a different animal model, possibly non-human primates, should be explored. In such a case, studies of naphthalene exposure in non-human primates, including assessment of both metabolism and toxicity, should be conducted.

Potential Studies to Fill Data Gaps

In order to make an appropriate assessment regarding naphthalene's human carcinogenic potential, research to address the above identified data gaps is required. Possible studies are suggested as follows.

Epidemiology. Appropriately conducted human cohort and case-control studies that demonstrate a possible association of exposure with the development of specific cancers will provide needed data to support a change in naphthalene's human carcinogenicity classification from 'possible' to 'likely.' However, if the study of populations with known naphthalene exposures or the study of specific case populations cannot demonstrate such an association, these data will strongly suggest that the findings of rodent naphthalene inhalation studies are not relevant to humans.

- **Cohort Studies.** Robust cohort studies that prospectively follow a population with known naphthalene exposures should be conducted. Such studies should evaluate the possible association of naphthalene exposure with development of all cancers, in addition to development of cancers specific to the nasal cavity and respiratory tract. Smokers should not be included in the cohorts to be studied because smoking confounds the incidence of respiratory tract cancers and higher naphthalene metabolite (1-naphthol and 2-naphthol) concentrations have been found in the urine of subjects with a history of smoking versus not smoking (Preuss et al., 2005). If in the conduct of such studies occupational exposures cannot be adequately assessed, then naphthalene exposure concentrations can be estimated through quantification of naphthalene concentrations in the breath or of naphthalene metabolites in the urine. Cohorts with likely naphthalene exposures include those involved in the distillation of coal tars, those working in occupations involving production or use of polyaromatic hydrocarbons, those in the military with exposure to military vehicle and jet fuel (jet propellant type-8; JP-8), and those working around commercial jet liners (Jet-A fuel exposures).
- **Case-control Studies.** Human epidemiological studies that retrospectively examine the naphthalene exposure histories of cancer cases and concurrently identified controls are needed. The cancer case populations of interest include those diagnosed with nasal

carcinomas and those with respiratory cancers. Each identified case population should be examined separately (i.e., cases of nasal and respiratory cancers should *not* be grouped together into one case population). Control populations should be identified concurrently for comparison purposes, ideally from the same hospitals as the cases. In addition to potential naphthalene exposures (likely as a result of occupation), smoking histories should also be noted, particularly since smoking has been suggested to affect naphthalene exposures, as noted above. Alternatively, omission of smokers from the case and control populations may be beneficial in order to minimize possible confounding.

Mode of Action.

- **Genotoxicity.** The EPA has recently focused efforts on identifying studies to assess the genotoxic potential of naphthalene (U.S. EPA, 2005). Although most studies to date do not support naphthalene genotoxicity, none have evaluated whether the nasal and respiratory tissues of rats and mice metabolize naphthalene to a genotoxic metabolite that is not produced using standard S9 liver fractions. To evaluate this possibility, participants in an EPA peer consultation workshop (U.S. EPA, 2005) recommended a tiered approach involving *in vitro* assays, followed by *in vivo* studies. If the recommended studies (discussed below) demonstrate genotoxicity, then a genotoxic mode of action for naphthalene can be assumed. However, if the recommended *in vitro* and *in vivo* studies fail to demonstrate genotoxicity, then a cytotoxic mode of action for naphthalene must be further investigated.
    - *In Vitro Assays.* Workshop participants agreed that positive results obtained using these assays would indicate that (1) naphthalene acted through a genotoxic mode of action in causing nasal tumors in rats and lung tumors in mice, and (2) further testing to assess the genotoxic potential of naphthalene would be unwarranted.
        - *Ames Assays.* Use of S9-activating fractions prepared from the nasal tissues of rats and lung tissues of mice in a standard Ames assay was recommended. Ames test strains TA102 and TA104 were suggested for these assays because they are sensitive to oxidative stress and should detect reactive oxygen species produced via naphthalene metabolism. In order to assure that these assays are working properly, positive control mutagens (i.e., agents known to be metabolized to a genotoxicant via the nasal and/or lung tissues) should be included in the testing scheme. The inclusion of such controls may also allow for quantification of the assay response to naphthalene.
        - *Other Assays.* Workshop participants suggested that lymphoblastoid cells expressing individual cytochrome P450 isoforms of interest (those expressed primarily in the nasal tissues of the rat or lung tissues of the mouse) could be used to assess genotoxicity *in vitro*. At this time, the primary CYP isoform of interest is CYP2F, which is preferentially expressed in mouse lung Clara cells (the primary site of naphthalene cytotoxicity in the mouse) and has been associated with naphthalene metabolism to the stereoisomer 1*R*,2*S*-naphthalene epoxide (Buckpitt et al., 1995; Nagata et al., 1990). However, naphthalene metabolism in target tissues (rat nasal tissues and mouse lung tissues) has not been fully characterized at this time, and other cytochrome P450 isoforms may also play a critical role in naphthalene metabolism. As such, such studies may

be shelved until the target tissues-specific critical pathways for naphthalene metabolism have been more fully elucidated. An *in vitro* assay to measure 8-oxo-2-deoxyguanosine (an indicator of oxidative DNA lesions) was also suggested; however, use of the TA102 and TA104 bacterial strains in the Ames assay should cover this possibility.

- *In Vivo Studies.* Whole animal studies to elucidate target-specific genotoxicity were also recommended at the EPA workshop. These studies would only be required if the *in vitro* investigations failed to detect genotoxicity.
  - *Covalent-Binding Studies.* Studies to measure covalent binding of naphthalene and its metabolites to the olfactory and respiratory epithelial tissues of rats and the lungs of mice were recommended. These studies could use either liquid scintillation counting, ^{32}P-postlabeling, or accelerator mass spectrometry. Only a few labs worldwide can perform accelerator mass spectrometry, however, which can be very expensive. In such studies, covalent binding to cellular proteins versus DNA (which results in formation of DNA adducts) will have to be distinguished. Covalent binding to DNA would be an indicator of DNA adducts formation, suggesting naphthalene genotoxicity.
  - *Transgenic Mutagenicity Studies.* Naphthalene inhalation studies conducted in transgenic rats and mice designed for *in vivo* mutagenicity studies were recommended. As suggested at the workshop, an exposure duration of one week likely will be needed to detect a direct-acting carcinogen while exposures of up to three months likely will be required to detect an indirect carcinogen (i.e., one not acting through a directly genotoxic mode of action). Such transgenic animal models include the Big Blue (BB) rat and BB mouse (both available through Stratagene, Inc.), and Mutamouse. These systems allow for the detection of point mutations and small genetic deletions that occur in the target tissues of interest. Briefly, the transgenic rodent model of choice would be treated with naphthalene via inhalation, DNA would be extracted from the isolated target tissue of interest (olfactory and nasal respiratory epithelium in the rat and lung respiratory epithelium in the mouse), and lambda DNA (from a phage vector that has been incorporated into the animal's genome as the target for mutagenesis) would be excised and packaged into a lambda head. Next, host DNA would be infected with the packaged DNA and plated onto agar to yield plaques. The number of blue plaques over the total number of plaques obtained reveals the mutation frequency. These mutations can be further isolated and characterized, as desired. Although these systems detect point mutations and small deletions, they cannot detect large genetic deletions or chromosomal breaks. For these types of genotoxicity, other assays will have to be incorporated into the experiment, including micronuclei formation (as an indicator of chromosomal damage), possibly in combination with measurement of BrdU incorporation, and the Comet assay using single cell electrophoresis (to detect single strand breaks, double strand breaks, and oxidative lesions). The types of damage detected using the Comet assay can be repaired *in vivo*. As such, measurements made at both early and late time

points following exposure may be required in order to assess the degree of repair that occurs. If mutations or other forms of genotoxicity were demonstrated using the *in vivo* mutagenicity models, then a genotoxic mode of action could be assumed for naphthalene administered via inhalation to rats and mice.

- **Cytotoxicity.** The design of studies to assess cytotoxicity was also considered at the EPA peer consultation workshop (U.S. EPA, 2005). In designing such studies, workshop participants acknowledged that much preliminary research may be needed – particularly with regard to actions in the rat. For example, the specific target cells in the rat are not known, the repair systems of the nasal olfactory tissue and respiratory epithelium are not well-characterized, and the specific cytochrome P450 isoforms involved in naphthalene metabolism in the nasal cavity have not been identified. In order to appropriately design the necessary studies, time points for the harvesting of relevant target cells will have to be determined for both animal models. Similarly, time points at which lesions occur, where they occur, and whether they show reversibility will have to be determined using repeat dosing, time-concentration studies. This information, once obtained, will be useful in the final design of acute and repeat inhalation studies, which should be conducted using a tiered approach. If such studies indicate that naphthalene acts via a cytotoxic mode of action to induce tumors in rats and mice, then these results would suggest that humans exposed to naphthalene at low concentrations will not be at risk for cancer. Generally, for tumors to develop via cytotoxicity, high concentrations of a chemical must be administered in order to overwhelm the normal detoxification and repair mechanisms of the body (Butterworth et al., 1995; Bogdanffy and Valentine, 2003). Thus, cytotoxicity usually does not result in tumor formation at low exposure concentrations, like those to which humans may be exposed.
    - *Acute Studies.* Single exposure inhalation studies should be conducted in both the rat and mouse (both sexes). The rat study should take precedence over that of the mouse because the EPA's draft cancer assessment for naphthalene relies heavily on rat tumor data. Additionally, because the rat tumor data are derived using the F344 rat, these studies be conducted using the same rat strain. The EPA workshop participants recommended that a range of naphthalene vapor concentrations be used, with the lowest concentration below one ppm. Exposure concentration range-finding studies may be necessary; alternatively, exposure concentrations may be determined from a review of the literature. The time course for induction of nasal lesions in the rat (and lung lesions in the mouse) should be assessed. Examination of blood/urine naphthalene metabolite concentrations for identification of potential biomarkers for later correlations among animals and humans may also be beneficial. The primary value of the acute studies is the provision of pertinent information needed for the final design of the repeat inhalation studies.
    - *Repeat Inhalation Studies.* Repeat inhalation studies will be necessary to demonstrate that naphthalene induces tumors via a cytotoxic mode of action. These studies should be conducted in both sexes of rat (F344 strain) and mouse. Three different naphthalene vapor concentrations, based on results from the acute study, and exposures over a 3-6 month duration were recommended by participants at the EPA workshop (U.S. EPA, 2005). It was further recommended that exposure concentrations should not exceed 10 ppm. Because repair processes

have been shown to affect the tissue response to repeat exposures (at least in the mouse), interim sacrifices will be required to examine the time course for histopathologic changes in these studies. Cell proliferation rates can be determined using BrdU incorporation; apoptosis can be assessed histopathologically. Also, blocks of target tissues can be saved for subsequent mapping studies (linking sites of lesions formation with areas of cytotoxicity). If naphthalene causes tumors via a cytotoxic mode of action, then naphthalene inhalation would be anticipated to produce cytotoxic lesions in the same areas/tissues as tumors develop, and repeat exposures would be anticipated to increase the incidence and severity of these lesions. As well, cell proliferation rates would be anticipated to increase upon repeat exposures in those tissues in which lesions develop.

- *Other Studies.* Cell culture studies using naphthalene and/or its metabolites may provide insight into whether these chemicals can affect cell-cycling mechanisms. While the conduct of such studies can be relatively straight forward, interpretation of their results may be difficult.

- **Threshold Carcinogen.** Once the pathways of naphthalene metabolism in the target tissues of interest have been more fully elucidated via the studies described above, studies to determine whether a threshold exists for naphthalene carcinogenicity can be conducted. Again, because research in this area is already being done in the mouse (Plopper et al., 2001; West et al., 2001), suggested studies will focus solely on furthering the understanding of mechanisms in the rat.
  - *Acute Studies.* F344 rats can be exposed via inhalation to increasing concentrations of naphthalene in single exposure studies. Concentrations of the primary naphthalene metabolites formed in rat target tissues can be measured, as described above. The aim of such studies should be to identify at what exposure concentration, if any, a shift in the naphthalene metabolic profile occurs. If this exposure concentration represents a true threshold at which certain metabolic pathways become saturated (resulting in increased formation of the toxic metabolite(s) of concern), then a proliferation of target tissue lesions will also be observed at this concentration level as well. To demonstrate unequivocally a threshold mode of action for naphthalene, the change in toxic metabolite concentration(s) will need to be well correlated with a change in the rate of lesions development. Additional studies to further characterize associated metabolic changes in target tissues (such as GSH depletion or cytochrome P450 inhibition) may also be useful.
  - *Repeat Exposure Studies.* Experiments discussed above using single naphthalene exposures should also be conducted using repeat exposure scenarios. As previously mentioned, studies have suggested that repeat exposures induce adaptive changes in target tissues, resulting in some degree of tolerance to subsequent naphthalene exposure. The mechanisms involved in these adaptive changes likely involve alteration to the pathways of naphthalene metabolism. As such, threshold concentrations may ultimately be higher under conditions of repeat naphthalene exposure. Based on studies done in the mouse, repeat exposure experiments likely will have to be conducted using exposure durations of a week or greater.

Metabolism. Studies to assess the role of metabolism in naphthalene's carcinogenicity in rodent inhalation studies were not considered at the EPA peer consultation workshop. A great deal of research has already been conducted regarding the target tissue-specific metabolism of naphthalene in the mouse lung (Plopper et al., 1991; Buckpitt et al., 1995). For these reasons, the following recommendations will focus solely on gaining a better understanding of the metabolic processes involved in naphthalene toxicity in the rat nasal cavity. The results of such studies will not directly address naphthalene's mode of action, but rather, will provide needed information regarding how naphthalene is handled by rat target tissues, which in turn, will be pertinent to understanding the relevance of findings from rat inhalation studies for humans.

