COURTNEY M. PRICE
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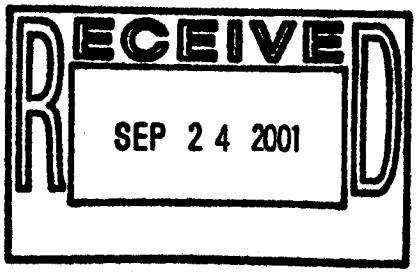
September 24, 2001

Via e-mail and FedEx



Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
79 Alexander Drive
Building 4401, Room 3118
P.O. Box 12233
Research Triangle Park, NC 27709

Re: National Toxicology Program; Call for Public Comment on 16 Substances, Mixtures and Exposure Circumstances Proposed for Listing in the Report on Carcinogens, Eleventh Edition; 66 Fed. Reg. 38430 (July 24, 2001)

Dear Dr. Jameson:

The Naphthalene Panel (Panel) of the American Chemistry Council submits these comments in response to the National Toxicology Program's (NTP) call for comments on the proposal to list a number of substances in the Eleventh Edition of the *Report on Carcinogens* (*RoC*). That notice lists naphthalene as one of the substances for which NTP is considering listing.

The Panel urges NTP not to list naphthalene as a carcinogen in the *RoC*. Naphthalene does not meet the criteria for listing in the *RoC*, for all of the reasons stated in the attached comments. As discussed more fully in the Panel's comments:

- The NTP mouse bioassay upon which NTP bases the proposed *RoC* listing provides insufficient evidence of carcinogenicity in the test animals for consideration under NTP's criteria, and any tumorigenic effect, if present in that study, would not be relevant to humans. Accordingly, the study does not show, as required by NTP's *RoC* listing criteria, that there is any increased incidence of malignant or a combination of malignant and benign tumors in "multiple species."

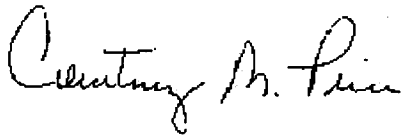
- The NTP rat bioassay, upon which the proposed *RoC* listing also is based, does not meet the standard for listing in the *RoC* because it does not indicate an increased incidence of malignant or a combination of malignant and benign tumors at multiple tissue sites, does not indicate an increased incidence of tumors to an unusual degree, and the observed increase in tumors represents a response that likely is not relevant to humans.

- The weight-of-the-evidence indicates that naphthalene is not genotoxic, and there is no other corroborative evidence known by the Panel that would support a listing in the *RoC*.

For all of these reasons, NTP should not list naphthalene in the *RoC*. If NTP nevertheless concludes that naphthalene warrants further consideration for listing, NTP should defer any such further consideration by NTP's RG2 Committee until after the International Agency for Research on Cancer (IARC) issues a monograph following its upcoming review of naphthalene. The Panel believes that such a modest deferral of NTP's further consideration of naphthalene, until IARC issues its monograph on naphthalene, would be appropriate and would avoid unnecessary duplication of efforts, especially as NTP is taking a lead role in the IARC review.

For further information, please contact the Naphthalene Panel Manager, Dr. Anne LeHuray at (703) 741-5630 or by e-mail: anne_lehuray@americanchemistry.com.

Sincerely yours,



Courtney M. Price
Vice President, CHEMSTAR

BEFORE THE NATIONAL TOXICOLOGY PROGRAM

COMMENTS OF THE NAPHTHALENE PANEL OF THE AMERICAN CHEMISTRY COUNCIL IN RESPONSE TO NTP'S REQUEST FOR COMMENTS ON THE NOMINATION OF NAPHTHALENE FOR POSSIBLE LISTING IN THE *REPORT ON CARCINOGENS*

National Toxicology Program; Call for Public Comments on 16 Substances, Mixtures and Exposure Circumstances Proposed for Listing in the Report on Carcinogens, Eleventh Edition; 66 Fed. Reg. 38430 (July 24, 2001)	))))))

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September 24, 2001

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EXECUTIVE SUMMARY

The Naphthalene Panel (Panel) of the American Chemistry Council submits these comments in response to the National Toxicology Program's (NTP) call for comments on the proposal to list naphthalene in the Eleventh Edition of the *Report on Carcinogens* (*RoC*). 66 Fed. Reg. 38430 (July 24, 2001). The Panel is comprised of the major domestic producers and importers of naphthalene.

