

## **Comments of the *ad hoc* Naphthalene Coalition (American Chemistry Council (ACC) Naphthalene Panel and others).**

### ***Comment 1. OEHHA SHOULD CONSIDER USEPA'S UPCOMING PEER-REVIEWED ASSESSMENT OF NAPHTHALENE.***

USEPA's IRIS program is in the final stages of a detailed assessment of cancer risk factors associated with naphthalene. According to USEPA, peer review of the assessment is expected to be completed by June this year. Further, the pesticidal uses of naphthalene are currently being evaluated, and subsequent risk assessments are being prepared as part of the USEPA reregistration program. Based on these extensive assessments – which span years of study evaluation and review - and the technical information presented below, OEHHA should not proceed with a unilateral effort to develop a unit risk factor for naphthalene, but instead, should collaborate with USEPA's IRIS evaluation, and work towards a harmonized assessment of naphthalene.

Such coordination is required under the 1996 Memorandum of Understanding (MOU) between California's Environmental Protection Agency (Cal/EPA) and the USEPA entitled *Memorandum of Understanding Between California EPA's Office of Environmental Health Hazard Assessment and the U.S. EPA's National Center for Environmental Assessment*. The MOU is intended to foster harmonization of the State and federal risk assessment programs, to reduce the potential for conflicting approaches and methods, to exchange work products, and to share resources more efficiently. The MOU was entered, in part, due to the parties' joint recognition that diminishing government resources necessitated better and more extensive coordination among federal and state agencies engaged in risk analyses. Given the State of California's fiscal crisis and the diminishing resources available to the USEPA, the utility of the MOU finds no better example than in its application here, which would compel OEHHA to suspend work on naphthalene until the IRIS assessment for naphthalene is completed.

The MOU is only one example of a long history of coordination between the USEPA and the State of California. For example, OEHHA's 1997 policy entitled *Improving the Scientific Basis of Risk Assessment Through Harmonization* confirms the importance the State of California places on coordinating with the USEPA and the need to conserve scarce resources. See <http://www.oehha.org/risk/raac/harmadv.html>. The policy states that harmonization of State and Federal risk assessment activities should be viewed as a two-way exchange of scientific analysis, methods, and approaches. The policy specifically notes that:

it has become increasingly apparent that different organizations using divergent risk assessment methodologies for the same chemical or comparable situations creates a difficult situation for risk managers, policy makers and stakeholders alike.

OEHHA states in the policy that harmonization should be viewed as “[m]aking the most effective use of our limited resources by information sharing.”

As a further example, the USEPA's Office of Pesticide Programs and California's Department of Pesticide Regulation have a long history of joint review and coordination on pesticide registrations. This collaboration has proved to save time and resources, and has minimized risks of developing inconsistent risk results or duplicative risk assessment procedures that challenge risk assessors and regulators alike.

Harmonization of risk assessment work was one important theme of the Risk Assessment Advisory Committee (RAAC), an external advisory Committee that reviewed the risk assessment practices of Cal/EPA.<sup>2</sup> RAAC made the following recommendation to the Cal/EPA in its final report:

Cal/EPA should endeavor to develop future risk assessments in concert with US EPA, especially for high volume and/or high risk compounds. Before Cal/EPA conducts an independent risk assessment for a substance, it should first review any existing US EPA risk assessment.

Naphthalene clearly falls within the description of a "high volume" compound. On March 3, 2004 the Coalition asked USEPA if the Agency could estimate when the IRIS peer review panel on naphthalene would convene. USEPA responded that the external peer review would likely convene in the next few months. Given the imminent timing of USEPA's IRIS peer review of naphthalene, which is expected to include a unit risk factor for naphthalene, OEHHA should coordinate with USEPA to develop its unit risk factor in concert with USEPA.

In light of the State of California's policy of coordinating with USEPA on the development of risk assessments, the imminent release of the IRIS peer review assessment on naphthalene, and OEHHA's MOU with USEPA on coordinating the development of risk assessments on molecules of shared interest, OEHHA should suspend work on naphthalene and resume it only after the results of the IRIS assessment of naphthalene can be integrated into the OEHHA review. All of the foregoing policies and practices, and the need for sensible regulation and fiscal prudence, require no less.

**RESPONSE:** *The comment proposes in essence that OEHHA waits for U.S. EPA's risk assessment for naphthalene, which they assert is about to be released for peer review.*

*The statutes of the state of California require the evaluation of the health risks of toxic air contaminants, including the development of quantitative evaluations of cancer risk. Naphthalene is on the Air Toxics Hot Spots list of chemicals for which emissions from stationary sources must be evaluated for potential public health impacts. The statutes governing Toxic Air Contaminants were enacted in the 1980's to address the slow pace of U.S. EPA in evaluating toxicants found in the air. Cal/EPA continues to exchange information with U.S. EPA on a number of evaluations particularly where timing coincides. In the case of naphthalene, we are in the process of completing our cancer potency estimate, while U.S. EPA has still to decide on whether it should be considered a carcinogenic hazard, and is soon to submit its first draft for public comment. While we believe that consistency between the two agencies is greatly valued, scientific judgement,*

*peer review, public comments and statutory requirements often result in some differences in the risk estimates, or differences in methodology.*

*The comment cites a previous Memorandum of Understanding between U.S. EPA and Cal/EPA that indicates U.S. EPA and Cal/EPA should work together on chemicals of mutual interest. While scientists often work closely together, the U.S. EPA process is lengthy and the time line is unpredictable.*

*The commenter believes that the IRIS evaluation of naphthalene is going to be released for review this Spring. The U.S. EPA staff we have spoken to about this cannot commit to a specific timetable. In our experience with U.S. EPA processes, the finalization of an IRIS assessment can take one to several years. The NTP published their evaluation of the carcinogenicity of naphthalene in 2000 and the California Air Resources Board has indicated to us that it is important to proceed with the quantification of that risk.*

*The comment quotes from the Risk Assessment Advisory Committee 1996 report (A Review of the California Environmental Protection Agency's Risk Assessment Practices, Policies, and Guidelines) stating that Cal/EPA should endeavor to develop future risk assessments in concert with U.S. EPA, and that we should first evaluate any existing U.S. EPA risk assessment. As indicated above, OEHHA and EPA scientists do exchange information. But it is important to keep in mind that there is no finalized risk assessment for naphthalene, and a final risk assessment date is unknown. Furthermore, a quantitative cancer risk assessment for naphthalene will not necessarily be included in the final IRIS summary.*

## **Comment 2. OEHHA's USE OF LOW DOSE LINEARITY TO DETERMINE A HUMAN UNIT RISK FACTOR IGNORES THE CURRENT WEIGHT OF EVIDENCE ON NAPHTHALENE.**

The Proposal uses the default assumption of low dose linearity because OEHHA believes the weight of the evidence “favors the interpretation” that the carcinogenicity of naphthalene is due to a reactive metabolic intermediate, which causes direct damage to DNA. The Proposal states:

Genetic toxicology results for naphthalene are mixed: *Salmonella* reverse mutation assays were generally negative, but some test results with eukaryotic systems *in vivo* or *in vitro* were positive (National Toxicology Program (NTP), 2000). However, it was considered on balance that the weight of evidence, including metabolism to 1-naphthol via an epoxide intermediate (NTP, 1992, citing Bock *et al.*, 1976 and others; NTP, 2000), and the reactivity of naphthoquinones to cellular components (Zheng *et al.*, 1997) favors the interpretation that the mechanism of naphthalene carcinogenicity likely involves a reactive metabolic intermediate which causes direct damage to DNA. A low dose linearity assumption is therefore appropriate when extrapolating from the point of departure to obtain an estimate of the cancer risk at low doses. (Proposal at page 14.)

This analysis simply ignores the considerable weight of evidence on naphthalene from other studies. The evidence as a whole does not support an assumption of low dose linearity. Both the genotoxicity and the metabolism of naphthalene have been extensively evaluated, as summarized below. The Naphthalene Coalition believes the weight of the scientific evidence favors the interpretation that the tumorigenic effects of naphthalene do not involve genotoxicity or direct damage to DNA. Therefore, the use of a low dose linear model for deriving a human risk factor for naphthalene is inappropriate.