- **Naphthalene Metabolic Profile.** Evidence strongly suggests that a metabolite is responsible for naphthalene's toxicity in rat target tissues (Lee et al., 1995). Very little is known, however, regarding naphthalene metabolism in these tissues upon inhalation exposure.
    - *In Vitro Studies.* To characterize potential naphthalene metabolites formed in rat target tissues, studies using microsomes isolated from the nasal olfactory and respiratory epitheliums of F344 rats are recommended. Following incubation of the microsomes with naphthalene (in the presence of an NADPH regenerating system, glutathione[GSH], and glutathione-*S*-transferases), specific naphthalene metabolites can be isolated using either high pressure liquid chromatography (HPLC) or gas chromatography, and identified using mass spectrometry. Specific naphthalene exposure concentrations and durations for these studies can be selected based on similar research done using mouse lung microsomes (Buckpitt et al., 1995; Shultz et al., 2001). Previous research in the mouse suggests that identification of stereoselective metabolites will be essential because only certain stereoisomers are thought to be associated with cytotoxicity (Buckpitt et al., 1995). Also, dose-response relationships should be examined to determine whether the metabolic profile for naphthalene is altered upon increasing exposure concentrations. A shift in the metabolism of naphthalene with increasing exposure concentrations may suggest saturation of particular metabolic pathways, which may, in turn, relate to a threshold carcinogenic response. These studies will elucidate the main naphthalene metabolites produced in the rat nasal olfactory and respiratory epitheliums, and provide possible evidence of a threshold response upon increasing exposure concentrations. This information will be important in assessing the relevance of rat inhalation studies to humans, as outlined below in the section on species relevance.
    - *In Vivo Studies.* Once target tissue-specific metabolites are identified using *in vitro* studies, research should be conducted in the F344 rat to assess whether the *in vivo* naphthalene metabolic profile of the nasal olfactory and respiratory epitheliums correlate with *in vitro* results. Following naphthalene exposure via inhalation, nasal tissues can be isolated, and metabolites determined using HPLC and mass spectrometry, as noted above. A possible shift in the naphthalene metabolic profile upon increasing exposure concentrations should be assessed. It may also be beneficial to look at blood and urinary metabolites of naphthalene as potential biomarkers for study in humans. Because data in the mouse suggest that repeat naphthalene exposure alters the metabolic response of target tissues, resulting in tolerance (Lakritz et al., 1996; West et al., 2000), the effects of repeat exposures should also be assessed in the rat nasal tissues. Finally, mouse studies

have suggested that GSH depletion may play a role in naphthalene cytotoxicity and that repeat exposures confer a degree of protection to target tissues by elevating GSH resynthesis mechanisms (West et al., 2000; Plopper et al., 2001). As such, the effects of naphthalene treatment on GSH concentrations in target tissues should be determined. Additionally, studies to assess whether pretreatment of animals with GSH prodrugs can ameliorate lesions development upon naphthalene exposure may be useful as confirmation of possible mode of action.

- *Additional Studies.* Studies to identify the specific cytochrome P450 isoforms involved in naphthalene metabolism in rat target tissues may be useful. Studies conducted in the mouse and rat suggest that CYP2F may be involved in naphthalene metabolism in the lung and nasal cavity (Shultz et al., 2001; Lee et al., 2005). Limited research has been done to ascertain the cytochrome P450 isoforms expressed in olfactory tissue (Minn et al., 2005; Ling et al., 2004), although not all expressed isoforms have been yet identified. Using isoform-specific anti-P450 antibodies, immunohistochemistry can be done on olfactory and nasal respiratory epitheliums from F344 rats exposed to naphthalene via inhalation in an attempt to correlate the expression of specific isoforms with the locations of lesions development. Such studies could also be done using isoform-specific cDNAs as probes for *in situ* hybridization (detecting mRNA versus protein expression). Once the specific cytochrome P450 isoforms of interest are identified, these proteins can be individually expressed in an *in vitro* system (for example, in baculoviruses), and microsomes prepared. Naphthalene treatment of such microsomes should result in formation of the specific metabolites catalyzed by each of the expressed cytochrome P450 isoforms. *In vivo* studies using isoform-specific inhibitors can also be done to demonstrate that inhibition prevents naphthalene metabolism (and subsequent lesions development). Alternatively, experiments can be conducted in transgenic cytochrome P450 knockout rats (or mice). If the transgenically-eliminated cytochrome P450 is involved in naphthalene metabolism in rat target tissues, then naphthalene exposure of the knockout animal should not result in formation of toxic metabolite(s) of concern, nor subsequent lesions development.

- **Identification of Metabolite(s) of Concern**. To identify the naphthalene metabolite(s) directly linked to toxicity, F344 rats can be exposed to each of the primary metabolites identified in the above *in vitro* and *in vivo* studies. Studies may use acute, single exposures, and efforts should be made to correlate development of cytotoxic lesions with exposure to specific individual naphthalene metabolite(s). While relatively expensive, such studies will elucidate the toxic metabolite(s) of concern. Additional metabolism studies can then address whether these metabolites are also formed in humans (as described below in the section on species relevance).
- **Identification of Covalent Adducts**. The toxic metabolite(s) of concern formed in the rat olfactory and nasal respiratory epitheliums upon naphthalene exposure are assumed to elicit lesions via disruption of specific cellular proteins (or DNA, a possibility that is being assessing using genotoxicity studies). Covalent binding of metabolites in rat target tissues can be assessed using methods similar to those being used to identify adducted proteins in mouse lung microsomes (Isbell et al., 2005). However, the results of such studies may not reflect the same population of protein adducts formed in intact tissues, as

illustrated in the study by Lin et al. (2005). Thus, methods may need to be developed to allow for protein covalent-binding studies to be conducted using intact nasal olfactory and respiratory epithelial tissues before these studies can be performed. The results of such protein covalent-binding studies will be useful in demonstrating a logical, biologically-plausible sequence of events from naphthalene exposure, to metabolic activation, covalent binding, biochemical changes, and finally, overt tissue damage.

Species Relevance. Showing human relevance of rodent tumor findings following naphthalene exposure will likely prove a difficult task – especially with regard to rat nasal tumors. Very little is known about the human nasal olfactory and respiratory epitheliums, and such information is somewhat limited by the scarce availability of tissues for study. For example, few, if any, human nasal tissue cell lines exist according to the American Type Tissue Collection (ATCC) website (www.lgcpromochem-atcc.com). Additionally, biopsy of human nasal tissues is likely uncommon except in cases when such tissues are removed due to a medical condition.

- **Naphthalene Deposition Patterns.** As previously mentioned, rats and mice differ greatly from humans in their manner of breathing and the anatomical structure of their respiratory systems, including nasal cavities. These differences likely affect how and where naphthalene vapors deposit in the nasal passages and airways, which is known to partly determine nasal injury patterns in rats (Lee et al., 2005). Naphthalene deposition studies in human airways are required to determine whether inhaled naphthalene vapor is likely to encounter, and thus interact with, target tissues identified in rat and mouse studies. Vapor deposition patterns in humans can be estimated using computational simulation models (Timchalk et al., 2001; Zhang et al., 2006).
- **Metabolic Activity of Human Target Tissues.** Once the key catabolic steps responsible for formation of the toxic naphthalene metabolite(s) of concern are deciphered for the target tissues of rats and mice, additional studies using human tissues will be required to determine whether these tissue-specific metabolic pathways also exist for humans. Expression and abundance of key enzymes involved in naphthalene metabolism (and subsequent detoxification of toxic metabolites) can be determined in surgical biopsy samples using immunohistochemistry and/or *in situ* hybridization. The concentrations of important substrates (e.g., GSH) can also be determined using the same tissue samples. Such studies can elucidate whether the enzymes shown to be important for naphthalene metabolism in rat and mouse target tissues also exist in corresponding human tissues and whether they are expressed at similar concentrations. However, some of the human cytochrome P450s may have differing substrate specificities than their rodent counterparts. For this reason, the specificity of the human enzyme isoforms *in vitro* will have to be examined as well as the rates of naphthalene metabolism in human tissues. As described above for the study of individual cytochrome P450 isoforms of the rat, human isoforms can be expressed using a baculovirus system and microsomes prepared for naphthalene metabolism studies. To examine *in situ* naphthalene metabolism, explant culturing methods are available for the *in vitro* study of human olfactory (Feron et al., 1998; Green et al., 2001; Hahn et al., 2005) and respiratory epitheliums. Isoform-specific cytochrome P450 inhibitors can be used in such studies to show that certain steps in the metabolism of naphthalene are catalyzed by particular P450 isoforms. Rates of formation of the toxic metabolite(s) of concern should be quantified. As well as, the production of any important protein covalent adducts identified in animal studies and the association between metabolism and lesions development (including time courses) can be examined.

These studies will determine whether naphthalene is metabolized in humans at the same rate and to the same metabolites as in rat and mouse target tissues and whether the same protein covalent adducts are formed as a result. If marked differences are observed between humans and rodents regarding naphthalene metabolism in target tissues, then it can be assumed that rats and mice are not appropriate models for assessing the carcinogenic potential of naphthalene inhalation for humans. Such *in vitro* studies (as well as the above immunohistochemistry and *in situ* hybridization studies) will likely have to be repeated using multiple human tissue samples. Such tissues are often only available following surgical biopsy, and the underlying medical conditions (which prompted surgical biopsy in the first place) may affect the tissue's enzyme expression patterns and associated metabolic capacity. Finally, if any blood and/or urine biomarkers of naphthalene toxicity are identified in animal studies, biomonitoring studies in human populations with known naphthalene exposures may be possible. Such studies will likely be of limited value, but may provide some information about the prevalence of toxicity pathways at low exposure concentrations.

- **Development of Relevant Animal Models.** If the above studies suggest that the rat or mouse is not an appropriate animal model for assessing the toxicity of naphthalene in humans, a more appropriate animal model may need to be developed. Certainly, non-human primates, because of their similarity to humans (especially in terms of respiratory system anatomy and inhalation patterns), are a prime candidate. However, before non-human primates are used to model possible risks of naphthalene exposure to humans, research must be done to confirm that they mimic humans in terms of airway deposition of inhaled naphthalene vapors, their olfactory and respiratory metabolism of naphthalene, and subsequent target tissue responses. Most likely, this research will primarily involve *in vitro* study using protocols as described above for assessing naphthalene disposition and metabolism in human target tissues. At the conclusion of such studies, comparisons among rats, mice, non-human primates, and humans can be made regarding the rates and pathways of naphthalene metabolism in target tissues, including the abundance of key catalytic enzymes and substrates, the production of toxic metabolite(s) of concern, formation of covalent protein adducts, and development of toxicity lesions. If, as a result of such comparisons, non-human primates are shown to be the most appropriate model for assessing the toxicity of naphthalene exposure to humans, only then should *in vivo* studies be conducted to demonstrate differences among species in their responses to naphthalene inhalation exposures.

Summary

The EPA is currently in the process of reassessing the human toxicological potential associated with naphthalene exposures. The Agency has proposed changing the chemical's human carcinogenicity classification from 'possible' to 'likely' based primarily on results from inhalation studies in mice and rats, conducted by the NTP (NTP, 1992, 2000). A number of data gaps regarding the understanding of naphthalene carcinogenicity in humans, its possible mode of action, and the relevance of naphthalene inhalation studies in rodents to humans have been outlined above. Additional studies to address these data gaps are described (see Table 3). Such studies will elucidate the possible mode of action for naphthalene carcinogenicity in rodents and its relevance to humans, including the toxic metabolite(s) of concern, the pathways responsible for their development, the mechanisms of action underlying toxicity, and the time course for lesions development. Completion of the suggested studies will permit better understanding of

the results from the rodent inhalation studies and their applicability to naphthalene's carcinogenic potential in humans.

Table 3. Potential Studies that Could Address Data Gaps in the Understanding of (1) How Naphthalene Causes Tumors in Rats and Mice, and (2) Whether Such Findings are Relevant in the Human Toxicity Assessment for Naphthalene.

Data Gap	Recommended Effort(s)	Information Gained
Evidence of Carcinogenicity in Humans	Robust cohort and case-control studies	Evidence of possible carcinogenicity in humans
Mode of Action – Possible Naphthalene Genotoxicity in Target Tissues	• Ames assay with S9-activating fractions from rat and mouse target tissues • *In vivo* covalent binding studies • Naphthalene inhalation studies in transgenic mutagenicity models, with incorporation of micronucleus and Comet assays	Evidence of target tissue-specific genotoxicity
Mode of Action – Possible Naphthalene Cytotoxicity in Target Tissues	Single and repeat inhalation studies using a range of naphthalene concentrations, with characterization of histopathology of lesions development, assessment of cell proliferation and apoptosis rates, and mapping of lesions to locations of tumor development	Characterization of cytotoxic response in target tissues, including histopathology, cell proliferation and apoptosis rats, and correlation of lesion sites with regions of tumor development
Mode of Action – Existence of a Possible Threshold	Single and repeat inhalation studies using a range of naphthalene concentrations, with efforts to correlate a shift in the rate of toxic metabolites formation with a change in the progress of lesions development	Evidence of a possible threshold, including rough estimation of threshold concentration under acute and chronic exposure conditions
Naphthalene Metabolism - Rodents	• Metabolism studies using microsomes prepared from rat target tissues • *In vivo* naphthalene metabolism studies in rats • Immunohistochemistry or *in situ* hybridization of specific cytochrome P450 isoforms in rat target tissues	• Identification of primary naphthalene metabolites in rat target tissues • Confirmation of primary metabolites • Correlation of expression of specific P450s with areas of lesions development

Table 3. (continued)

Data Gap	Recommended Effort(s)	Information Gained
Naphthalene Metabolism – Rodents (continued)	• Naphthalene metabolism studies using microsomes expressing individual P450 isoforms • Rat inhalation studies using isoform-specific P450 inhibitors • Rat inhalation studies with exposure to primary naphthalene metabolites • *In vitro* and *in vivo* protein adduct studies	• Identification of specific P450s involved in formation of toxic metabolite(s) • Identification of isoforms associated with lesions development • Identification of toxic metabolite(s) of concern for lesions development • Identification of possible biochemical changes associated with lesions development in rat target tissues
Species Relevance – Naphthalene Deposition	Computational simulation models for naphthalene vapors	Assessment of likely vapor deposition patterns in humans
Species Relevance – Naphthalene Metabolism in Humans	• Immunohistochemistry or *in situ* hybridization of specific cytochrome P450 isoforms • Naphthalene metabolism studies using microsomes expressing individual human cytochrome P450 isoforms • *In situ* naphthalene metabolism studies using human nasal and respiratory tissues in explant culture and isoform-specific P450 inhibitors	• Identification of isoforms expressed in human nasal and respiratory tissues • Identification of specific P450s involved in naphthalene metabolism in human nasal and respiratory tissues • Confirmation of P450s involved in naphthalene metabolisms in human nasal and respiratory tissues
Species Relevance – Identification of a More Appropriate Animal Model	Repeat of studies done to assess naphthalene deposition and metabolism in humans using non-human primates	Information on vapor deposition patterns and naphthalene metabolism in non-human primates

References

Abdo, KM, Grumbein, S, Chou, BJ, and Herbert, R. (2001). Toxicity and carcinogenicity study in F344 rats following 2 years of whole-body exposure to naphthalene vapors. *Inhalation Toxicology* **13**:931-950.