Naphthalene has been nominated for listing in the *RoC* based on the results of an NTP bioassay that reported clear evidence of carcinogenicity in male and female rats and an NTP bioassay on mice that reported some evidence of carcinogenicity in female mice. For the reasons provided below, the Panel believes that neither of these bioassays, nor, to the Panel's knowledge, other evidence, provides a basis for listing naphthalene under NTP's "reasonably anticipated to be a human carcinogen" listing criteria. Specifically, there is insufficient evidence of carcinogenicity either in humans or from studies on experimental animals to conclude that naphthalene is "reasonably anticipated to be a human carcinogen" under the NTP criteria for listing in the *RoC,* and no other supplementary data meet the listing criteria.

The Panel bases this conclusion on the following considerations:

- The NTP mouse bioassay provides insufficient evidence of carcinogenicity in the test animals for consideration under NTP's criteria, and any tumorigenic effect, if present in that study, would not be relevant to humans. Accordingly, there is no increased incidence of malignant or a combination of malignant and benign tumors in "multiple species."

- The NTP rat bioassay does not meet the standard for listing in the *RoC* because it does not indicate an increased incidence of malignant or a combination of malignant and benign tumors at multiple tissue sites, does not indicate an increased incidence of tumors to an unusual degree, and the observed increase in tumors represents a response that likely is not relevant to humans.

- The weight-of-the-evidence indicates that naphthalene is not genotoxic, and there is no other corroborative evidence that would support a listing in the *RoC*.

If following the RG1 review, NTP nevertheless concludes that naphthalene warrants further consideration for listing, NTP should defer any such further consideration by the RG2 Committee until after the International Agency for Research on Cancer issues a monograph following its upcoming review of naphthalene. The Panel believes that such a modest deferral of NTP's further consideration of naphthalene, until IARC issues its monograph on naphthalene, would be appropriate and would avoid unnecessary duplication of efforts, especially as NTP is taking a lead role in the IARC review.

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INTRODUCTION

The Naphthalene Panel (Panel) of the American Chemistry Council submits these comments in response to the National Toxicology Program's (NTP) call for comments on the proposal to list naphthalene in the Eleventh Edition of the *Report on Carcinogens* (*RoC*). 66 Fed. Reg. 38430 (July 24, 2001). The Panel is comprised of the major domestic producers and importers of naphthalene.

Naphthalene has been nominated for listing in the *RoC* based on the results of a NTP rat bioassay[1] that reported clear evidence of carcinogenicity in male and female rats and a NTP bioassay on mice[2] that reported some evidence of carcinogenicity in female mice.[3] For the reasons provided below, neither of these bioassays, nor, to the Panel's knowledge, any other evidence, provides a basis for listing naphthalene under NTP's listing criteria.

I. THE NTP REQUIRES THAT BEFORE A SUBSTANCE MAY BE LISTED IN THE *RoC* THAT SUBSTANCE MUST BE DETERMINED TO BE "REASONABLY ANTICIPATED TO BE A HUMAN CARCINOGEN" UNDER SPECIFICALLY DELINEATED CRITERIA

Chemicals may be listed in the *RoC* if NTP determines they are "known to be human carcinogens" or "reasonably anticipated to be human carcinogens."[4] The applicable criteria for listing are as follows:[5]

- Studies in humans indicate either: (1) there is sufficient evidence of carcinogencity from studies in humans which indicates a causal relationship between exposure to the agent, substance, or mixture and human cancer ("known to be human carcinogen") or (2) there is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible, but that alternative explanations, such as

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1 NTP, *Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in F344/N Rats (Inhalation Studies)* (Dec. 2000), Technical Report No. 500 (NTP Rat Bioassay).

2 NTP, *Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in B6C3F$_1$ Mice (Inhalation Studies)* (Apr. 1992), Technical Report No. 410 (NTP Mouse Bioassay).

3 66 Fed. Reg. at 38432.

4 61 Fed. Reg. 50499-50500 (Sept. 26, 1996).