### **2a. Naphthalene and its metabolites should not be considered in overly broad comparisons of genotoxicity and carcinogenicity associated with Polynuclear Aromatic Hydrocarbons (PAHs)**

The draft naphthalene health effects summary document states (page 2, paragraph 2) that, “if information about the carcinogenicity of naphthalene had been available at the time, the carcinogenicity of naphthalene would have been evaluated in conjunction with benzo(a)pyrene and other carcinogenic PAHs.”

While some chemists would agree that naphthalene can be technically classified as a PAH for purposes of definitional nomenclature, the importance of PAHs as a group is associated with their biological activity. Biologically active PAHs share a common mechanism for genotoxicity and carcinogenicity based on their structure, which allows for metabolic conversion via the cytochrome P450 enzyme, CYP1A1, to an active dihydrodiol-epoxide.

Although planar fused ring compounds (PAHs) vary considerably in their biological activity, genotoxic PAHs are indirect-acting or promutagens, such that genotoxicity is only expressed following metabolic conversion of the PAH to an active species. The mechanism by which PAHs are thought to induce tumor formation is via interaction with genetic material within target cells, either frank mutagenicity or interference with normal genetic biology as a result of PAH-adduct formation with nuclear material. Accordingly, it is generally observed that the genotoxic potency of PAHs closely parallels the carcinogenic potency. However, this relationship is based on experience with PAHs having greater than two fused rings and information suggests that the active structure of some PAHs is a reactive arene oxide, in older literature termed the bay region diol-epoxide. A bay region diol-epoxide is formed in a PAH when three rings are fused in a way to create a pocket, the "bay". Bay region diol epoxides are formed enzymatically in humans by CYP1A1. The ability and ease of a PAH to form a bay region diol epoxide can be calculated. This has led to a great deal of work in structure-activity-relationship (SAR) assessment of the potential for carcinogenicity of PAH compounds - but only PAH compounds with three or more fused rings.

In addition to the recognition of the importance of the bay region to genotoxicity and carcinogenicity of PAHs, it has been observed that the addition of a substituent group, almost always a methyl group, in or opposite to the bay region containing the epoxide impacts on PAH biologic activity. There are numerous examples of the alkylation of the PAH (with a methyl group) both enhancing and eliminating PAH tumorigenicity and mutagenicity (Saas, 1996; Slaga, 1979, Thakker, 1979).

Naphthalene is both biologically and structurally distinct from the biologically active genotoxic and carcinogenic PAHs. Naphthalene does not have greater than two fused rings and, as discussed below, the weight of evidence supports the conclusion that naphthalene is not mutagenic. Unlike the PAHs, the metabolism of naphthalene is under the control of the CYP2F enzyme family, not the CYP1A family, and does not lead to the formation of a dihydrodiolepoxide but instead forms naphthalene-1,2-oxide. Further, the large and long-standing body of information relating to carcinogenic characteristics of PAHs, whether it be induction or suppression of genotoxic/ carcinogenic activity, has not been associated with naphthalene.

To date, a unified SAR theory does not exist to account for the observations of PAH carcinogenicity, particularly for PAHs that are substituted beyond the methyl state (nitroaromatics and branched chain alkylated PAHs, for instance). Various illuminating bodies of work have evaluated the carcinogenic effect of methyl-, ethyl-, and propyl-substitutions on fused-ring PAHs such as chrysene. Methylation has been shown to transform inactive PAHs to active and to de-active carcinogenic PAHs. For example, methylchrysene is a more potent lung carcinogen than chrysene, but ethyl- and propylchrysene are less potent. Similarly, bay region methylation of dimethylbenzanthracene, a potent mutagen and carcinogen, completely blocks mutagenic and carcinogenic activity. However, none of these observations characteristic of PAH carcinogenicity have been found applicable to "PAHs" with less than three fused rings. In fact, no approach to PAH carcinogenic SAR, whether involving electron cloud density theories or methods of

analysis involving statistics and artificial intelligence, includes naphthalene in the paradigm.

**RESPONSE:** *OEHHA agrees that “Naphthalene and its metabolites should not be considered in overly broad comparisons of genotoxicity and carcinogenicity associated with PAHs”, and indeed makes no such comparisons (broad or otherwise) in the summary describing the derivation of the unit risk for naphthalene. The sole reference to PAHs in the introductory section of the document points out that naphthalene, along with the other polycyclic aromatic hydrocarbons (PAHs), is included in the class of chemicals designated in the US EPA Hazardous Air Pollutant (HAP) list as polycyclic organic matter (POM). The Air Resources Board (ARB) has designated all HAPs as Toxic Air Contaminants (TACs). Therefore, naphthalene is included in the TAC list. However, naphthalene was not automatically considered to be a genotoxin or carcinogen just because many PAHs are in fact genotoxins and/or carcinogens. Naphthalene and its metabolites were evaluated for genotoxicity and carcinogenicity solely on the basis of empirical data on the effects of those chemicals. In fact, naphthalene was specifically excluded from consideration alongside the 3-ring or larger PAHs in the original document (OEHHA, 1993), because the structural analogy, which the commenter disavows, was not considered sufficiently persuasive in spite of US EPA’s inclusion of naphthalene within the larger class of POM. This optimistic conclusion has, unfortunately, had to be reversed in the light of more recent data, especially NTP (2000). Notwithstanding this overall consistency between the comment and the process of development of the naphthalene unit risk, OEHHA offers the following comments on specific points:*

*Naphthalene is metabolized to 1,2-naphthoquinone and 1,4-naphthoquinone by F344 rats (Waidyanatha et al., 2002) and human hepatic microsomes (Wilson et al., 1996). These metabolites have been shown to bind to macromolecules (Waidyanatha et al., 2002), and to induce sister chromatid exchange (SCE) in vitro in human lymphocytes (Wilson et al., 1996). These data suggest that metabolism of naphthalene to a dihydrodiolepoxide is not a requisite step in the mechanism of its genotoxicity.*

*If no approach to PAH carcinogenic SAR, whether involving electron cloud density theories or methods of analysis involving statistics and artificial intelligence, includes naphthalene in the paradigm, then a new paradigm will have to be developed. Empirical data should not be discarded because it does not fit an existing model; rather, models should be altered to fit empirical data.*

## **2b. The weight of evidence strongly supports the conclusion that naphthalene is not genotoxic**

The results of the genotoxicity studies of naphthalene are primarily negative, and reviews by others of these data support this interpretation. Recently, the genotoxicity of naphthalene encompassing both the published literature and several unpublished studies was reviewed (Schreiner, 2003). The review contained results from 16 bacterial assays, 9

cytogenetic assays (7 in vitro, 2 in vivo) and 13 other assays, including 6 cell transformation assays, 3 unscheduled DNA synthesis assays, 2 alkaline elution assays, one Drosophila assay, and a human cell gene mutation assay. Tables summarizing the data presented in Schreiner (2003) are included here as Attachment B. Naphthalene did not induce positive responses in any of the 30 in vitro assays conducted with nonmammalian and mammalian cells and gave negative results in all 4 assays in which animals were directly dosed and evaluated. Positive responses were only seen in 4 in vitro assays: the NTP chromosomal aberration assay in CHO cells, an in vitro micronucleus assay in a human lymphoblastoid cell line, a mouse embryo chromosome assay and the Drosophila assay. It should be noted that two of the negative in vivo assays were micronucleus assays.

The genotoxicity of naphthalene has also been considered by at least four national and international agencies over the last few years.

1. The authors of the NTP study report (NTP, 2000 at page 20) stated:

There is little evidence for mutagenic potential of naphthalene in the most widely used genotoxicity assays.