Ajao, OG, Adenuga, MO, Ladipo, JK. (1988). Colorectal carcinoma in patients under the age of 30 years: a review of 11 cases. *Journal of the Royal College of Surgeons of Edinburgh* **33**:277-9.

Baldwin, RM, Shultz, MA, and Buckpitt, AR. (2005). Bioactivation of the pulmonary toxicants naphthalene and 1-nitronaphthalene by rat CYP2F4. *Journal of Pharmacology and Experimental Therapeutics* **312**:857-865.

Bogdanffy, MS, and Valentine, R. (2003). Differentiating between local cytotoxicity, mitogenesis, and genotoxicity in carcinogen risk assessments: The case of vinyl acetate. *Toxicology Letters* **140-141**:83-98.

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

Buckpitt, A, Chang, AM, Weir, A, Van Winkle, L, Duan, X, Philpot, R, and Plopper, C. (1995). Relationship of cytochrome P450 activity to Clara cell cytotoxicity. IV. Metabolism of naphthalene and naphthalene oxide in microdissected airways from mice, rats, and hamsters. *Molecular Pharmacology* **47**:74-81.

Butterworth, BE, Conolly, RB, and Morgan, KT. (1995). A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. *Cancer Letters* **93**:129-146.

DeSesso, JM. (1993). The relevance to humans of animal models for inhalation studies of cancer in the nose and upper airways. *Quality Assurance: Good Practice, Regulations, and Law* **2**:213-231.

Feron, F, Perry, C, McGrath, JJ, and Mackay-Sim, A. (1998). New techniques for biopsy and culture of human olfactory epithelial neurons. *Archives of Otolaryngology – Head and Neck Surgery* **124**:861-6.

Green, T, Lee, R, Toghill, A, Meadowcroft, S, Lund, V, and Foster, J. (2001). The toxicity of styrene to the nasal epithelium of mice and rats: studies on the mode of action and relevance to humans. *Chemico-Biological Interactions* **137**:185-202.

Hahn, CG, Han, LY, Rawson, NE, Mirza, N, Borgmann-Winter, K, Lenox, RH, and Arnold, SE. (2005). In vivo and in vitro neurogenesis in human olfactory epithelium. *Journal of Comparative Neurology* **483**:154-163.

Isbell, MA, Morin, D, Boland, B, Buckpitt, A, Salemi, M, and Presley, J. (2005). Identification of proteins adducted by reactive naphthalene metabolites in vitro. *Proteomics* **5**:4197-4204.

Kup, W. (1978). Work-related origin of cancer in the nose, mouth, throat, and larynx. *Akad Wiss* (German) **2**:20-25.

Lakritz, J, Chang, A, Weir, A, Nishio, S, Hyde, D, Philpot, R, Buckpitt, A, and Plopper, C. (1996). Cellular and metabolic basis of Clara cell tolerance to multiple doses of cytochrome P450-activated cytotoxicants. I. Bronchiolar epithelial reorganization and expression of cytochrome P450 monooxygenases in mice exposed to multiple doses of naphthalene. *Journal of Pharmacology and Experimental Therapeutics* **278**:1408-1418.

Lee, MG, Phimister, A, Morin, D, Buckpitt, A, and Plopper, C. (2005). In situ naphthalene bioactivation and nasal airflow cause region-specific injury patterns in the nasal mucosa of rats exposed to naphthalene by inhalation. *Journal of Pharmacology and Experimental Therapeutics* **413**:103-110.

Lin, CY, Isbell, MA, Morin, D, Boland, BC, Salemi, MR, Jewell, WT, Weir, AJ, Fanucchi, MV, Baker, GL, Plopper, CG, and Buckpitt, AR. (2005). Characterization of a structurally intact in situ lung model and comparison of naphthalene protein adducts generated in this model vs lung microsomes. *Chemical Research in Toxicology* **18**:802-813.

Ling, G, Gu, J, Genter, MB, Zhuo, X, and Ding, X. (2004). Regulation of cytochrome P450 gene expression in the olfactory mucosa. *Chemico-Biological Interactions* **147**:247-258.

Long, PH, Herbert, RA, Peckham, JC, Grumbein, SL, Shackelford, CC, and Abdo, K. (2003). Morphology of nasal lesions in F344/N rats following chronic inhalation exposure to naphthalene vapors. *Toxicologic Pathology* **31**:655-664.

Minn, AL, Pelczar, H, Denizot, C, Martinet, M, Heydel, JM, Walther, B, Minn, A, Goudonnet, H, and Artur, Y. (2005). Characterization of microsomal cytochrome P450-dependent monooxygenases in the rat olfactory epithelium. *Drug Metabolism and Disposition* **33**:1229-1237.

Nagata, K, Martin, BM, Gillette, JR, and Sasame, HA. (1990). Isozymes of cytochrome P-450 that metabolize naphthalene in liver and lung of untreated mice. *Drug Metabolism and Disposition* **18**:557-564.

NTP. (1998). Toxicology and Carincogenesis Studies of Naphthalene (CAS No. 91-20-3) in B6C3F1 Mice (Inhalation Studies). NTP TR 410 168 pp.

NTP. (2000). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in F344/N Rats (Inhalation Studies). NTP TR 500. December 2000 170 pp.

O'Brien, KA, Smith, LL, and Cohen, GM. (1985). Differences in naphthalene induced toxicity in the mouse and rat. *Chem. Biol. Interact* **55**:109-122.

Plopper, CG, Chang, AM, Pang, A, and Buckpitt, AR. (1991). Use of microdissected airways to define metabolism and cytotoxicity in murine bronchiolar epithelium. *Experimental Lung Research* **17**:197-212.

Plopper, CG, Suverkropp, C, Morin, D, Nishio, S, and Buckpitt, A. (1992). Relationship of cytochrome P450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract in mice after parenteral administration of naphthalene. *Journal of Pharmacology and Experimental Therapeutics* **261**:353-363.

Plopper, CG, Van Winkle, LS, Fanucchi, MV, Malburg, SR, Nishio, SJ, Chang, A, and Buckpitt, AR. (2001). Early events in naphthalene-induced acute Clara cell toxicity. II. Comparison of glutathione depletion and histopathology by airway location. *American Journal of Respiratory Cell and Molecular Biology* **24**:272-281.

Preuss, R, Drexler, H, Böttcher, M, Wilhelm, M, Brüning, T, and Angerer, J. (2005). Current external and internal exposure to naphthalene of workers occupationally exposed to polycyclic aromatic hydrocarbons in different industries. *International Archives of Occupational and Environmental Health* **78**:355-362.

Reznik, GK. (1990). Comparative anatomy, physiology, and function of the upper respiratory tract. *Environmental Health Perspectives* **85**:171-176.

Schreiner, CA. (2003). Genetic toxicity of naphthalene: A review. *Journal of Toxicology and Environmental Health, Part B* **6**:161-183.

Shultz, MA, Choudary, PV, and Buckpitt, AR. (1999). Role of murine P-450 2F2 in metabolic activation of naphthalene and metabolism of other xenobiotics. *Journal of Pharmacology and Experimental Therapeutics* **290**:281-288.

Shultz, MA, Morin, D, Chang, AM, and Buckpitt, A. (2001). Metabolic capabilities of CYP2F2 with various pulmonary toxicants and its relative abundance in mouse lung subcompartments. *Journal of Pharmacology and Experimental Therapeutics* **296**:510-519.

Timchalk, C, Trease, HE, Trease, LL, Minard, KR, and Corley, RA. (2001). Potential technology for studying dosimetry and response to airborne chemical and biological pollutants. *Toxicology and Industrial Health* **17**:270-276.

U.S. EPA. (1998). Toxicological Review of Naphthalene (CAS No. 91-20-3). In Support of Summary Information on the Integrated Risk Information System (IRIS). August 1998. 116 pp.

U.S. EPA. (2004). External Peer Review for the IRIS Reassessment of the Inhalation Carcinogenicity of Naphthalene. August 2004. 27 pp.

U.S. EPA. (2005). Peer Consultation Workshop on Research Needs Related to the IRIS Draft Toxicological Review of Naphthalene. EPA/635/R-05/0003. August 2005. 36 pp.

West, JA, Buckpitt, AR, and Plopper, CG. (2000). Elevated airway GSH resynthesis confers protection to Clara cells from naphthalene injury in mice made tolerant by repeated exposures. *Journal of Pharmacology and Experimental Therapeutics* **294:**516-523.

West , JA, Pakehham, G, Morin, D, Fleschner, CA, Buckpitt, AR, and Plopper, CG. (2001). Inhaled naphthalene cases dose dependent Clara cell cytotoxicity in mice but not in rats. *Toxicology and Applied Pharmacology* **173**:114-119.

Wilson, AS, Davis, CD, Williams, DP, Buckpitt, AR, Pirmohamed, M, and Park, BK. (1996). Characterization of the toxic metabolite(s) of naphthalene. *Toxicology* **114**:233-242.

Wolf, O. (1976). Cancer diseases in chemical workers in a former naphthalene cleaning plant. *Deutsch Gesundheitswes* (German). **31**:996-999.

Zhang, Z, Kleinstreuer, C, and Kim, CS. (2006). Transport and uptake of MTBE and ethanol vapors in a human upper airway model. *Inhalation Toxicology* **18**:169-184.

University of Nebraska

THE UNIVERSITY OF NEBRASKA CENTER FOR ENVIRONMENTAL TOXICOLOGY

January 25, 2006

George Gray, Ph.D., Assistant Administrator
Environmental Protection Agency
Office of Research & Development
1300 Pennsylvania Ave., N.W.
Washington, D.C. 20004

RE: UNIVERSITY OF NEBRASKA NAPHTHALENE STATE-OF-THE-SCIENCE SYMPOSIUM

Dear Dr. Gray:

The Center for Environmental Toxicology at the University of Nebraska (UN) is today announcing its sponsorship of the Naphthalene State-of-the-Science Symposium (NS^3), to be held May 7-10, 2006, in Napa, California. I am writing to seek your support in making this event a success.

What is naphthalene, how is it used, and how are people exposed?

Naphthalene is a bicyclic aromatic hydrocarbon with the chemical formula $C_{10}H_8$. Pure naphthalene is a white, water-insoluble solid at room temperature. It is produced by distillation and fractionation of either petroleum or coal tar. Naphthalene's principal use is as an intermediate in the production of phthalic anhydride, a chemical important in the manufacture of phthalate plasticizers, resins, dyes, and insect and animal repellents. Naphthalene is also used in the manufacture of synthetic leather tanning agents and the insecticide carbaryl. Naphthalene has been used as a moth repellent and as a deodorizer for diaper pails and toilets. A large number of hazardous waste sites included on the U.S. Environmental Protection Agency's (U.S. EPA's) National Priorities List have detectable levels of naphthalene that heretofore have not been believed to pose any appreciable risk to human health. Naphthalene exposure is widespread in the environment at part per billion (ppb) levels.

Does naphthalene cause cancer in humans?

Historically, naphthalene in the environment has been believed to present no material human cancer risk. This view was based on the fact that ambient environmental levels are in the ppb range, and there have been no credible scientific studies showing that naphthalene caused cancer in animals even at doses more than a thousand times greater. In 2001, however, the U.S. National Toxicology Program (NTP) released the results of a two-year bioassay showing that some rats exposed to doses of 10, 30 and 60 parts per million (ppm) exhibited rare cancers in the nasal epithelium. Based on this study, U.S. EPA's upcoming National Air Toxics Assessment is widely expected to extrapolate that as much as 5% of all cancers from air toxics to naphthalene.

Ercole L. Cavalieri, Director
986805 Nebraska Medical Center / Omaha, NE 68198-6805
402-559-8924 / FAX: 402-559-8068 / uncetox@unmc.edu / www.unmc.edu/toxcenter

Why is science important?

The protection of public health is our primary concern. If naphthalene at environmental concentrations does cause cancer in humans at the rate implied by the various risk factors that have been proposed based on this NTP study, then thousands more Americans experience cancer over their lifetimes than if these exposures were prevented. On the other hand, if naphthalene at environmental doses causes cancer in humans at less than these implied rates (or perhaps not at all), then resources expended to avoid human exposure will not reduce the number of persons who experience cancer, but they will diminish national and individual wealth, including the capacity to invest in other goods, services, and activities that reduce or eliminate cancer and other human health risks.