5 *Id. See also* 66 Fed. Reg. at 38430; NTP, *Report on Carcinogens, Ninth Edition, Carcinogen Profiles 2000*, at I-2.

chance, bias, or confounding factors, could not adequately be excluded ("reasonably anticipated to be human carcinogen").[6]

- Sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors ("reasonably anticipated to be human carcinogen"):
  - In multiple species or at multiple tissue sites;
  - By multiple routes of exposure; or
  - To an unusual degree with regard to incidence, site, or type of tumor or age at onset.

- When there is less than sufficient evidence of carcinogenicity in humans or laboratory animals, a chemical may nevertheless be found to be "reasonably anticipated to be a human carcinogen" based on other considerations concerning structure and mechanism. For example, a substance may be listed if it belongs to a well-defined, structurally related class of substances whose members are listed in a previous *RoC* as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen.

- Conclusions regarding carcinogenicity are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, metabolism, and pharmacokinetics. Importantly, substances for which there is evidence of carcinogenicity in laboratory animals are not considered "reasonably anticipated to cause cancer in humans" where there are compelling data indicating that the agent acts through mechanisms which do not operate in humans.

For the reasons discussed below, available studies and data on naphthalene do not satisfy NTP's own criteria for listing.

II. THERE ARE INSUFFICIENT HUMAN DATA TO RAISE ANY ISSUE AS TO WHETHER NAPHTHALENE IS KNOWN OR IS REASONABLY ANTICIPATED TO BE A HUMAN CARCINOGEN

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[6] Napthalene has not been nominated based on human studies, and as discussed below, there is insufficient human data to raise an issue as to whether naphthalene may be listed based on human studies.

The nominating body for naphthalene, the National Institute of Environmental Health Sciences (NIEHS), does not base its nomination of naphthalene on any human data.[7] Further, to the Panel's knowledge, there exist no human studies that raise any issue as to whether naphthalene should be listed. EPA's Integrated Risk Information System (IRIS) database for naphthalene, last updated in September 1998, concludes with respect to human carcinogenicity data: "Available data are inadequate to establish a causal association between exposure to naphthalene and cancer in humans. Adequately scaled epidemiological studies designed to examine a possible association between naphthalene exposure and cancer were not located. Overall, no data are available to evaluate the carcinogenic potential in exposed human populations."[8] In addition, the Draft UK Health and Safety Executive (HSE) Risk Assessment Document for Naphthalene (Draft HSE Risk Assessment) concludes that no conclusions can be drawn about the carcinogenicity of naphthalene from the limited information available in humans.[9]

III. NEITHER THE NTP MOUSE BIOASSAY NOR THE NTP RAT BIOASSAY, SEPARATELY OR IN COMBINATION, INDICATES THAT NAPHTHALENE MAY BE DETERMINED "REASONABLY ANTICIPATED TO BE A HUMAN CARCINOGEN" UNDER THE NTP CRITERIA FOR LISTING IN THE ROC

A. The NTP Mouse Bioassay Provides Insufficient Evidence of Carcinogenicity in the Test Animals for Consideration Under NTP's Criteria, and Any Tumorigenic Effect, If Present in That Study, Would Not Be Relevant to Humans; Accordingly, There Is No Increased Incidence of Malignant or a Combination of Malignant and Benign Tumors in "Multiple Species"

The NTP Technical Report for the mouse bioassay on naphthalene found only that there was "some evidence of carcinogenic activity" of naphthalene in female $B6C3F_1$ mice, based on increased incidences of pulmonary alveolar/bronchiolar adenomas in the high dose group.[10] The Technical Report did not make a finding of "clear evidence of carcinogenicity" in the test animals. An NTP study that finds that only "some evidence" of carcinogenicity, as

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7 *See* 66 Fed. Reg. at 38432.

8 EPA, IRIS Substance File for Naphthalene, at Section II.A.2., available at http://www.epa.gov/iris/subst/0436.htm (last visited on Sept. 5, 2001).

9 EU, *Draft Risk Assessment Document for Naphthalene*, at Sections 4.1.2.8.2, 4.1.2.8.3, and 5.3.1 (August, 2001). The Draft EU Risk Assessment contains the "final agreed" text, scheduled to become effective in January, 2002.

10 NTP Mouse Bioassay at 36.

opposed to "clear evidence," should be deemed insufficient in weight to warrant consideration under the NTP "reasonably anticipated to be a human carcinogen" standard.