2. USEPA's Toxicological Review of Naphthalene (USEPA, 1998 at page 24), developed in support of its IRIS database, concluded:

The available data suggest that the genotoxic potential of naphthalene and/or its metabolites is weak, at best

3. The monograph documenting the review of naphthalene by the International Agency for Research on Cancer (IARC, 2002 at page 418) states:

There is little evidence for induction of gene mutations by naphthalene. In contrast, positive results were obtained in assays for micronucleus formation, chromosomal aberrations, and chromosomal recombinations in vitro, which are consistent with a clastogenic potential.

4. In the European Union (EU) Risk Assessment Report for Naphthalene (EU, 2003 at page 155) document, it was concluded:

Overall, the balance of evidence indicates that naphthalene is not genotoxic.

Collectively, the weight of evidence strongly favors the interpretation that naphthalene is not genotoxic.

**Response:** *The comment highlighted certain authorities that reached conclusions "overall" that naphthalene was not genotoxic; however, this does not mean that these authorities found no evidence of genotoxicity. Examples and discussion of specific instances of mutagenicity of naphthalene and its metabolites are presented in the*

*response to comment 2c below. In any case, the key question in this case is not whether the overall weight of evidence favors genotoxicity (or some defined subset of that range of different actions) of naphthalene at greater than 50% confidence level. Instead, in the analysis presented in the toxicity summary the key question is whether the preponderance of negative findings is so great, and the affirmative (rather than merely negative) evidence in favor of a mechanism of action not involving direct genotoxicity is so convincing, that departure from the default linear extrapolation procedure for low-dose risk estimation is appropriate. OEHHA's conclusion, based on the positive genotoxicity findings in some assays (see response to comment 2c), and the lack of support for a non-genotoxic mechanism other than a general assertion of cytotoxicity (see response to comment 3), is that such a departure from the default cannot be justified.*

### **2c. Studies indicate that naphthalene metabolites are not genotoxic**

The metabolism of naphthalene has been extensively investigated and reported in the literature. It has been demonstrated that naphthalene metabolites are responsible for the cytotoxicity noted in the respiratory tract in mice and rats (Buckpitt et al., 1992; Buckpitt et al., 1995; Plopper et al., 1992). The primary step in the metabolism of naphthalene in mammalian species is oxidation, catalyzed by cytochrome P450 oxygenases (CYP2F family) to naphthalene-1,2-epoxide; both the 1R2S and 1S2R enantiomers may be formed. The epoxide has a very short half-life of 3.6 minutes (Buonarati et al., 1989) and may spontaneously rearrange to form naphthols (primarily 1-naphthol) and eventually form naphthalene diols and naphthoquinones. The epoxide can also be enzymatically conjugated with glutathione by glutathione S-transferases to form a variety of glutathione conjugates that are excreted in the urine as n-acetylcysteine. Naphthalene-1,2-epoxide can also be enzymatically hydrated by epoxide hydrolase to form naphthalene-1,2-dihydrodiol, which can be conjugated with sulfate and glucuronic acid, or converted to naphthalene-1,2-dihydrodiol by catechol reductase, forming naphthoquinone via oxidation (USEPA, 1998). Further hydroxylation of naphthols, catalyzed by microsomal O<sub>2</sub>/NADPH<sub>2</sub>-dependent monooxygenases, results in naphthalenediols which, via enzymatic and autocatalytic oxidation, form 1,2- and 1,4-naphthoquinones.

The genotoxicity studies of naphthalene also evaluated the metabolites of naphthalene; the results do not support the conclusion that the metabolic products of naphthalene are mutagenic. Naphthalene metabolism and the potential for metabolites to induce genotoxicity were considered in many of the genotoxicity evaluations included in the Schreiner (2003) review. The majority of the in vitro genetic toxicology tests included a "metabolic activation" component. Many compounds are not mutagenic or carcinogenic but can be converted to mutagens (promutagens) or carcinogens (procarcinogens) by mammalian metabolism (Casarett and Doull, 1995). Unlike in vivo assays, the short term in vitro assays require exogenous metabolic activation to detect promutagens. The most common means to provide metabolic activation is the addition of a postmitochondrial supernatant from a rat liver homogenate (S9 mixture). The results of the in vitro genotoxicity assays with metabolic activation were generally negative. Further, rat hepatocytes, which are metabolically active, were evaluated in two in vitro unscheduled DNA synthesis assays and an alkaline elution assay. Naphthalene was not mutagenic in these assays. In vivo assays, which permit the metabolism of naphthalene, were also

negative. The weight of evidence from these studies supports a conclusion that naphthalene metabolites produced in situ do not result in a mutagenic response. This conclusion is supported by mutagenicity studies conducted with naphthalene metabolites per se.

In several studies, the naphthalene metabolites, 1-naphthol and 2-naphthol, were not mutagenic in *S. typhimurium* with or without metabolic activation (Florin et al., 1980; McCann et al., 1975, Narbonne et al., 1987). Further, naphthoquinone was not mutagenic in several strains of *S. typhimurium* with or without metabolic activation (Sakai et al., 1985). Flowers-Geary et al. (1994) reported that naphthalene-1,2-dione was mutagenic in strains of *S. typhimurium* without metabolic activation. The naphthalene metabolite 1-naphthol failed to produce positive results in several other genotoxicity assays, including tests for sex-linked recessive lethal mutations in *D. melanogaster* (Gocke et al., 1981), mutations in mouse L5178Y cells (Amacher and Turner, 1982), unscheduled DNA synthesis in cultured rat hepatocytes (Probst and Hill, 1980) and induction of micronuclei in bone marrow cells in mice (Gocke, 1981) and rats (Hossack and Richardson, 1977) after in vivo exposures.

In consideration of the above, the assumption that naphthalene is genotoxic is contrary to the weight of evidence and at variance with conclusions reached by federal and international government agencies that have recently reviewed naphthalene. The underlying basis for assuming low dose linearity to determine the human unit risk factor is seriously flawed and should be reconsidered. The weight of the scientific evidence does not favor the interpretation that the mechanism of naphthalene carcinogenicity likely involves a reactive metabolic intermediate which causes direct damage to DNA. Therefore, it is not appropriate to assume low dose linearity in estimating a unit risk factor for naphthalene. The Naphthalene Coalition thus urges OEHHA to withdraw the Proposal and consider alternative models that are consistent with the most likely mode of action of naphthalene, as discussed in the next section.

**RESPONSE:** *Naphthalene has generated negative results in bacterial gene mutation, single strand DNA breaks, and unscheduled DNA synthesis assays (Schreiner, 2003), and in one human lymphocyte gene mutation assay (Sasaki et al., 1997). However, the naphthalene metabolite naphthalene-1,4-diol (1,4-DHN) did induce mutations in Salmonella strain TA2637 in the presence of rat liver S9, and in TA104 (an oxidative mutagen-sensitive strain) in the absence of rat liver S9 (Hakura et al., 1996). The addition of catalase, which degrades hydrogen peroxide, to the culture medium inhibited the mutagenicity of 1,4-DHN by approximately 50%. These data suggest that 1,4-DHN may induce mutations through oxidative DNA damage. Naphthalene has also been demonstrated to induce superoxide anion production, lipid peroxidation, and DNA fragmentation in a dose-dependent manner in a cultured mouse macrophage cell line (Bagchi et al., 1998), and in mouse hepatic and brain tissue in vivo (Bagchi et al., 2002).*

*Additionally, naphthalene and its metabolites have been demonstrated to be clastogenic in several in vitro and in vivo assays. Naphthalene has been shown to induce SCEs in the presence and absence of rat liver S9 and chromosomal aberrations in the presence of rat liver S9 in Chinese hamster ovary (CHO) cells (NTP, 1992). Naphthalene induces*

*micronuclei (indicative of chromosomal damage) in human MCL-5 lymphoblastoid cells (Sasaki et al., 1997), and was positive in the Drosophila melanogaster somatic mutation and recombination wing spot assay (Delgado-Rodriguez et al., 1995). The naphthalene metabolites 1,2-naphthoquinone (1,2-NQ) and 1,4-naphthoquinone (1,4-NQ) induced SCEs in human peripheral mononuclear leukocytes (Wilson et al., 1996), and 1,4-NQ induced micronuclei in human MCL-5 lymphoblastoid cells (Sasaki et al., 1997). The enumeration of negative genotoxicity results for naphthols should be augmented by the following quotation from NTP (2000):*