What is NS^3 about?

Our goal is first to provide a circumspect accounting of what is known and unknown about fundamental scientific issues related to the human carcinogenic risk posed by exposure to naphthalene at environmentally relevant doses. Second, we seek to provide an independently validated agenda for research having a high level of policy-relevant information value.

In greater detail, NS^3 is designed to:

- *Share openly*, with participation invited from all interested parties, all salient knowledge regarding the specific scientific issues that will be addressed, with formal presentations by leading investigators;
- *Report the collective scientific wisdom* of a panel of independently-selected and universally-recognized scientific experts; and
- *Propose an agenda for research* that if conducted, will resolve critical remaining scientific uncertainties.

NS^3 will therefore provide valuable information to basic research scientists, applied scientists and risk assessors, public- and private-sector research sponsors, risk managers, and advocates of all persuasions who are committed to public policies informed by science.

NS^3 will review five fundamental science issues related to the quantification of human cancer risk from naphthalene exposure at environmentally relevant levels. Researchers who performed the most important recent scientific studies in animal systems on metabolism, biochemistry, animal-to-human extrapolation, and mechanism of action will present their work. A review of the literature on human exposure, occupational and environmental epidemiology, and the incidence in humans of nasal epithelial tumors (the specific tumors reported in the NTP bioassay) also will be presented.

NS^3 will be held for scientists, consultants, regulatory agency officials, and others interested in learning the latest about naphthalene first hand from scientists who have performed and published the most relevant scientific research. We will bring together distinguished, independent scientists who will provide their informed insights on these subjects and help craft consensus reports on the state of the science. In addition, our expert panels will identify gaps in scientific knowledge and help craft specific, targeted, cost-effective research projects that could be performed in a reasonable amount of time and would resolve remaining scientific uncertainties. Our goal is to produce a research agenda that, if implemented, would enable human health risk assessment and risk management to be informed by the best available science.

The five fundamental science issues to be addressed by NS^3 are:

1. Animal data. The most important laboratory study on naphthalene was performed by the NTP and published in 2001. How was this study performed? What did this study reveal? What inferences are scientifically appropriate to make from this study?

2. Human data. What is known about human exposure to naphthalene? What does the body of occupational and environmental epidemiology tell us? What is the incidence in humans of the specific types of cancer observed in the most relevant animal study?
3. Species differences. Much of what is known about naphthalene's mode of action comes from laboratory experiments in animals. What are the relevant similarities and differences between animals and humans? What do we know (and don't know) about developmental and gender differences in animals? To what extent can these differences be quantified?
4. Metabolism. Naphthalene appears to be metabolized in tissues at or near the point of contact in the respiratory system. What is known (and unknown) about naphthalene metabolites? How much metabolism occurs, and under what conditions? To what extent can the production of metabolites be related to their carcinogenic potential?
5. Mechanisms of carcinogenesis. Alternative mechanisms of carcinogenesis have been suggested, including both genotoxicity and cytotoxicity. What data exist that support or contradict each of these alternative mechanisms? Do they operate at differential intensities at different doses? To what extent can carcinogenic potential be quantified?

For each module a pool of recognized experts has been assembled from which it is expected that all expert panelists will be drawn. Each individual listed is widely respected for intellectual rigor, scholarship, independence and open-mindedness, and was identified by consensus of the symposium Planning Committee. The identities and affiliations of members of the Planning Committee are reported in Attachment A.

Attachment B provides an outline of the NS^3 program, including the scientific topics to be addressed, the identities and affiliations of the invited research speakers, and the names and affiliations of members of the expert panel pool. At this time we are unaware of any conflict-of-interest concerns that would cast doubt on the ability of any scientist on this list to capably and fully perform the duties assigned. Of course, if you are aware of any information that you believe might be potentially disqualifying, the Planning Committee is ready and willing to reconsider whether the individual in question should be removed from our pool.

What is NS^3 not about?

NS^3 is not about policy. Our focus is strictly on science, and in particular, primary scientific data and opportunities for future scientific research. For that reason, NS^3 will not review the adequacy or sufficiency of risk assessment documents prepared by various government agencies or private stakeholders, nor will we debate the merits of public policy judgments that are (or could be) contained in such risk assessments. That is, NS^3 will not take sides on policy issues. The purpose of NS^3 is to inform the policy debate with science, not to prescribe what policy choices ought to be made.

What is the symposium format?

Each scientific issue will be addressed by one or more scientists who have performed primary research and published extensively on the subject. Research speakers will present and summarize the data they have collected and explain what inferences they think ought to be drawn from their work.

For each of the five scientific modules, a panel of recognized independent experts will review the data and develop consensus reports on the state of the science. They will deliver preliminary reports on their deliberations at the end of the Symposium, and shortly thereafter craft written reviews suitable for publication in an appropriate scholarly journal.

In addition to providing consensus reports on the state of the science, expert panels will identify gaps in scientific knowledge that currently give rise to the use of default assumptions in human health risk assessment and propose specific, targeted, cost-effective research projects that would, if performed, resolve these scientific uncertainties.

Research speakers, who are likely to be most familiar with the existing data and have well developed ideas concerning areas of promising future research, will be encouraged to suggest research ideas for consideration by these panels. The goal is to obtain a broad-based scientific consensus on a research agenda that is financially feasible, can be accomplished without undue delay, and will enable risk assessment and risk management to be maximally informed by science.

Unlike many scientific conferences and symposia, NS^3 will be highly interactive. There will be extensive opportunity for public participation—in fact, there will be at least one hour allotted to question-and-answer time for each hour of scientific presentations delivered. At the same time, because NS^3 will be strictly scientific, policy matters (such as how much public health protection is appropriate or how precautionary risk managers ought to be) will be strictly excluded from the discussion.

What financial support do we need?

To be successful, NS^3 requires that we obtain significant, external and diversified financial support. Financial sponsorship provides your organization recognition that it supports independent scientific review of the highest quality, the development of an equally independent scientific research agenda, and is committed to maximizing the role of science in risk assessment. Organizations and agencies that support NS^3 will have their logos prominently displayed on event announcements, meeting materials, and on the Symposium website. The Planning Committee encourages sponsors to promote attendance to the relevant employees within their organizations, and among those elsewhere in government, industry, and the NGO community whom they know to have an interest in human health risk assessment for naphthalene.

Your sponsorship of this vital event is very important. Members of the Planning Committee will follow up to ensure that you have all the information you need to make this decision. Of course, if you have any questions please do not hesitate to call me.

Sincerely,

Ercole Cavalieri

Ercole L. Cavalieri, D.Sc.
Director, University of Nebraska Center for Environmental Toxicology
Professor, Eppley Institute for Research in Cancer and Allied Diseases
University of Nebraska Medical Center

Attachments (2)

cc: Peter Preuss, Ph.D., Director
National Center for Environmental Assessment
Environmental Protection Agency

Marcus Peacock, Deputy Administrator
Administrator's Office
Environmental Protection Agency

ATTACHMENT A:

NAPHTHALENE STATE-OF-THE-SCIENCE SYMPOSIUM
PLANNING COMMITTEE

Ercole Cavalieri DSc
Director, University of Nebraska Center for Environmental Toxicology
Professor, Eppley Institute for Research in Cancer and Allied Diseases
University of Nebraska Medical Center
Omaha, Nebraska

William O. Berndt PhD
Special Assistant to the Dean
College of Medicine
Professor Emeritus
Department of Pharmacology
University of Nebraska Medical Center
Omaha, Nebraska

Steven C. Lewis PhD DABT
President and Principal Scientist
Integrative Policy & Science, Inc.
Washington, New Jersey
Adjunct Professor of Occupational and Environmental Medicine
Robert Wood Johnson School of Medicine and Dentistry
New Brunswick, NJ

Richard C. Pleus PhD
Director
Intertox, Inc.
Seattle, Washington
Adjunct Associate Professor of Pharmacology
University of Nebraska Medical Center
Omaha, Nebraska

Richard B. Belzer PhD
President
Regulatory Checkbook
Mt. Vernon, Virginia

ATTACHMENT B:

NAPHTHALENE STATE-OF-THE-SCIENCE SYMPOSIUM

MAY 7-10, 2006

PROGRAM AGENDA

⌘

OBJECTIVES

The Naphthalene State of the Science Symposium (NS^3) will:

- Develop brief publishable quality consensus summaries of the state of the science with respect to highly specific technical issues crucial to the assessment of human cancer risk from exposure to naphthalene at environmentally relevant levels.
- Identify areas where scientific uncertainty impedes the development of a consensus on the state of the science.
- Design carefully targeted, cost-effective and timely research projects that if performed would resolve specific elements of scientific uncertainty, thereby supplanting the use of default assumptions in human health risk assessment.

⌘

MODULE STRUCTURE

Four scientific issues will be addressed

- Animal bioassays
- Human exposure, epidemiology and incidence of epithelial respiratory cancers
- Quantitation of cytotoxic modes of action, with emphasis on
    - Species, developmental and gender differences
    - Metabolism
- Quantitation of genotoxic modes of action and human carcinogenesis

Each module will have 1-2 presentations by distinguished experts who have performed and published significant relevant research.

Each module will have a panel consisting of 3 to 5 distinguished, independent scientists who are expert in the underlying scientific disciplines, but who may or may not have significant prior knowledge of naphthalene. The pool of panelists under consideration for each module is provided below in alphabetical order. Potential NS^3 co-sponsors will be asked to identify any individual whose independence they believe is insufficient.

Some but not all individuals have been contacted by the Planning Committee. Final selections will be based primarily on availability. Because of the level of effort involved, expert panelists will be awarded fixed honoraria for their service, subject to any constraints imposed by their employers.

Panelists for each module will be asked to:

- develop brief publishable quality consensus summaries of the state of the science with respect to highly specific technical issues crucial to the assessment of human cancer risk from exposure to naphthalene at environmentally relevant levels.
- identify areas where scientific uncertainty impedes the development of a consensus on the state of the science.
- design carefully targeted, cost-effective and timely research projects that if performed would resolve specific elements of scientific uncertainty, thereby supplanting the use of default assumptions in human health risk assessment.

On the last day of the program, a representative expert from each module will be asked to deliver a preliminary presentation of the panel's consensus scientific judgment and research recommendations.

Final reports will be expected within 30 days of the conclusion of the report. These reports will not be edited by the Planning Committee or any other third parties.

⌘

May 7, 2006
Evening

Arrival and registration

Private reception and dinner for Research Speakers, Expert Panelists, and the Planning Committee

⌘

May 8, 2006
0830 to 1030

MODULE A
ANIMAL BIOASSAYS

RESEARCH SPEAKER
Dr. Kamal Abdo PhD
NIEHS
P.O. Box 12233, MD ED-35
Research Triangle Park, NC 27709
Phone: (919) 541-7819
Fax:
Email: abdok@niehs.nih.gov
http://dir.niehs.nih.gov/dirtob/abdo.htm

Backup:
Ronald A. Herbert DVM PhD
Director, Pathology Support Group
Laboratory of Experimental Pathology, DIR, NIEHS
Phone:
Fax:

Email: herbert1@niehs.nih.gov
http://dir.niehs.nih.gov/dirlep/home.htm

EXPERT PANELIST POOL	
Janet Benson, PhD DABT	Lovelace Respiratory Research Institute 2425 Ridgecrest Drive SE Albuquerque, NM 87108-5127 Phone: (505) 348-9400 Fax: Email: jbenson@LRRI.org http://www.lrri.org/staff/directoryofscientists/benson.html
Michael Gallo PhD	Environmental and Occupational Health Sciences Institute University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School and Director, NIEHS Center of Excellence Director, Toxicology Division Phone: (732) 445-0175 Fax: (732) 445-4161 Email: magallo@eohsi.rutgers.edu
Per Gerde PhD	Lovelace Respiratory Research Institute 2425 Ridgecrest Drive SE Albuquerque, NM 87108-5127 Phone: (505) 348-9400 Fax: Email: Per.gerde@imm.ki.se http://www.lrri.org/staff/directoryofscientists/gerde.html
Jerry Hardesty PhD	Experimental Pathology Laboratories, Inc. (EPL) PO Box 474 Herndon, VA 20172-0474 Phone: (703) 471-7060 Fax: Email: http://www.epl-inc.com/s-tp05.html
Jack Harkema DVM PhD DACVP	Department of Pathobiology and Diagnostic Investigation College of Veterinary Medicine 212 Food Safety & Toxicology Building Michigan State University Lansing, MI 48910-8107 Phone: (517) 353-8627 Fax: Email: harkemaj@msu.edu http://cvm.msu.edu/vetpath/bios/harkemabio.htm
Rogene Henderson, PhD, DABT	Lovelace Respiratory Research Institute 2425 Ridgecrest Drive SE Albuquerque, NM 87108-5127 Phone: Fax: Email: rhenders@LRRI.org http://www.lrri.org/staff/directoryofscientists/henderson.html

EXPERT PANELIST POOL	
Charles Hobbs DVM DABT DABVT	Lovelace Respiratory Research Institute 2425 Ridgecrest Dr. SE Albuquerque, NM 87108 Phone: (505) 348-9413 Fax: (505) 348-4983 Email: chobbs@LRRI.org https://www.lrri.org/staff/directoryofscientists/hobbs.pdf
Ernest Eugene McConnell DVM DABT	Tox Path, Inc. 3028 Ethan Lane Raleigh, NC 27613 USA Phone: (919) 848-1576 Fax: (919) 848-1576 Email: toxpathmcc@bellsouth.net
John Morris PhD	Professor of Pharmacology and Toxicology Head, Department of Pharmaceutical Sciences University of Connecticut School of Pharmacy 69 North Eagleville Road, Unit 3092 Storrs, CT 06269-3092 Phone: (860) 486-3590 Fax: (860) 486-4998 Email: john.morris@uconn.edu http://web.uconn.edu/pharmacy/FacultyPages/Morris.html