Further, the statements in the current IRIS database on naphthalene confirm that the NTP mouse study provides insufficient evidence of the carcinogenicity of naphthalene in mice. Addressing the NTP mouse study, it states: "An inhalation unit risk estimate for naphthalene was not derived because of the weakness of the evidence (observations of predominant benign respiratory tumors in mice at high dose only) that naphthalene may be carcinogenic in humans."[11] Indeed, only a single alveolar/bronchiolar carcinoma appeared among the 135 high dose female mice. The NTP criteria regarding an increased incidence of malignant and/or combination of malignant and benign tumors clearly are not intended to pertain to an increased incidence of tumors that are so predominantly benign as in the case of the NTP mouse study.

The NTP mouse study should not be considered by NTP for purposes of listing for the additional reason that "the pattern of toxicological evidence indicates that the mouse is more susceptible to the pulmonary toxicity of naphthalene than other species, and therefore the observed pulmonary adenomas seen in mice at [the high dose in the NTP study] are not considered to be of relevance to human health."[12]

B. The NTP Rat Bioassay Does Not Meet the Standard for Listing in the *RoC* Because It Does Not Indicate an Increased Incidence of Malignant or a Combination of Malignant and Benign Tumors At Multiple Tissue Sites, Does Not Indicate an Increased Incidence of Tumors to an Unusual Degree, and the Observed Increase in Tumors Represents a Response That Likely Is Not Relevant to Humans[13]

Naphthalene can meet the NTP standard for listing in the *RoC* only if the NTP rat bioassay indicates a significant increase in malignant or combined malignant and benign tumors in multiple tissue sites or an increase in such tumors to an unusual degree. As discussed below, neither of these criteria are met by the rat bioassay. Moreover, as further discussed, other data indicate that naphthalene likely acts through mechanisms in inducing rat tumors that would not be anticipated to operate in humans under reasonably anticipated patterns of use.

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11 IRIS Substance File for Naphthalene at Section II.C.

12 Draft EU Risk Assessment at Section 4.1.2.8.3.

13 There are no scientifically sound studies indicating that naphthalene increases tumors by routes of exposure other than inhalation. *See* IRIS Substance File for Naphthalene, at Section II.A.3.

1. The NTP Rat Bioassay Does Not Indicate an Increase in Malignant or a Combination of Malignant and Benign Tumors in Multiple Tissue Sites

The Technical Report on the NTP rat bioassay on naphthalene states that the incidences of neuroblastomas of the olfactory epithelium occurred with positive trends in male and female rats and that the incidence in the high dose females was statistically significant compared to controls. The Technical Report also reports a statistically significant increase in adenomas of the respiratory epithelium, a benign tumor, in the male rats and an increase in that tumor that was not statistically significant in the mid and high dose female rats.[14] While these results indicate an increase in tumors in two different types of tissue, the tumors all occurred in the nasal cavity. Therefore, it is clear that there was not an increase in malignant and/or a combination of malignant and benign tumors in multiple tissue sites *both* because the nasal cavity is a single tissue site *and* because there was an increase only in benign tumors, not a combination of benign and malignant tumors, in the respiratory epithelium.[15]

2. The NTP Rat Study Does Not Report an Increase of Malignant or a Combination of Malignant and Benign Tumors to an Unusual Degree

The only malignant tumor increased in the NTP rat study that possibly could be found to be induced to an unusual degree are the neuroblastomas of the olfactory epithelium. The NTP report for that study notes that neuroblastomas of the nasal olfactory epithelium are rare neoplasms in rodents and humans. In addition, the report states that this tumor was not observed in the concurrent controls nor in NTP historical control databases. Several considerations, however, establish that these tumors should not be considered unusual under the NTP criteria for *RoC* listing. First, the number of historical controls in which rats were fed the NTP-2000 diet, the diet used in the NTP rat bioassay on naphthalene, is relatively small.[16] Second, as the Draft EU Risk Assessment concludes, given that the weight-of-the-evidence indicates that naphthalene is non-genotoxic (see discussion below) and the tumors develop only at the sites where non-neoplastic inflammatory changes also occur (changes such as atrophy, hyperplasia, and metaplasia), the development of the nasal tumors is apparently a consequence of chronic tissue injury, for which an identifiable threshold of effect will exist.[17] Tumors induced

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14 NTP Rat Bioassay at 36.