*The metabolites of naphthalene, 1,2-dihydro-1,2-dihydroxy naphthalene, 1-naphthol, and 2-naphthol, were nonmutagenic in S. typhimurium (Narbonne et al., 1987; Florin et al., 1980; Probst et al., 1981), but 2-naphthol was shown to induce growth inhibition in DNA repair-deficient strains of E. coli (Suter and Jaeger, 1982) and Bacillus subtilis (Tanooka, 1977; Kawachi et al., 1980; Suter and Jaeger, 1982), presumably through induction of DNA damage.*

*These data suggest that naphthalene may cause gene mutations through oxidative damage to DNA by metabolites, and that naphthalene and certain naphthalene metabolites do cause chromosomal damage, and therefore have genotoxic potential. The comment highlighted certain authorities that suggest “generally” negative results in genotoxic assays; however, this does not mean that these authorities found no evidence of genotoxicity. For instance, although the quotation from NTP (2000) appears as given (and accompanied with a number of references to negative results, especially in bacterial mutation assays), it is then followed by the word “However,” and the enumeration of several of the positive results cited in this response. Evaluation of the weight of evidence in assessing genotoxic potential requires consideration of the number and nature of different endpoints where positive results have been found, rather than merely the enumeration of negative results in test systems that have been adequately demonstrated not to respond to the particular chemical under consideration.*

*In any event, the purpose of the brief consideration of genotoxicity in the summary is to determine whether there are any grounds for departure from the default methodology of linear extrapolation to low doses from the established point of departure. The US EPA (2003) draft carcinogen risk assessment guidelines state that departure from this default is only undertaken when an alternative mechanism, which can be shown to follow a non-linear dose response relationship, can be clearly established. They represent a reasonable summary of current scientific opinion, and may be taken as an indication of OEHHA’s views on this particular issue. The comment appears to assert firstly that naphthalene is non-genotoxic, and secondly that this fact alone is therefore sufficient grounds for departure from the linear extrapolation approach. OEHHA disagrees with the first assertion at least to the extent that there are several positive findings that disallow an unqualified conclusion of non-genotoxicity. Indeed, the extensive discussion of metabolism and cytotoxicity in a succeeding comment may be taken as further evidence against such a conclusion. The second assertion, that negative genotoxicity studies alone support a non-linear model, is not consistent with the US EPA (2003) guidelines, or with OEHHA’s practice in using the benchmark dose methodology.*

### **Comment 3. USE OF A GENOTOXIC RATHER THAN A CYTOTOXIC MODEL TO EVALUATE MODE-OF-TUMORIGENIC-ACTION FOR NAPHTHALENE IS INCONSISTENT WITH THE FINDINGS OF KEY MECHANISTIC STUDIES.**

Mode of action for induction of tumors is a key element in contemporaneous carcinogen risk assessment (USEPA, 2003). Mechanistic studies have been conducted in experimental animals and tissues to determine the mode of action for naphthalene toxicity and possible carcinogenicity (Buckpitt *et al.*, 1992; Buckpitt *et al.*, 1995; Plopper *et al.*, 1992). Overall, these studies suggest a cytotoxic mode of action for naphthalene.

In the NTP mouse study (NTP, 1992), exposure-related increases in the incidences of chronic inflammation, metaplasia of the olfactory epithelium and hyperplasia of the respiratory epithelium were the predominant nonneoplastic changes observed. Neoplastic findings were limited to an increased incidence of alveolar/bronchiolar adenomas in female mice exposed at the highest concentration (30 ppm) only.

In rats, non-neoplastic lesions were seen in the nasal tissues of both males and females (NTP, 2000). The findings consisted of hyperplasia, atrophy, chronic inflammation and hyaline degeneration of the olfactory epithelium and hyperplasia, squamous metaplasia, hyaline degeneration and goblet cell hyperplasia of the respiratory epithelium. Neoplastic findings were limited to these tissues. Exposure-related increases in respiratory epithelial adenomas and in olfactory epithelial neuroblastomas were seen in both sexes of rats.

#### **a. Site and species differences in naphthalene toxicity correlate with higher rates of metabolism in mouse lung and rat nasal tissue**

The NTP studies and the published literature support the conclusion that varying high-dose exposures to naphthalene cause cellular injury and increased cell replication rates in the ciliated and the Clara cells of the bronchiolar epithelium in mice and in the nasal epithelium of rats and mice. Intraperitoneal administration of naphthalene produces injury (swelling, vacuolization, exfoliation and necrosis of the tracheobronchial epithelial Clara cells of mice but not rats (Plopper *et al.*, 1992). In this same study, naphthalene was also cytotoxic to the olfactory epithelium of both rats and mice; however, the effects in mice occurred at much higher doses than rats, which suggests increased sensitivity of the nasal tissues in rats. These site and species differences in toxicity correlate well with higher rates of metabolism by mouse lung tissue and rat nasal tissue. Investigation of metabolism by lung or liver microsomes demonstrated that metabolism of naphthalene to a covalently bound protein product and to 1-naphthol and naphthalene-1,2-dihydrodiol was 10-fold greater in mouse tissue than in rat tissue.

The ratio of 1-naphthol to 1,2-dihydrodiol in mouse lung was 17-fold higher than in mouse liver (Buckpitt *et al.*, 1984; Tingle *et al.*, 1993). Buckpitt *et al.* (1992) characterized the stereochemistry of naphthalene epoxidation in preparations of nasal mucosa, lung and liver of mouse, rat, hamster and monkey. The highest metabolic rates were observed in mouse lung and liver microsomal incubation mixtures: rat, hamster and

monkey lung preparations metabolized naphthalene at 12%, 37% and 1%, respectively, of the rate in mouse lung. Murine microsomal fractions were characterized by an excessive, stereospecific formation of the 1R,2S-naphthalene epoxide from naphthalene with 1R,2S:1S,2R ratios of 10:1 to 30:1 in incubations with lung microsomes and 1:1 to 5:1 in liver microsomes depending on the initial naphthalene concentration. In lung microsomal preparations from rat, hamster and monkey, enzymes yielded 1R,2S:1S,2R ratios of 0.48, 0.61 and 0.12, respectively. Subsequent investigation of the role of cytochrome P-450 (CYP) monooxygenases in the mouse lung demonstrated that CYP 2F2 catalyzes the stereospecificity of naphthalene metabolism to 1R,2S-oxide in nonciliated cells at all airway levels and is a critical determinant of species-specific and region-specific cytotoxicity of naphthalene in mice (Buckpitt et al., 1995; Shulz et al., 1999). Since mice are prone to developing alveolar/bronchial adenomas, continuous damage to Clara cells by chronic naphthalene exposure and subsequently high levels of 1R,2S-epoxide could stimulate increased expression of these tumors.

In the rat, an obligate nose breather, the olfactory epithelium contains the greatest amounts of CYP protein of all tissues studied in the rat (Baldwin et al., 2004). As noted for the mouse lung, cytotoxicity is most prevalent in tissues with the highest capacity for metabolizing naphthalene. Thus, the high levels of CYP protein in the olfactory epithelium of the rat may explain the sensitivity of this tissue to the cytotoxicity from naphthalene exposure. It is postulated that the significant cell damage in the rat nasal epithelium is followed by cell proliferation and repair, which frequently includes mutational events secondary to the induced toxicity and unrelated to direct genotoxicity. Based on the metabolic differences in this tissue, the tumors are considered species specific as no nasal tumors were noted in exposed mice.