⌘

May 8, 2006
1030 to 1230

MODULE B
EXPOSURE, EPIDEMIOLOGY AND HUMAN CANCER INCIDENCE

COMMISSIONED RESEARCH SPEAKER

Paul Lioy PhD
Deputy Director for Government Relations, Director EMAD
Professor
Exposure Science Division
Environmental and Occupational Health Sciences Institute
Robert Wood Johnson Medical School
Phone: (732) 445-0155
Fax: (732) 445-0116
Email: plioy@eohsi.rutgers.edu
http://eohsi.rutgers.edu/facultystaff/view.php?id=90

Backup:
Ellen Silbergeld PhD
Professor
Bloomberg School of Public Health
Johns Hopkins University
615 N. Wolfe St., E6644

Baltimore, MD 21205
Phone: (410) 955-8678
Fax: (443) 287-6414
Email: esilberg@jhsph.edu
http://faculty.jhsph.edu/?F=Ellen&L=Silbergeld

Backup:
Michael Gochfeld MD PhD
Professor
Department of Environmental and Community Medicine
University of Medicine and Dentistry of New Jersey/
Robert Wood Johnson Medical School
Environmental and Occupational Health Sciences Institute
Phone: (732) 445-0123 x627
Fax: (732) 445-0130
Email: gochfeld@eohsi.rutgers.edu
http://www.eohsi.rutgers.edu/facultystaff/view.php?id=51

EXPERT PANELIST POOL	
Richard Albertini MD PhD	Research Professor, Pathology Vermont Cancer Center 655 Spear St. Bldg C Burlington, VT 05405 Department of Microbiology and Molecular Genetics University of Vermont Medical School 32 N. Prospect Burlington, VT 05405 Office Phone: (802) 656-8346 Lab Phone: (802) 656-5443 Fax: (802) 656-8333: E-mail: richard.albertini@uvm.edu http://www.vermontcancer.org/getpage.php?pid=163 http://www.uvm.edu/cmb/faculty_details.php?people_id=28
Peter Gann MD ScD	Robert H. Lurie Comprehensive Cancer Center Northwestern University 675 North St. Clair Chicago, Illinois 60611 Phone: Fax: Email: pgann@northwestern.edu http://www.cancer.northwestern.edu/Research/members_bio.cfm?ID=115\
Richard Hayes DDS PhD	Senior Investigator Division of Cancer Epidemiology and Genetics Executive Plaza South, Room 8114 Phone: 301-435-3973 Fax: 301-402-1819 Email: hayesr@mail.nih.gov http://dceg.cancer.gov/people/HayesRichard.html

EXPERT PANELIST POOL	
Suresh Moolgavkar PhD MBBS	Fred Hutchinson Cancer Research Center Box 358080 MP-665 1100 Fairview Ave N P.O. Box 19024 Seattle, WA 98109-10 Phone: (206) 667-4273 Fax: Email: smoolgav@fhcrc.org http://depts.washington.edu/epidem/fac/facBio.shtml?Moolgavkar_Suresh
Charles Poole PhD	Associate Professor Department of Epidemiology School of Public Health University of North Carolina at Chapel Hill CB#7435 2104A McGavran-Greenberg Hall Chapel Hill , North Carolina 27599-7435 Phone: 919-966-9294 Fax: 919-966-2089 E-mail: cpoole@unc.edu http://www.sph.unc.edu/epid/facstaff/?fuseaction=profile_detail&subject=epid&profile_id=1296&dropnull=1
Nathaniel Rothman MD MPH, MHS	Senior Investigator Division of Cancer Epidemiology and Genetics National Cancer Institute Executive Plaza South, Room 8116 Phone: (301) 496-9093 Fax: Email: rothmann@mail.nih.gov http://dceg.cancer.gov/people/RothmanNathaniel.html
Edo Pellizzari PhD	Senior Fellow, Analytical and Environmental Health Sciences Research Triangle Institute 3040 Cornwallis Road Post Office Box 12194 Research Triangle Park, NC 27709-2194 Phone: (919) 541-6579 Fax: (919) 541-6161 Email: edp@rti.org http://www.rti.org/experts.cfm?objectid=DE021822-312B-4A5A-9D6913EA361C89B7

EXPERT PANELIST POOL	
Ellen Silbergeld PhD	Professor Bloomberg School of Public Health Johns Hopkins University 615 N. Wolfe St., E6644 Baltimore, MD 21205 Phone: (410) 955-8678 Fax: (443) 287-6414 Email: esilberg@jhsph.edu http://faculty.jhsph.edu/?F=Ellen&L=Silbergeld
Douglas Weed MD PhD	Dean, Education and Training Chief, Office of Preventive Oncology Division of Cancer Prevention National Cancer Institute EPS T-41 6130 Executive Blvd. Bethesda, MD 20892-7105 Phone: 301-496-8640 Fax: 301-402-4863 Email: dw102i@nih.gov http://www3.cancer.gov/prevention/pob/about/weed.html

⌘

May 8, 2006
Lunch Provided
1230 to 1330

⌘

May 8, 2006
1330 to 1730

MODULE C
QUANTITATION OF CYTOTOXIC MODES OF ACTION

Part 1. Species, developmental and gender differences, and susceptibility

RESEARCH SPEAKERS

Species differences and developmental differences

Charles Plopper PhD
Professor
Department of Anatomy and Pharmacology
School of Veterinary Medicine
2228 Haring Hall
University of California, Davis, California
Phone: (530) 752-7067
Fax:
Email: cgplopper@ucdavis.edu

http://faculty.vetmed.ucdavis.edu/faculty/cgplopper/

Backup on developmental differences:
Michelle V. Fanucchi PhD
Assistant Researcher, Cell Biologist
Department of Anatomy, Physiology and Cell Biology
School of Veterinary Medicine
University of California, Davis
2216 Haring Hall
Davis, CA
Phone: (530) 754-8141
Fax:
Email: mvfanucchi@ucdavis.edu
http://faculty.vetmed.ucdavis.edu/faculty/mvfanucchi/

Gender differences:

Laura Van Winkle PhD
Associate Adjunct Professor
Department of Anatomy, Physiology and Cell Biology
Center for Comparative Respiratory Biology and Medicine
School of Veterinary Medicine
University of California, Davis
CHE Room 508
Davis, CA
Phone: (530) 754-7547
Fax:
Email: lsvanwinkle@ucdavis.edu
http://www.envtox.ucdavis.edu/ptx/subpage/faculty/lsvanwinkle.html
http://faculty.vetmed.ucdavis.edu/faculty/lsvanwinkle/
http://www.vetmed.ucdavis.edu/research/Publications/newsletters/summer2005.

⌘

Evening Recess

⌘

May 9, 2006
0830 to 1230

MODULE C, continued
QUANTITATION OF CYTOTOXIC MODES OF ACTION

Part 2. Metabolism

RESEARCH SPEAKERS
Alan Buckpitt PhD
Professor Department of Molecular Biosciences
Veterinary Medicine
220 Everson Hall

UC Davis
Davis, CA
Phone: (530) 752 7674
Fax:
Email: arbuckpitt@ucdavis.edu
http://www.envtox.ucdavis.edu/ptx/subpage/faculty/arbuckpitt.html

Steven Rappaport PhD MSPH
Professor of Occupational Health
Department of Environmental Sciences and Engineering
School of Public Health
University of North Carolina
Chapel Hill, NC
Phone: (919) 966-5017
Fax:
Email: stephen_rappaport@unc.edu
http://www.unc.edu/~rappapor/

EXPERT PANELIST POOL	
Janet Benson PhD DABT	Lovelace Respiratory Research Institute 2425 Ridgecrest Drive SE Albuquerque, NM 87108-5127 Phone: (505) 348-9400 Fax: Email: jbenson@LRRI.org http://www.lrri.org/staff/directoryofscientists/benson.html
Lynn Flowers PhD	National Center for Environmental Assessment Office of Research and Development U.S. Environmental Protection Agency USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N.W. Mail Code: 8601D Washington, DC 20460 Phone: (202) 564-1537 Fax: Email: flowers.lynn@epa.gov
Po-Gek Forkert PhD	Professor Emeritus Department of Anatomy and Cell Biology Botterell Hall Stuart Street Kingston, ON K7L 3N6 Canada Queens University Phone: Fax: Email: forkertp@post.queensu.ca http://www.cancer.ca/ccs/internet/standard/0,3182,3543_31 8933680_376268752_langId-en,00.html http://www.tera.org/peer/VCCEP/VDC/VDCAdHoc.htm#p oh

EXPERT PANELIST POOL	
Mary Beth Genter PhD	Associate Professor Department of Environmental Health University of Cincinnati 2600 Clifton Ave. 144 Kettering Cincinnati, OH 45221 Phone: (513) 558-6266 Fax: Email: gentermb@ucmail.uc.edu
Jay Goodman PhD	Professor Department of Pharmacology and Toxicology Michigan State University Phone: (517) 353-9346 Fax: (517) 353-8915 E-mail: goodman3@msu.edu http://www.phmtox.msu.edu/faculty/goodman.php
Jack Hinson PhD	Professor and Director, Division of Toxicology Department of Pharmacology and Toxicology University of Arkansas for Medical Sciences 4301 West Markham St., Slot 638 Little Rock, AR 72205 Phone: (501) 686-5766 Fax: (501) 686-8970 E-mail: jahinson@uams.edu
Charles Hobbs DVM DABT DABVT	Lovelace Respiratory Research Institute 2425 Ridgecrest Dr. SE Albuquerque, NM 87108 Phone: (505) 348-9413 Fax: (505) 348-4983 Email: chobbs@LRRI.org https://www.lrri.org/staff/directoryofscientists/hobbs.pdf
James Klaunig PhD	Professor of Toxicology Director of Division of Toxicology Department of Pharmacology and toxicology Indiana University School of Medicine 635 Barnhill Drive, Room MS 548 Indianapolis, IN 46202 Phone: (317) 274.7844 E-mail jklauni@iupui.edu http://pharmtox.iusm.iu.edu/klaunig.htm

EXPERT PANELIST POOL	
Raymond Novak PhD	Professor of Pharmacology Wayne State University Director, Institute of Environmental Health Sciences Director, Environmental Health Sciences Center in Molecular and Cellular Toxicology with Human Applications Room 4000 2727 Second Ave. Detroit, MI 48201 Phone: (313) 577-0100 Fax: (313) 577-0082 E-mail: r.novak@wayne.edu http://www.med.wayne.edu/pharm/novak.htm

⌘

Lunch Provided
1230 to 1330

⌘

May 9, 2006
1330 to 1730

MODULE D
GENOTOXICITY, MUTAGENESIS, CARCINOGENESIS

RESEARCH SPEAKERS

Ercole L. Cavalieri DSc
Professor
The Eppley Institute for Research in Cancer and Allied Diseases
University of Nebraska Medical Center
986805 Nebraska Medical Center
Omaha, NE 68198-6805
Phone: (402) 559-7237
Fax: (402) 559-8068
Email: ecavalie@unmc.edu

Joseph B. Guttenplan PhD
Professor
Basic Science & Craniofacial Biology
New York University, David B. Kriser Dental Center
Schwarts Hall, Mail Code 9436
345 E 24th Street
New York, NY 10010
Phone: (212) 998 9604
Fax: (212) 443 0418
Email: joseph.guttenplan@nyu.edu

EXPERT PANELIST POOL

EXPERT PANELIST POOL	
Richard Albertini MD PhD	Research Professor, Pathology Vermont Cancer Center 655 Spear St. Bldg C Burlington, VT 05405 Department of Microbiology and Molecular Genetics University of Vermont Medical School 32 N. Prospect Burlington, VT 05405 Office Phone: (802) 656-8346 Lab Phone: (802) 656-5443 Fax: (802) 656-8333: E-mail: richard.albertini@uvm.edu http://www.vermontcancer.org/getpage.php?pid=163 http://www.uvm.edu/cmb/faculty_details.php?people_id=28
James Bond PhD	Editor, *Chemico-Biological Interactions* 5505 Frenchman's Creek Durham, NC 27713 Phone: (919) 544-6384 Fax: (919) 544-6384 Email: toxcom@earthlink.net
Michael Boyd MD PhD	Abraham Mitchell Chair and Director University of South Alabama Cancer Research Institute Professor of Medicine and Pharmacology University of South Alabama College of Medicine Medical Sciences Building Room 2015 307 N. University Blvd. Mobile, AL 36688-0002 Phone: (251) 460-7307 Fax: (251) 460-6994 Email: mboyd@usouthal.edu http://www.southalabama.edu/cri/faculty/mboyd.html
David Eaton PhD	Professor of Environmental and Occupational Health Sciences, Toxicology Program Associate Dean of Research, School of Public Health & Community Medicine Director, Center for Ecogenetics and Environmental Health Department of Environmental Health University of Washington 4225 Roosevelt Way, N.E. Suite 100 Seattle, WA 98105-6099 Phone: (206) 685-3785 Fax: (206) 685-4696 Email: deaton@washington.edu http://depts.washington.edu/envhlth/about/facultypage/eato_page.html

EXPERT PANELIST POOL	
Thomas Kensler PhD	Professor, Department of Environmental Health Sciences Johns Hopkins Bloomberg School of Public Health Room 7032 615 N. Wolfe St. Baltimore, MD 21205 Phone: (410) 955-4712 Fax: (410) 955-0116 Email: tkensler@jhsph.edu http://faculty.jhsph.edu/?F=Thomas&L=Kensler
Raymond Loehr PhD	Hussein M. Alharthy Centennial Chair and Professor of Civil Engineering Civil, Architectural and Environmental Engineering Department-EWRE The University of Texas at Austin 1 University Station C1786 Austin, TX 78712-0273 Phone: (512) 471-4624 Fax: 512) 471-5870 Email: r.loehr@mail.utexas.edu http://www.ce.utexas.edu/profile.cfm?profilePK=106 http://www.utexas.edu/opa/experts/profile.php?id=252 http://www.icisnyu.org/inst_peo_detail.cfm?ID=46 http://www.ce.utexas.edu/newsreader.cfm?articlePK=308&&namedpagePK=1&articleType=externalNews
Suresh Moolgavkar PhD MBBS	Fred Hutchinson Cancer Research Center Box 358080 MP-665 1100 Fairview Ave N P.O. Box 19024 Seattle, WA 98109-10 Phone: (206) 667-4273 Fax: Email: smoolgav@fhcrc.org http://depts.washington.edu/epidem/fac/facBio.shtml?Moolgavkar_Suresh
Curt Omiecinski PhD	Professor of Veterinary Science H. Thomas and Dorothy Willits Hallowell Chair The Department of Veterinary Science Agricultural Sciences & Industries Bldg., Room 122 Penn State University University Park, PA 16802 Phone: (814) 863-1625 Fax: (814) 863-6140 Email: cjo10@psu.edu http://www.personal.psu.edu/faculty/c/j/cjo10/

EXPERT PANELIST POOL	
James Popp, DVM PhD DAVP	Stratoxon LLC 1853 William Penn Way Suite 2 Lancaster, PA 17601 Phone: (717) 735-3646, 3647 Fax: (717) 293-4470 E-mail: popp@stratoxon.com http://www.stratoxon.com/about1.asp
Iain Purchase PhD	Professor Faculty of Life Sciences University of Manchester Phone: Fax: Email: iain.f.purchase@manchester.ac.uk http://www.iutox.org/meritaward2004.asp
Cenwein Schreiner PhD FATS	C&C Consulting 1950 Briarcliff Avenue Meadowbrook, PA 19046 Phone: (215) 947-9321 Fax: (215) 947-9321 E-mail: castox@comcast.net

⌘

May 10, 2006

0900 to 1200

PRELIMINARY EXPERT PANEL REPORTS

⌘

Adjournment

NAPHTHALENE: UNRESOLVED SCIENCE ISSUES

Issues not raised by EPA in its "Charge" questions to its peer review panel.

SPECIES RELEVANCE

1. Multiple peer-reviewed studies point to important differences between mouse, rat, and primate in susceptibility to naphthalene.
2. The rat nose has high metabolic capacity associated with the acute sense of olfaction.

SPECIES SENSITIVITY

3. The rat nose has a proportionately greater susceptible surface area (~50% olfactory epithelium in rats, ~10% in humans).
4. The rat nose is highly convoluted to maximize sensitivity of olfaction by maximizing contact of inhaled air with the olfactory epithelium. Fluid dynamic studies of naphthalene disposition in the rodent and primate nose are needed.

METABOLISM

5. Evidence points to important differences between rodents and primates in their ability to metabolize naphthalene to a toxic intermediate. Rates of metabolic turnover in primates are 10-100x lower than in rodents.
6. The patterns of injury in the rodent respiratory tract correlate with areas of highest naphthalene metabolism. Additional efforts to map lesions in the rat and primate respiratory tract would help to identify potential sites of susceptibility in the primate respiratory tract.
7. Are the metabolites generated at the site of injury the same in rats and primates?
8. Need more characterization of the velocity and affinity of the important metabolic enzymes in mice, rats, primates, and in different tissues. This data would help inform a data-driven PBPK model.

MODE OF ACTION

9. Evidence for cytotoxicity is overwhelming; there is no evidence of carcinogenicity without cytotoxicity. Suggestive of a threshold response.
10. *Evidence for genotoxicity is very limited. EPA assumes genotoxicity absent evidence of undisclosed strength showing that the assumption is false.*
11. Need to characterize tissue reaction in response to naphthalene injury in both rodents and primates.

SATURATION EFFECTS

12. Evidence suggests that higher doses of naphthalene overwhelm protective and repair mechanisms in the cell.

13. Need to understand the dose-response relationship for saturation of protective mechanisms.

14. Need to understand differences between rodent and human capability of protection.

ABSENCE OF SUPPORTING EPIDEMIOLOGY

15. If EPA's cancer slope factor is correct, naphthalene is 20 times as potent as benzene, an established carcinogen. Given the breadth of historic human exposure to both, we would expect there to be epidemiological evidence of nasal tumors in humans.

ADMINISTERED VS. ACTUAL DOSE

16. Is aerosol formation and deposition exacerbating tissue irritation? What is the appropriate relationship between vapor-only based dose and response.

EPA-IRIS REASSESSMENT OF THE INHALATION CARCINOGENICITY OF NAPHTHALENE

Summary of issues highlighted by EPA-NCEA in two recent briefs

EPA's Risk Assessment

- Foundation
  - Critical Study: NTP inhalation 2 yr bioassay in rats (2000)
    - Rare neuroblastoma in olfactory epithelium – statistically significant trend
    - Adenoma in respiratory epithelium – statistically significant trend
    - No such tumors in controls – clear evidence of carcinogenicity
  - Supporting Study: NTP inhalation 2 yr bioassay in mice (1992)
    - Pulmonary adenomas, elevated incidence vs controls in females
    - No evidence of carcinogenicity in males
  - Genotoxicity data
    - Ames assays – negative
    - SCE, chromosome aberration assays – positive
    - Metabolite genotoxicity – possible
  - LMS model – predicate for risk assessment
- EPA's issues
  - Are rat and mouse tumors relevant to an assessment of human carcinogenicity?
  - Best and sufficient data set for deriving naphthalene's IU?
  - Mode of action:
    - Are the pertinent events, D/R, temporal and biological relationships described?
    - Is the "mode of action" sufficiently described and supportive of the LMS model for deriving the IU?
    - Is the mode of action relevant to humans and who/how/when are we susceptible to it?
    - Is there more than one MOA?
  - Rat vs mouse vs primate – are differences relate to metabolic differences in lungs and airways?
    - Lung microsomes – mice 100X > primates; rats 10X > primates
    - Nasal CYP2F protein – mice 2X > rats; mice 20X > primates; rats 10X > primates
  - MOA: Mutagenicity – evidence ambiguous
  - MOA: Cytotoxicity & Hyperplasia
    - Rodent lung & nasal cytotoxicity lead to injury, repair, hyperplasia (esp olfactory epithelium)
    - Primate effects – none mentioned [not researched?]
  - MOA issues
    - What key events lead to tumors in rodents?
      - ◊ metabolic activation, protein binding, mutagenicity, cytotoxicity, GSH depletion?
      - ◊ why do rats and mice exhibit nasal cytotoxicity; but, only rats develop tumors?
- Research needs
  - Identify key metabolites & distribution
  - Mutagenicity of naphthalene in lung and nasal tissue
  - Map & correlate lesions with tumor formation
  - Determine time course & dose-response for lesion formation
  - Determine whether cytotoxicity is necessary & sufficient for tumor formation

[No correlation of rodent with primate effects? Pertinence of model to human risk assessment?]

JPH: 15May06

NTP INHALATION BIOASSAY

Mouse – incidence of survival; incidence of lesions (both sexes combined) expressed as %

Lesions	Exposure Conc (ppm):	0	10	30
survival		61	81	81
chronic nasal inflammation		1	99	99
hyperplasia of respiratory epithelium		0	98	100
metaplasia of olfactory epithelium		0	98	100
chronic lung inflammation		2	25	40
alveolar/bronchiolar adenomas		*9*	*13*	*20*
alveolar/bronchiolar carcinomas		*0*	*2*	*3*

==========

Observations

- Survival
  - overall survival adequate – roughly comparable across sexes
  - control survival lower than expected due to males fighting – not apparent in treatment groups (why?)

- Lesions
  - incidence of cytotoxic effects, except lung inflammation, ~100% across all groups & sexes
  - lung inflammation suggests dose-response across groups and sexes
    - ambiguous apparently higher incidence in males
  - alveolar/bronchiolar adenomas
    - ambiguous incidence in controls
    - ambiguous dose-response across treatment groups
  - alveolar/bronchiolar carcinomas
    - no incidence in controls
    - modest incidence in treatment groups
  - exposure levels too high
    - excessive incidence of cytotoxic effects
    - exceeds environmentally relevant concentrations

Assessment

Supporting, but not the determinative, study in the EPA-IRIS 2004 risk assessment of naphthalene's carcinogenic potential.

For the most part, across treatment groups and both sexes, the mice exhibited a uniformly high – and entirely too high – incidence of cytotoxic effects. The exposure levels were seemingly so high that the animal's reactions obviate any assessment of dose-response. Lung inflammation does suggest a dose-response; the apparent differences between sexes is ambiguous.

As was the case with the determinative rat study, the exposure concentrations appear to have been excessive and well over environmentally relevant levels. Exposure levels for both the mouse and rat studies were set to range at and below the MTD for naphthalene, with the MTD determined by the saturation limit(s) of naphthalene in air rather than the effects in the test animals. No dose ranging studies appear to have been conducted prior to the rat and mouse bioassays.

NTP INHALATION BIOASSAY

Rat – incidence of survival and incidence of lesions (both sexes combined) both expressed as %.

Lesions	Exposure Conc (ppm):	0	10	30	60
survival		53	44	52	46
Olfactory Epithelium					
atypical hyperplasia		0	98	95	91
atrophy		3	100	99	96
chronic inflammation		0	98	97	95
hyaline degeneration		16	94	92	86
neuroblastoma		*0*	*2*	*7*	*15*
Respiratory Epithelium					
hyperplasia		3	40	53	54
squamous metaplasia		0	37	41	34
hyaline degeneration		8	54	55	48
goblet cell hyperplasia		0	42	60	47
adenoma		*0*	*6*	*12*	*18*
Nasal Glands					
hyperplasia		1	99	99	93
squamous metaplasia		0	5	35	47

==========

Observations

- Olfactory Lesions:
  - incidence of cytotoxic effects, 90-100% irrespective of dose
  - cytotoxic effects exhibit no evidence of dose/response
  - only CA effect (neuroblastoma) exhibits dose-response at modest rate
  - exposure levels too high
    - excessive incidence of cyto effects
    - exceeds environmentally relevant concentrations
- Respiratory Lesions:
  - incidence of cytotoxic effects, high but not total
  - cytotoxic effects exhibit no consistent evidence of dose response
  - only CA effect (adenoma) exhibits dose-response at modest rate
  - exposure levels too high
    - excessive incidence of cyto effects
    - exceeds environmentally relevant concentrations
- Nasal Glands
  - incidence of cytotoxic effects, 90-100% irrespective of dose
  - cytotoxic effect exhibits no evidence of dose/response
  - pre-CA effect (squamous metaplasia) exhibits dose-response
  - exposure levels too high
    - excessive incidence of cyto effects
    - exceeds environmentally relevant concentrations

Assessment

To better identify both the *threshold* and *time course* of response for cytotoxicity as well as cancer effects, lower exposure levels are needed. In 2-wk exposure studies, Buckpitt recently demonstrated an increased incidence of cytotoxic effects with decreases in P-450 levels as concentrations increase from 1.5 to 15 ppm. In addition to mechanistic studies, naphthalene's data base arguably needs a long-term bioassay at concentrations =/< 10 ppm that includes interim sample collections. Use 10 ppm as high dose and range remaining exposure groups down to 1 or 0.1 ppm. Any modeling of the CA slope (whether threshold- or LMS-based) would presumably start at some conc below 10 ppm resulting in a different slope factor for naphthalene.

If mechanistic-based issues argue that rats are an inappropriate, or otherwise non-predictive, model for human risk assessment, then primate-based studies may be needed. This begs the question of whether a primate chronic-onco bioassay is needed for an informed evaluation of naphthalene's non-CA and CA thresholds and effects that is more pertinent for human risk assessment.

Furthermore, existing data (cancer in the NTP study at 10 ppm) suggests that present OELs of 10 ppm are unsustainable.

Results of these requisite studies support three goals: improved CA risk assessment, improved non-CA risk assessment, amended (lowered) OEL development. Preuss et al (2003) reviewed the EU literature and argued for an OEL of ~0.3 ppm.

Occupational Epidemiological Studies Relevant to Naphthalene do not Indicate an Increased Risk of Nasal or Lung Tumors in Humans

The NTP studies of nasal cancer in rats and lung cancer in mice resulting from high dose naphthalene inhalation are very consistent with a localized response. Although the responses in the rat and mouse are at different sites, they correlate closely with sites of both extensive tissue damage and high metabolism of naphthalene to toxic intermediates. Studies in primates have failed to identify corresponding high levels of naphthalene metabolism in either the nose or the lung. In addition, large-scale epidemiological studies of workers exposed to naphthalene and other compounds do not show elevated levels of nasal and lung tumors. This human and primate data strongly suggest that the NTP study measured a mechanism that is specific to rodents and that has little applicability to public health protection.

Although no studies have been reported among workers exposed to pure naphthalene, naphthalene is a common constituent of petroleum products such as gasoline, diesel, and jet fuel and of lighter coal tar products such as creosote. Multiple large epidemiological studies have been conducted among refinery workers [1-24] and in creosote workers or workers distilling coal tar[25-29]. All of these studies involved complex mixtures of hydrocarbons that would be expected to contain significant naphthalene. Although none of these studies were designed to specifically study nasal tumors, because nasal tumors are rare in humans, four studies report nasal tumors and ten others report "respiratory tumors" which includes lung cancer as well as nasal cancer.