#### **b. Primates have low pulmonary metabolic capacity for naphthalene**

The observations may also have relevance to man. In vitro metabolism studies of naphthalene using pulmonary tissue fractions from humans and nonhuman primates show that the metabolic capacity is 1 to 2 orders of magnitude lower than that in rodents. Further, recent research shows that the nasal epithelium of nonhuman primates contains levels of CYP2F, the primary microsomal enzyme involved in naphthalene metabolism in the rat nasal epithelium, that are roughly 10- and 20- fold less than the corresponding tissues in rats and mice, respectively.

Collectively, these results strongly demonstrate that tumors induced by naphthalene are related to the metabolic capacity of the specific tissue and that ultimate induction of cytotoxicity in that tissue potentially leads to a tumorigenic event. Current research suggests that the metabolic capacity of the lung and nasal tissues of humans and nonhuman primates are an order of magnitude lower than the rodent species and, therefore, these tissues may not be susceptible to the effects of naphthalene.

In light of the relevant metabolic differences between rodent species and humans, the Naphthalene Coalition urges OEHHA to withdraw the Proposal and reconsider the mode of turmeric action using alternative models that are consistent with the likely cytotoxic

mode of action of naphthalene. Ideally, this model should account for differences in metabolism and tissue susceptibility between rodents and humans.

**Response:** *It is entirely possible, and in fact likely, that site and species differences in naphthalene toxicity correlate with higher rates of metabolism in mouse lung and rat nasal tissue. Reactive metabolites of naphthalene such as 1,2- and 1,4-naphthoquinone (1,2-NQ, 1,4-NQ) have been demonstrated to bind to cellular macromolecules (Waidyanatha et al., 2002) and have cytotoxic and genotoxic effects (Wilson et al., 1996). However, the fact that human hepatic microsomes have been demonstrated to bioactivate naphthalene and several of its metabolites (Wilson et al., 1996) suggests that it is unlikely that the cytotoxic and genotoxic effects of naphthalene are either site or species specific.*

*Office of Environmental Health Hazard Assessment cancer risk assessment procedures are outlined in "Guidelines for Chemical Carcinogen Risk Assessments and their Scientific Rationale" (CDHS, 1985) and in the Air Toxics Hot Spots Risk Assessment Guidelines Part II (OEHHA, 1999). The procedures used to extrapolate low-dose human cancer risk from higher dose animal carcinogenicity data assume that a carcinogenic change induced in a cell is transmitted to successive generations of cells descended from that cell, and that the initial change in the cell is an alteration (e.g. mutation, rearrangement, etc.) in the cellular DNA. Non-threshold models are used to extrapolate to low-dose human cancer risk from animal carcinogenicity data. Exceptions to this procedure could occur when carcinogenicity can be definitively shown to occur as the result of a mechanism which has a threshold effect or which is not relevant to humans. The demonstrated genotoxicity of naphthalene, the ability of human hepatic microsomes to bioactivate naphthalene, and the bioactivation of several of naphthalene's metabolites make it unlikely that naphthalene carcinogenicity has a threshold effect or is not relevant to humans. Therefore, use of a non-threshold model to extrapolate low-dose human cancer risk from animal naphthalene carcinogenicity data is appropriate.*

**Comment 4. IT IS NOT APPROPRIATE TO COMBINE UNRELATED TUMOR TYPES TO CALCULATE A UNIT RISK FACTOR.**

The Proposal combines Nasal Respiratory Epithelial Adenomas (NREA) and Nasal Olfactory Epithelial Neuroblastoma (NOEN) incidence rates to calculate a unit risk factor for naphthalene. Although NREA and NOEN were considered together in NTP's conclusion of "Clear Evidence for Carcinogenicity," this was based on a weight of evidence approach. It is **not** appropriate for OEHHA to combine NREA and NOEN for the purposes of a quantitative potency calculation as a basis for proposing the naphthalene unit risk factor. The tumor types should not be combined because they are pathologically unrelated.

OEHHA has developed and adopted guidelines for calculating cancer potency factors based on animal data. [OEHHA. (2002) Air Toxics Hot Spots Program Risk Assessment Guidelines. Part II. Technical Support Document for Describing Available Cancer Potency Factors. December, 2002.] These guidelines specifically address the issue of combining tumors as follows (OEHHA, 2002 at page 13):

Where both benign and malignant tumors are induced *at the same site* and the malignant tumors are significantly increased, the data on both types of tumors may be combined to form the basis for risk assessment. [emphasis added]

The tumor types combined in the Proposal are not tumors "at the same site." Although NREA and NOEN occur in the same general region of the body, they are histologically distinct, and they are certainly not induced "at the same site." By analogy, liver adenoma and lung carcinoma occur in the same general region of the body (*i.e.*, the abdominal cavity); however, it would not be appropriate to combine these tumors because they do not occur "at the same site."

When combining tumors, it is also important to consider whether benign tumors have the potential to progress to the associated malignancies of the same histologic origin. Benign and malignant tumors are combined only when the benign tumor is expected to progress to the malignant tumor observed at an increased incidence. OEHH (2002 at page 9) has described USEPA's approach to combining benign and malignant tumors as follows:

US EPA separates tumor incidence data according to organ sites or tumor types. The incidence of benign and malignant tumors is combined whenever scientifically defensible. US EPA considers this incidence combination scientifically defensible *unless the benign tumors are not considered to have the potential to progress to the associated malignancies of the same histogenic origin.* [emphasis added]

Based on these criteria, it is inappropriate to combine the tumor types in the rat bioassay of naphthalene. There is no reasonable basis for concluding that NREA has the potential to progress to NOEN. The two tumor types are histologically distinct and unrelated; one

cannot progress to the other. Therefore, NREA and NOEN should not be combined to calculate a cancer potency factor for naphthalene, as in the Proposal.

**RESPONSE:** *OEHHA agrees that, since the nasal respiratory epithelial adenomas and nasal olfactory epithelial neuroblastoma tumors evidently arise from different cell types, it is inappropriate to combine the incidences in the way that is usually done for tumor types of common origin, i.e. to go to the individual animal data and determine a combined incidence (individuals affected/individuals at risk). We therefore deliberately refrained from doing this in the naphthalene case. There is, however, a problem with simply calculating cancer potency based on the incidence of each tumor type separately and then taking the larger potency value, or averaging the potency values, when there are two or more independent sites at which tumor induction occurs to a substantial degree. The public health question obviously is "what is the overall risk of cancer of some sort, on a per individual (human or animal) basis?" In other words, independent risks (at different sites or of different origins) each contribute to the overall risk of cancer to the individual. In addressing the question of overall risk, however, one cannot simply add the upper 95% confidence bounds; that approach would over predict the likely range of risks. So the procedure used by OEHHA statistically adds the cancer slopes, represented by probability distributions, for the independent sites using Monte Carlo sampling. The upper 95% confidence bound of the resulting distribution is taken as the cancer potency value. OEHHA believes that this is a mathematically correct way of determining overall risk of tumor induction in the case where tumors independently arise from different cell types.*

*The U.S. EPA also addresses this issue by offering a variety of options for consideration in their Proposed guidelines for carcinogen risk assessment (2003; similar text appears in the 1996 and 1999 versions):*

### **3.3.5. Comparing and Combining Multiple Extrapolations**

*When multiple estimates can be developed, all datasets should be considered and a judgment made about how best to represent the human cancer risk. Some options for presenting results include*

- *adding risk estimates derived from different tumor sites (NRC, 1994),*
- *combining data from different datasets in a joint analysis (Stiteler et al., 1993; Vater et al., 1993),*
- *combining responses that operate through a common mode of action,*
- *representing the overall response in each experiment by counting animals with any tumor showing a statistically significant increase,*
- *presenting a range of results from multiple datasets (in this case, the dose-response assessment includes guidance on how to choose an appropriate value from the range),*
- *choosing a single dataset if it can be justified as most representative of the overall response in humans, or*
- *a combination of these options.*

*The text of the toxicity summary document will be modified to clarify the explanation of this issue.*

*With regard to the question of benign versus malignant tumors, this is an issue that is considered as part of the hazard identification process. Once an agent is determined to be a carcinogen, OEHHA's dose-response analysis focuses on the overall risk of tumor induction.*

**Comment 5. THE DATA USED FOR THE CALCULATIONS DID NOT INCLUDE ALL OF THE RATS FROM THE NTP STUDIES AND, THEREFORE, THE SUBSEQUENT CALCULATIONS ARE INCORRECT.**

The calculations in the Proposal excluded certain animals within the exposure groups, so subsequent calculations are incorrect.