Seven of these studies[1,5,6,9,10,18,22] report nasal tumors either directly or indirectly. In one study[18], nasal cancer reached statistical significance ($p=0.04$) while in the other six, statistical significance was not reached. In addition, in one of the creosote studies[27], no nasal tumors were observed but this was not reported in the manuscript (personal communication with Otto Wong). In an additional six studies[4,15,16,17,23,24], tumors of the respiratory system (including lung, larynx and nose) are reported and there were no statistical increases. Overall in the studies of petroleum workers, there appears to be a consistent pattern of decreased overall cancer mortality as well as lung cancer mortality which is borne out in two studies that are meta-anlayses[2,8]. Although naphthalene exposures were not documented in these studies, they were likely not insignificant relative to EPA's unit risk factor of 0.1 per mg/m^3 per lifetime.

In addition to the studies referenced above, the U.S. Air Force is currently completing a nested case control study among Air Force personnel with exposure to JP-8 (stratified by high, medium and low exposure) based on invasive cancer cases identified through the Armed Forces Institute of Pathlology Automated Tumor Registry between 1989 and 2003[29]. Jet fuel is a good indicator of naphthalene exposure since JP8 is 1-3 percent naphthalene. In all, 2,754 Air Force personnel met the criteria for study inclusion. There were no significant relationships between specific types of cancer and jet fuel exposure including lung and nasal cancers. Additionally, there is no overall increased tumor incidence associated with JP-8 exposure.

While none of these studies can individually rule out nasal cancer or lung cancer excess in response to naphthalene inhalation, it is significant that as a whole, these studies do not demonstrate an elevated nasal, lung or overall cancer risk among exposed workers. If the animal studies were relevant to humans, one would expect to see some increase in nasal, lung or overall cancers. Clearly, there appears to be a disconnect between the predicted excess burden of nasal tumors based on time-to-tumor modeling of the recent NTP rat bioassay results and what is observed in humans.

If the rodent studies are relevant to human cancer risk assessment, the cancer risk may be to sites other than the nasal cavity or the lung. The studies cited above can not rule this possibility out. If this is indeed the case, the naphthalene would have to be first absorbed systemically and distributed through the blood stream. EPA (2003)[30] indicates that their PBPK modeling predicts that naphthalene is readily absorbed into the bloodstream through both the oral and inhalation routes. If the NTP rodent studies are relevant to human cancer risk assessment and there is not site concordance, then naphthalene would need to be considered carcinogenic through both routes of exposure.

#	Reference	A	L	R	N
1	**Wong O**, Morgan RW, Bailey WJ, Swencicki RE, Claxton K, Kheifets L. An epidemiological study of petroleum refinery employees. Br J Ind Med. 1986 Jan;43(1):6-17.	↓	↓	↓	↔
2	**Wong O**, Raabe GK. Critical review of cancer epidemiology in petroleum industry employees, with a quantitative meta-analysis by cancer site. Am J Ind Med. 1989;15(3):283-310.	↓	↓		
3	Dagg TG, Satin KP, Bailey WJ, **Wong O**, Harmon LL, Swencicki RE.An updated cause specific mortality study of petroleum refinery workers. Br J Ind Med. 1992 Mar;49(3):203-12.	↓	↓		
4	Satin KP, **Wong O**, Yuan LA, Bailey WJ, Newton KL, Wen CP, Swencicki RE. A 50-year mortality follow-up of a large cohort of oil refinery workers in Texas. J Occup Environ Med. 1996 May;38(5):492-506.	↔	↔	↔	
5	Collingwood KW, Raabe GK, **Wong O**. An updated cohort mortality study of workers at a northeastern United States petroleum refinery. Int Arch Occup Environ Health. 1996;68(5):277-88.	↔	↓	↓	↔
6	Raabe GK, Collingwood KW, **Wong O**. An updated mortality study of workers at a petroleum refinery in Beaumont, Texas. Am J Ind Med. 1998 Jan;33(1):61-81.	↔	↔	↔	↔
7	Rosamilia K, **Wong O**, Raabe GK.A case-control study of lung cancer among refinery workers. J Occup Environ Med. 1999 Dec;41(12):1091-103.	↔			
8	**Wong O**, Raabe GK. A critical review of cancer epidemiology in the petroleum industry, with a meta-analysis of a combined database of more than 350,000 workers. Regul Toxicol Pharmacol. 2000 Aug;32(1):78-98.	↓	↓		
9	**Wong O**, Harris F, Rosamilia K, Raabe GK. An updated mortality study of workers at a petroleum refinery in Beaumont, Texas, 1945 to 1996. J Occup Environ Med. 2001 Apr;43(4):384-401	↓	↓	↓	↔
10	**Wong O**, Harris F, Rosamilia K, Raabe GK. Updated mortality study of workers at a petroleum refinery in Torrance, California, 1959 to 1997. J Occup Environ Med. 2001 Dec;43(12):1089-102.	↓	↓	↓	↔
11	**Schnatter AR**, Theriault G, Katz AM, Thompson FS, Donaleski D, Murray N. A retrospective mortality study within operating segments of a petroleum company. Am J Ind Med. 1992;22(2):209-29.	↔	↔		
12	**Schnatter AR**, Katz AM, Nicolich MJ, Theriault G.A retrospective mortality study among Canadian petroleum marketing and distribution workers. Environ Health Perspect. 1993 Dec;101 Suppl 6:85-99.	↔	↔		
13	Lewis RJ, **Schnatter AR**, Katz AM, Thompson FS, Murray N, Jorgensen G, Theriault G. Updated mortality among diverse operating segments of a petroleum company. Occup Environ Med. 2000 Sep;57(9):595-604.	↓	↓		
14	Lewis RJ, **Schnatter AR**, Drummond I, Murray N, Thompson FS, Katz AM, Jorgensen G, Nicolich MJ, Dahlman D, Theriault G.Mortality and cancer morbidity in a cohort of Canadian petroleum workers. Occup Environ Med. 2003 Dec;60(12):918-28.	↓	↓		↔
15	Tsai SP, Gilstrap EL, Cowles SR, Snyder PJ, Ross CE. A cohort mortality study of two California refinery and petrochemical plants. J Occup Med. 1993 Apr;35(4):415-21.	↓	↓	↓	
16	Tsai SP, Gilstrap EL, Cowles SR, Snyder PJ, Ross CE. Long-term follow-up mortality study of petroleum refinery and chemical plant employees. Am J Ind Med. 1996 Jan;29(1):75-87.	↓	↓	↓	
17	Tsai SP, Chen VW, Fox EE, Wendt JK, Cheng Wu X, Foster DE, Fraser AE. Cancer incidence among refinery and petrochemical employees in Louisiana, 1983-1999. Ann Epidemiol. 2004 Oct;14(9):722-30.	↓	↓	↓	
18	**Rushton L**, Alderson M. The influence of occupation on health--some results from a study in the UK oil industry. Carcinogenesis. 1980 Sep;1(9):739-43.	↓	↓		↑
19	**Divine BJ**, Hartman CM, Wendt JK. Update of the Texaco mortality study 1947-93: Part I. Analysis of overall patterns of mortality among refining, research, and petrochemical workers. Occup Environ Med. 1999 Mar;56(3):167-73.	↓	↓		
20	**Divine BJ**, Hartman CM. Update of a study of crude oil production workers 1946-94. Occup Environ Med. 2000 Jun;57(6):411-7.	↓	↓		
21	Gun RT, Pratt NL, Griffith EC, Adams GG, Bisby JA, Robinson KL. Update of the Texaco mortality study 1947-93: Part I. Analysis of overall patterns of mortality among refining, research, and petrochemical workers. Occup Environ Med. 1999 Mar;56(3):167-73.	↓	↓		
22	Sorahan T, Nichols L, Harrington JM. Mortality of United Kingdom oil refinery and petroleum distribution workers, 1951-1998. Occup Med (Lond). 2002 Sep;52(6):333-9.	↓	↓		↔

23	Sathiakumar N, Delzell E, Rodu B, Beall C, Myers S. Cancer incidence among employees at a petrochemical research facility. J Occup Environ Med. 2001 Feb;43(2):166-74.	↓	↓	↓	
24	Jarvholm B, Mellblom B, Norrman R, Nilsson R, Nordlinder R. Cancer incidence of workers in the Swedish petroleum industry. Occup Environ Med. 1997 Sep;54(9):686-91.	↔		↓	
25	Karlehagen S, Andersen A, Ohlson CG. Cancer incidence among creosote-exposed workers. Scand J Work Environ Health. 1992 Feb;18(1):26-9.	↔	↔		
26	Swaen GM, Slangen JM. Mortality in a group of tar distillery workers and roofers. Int Arch Occup Environ Health. 1997;70(2):133-7.	↔	↔		
27	Wong O, Harris F. Retrospective cohort mortality study and nested case-control study of workers exposed to creosote at 11 wood-treating plants in the United States. J Occup Environ Med. 2005 Jul;47(7):683-97.	↔	↔	↔	
28	Moulin JJ, Mur JM, Wild P, Demonchy A, Eloy E, Jeannot A. Epidemiologic study of the mortality among the employees of a coal tar distillery Rev Epidemiol Sante Publique. 1988;36(2):99-107.		↔		
29	D'Mello TA Cancer and Jet Fuel Occupational Exposure in the U.S., Air Force: 1989-2003. Abstract, presented at the 77th Aerospace Medical Association meeting, 2005	↔	↔		↔
30	USEPA (2003) http://www.epa.gov/OGWDW/ccl/pdfs/reg_determine1/support_ccl_naphthalene_healtheffects.pdf				

A All cancers

L Lung Cancer (162)

R Respiratory Cancer (160-162)

N Nose & Sinus Cancer (160)

↔ SMR or SIR not statistically different from expected

↓ SMR or SIR statistically lower than expected

↑ SMR or SIR statistically higher than expected

K/MG:2006

OBSERVED vs PREDICTED NASAL CANCER INCIDENCE BASED ON THE EPA CANCER POTENCY FACTOR FOR NAPHTHALENE

The nasal cavity and the paranasal sinuses are lined by a layer of mucous producing tissue called mucosa[1]. The mucosa has multiple types of cells including:

- Squamous epithelial cells which are lining cells and form the majority of the mucosa,
- Glandular cells such as minor salivary gland cells, which produce mucus and other fluids,
- Nerve cells which are responsible for sensation and the sense of smell in the nose
- Infection-fighting cells which are part of the immune system, blood vessel cells, and other supporting cells.

There are many types of nasal (ICD•9 160.0–160.9) All of the cells that make up the mucosa can become cancerous and each type behaves or grows differently. The types of tumors formed when these cell types become cancerous include:

- **Squamous cell carcinoma** (cancer of squamous cells of the nasal cavity and sinus lining layer) is the most common type of cancer in the nasal cavity and paranasal sinuses. It makes up about 60%-70% of cancers of these areas.
- Papillomas (wart-like growths that are not cancer, but can be destructive) have a small chance of developing into squamous cell carcinoma. A subtype called inverting (sunken) papilloma, has a tendency to recur or come back. Inverting papilloma is often called a benign tumor, but can invade surrounding tissue and act like a malignant tumor. It needs to be treated like a cancer in many cases.
- **Adenocarcinomas and mucoepidermoid cancers** (cancers arising from gland cells) are the next most frequent type, making up about 10%-20%.
- **Malignant lymphomas** (cancer arising from lymph or immune system cells) make up about 5% of cancers of the nasal cavity and paranasal sinuses.
- **Malignant melanoma** (cancer of pigment or skin color containing cells) is an aggressive cancer that comprises about 3% of these tumors.
- **Esthesioneuroblastomas** come from the olfactory nerves (nerves which govern the sense of smell). They are sometimes mistaken for undifferentiated carcinoma (another rapidly growing cancer) or lymphoma. These cancers usually occur on the roof of the nasal cavity and involve a structure called the cribriform plate, which is a bone deep in the skull, between the eyes, and above the ethmoid sinuses.
- Tumors of muscle, bone, cartilage, and fibrous cells may also occur.

Cancers of the nasal cavity and paranasal sinuses are rare. About 2,000 people in the United States develop cancer of the nasal cavity and paranasal sinus each year. Men are about 50% more likely than women to get this cancer. Nearly 80% of the people who get this cancer are between the ages of 45 and 85. These cancers also occur much more often in certain areas of the world such as Japan and South Africa.

Table 1

Average Annual Cancer Incidence (per 100,000 individuals) in the United States

Nasal and Nasopharyngeal Cancers

	45–49 Years of Age			50–54 Years of Age			55–59 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Nose, Nasal Cavity, and Mid-ear									
Males	0.9	0.7	1.6	1.2	1.2	1.1	1.7	1.6	2.5
Females	0.4	0.4	0.7	0.7	0.7	0.6	0.8	0.8	1.2

SEER nine standard registries, crude age-specific rate, 1993–1997.

Scientists have found many occupational risk factors that make a person more likely to develop nasal cavity and paranasal sinus cancer. Most of these risk factors are associated with substances in the work environment that are inhaled. These include occupational exposure to dusts from wood, textiles, and leather and even perhaps flour. Other substances linked to this type of cancer are glues, formaldehyde, solvents used in furniture and shoe production, nickel and chromium dust, mustard gas, isopropyl ("rubbing") alcohol, and radium. Smoking is a risk factor for nasal cavity cancer.

No studies have been reported among workers exposed to pure naphthalene, however, naphthalene is a common constituent of petroleum products such as gasoline, diesel and jet fuel as well as a common constituent of lighter coal tar products such as creosote. Multiple large epidemiological studies have been conducted among refinery workers [2-8] and creosote workers[9-10].