**Table A**

**Exposure, number of animals on test, and response**

Chamber Concentration (ppm)	Average Concentration (mg/m <sup>3</sup> )	Animals on Test (NTP, 2000)	Animals used in OEHHA Analyses	Animals with Epithelial Adenoma
0	0	49	44	0
10	9.67	49	42	6
30	29.0	48	44	8
60	58.0	48	41	15

The Proposal considered only animals alive after the first occurrence of a tumor (see footnote b in Table 3 of the Proposal). As a result, fewer animals were included in their analyses. This practice is incorrect for at least two reasons:

- 1. Exclusion of animals within the exposure groups from the statistical analyses violates the assumptions of the statistical models.** The statistical analyses used in the Proposal assume that exposure groups are statistically independent. If the number of animals in each group is based on the animals alive after the first occurrence of a tumor, then the number of animals in each group is dependent on the common value of the first occurrence of a tumor and therefore the groups are not independent. The assumption of independence for the statistical analyses (LMS and BMD) is fundamental, and if the assumption is not met the resulting calculations are incorrect.
- 2. Exclusion of animals within the exposure groups creates a non-random sampling.** If the result of the animal study is to be extrapolated to humans, the sampling scheme has to be similar. A complete random sample of animals is the only reasonable choice because there is not a corresponding selection of humans where you only consider the tumor rate in humans after the appearance of the first tumor. An often-stated reason for using the reduced animal count is that it accounts for differences in survivorship. In the current analysis the survivorship was not different among the groups (2<sup>nd</sup> full paragraph, page 8 of the Proposal). Therefore, there is no compelling reason to use the reduced numbers in the analyses. In fact, reducing the sample size for the calculations produces incorrect results.

**RESPONSE:** *Contrary to the assertion in the comment, the statistical models used for both significance testing (hazard identification) and dose response assessment define*

*“incidence” (the key numeric input to statistical or biological models of tumor induction) as the ratio of the number of individuals (animals in this case) in which a tumor was observed to the number of individuals at risk. Since all models of tumor causation include a delay before appearance of a detectable tumor (often called “latency”), it follows that there are times early in the study when observation of a tumor is extremely unlikely or impossible. If an animal is removed from the study by some competing risk (i.e. early death from non-tumor related causes) before a tumor could be observed, then by definition it is not at risk, and should not be considered part of the study population. U.S. EPA and the State of California recognize this in numerous risk assessments, and it has been explicitly discussed by IARC in the Preamble to the Monographs, where it is stated:*

*"When there is no difference in survival between control and treatment groups, the Working Group usually compares the proportions of animals developing each tumor type in each of the groups. Otherwise, consideration is given as to whether or not appropriate adjustments have been made for differences in survival. These adjustments can include: comparisons of the proportions of tumor-bearing animals among the effective number of animals (alive at the time the first tumor is discovered), in the case where most differences in survival occur before tumors appear; ..."*

*Thus this adjustment is routinely used in cases where differential survival appears to be a problem in the bioassay data. This statistical procedure is discussed at greater length by Gart et al. (Gart JJ, Krewski D, Lee PN, Tarone RE, Wahrendorf J, 1986. *Statistical Methods in Cancer Research, Volume III – The design and analysis of long-term animal experiments*. IARC Scientific publications No. 79. IARC, Lyon, France. See page 75 et seq.). They note as permissible the use of an overall cutoff date for all groups, evidently not considering that this compromises any statistical assumption of independence or randomness of sampling for the different dose groups. They note that their alternative suggestion of using a different cutoff date for each group is often difficult to apply. This generally would provide a more extreme correction than the more cautious overall method used by OEHHA in this case. Alternatives which produce similar or greater effects in adjusting the overall time and number of individuals at risk have also been proposed, such as life table methods in hazard identification (Tarone RE, 1975. *Tests for trend in life table analysis*. *Biometrika* **62**:679-682) or fully time-dependent models based on individual animal data for dose-response assessment (Krewski D, Crump KS, Farmer J, Gaylor GW, Howe R, Portier C, Salsburg D, Sielken RL, Van Ryzin J, 1983. *A comparison of statistical methods for low dose extrapolation using time-to-tumor data*. *Fund Appl Toxicol* **3**:140-160). OEHHA concluded that this latter approach was unnecessary in this case, since the simpler methods resulted in adequate model fits to the data sets (see following comments and responses).*

## **Comment 6. THE BENCHMARK DOSE (BMD) ANALYSIS DID NOT USE THE OPTIMUM MODEL.**

There are two key problems with the BMD analysis presented in Table 6 of the Proposal. First, the Naphthalene Coalition assessed the modeling and found that an optimum fitting quantal-linear model could not be determined. This is illustrated in Attachment C, which shows that one of the terms in the model could not be estimated and was replaced by a boundary value. Second, the fitted model was not statistically different from a mean model. In other words, the model is not effective (it yields a p-value of 0.4943). As seen in Attachment D, the model still does not adequately fit the data even if all of the animals in each group are analyzed (p-value of 0.5166).

The third column of Table 6 of the Proposal indicates the epithelial adenomas analysis model fit was adequate. These values describe the differences between observed and predicted values, but not if the model is statistically different from a mean model (a model that predicts only the mean without considering exposure).

The quantal-quadratic model is the best-fit model for the data, as seen in Attachment E ( $p < 0.016$ ). In this model all parameters are estimated by the data and the  $LED_{10}$  is 25.30  $mg/m^3$ . A similar analysis with the reduced data set has a model fit p value of 0.0156 and a  $LED_{10}$  of 23.00  $mg/m^3$ .

Using the optimum  $LED_{10}$  value 25.30  $mg/m^3$ , the estimated human unit risk factor would be 0.017  $(mg/m^3)^{-1}$ .

In summary, the quantal linear model used in the Proposal is not a statistically significantly fitting model, whereas the quantal-quadratic model is a statistically significantly fitting model and estimates a human unit risk value of 0.017  $(mg/m^3)^{-1}$ .

**RESPONSE :** *This comment is based on an incorrect premise, namely that all of the test animals should be included in the denominator of the quantal tumor incidence for the purpose of dose response assessment. As noted elsewhere in these responses it has been OEHHA practice for many years only to include only those animals actually at risk of developing tumors in the quantal denominators. Animals that died before the appearance of the first tumor were considered not at risk and hence removed. If this correct approach is taken it is clear that the dose response is adequately fit by the quantal linear (QL) model but not by the quantal quadratic (QQ) model. Figure 1a gives the fit statistics for the QL model and the male rat nasal respiratory epithelial adenomas induced by inhaled naphthalene. The most important statistic here is the overall model fit indicated by the chi-square goodness of fit statistic which exceeds the fit criterion of  $P \geq 0.1$ . However, also of importance is the visual inspection of the fitted line in the critical region of the point of departure. As can be seen in Figure 1b the fit is good in the critical region. Conversely the QQ model fit to this correct data set does not meet the 0.1 P fit criterion and gives a poor fit in the critical region (Figs 2a and 2b, respectively). Thus in*

*contrast to the commenter who used incorrect denominators to claim an acceptable quadratic fit to this data set the use of the correct denominators gives an acceptable linear fit.*

Figure 1a. Benchmark Dose Analysis of Rat Nasal Respiratory Epithelial Adenoma using the Quantal Linear Model

```
=====
Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17
22:27:16 $
Input Data File: C:\BMDS\NAPNREA.(d)
Gnuplot Plotting File: C:\BMDS\NAPNREA.plt
Wed Mar 10 08:56:48 2004
=====
```