All of these studies involved complex mixtures of hydrocarbons. Although none of these studies were designed to specifically study nasal tumors, because nasal tumors are rare in humans, it is very likely that the presence of such tumors would have been reported in most or all of these studies had they been seen. None of these studies presents any cases of nasal cancer and no consistent pattern suggestive of increased overall cancer mortality. If a rare cancer such as nasal cancer was caused by naphthalene, it is likely that it would have been reported in some of these studies. Although naphthalene exposures were not documented in these studies, they were likely not insignificant relative to EPA's unit risk factor of 0.1 per mg/m^3 per lifetime.

The U.S. Air Force is currently completing a cancer incidence study among Air Force personnel with exposure to JP-8 (stratified by high, medium and low exposure) based on DOD tumor registry data between 1988 and 2003 (personal communication from Col. Yamane Grover). In addition, they are conducting a nested case control study for each invasive tumor. Although 14 nasal tumors are in the database, all were among the lowest exposure group. There is no overall increased tumor incidence associated with JP-8 exposure.

According to census statistics from 2000, the population of the United States is assumed to be 281,000,000. EPA (2003)[11] assume *"an average ambient concentration level of*

5.19 μg naphthalene/m^3 and an average inhalation rate of 15.2 m^3/day (U.S. EPA, 1996c), an average daily dose of 1,127 ng/kgday can be calculated for a 70-kg adult. An estimated average daily dose of 4,515 ng/kg-day can be calculated for a 10-kg child assuming an inhalation rate of 8.7 m^3/day (U.S. EPA, 1996c). Individual intake will vary depending on factors including activity, geographic location, and inhalation rate."

ATSDR (2005)[12] states that: *"The largest source of emission (more than 50%) is through inadvertent releases due to residential combustion of wood and fossil fuels (EPA 1982d). Naphthalene emissions from unvented kerosene space heaters have been reported (Traynor et al. 1990).*

The second greatest contribution comes from the use of naphthalene as a moth repellent (EPA 1982d). Because it volatilizes appreciably at room temperature, virtually all of the naphthalene contained in moth repellent is emitted to the atmosphere. Thus, in 1989, about 12 million pounds of naphthalene were released to air from moth repellent use."

We can estimate the magnitude of these additional exposures and their potential impact on nasal cancer incidence based on EPA's cancer potency factor for naphthalene. According to High and Skog[13], approximately 29% of residential household had wood burning stoves or fireplaces in 1980 and in 2000. Hawthorne et al 1985[14] reported that people who live in homes that have fireplaces, wood stoves or kerosene heaters are exposed to an average concentration of 46 μg/m^3.

EU (2003)[15] cites 1995 mothball exposure data from Reochem. They state that in a controlled experiment on mothball use, "typical household situations following the product label directions," Reochem found 1000 to 12000 μg/m^3 in closed areas. EU (2003) evaluates the exposure by saying that someone could be exposed to 12,000 μg/m^3 for 1 hour per day and 820 μg/m^3 for 23 hours. This yields 1300 μg/m^3 on average over a day.

If one assumes that:

a) half of the naphthalene released from moth balls reported by ATSDR was used in domestic homes
b) a box of moth balls weighs 1 pound
c) one box is used yearly
d) the "average family" is 2.5 persons,

Then one can estimate that 15,000,000 Americans are potentially exposed to mothballs.

Given the current incidence of 2,000 new cases of nasal cancer annually in the U.S., one might expect 150,000 "lifetime" cases from all causes, (assuming a 75 year life span.).

Table 2 contains estimates of excess nasal tumors based on the USEPA cancer potency factor for naphthalene.

Table 2

	Air Conc. $\mu g/m^3$	Incremental Risk	Estimate of Exposed Population	Expected Incremental Lifetime Cancers
Moth ball users	1,300	0.13	15,000,000	1,950,000
Combustion indoors	46	0.0046	77,000,000	354,200
General U.S population	5	0.0005	189,000,000	94,5000
Total Number of Lifetime Cancers Expected in the U.S.				**2,398,700**

We offer this screening level analysis of the predicted excess burden of nasal tumors based on time-to-tumor modeling of the recent NTP rat bioassay results. Clearly, there is a disconnect between the nasal tumors predicted to be due to naphthalene and those reported.

If the rodent studies are relevant to human cancer risk assessment, the cancer risk may be to sites other than the nasal cavity. If this is indeed the case, the naphthalene would have to be absorbed systemically and distributed through the blood stream. EPA (2003)[11] indicates that their PBPK modeling predicts that naphthalene is readily absorbed into the bloodstream through both the oral and inhalation routes. If the NTP rodent studies are relevant to human cancer risk assessment and there is not site concordance, then naphthalene must be considered carcinogenic through both routes of exposure.

References

1) http://www.nlm.nih.gov/medlineplus/nasalcancer.html

2) Lewis RJ, Schnatter AR, Katz AM, Thompson FS, Murray N, Jorgensen G, Theriault G. Updated mortality among diverse operating segments of a petroleum company. Occup Environ Med. 2000 Sep;57(9):595-604.

3) Wong O, Harris F, Rosamilia K, Raabe GK.Updated mortality study of workers at a petroleum refinery in Torrance, California, 1959 to 1997. J Occup Environ Med. 2001 Dec;43(12):1089-102.

4) Schnatter AR, Katz AM, Nicolich MJ, Theriault G.A retrospective mortality study among Canadian petroleum marketing and distribution workers. Environ Health Perspect. 1993 Dec;101 Suppl 6:85-99.

5) Wong O, Harris F, Rosamilia K, Raabe GK. An updated mortality study of workers at a petroleum refinery in Beaumont, Texas, 1945 to 1996. J Occup Environ Med. 2001 Apr;43(4):384-401.

6) Sorahan T, Nichols L, Harrington JM. Mortality of United Kingdom oil refinery and petroleum distribution workers, 1951-1998. Occup Med (Lond). 2002 Sep;52(6):333-9.

7) Gun RT, Pratt NL, Griffith EC, Adams GG, Bisby JA, Robinson KL. Update of the Texaco mortality study 1947-93: Part I. Analysis of overall patterns of mortality among refining, research, and petrochemical workers. Occup Environ Med. 1999 Mar;56(3):167-73.

8) Satin KP, Wong O, Yuan LA, Bailey WJ, Newton KL, Wen CP, Swencicki RE. A 50-year mortality follow-up of a large cohort of oil refinery workers in Texas. J Occup Environ Med. 1996 May;38(5):492-506.

9) Wong O, Harris F. Retrospective cohort mortality study and nested case-control study of workers exposed to creosote at 11 wood-treating plants in the United States. J Occup Environ Med. 2005 Jul;47(7):683-97.

10) Karlehagen S, Andersen A, Ohlson CG. Cancer incidence among creosote-exposed workers. Scand J Work Environ Health. 1992 Feb;18(1):26-9.

11) USEPA (2003) http://www.epa.gov/OGWDW/ccl/pdfs/reg_determine1/support_cc1_naphthalene_healtheffects.pdf

12) ATSDR (2005) http://www.atsdr.cdc.gov/toxprofiles/tp67.pdf

13) High C, Skog K. Current and Projected Wood Energy Consumption in the United States, it Klass, DL, Ed. Energy from Biomass and Wastes, Proceedings of IGT's Conference, 1989 pages 229-260

14) Hawthorne AR, Gammage RB, Dudney CS. An Indoor Study of 40 East Tennessee Homes. Environment International 1985 12:221-239

15) EU (2003) European Union Risk Assessment Report, Naphthalene, United Kingdom, Final Report, March 2002

CANCER AND JET FUEL OCCUPATIONAL EXPOSURE IN THE U.S. AIR FORCE: 1989—2003

Tiffany A. D'Mello, MPH, Grover K. Yamane, MD, MPH, Col, USAF, MC, SFS, Epidemiology Services Branch, Air Force Institute for Operational Health, Brooks City-Base, TX, 78235-5116

Introduction

This study attempted to measure the association between invasive cancer and jet fuel occupational exposure in U.S. Air Force Active Duty (AFAD) personnel.

Methods

A nested case-control study design was used, with the cohort defined as personnel who had ≥ 1 yr of AFAD service between 1 January 1988 and 31 December 2003. Case subjects with incident invasive cancer (excluding skin squamous cell and basal cell cancers) diagnosed between 1 January 1989 and 31 December 2003 were obtained from the Armed Forces Institute of Pathology Automated Tumor Registry. Control subjects, 4 for each respective case subject and matched on year of birth, gender, and race, were randomly selected from the Air Force Personnel Center main personnel database. Occupational data were extracted from the latter. Fuel exposure was classified as high, moderate or low based on job descriptions and previous research. Jobs whose descriptions indicated direct and frequent contact with jet fuel were classified as high exposure, whereas those involved with fuel equipment and indirect fuel contact were classified as moderate. All other positions were classified as low exposure. Conditional logistic regression was used to obtain odds ratios (ORs) and 95% confidence intervals (95%CIs).

Results

During this 15-yr period, 2,754 AFAD personnel were diagnosed with invasive cancer and met the cohort definition for study inclusion. Using low jet fuel exposure as the reference, crude ORs for moderate and high exposure levels were 0.84 (95%CI: 0.65-1.09) and 0.73 (95%CI: 0.32-1.64), respectively. There were no significant relationships between specific types of cancer and jet fuel exposure. Adjustment for military rank at time of diagnosis did not significantly alter any of these associations

Conclusions

A non-significant negative association between occupational jet fuel exposure and incident invasive cancer was observed. Future work can therefore focus on factors that may influence this relationship.

Educational Objectives: The association between incident invasive cancer and jet fuel occupational exposure in the U.S. Air Force Active Duty population is described.

U.S. AIR FORCE

Integrity - Service - Excellence

Occupational Jet Fuel Exposure and Invasive Cancer Occurrence

Tiffany A. D'Mello, MPH

Grover K. Yamane, Col, USAF, MC, SFS

AFIOH

Air Force Institute for Operational Health

Sustaining Readiness through Healthy Communities

U.S. AIR FORCE

Background

- JP-8 is the primary aircraft, vehicle and equipment fuel used in the USAF
- Jet fuel is the greatest source of chemical exposure for these personnel
- Among fuel-exposed workers
  - Acute adverse health effects have been well-documented
  - Long-term effects have not been widely studied

Purpose

- To conduct an exploratory study measuring the association between occupational jet fuel exposure and invasive cancer occurrence in USAF personnel
- USAF is an ideal population for data records analysis because of the detailed information that is routinely collected
  - Automated Central Tumor Registry (ACTUR)
  - Air Force Personnel Center

Methodology

Nested Case-Control Study Design

USAF personnel with ≥1 year active duty between 1 Jan 88 and 31 Dec 03

CASES
USAF diagnosed with cancer between 1 Jan 89 and 31 Dec 03 (2,754 people)

compared to

CONTROLS
USAF with same year of birth, race and gender as each respective case (11,016 people)

Total Sample = 13,770

Exposure Assessment

- Categorized based on current and previous USAF occupations:
  - **High** (N=45)
    - Aircraft fuel systems workers
    - Direct and frequent fuel contact
  - **Moderate** (N=428)
    - Fuel storage and distribution systems
    - Indirect and/or intermittent fuel contact
  - **Low** (N=13,297)
    - All other occupations
    - Little or no fuel contact

Fuel Systems Workers

Sample Characteristics

Distribution of Select Sample Characteristics		
AGE AT DIAGNOSIS	Range Median	18 - 61 years 37 years
GENDER	Female Male	27.1% 72.9%
RACE	White (Hispanic & Non-Hispanic) Black Other	84.6% 11.4% 3.2%

Sample Characteristics

Distribution of Jet Fuel Exposure Levels			
Level of Occupational Jet Fuel Exposure	Cases %	Controls %	Total %
High	0.3	0.3	0.3
Moderate	2.7	3.2	3.1
Low	97.0	96.5	96.6

Results

Jet Fuel Exposure and Odds Ratio for Cancer			
Level of Occupational Jet Fuel Exposure	Odds Ratio	95% CI	p-value
High	0.73	0.32-1.64	0.44
Moderate	0.84	0.65-1.09	0.19
Low	Reference	--	--
Exposed	0.83	0.65-1.06	0.14
Unexposed	Reference	--	--

Specific Cancer Types

Cancer Type	Cases N	OR	95% CI
Acute Myeloid Leukemia	26	0.48	0.06-4.01
All Leukemias	71	0.55	0.12-2.52
Urinary Bladder	48	0.70	0.10-5.07
Breast Adenocarcinoma	217	0.49	0.11-2.17
Hodgkin's Lymphoma	135	0.44	0.10-1.91
Lung (Small & Non-Small cell)	42	0.79	0.09-7.28
Multiple Myeloma	17	1.33	0.14-12.82
Non-Hodgkin Lymphoma	145	1.00	0.33-3.03
Renal Clear Cell	49	0.83	0.21-3.32

Frequencies were too small for valid comparisons of ALL, CLL, CML, dermatofibrosarcoma, hepatocellular and nasal cancers

Discussion - Strengths

- All data were abstracted from surveillance databases that utilized standardized reporting procedures
  - Minimizes chance of recall bias
  - All cancer cases analyzed, coded and entered by trained registrars
- Novel study
  - Addresses gap in the literature and may be useful for future work

Discussion - Limitations

- Individual exposure assessment
- Analysis of specific fuels/chemicals
- Adjust for other cancer risk factors
- Healthy worker effect
- Small number of some cancer types

Discussion - Improvements

- Environmental Sampling
- Biological Markers
- Personal Surveys/Questionnaires
- Measure a more prevalent and/or earlier onset outcome

Conclusion

- No association observed between occupational jet fuel exposure and invasive cancer occurrence
  - Similar findings reported in other studies
- No red flags

Acknowledgements

- Mr. John Hinz
- Dr. Donald Goodwin
- Oak Ridge Institute for Science and Education (ORISE)

Questions?