BMDS MODEL RUN  
~~~~~

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{slope} * \text{dose})]$$

Dependent variable = COLUMN3  
Independent variable = COLUMN1

Total number of observations = 4  
Total number of records with missing values = 0  
Maximum number of iterations = 250  
Relative Function Convergence has been set to: 1e-008  
Parameter Convergence has been set to: 1e-008

```
Default Initial (and Specified) Parameter Values
Background = 0.0111111
Slope = 0.00774744
Power = 1 Specified
```

Asymptotic Correlation Matrix of Parameter Estimates

( \*\*\* The model parameter(s) -Background -Power  
have been estimated at a boundary point, or have been  
specified by the user,  
and do not appear in the correlation matrix )

Slope

Slope 1

Parameter Estimates

| Variable   | Estimate   | Std. Err.  |
|------------|------------|------------|
| Background | 0          | NA         |
| Slope      | 0.00843096 | 0.00157481 |

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

| Model         | Log(likelihood) | Deviance | Test DF | P-value |
|---------------|-----------------|----------|---------|---------|
| Full model    | -65.0122        |          |         |         |
| Fitted model  | -66.2104        | 2.39643  | 3       | 0.4943  |
| Reduced model | -77.8454        | 25.6665  | 3       | <.0001  |

AIC: 134.421

Goodness of Fit

| Dose     | Est._Prob. | Expected | Observed | Size | Scaled |
|----------|------------|----------|----------|------|--------|
| Residual |            |          |          |      |        |
| 0.0000   | 0.0000     | 0.000    | 0        | 44   |        |
| 0        |            |          |          |      |        |
| 9.6700   | 0.0783     | 3.288    | 6        | 42   |        |
| 1.558    |            |          |          |      |        |
| 29.0000  | 0.2169     | 9.544    | 8        | 44   | -      |
| 0.5647   |            |          |          |      |        |
| 58.0000  | 0.3868     | 15.857   | 15       | 41   | -      |
| 0.2748   |            |          |          |      |        |

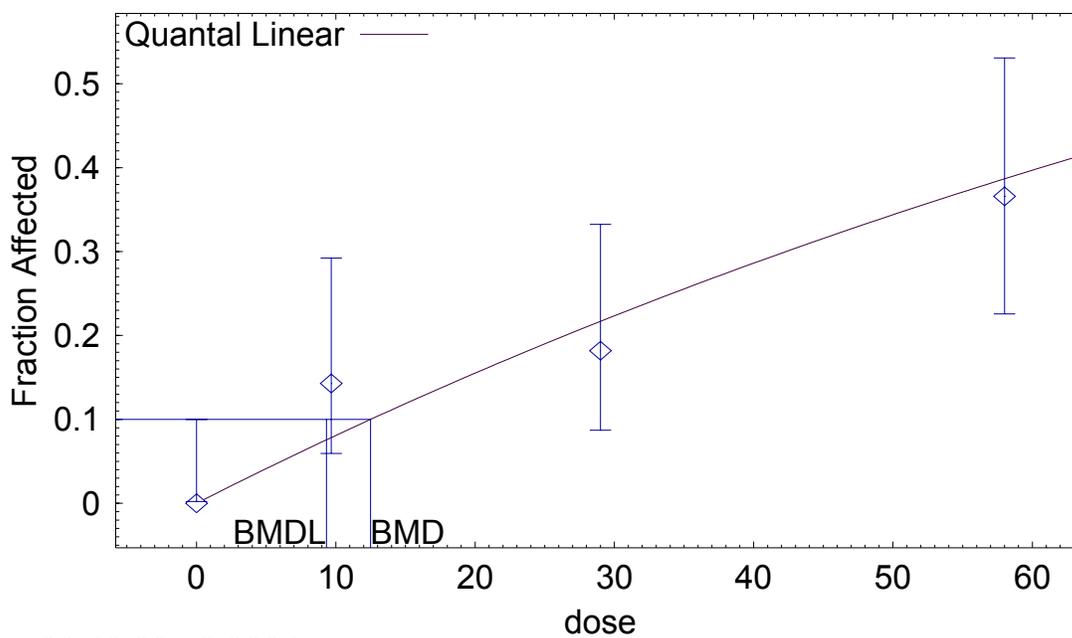
Chi-square = 2.82 DF = 3 P-value = 0.4201

Benchmark Dose Computation

Specified effect = 0.1  
Risk Type = Extra risk  
Confidence level = 0.95  
BMD = 12.4969  
BMDL = 9.3263

**Figure 1b. Naphthalene Male Rat Nasal Respiratory Epithelial Adenomas (Tumor Incidence v. Dose  $\text{mg}/\text{m}^3$ )**

Quantal Linear Model with 0.95 Confidence Level



08:40 03/12 2004

Figure 2a. Benchmark Dose Analysis of Rat Nasal Respiratory Epithelial Adenoma using the Quantal Quadratic Model

=====  
Quantal Quadratic Model \$Revision: 2.2 \$ \$Date: 2000/03/17  
22:27:16 \$

Input Data File: C:\BMDS\NAPNREA.(d)

Gnuplot Plotting File: C:\BMDS\NAPNREA.plt

Wed Mar 10 09:35:58 2004  
=====

BMDS MODEL RUN  
~~~~~

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-\text{slope} * \text{dose}^2)]$$

Dependent variable = COLUMN3

Independent variable = COLUMN1

Total number of observations = 4

Total number of records with missing values = 0

Maximum number of iterations = 250

Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values

Background = 0.0111111

Slope = 0.000133577

Power = 2 Specified

Asymptotic Correlation Matrix of Parameter Estimates

( \*\*\* The model parameter(s) -Power  
have been estimated at a boundary point, or have been  
specified by the user,  
and do not appear in the correlation matrix )

	Background	Slope
Background	1	-0.48
Slope	-0.48	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0617332	0.0288511
Slope	0.000129077	3.86179e-005

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-65.0122			
Fitted model	-69.1727	8.32095	2	0.0156
Reduced model	-77.8454	25.6665	3	<.0001
AIC:	142.345			

Goodness of Fit

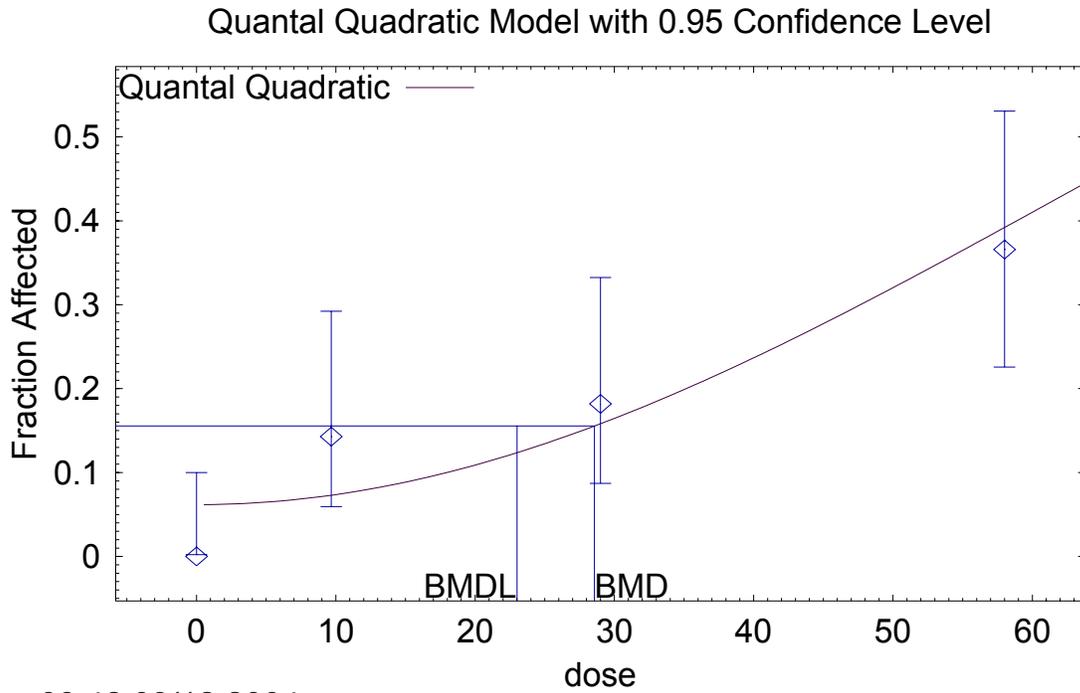
Dose	Est._Prob.	Expected	Observed	Size	Scaled
Residual					
0.0000	0.0617	2.716	0	44	-
1.701					
9.6700	0.0730	3.066	6	42	
1.741					
29.0000	0.1583	6.963	8	44	
0.4283					
58.0000	0.3922	16.081	15	41	-
0.3457					

Chi-square = 6.23      DF = 2      P-value = 0.0444

Benchmark Dose Computation

Specified effect = 0.1  
 Risk Type = Extra risk  
 Confidence level = 0.95  
 BMD = 28.5703  
 BMDL = 23.0058

**Figure 2b. Naphthalene Male Rat Nasal Respiratory Epithelial Adenomas (Tumor Incidence v. Dose mg/m<sup>3</sup>)**



## **Comment 7. THE LINEAR MULTISTAGE (LMS) MODEL DOES NOT ADEQUATELY FIT THE DATA.**

The Naphthalene Coalition ran the LMS analysis using MSTAGE with the reduced and full data sets and found that a full model fit was not possible because the LMS model requires that the coefficients be non-negative. This is similar to the problems in the fitting of the quantal-linear model in Table 6 of the Proposal (noted above). The two statistical models (the LMS and quantal-linear) are similar in mathematical form.

To test if the fitting problems were unique to the MSTAGE computer program the data and LMS model were fit using other computer programs such as TOXRISK and an LMS program from Crump Associates. These programs were unable to estimate terms in the LMS model with these data because the programs failed to converge. Thus, these results indicate the LMS model is not appropriate for these data.

Note that the value 0.01919 as the  $q_{\text{animal}}$  estimate for the epithelial adenomas analysis in Table 5 of the Proposal is incorrect. Based on a reassessment of the reduced data set, the value should be 0.01191 (the upper confidence limit on parameter 1; see Attachment F), which corresponds to a human unit risk factor of  $0.018 \text{ (mg/m}^3\text{)}^{-1}$ . If the full data set is used with this incorrect method the value of  $q_{\text{animal}}$  should be 0.01015 with a corresponding human unit risk factor of  $0.016 \text{ (mg/m}^3\text{)}^{-1}$ .

In summary, the LMS model does not adequately fit the data. If the LMS model is applied in spite of this, the resulting human unit risk factor is  $0.016 \text{ (mg/m}^3\text{)}^{-1}$ .

**RESPONSE:** *OEHHA thanks the commenter for confirming the well-known requirement of the LMS model for non-negative coefficients in the multistage polynomial, and for presenting the conclusion that this model (in its non-time-dependent form) does not fit the uncorrected data, a point of which OEHHA was aware but did not consider necessary to include in the toxicity summary. Also noteworthy is that, as also discussed in the previous response, although the quantal quadratic model provides a fit to the uncorrected data which meets the statistical criteria (which tend to emphasize the higher dose group results in this particular case), it is evident by inspection of the fitted curves and the data that the fit to the critical low-dose range is poor for this model also. For the data with the proper correction applied, the quantal quadratic model fit fails both the statistical and inspection tests. The commenter is referred to the earlier discussion of the correction that was applied to allow for early mortality in the bioassay. It is evident that this correction is both necessary and appropriate, since it results in an adequate fit by a plausible model, as opposed to the uncorrected data set where inadequacies of fit are seen for both the models described.*

*With regard to the assertion that the  $q_{\text{animal}}$  estimate for the analysis of nasal respiratory epithelial adenomas in rats is incorrect, the commenter is mistaken. The commenter does not give the output for calculation of this number [Attachment F only provides the calculation on the uncorrected data set]. The calculation of the  $q_{\text{animal}}$  estimate of  $0.01919 \text{ (mg/kg-day)}^{-1}$ , as reported in Table 5, is described below.*

The calculation of the  $q_{animal}$  in units  $(\text{mg}/\text{kg}\cdot\text{day})^{-1}$  requires conversion of the average concentrations into lifetime average doses in units  $\text{mg}/\text{kg}\cdot\text{day}$ . Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the appropriate animal breathing rate divided by the corresponding animal body weight. [The dosing period in the NTP (2000) rat studies was 105 weeks, which exceeds the assumed lifespan for rats of 104 week; thus no adjustment for less than lifetime exposure was required.] For male rats, the breathing rate was estimated to be  $0.262 \text{ m}^3/\text{day}$  and the body weight  $0.445 \text{ kg}$ . The dose calculations for the male rat bioassay are shown below:

$$9.67 \text{ mg}/\text{m}^3 \times \frac{0.262 \text{ m}^3/\text{day}}{0.445 \text{ kg}} = 5.69 \text{ mg}/\text{kg} \cdot \text{day}$$

$$29.0 \text{ mg}/\text{m}^3 \times \frac{0.262 \text{ m}^3/\text{day}}{0.445 \text{ kg}} = 17.1 \text{ mg}/\text{kg} \cdot \text{day}$$

$$58.0 \text{ mg}/\text{m}^3 \times \frac{0.262 \text{ m}^3/\text{day}}{0.445 \text{ kg}} = 34.1 \text{ mg}/\text{kg} \cdot \text{day}$$

The Tables in the summary have been amended to clearly indicate the data used to generate the  $q_{animal}$  estimates in units of  $(\text{mg}/\text{kg}\cdot\text{day})^{-1}$ .

The MSTAGE output for the respiratory epithelial adenomas is reproduced below:

```
*****
**                               **
**      MSTAGE                   **
**      Version 2.01             **
**                               **
*****
```

Copyright 1992

Edmund A.C. Crouch	and	Cambridge Environmental Inc.
62 Buena Vista Road		58 Charles Street
Arlington, MA 02174		Cambridge, MA 02141

Phone: 617-225-0810 (days)

617-648-5343 (eves)

FAX: 617-225-0813

If you have any problems with this program, please give me a call

Give the doses d[1], d[2], .... in order. Terminate with the word "end".

```
d[1]----> 0
d[2]----> 5.69
d[3]----> 17.1
d[4]----> 34.1
d[5]----> end
```

Enter results as r[1] n[1] r[2] n[2]....

0 44 6 42 8 44 15 41

That is 4 values

Summary of input data

#	Dose value	Number w. tumor	tested	Fisher exact comparison
1	0.000E+0000	0	44	
2	5.690E+0000	6	42	1.116E-0002
3	1.710E+0001	8	44	2.757E-0003
4	3.410E+0001	15	41	3.527E-0006

That's 4 doses and 4 parameters. OK? y  
 Do you want to set any parameters? n

Parameter number	Status	Value	Approximate p value	Gradient of Loglikelihood
0	Set to zero	0	1.778E-0005	-1.871E+0001
1	Optimized	1.43245E-0002	7.532E-0003	1.776E-0015
2	Set to zero	0	1.000E+0000	-3.023E+0000
3	Set to zero	0	1.000E+0000	-2.928E+0000

Fit: Chisquared 2.826E+0000 with 3 d.f., p = 4.192E-0001

Do you want to remove the topmost dose? n

Do you want to calculate confidence limits? y

Confidence limit (one-sided, percent)? ----> 95

On what parameter? ---> 1

95.00 % one-sided confidence limits ( 90.00% confidence interval) on stage 1 are: 6.93622E-0003 to **1.91942E-0002** (Optimum value: 1.43245E-0002)

Parameter values at these confidence limits are:

number	Lower	Optimum	Upper
0	2.61523E-0002	0.00000E+0000	0.00000E+0000
1	6.93622E-0003	1.43245E-0002	1.91942E-0002
2	1.83117E-0004	0.00000E+0000	0.00000E+0000
3	0.00000E+0000	0.00000E+0000	0.00000E+0000