15 It is apparent that the combination of malignant and benign tumors is intended to refer to tumors that are derived from a single type of tissue and only where the malignant tumor is considered to be a progression from the benign tumor.

16 NTP Rat Bioassay at 28-29, 38 (Table 6, note "c").

17 Draft EU Risk Assessment, at Section 4.1.2.8.3.

by such a common and non-specific mechanism of action should not be considered unusual, particularly when they occur at a site, as in the case of the nasal airway of the rat, where exposure to any irritating agent would be expected to cause inflammatory changes. Third, neuroblastomas of the nasal olfactory epithelium have been induced by oral, inhalation, or peritoneal exposure to several structurally unrelated chemicals, and in several of these studies, the induction of the tumors occurred in conjunction with olfactory epithelial non-neoplastic lesions, as in the bioassay on naphthalene.[18]

3. There Is Sufficient Question as to the Relevance of the Nasal Tumors Observed in the NTP Rat Bioassay to Humans That the Reported Increase in the Olfactory Epithelium Neuroblastomas (as well as the Respiratory Adenomas) Should Not Constitute Grounds for Concluding That Naphthalene Is Reasonably Anticipated to Be a Human Carcinogen

As discussed below and more fully in the appended white paper, anatomical, physiological, and metabolic differences between the rat and humans raise substantial questions as to the relevance of the rat nasal tumors to humans.[19] Human nasal physiology is greatly different from that of rodents. A primary site of action for toxic effects in rats is the olfactory epithelium, which comprises a significant portion of the total nasal cavity. The rat is an obligatory nose breather and must rely on olfaction for survival. The olfactory mucosa in rats is a highly developed system of cellular structures that performs complicated integration of olfaction and air humidification. The vast majority (approximately 50% of the total surface area) of the posterior region of the rat nasal cavity is comprised of the olfactory epithelium.[20] Inhaled vapors need traverse only a few millimeters past the resistant respiratory epithelium to reach the sensitive olfactory tissue in rats.

By comparison, the total surface area for chemical exposure is much less in humans (by a factor of five) since human nasal turbinates are much less convoluted than in the rodent. The olfactory epithelium comprises only about 10% of the human nasal cavity and is

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18 NTP Rat Bioassay at 42.

19 Vincent Piccirillo, Ph.D., DABT, "Naphthalene Nasal Tumors in Rats -- Relevance to Humans" (Feb. 1, 2001); Included as an attachment to these comments.

20 Gross, E.A., Swenberg, J.A., Fields, S., Pop, J.A. (1982). "Comparative morphometry of the nasal cavity in rats and mice." *J. Anat.* 135:83-88; Uriah, L.C. and Maronpot, R.R. (1990). "Normal histology of the nasal cavity and application of special application of special techniques." *Environ. Health Perspect.* 85:187-208.

confined to the posterior dorsal region of the nasal cavity.[21] The ciliated respiratory epithelium is the major lining of the human nasal cavity. In humans, inhaled vapors must traverse several centimeters through the ciliated respiratory epithelium before reaching the olfactory epithelium. Through mucociliary actions, the respiratory epithelium provides a protective system for the olfactory epithelium and other respiratory tissues. As a result of these differences, the efficiency of extracting chemicals from air inhaled through the nose is much less in humans than in rodents, which rely heavily on their sense of smell to locate food. The resulting dose deposited to the human olfactory epithelium, in particular, from inspired air is far less than for rodents for any given naphthalene concentration in air.

As noted above, irritation occurred in the nasal olfactory and respiratory epithelium in the NTP rat study (as well as in the NTP mouse study). Also as explained, it is likely that irritation plays a central role in the induction of nasal tumors seen in the rat. This conclusion is supported by the fact that naphthalene is largely negative in genotoxicity studies. Moreover, both the Draft EU Risk Assessment referenced above,[22] as well as the EU Scientific Committee on Occupational Exposure Limits,[23] concur that chronic cytotoxicity is the likely mechanism for the tumorigenic effects of naphthalene in the rat nasal cavity. Given the factors discussed above, it appears unlikely that such chronic cytotoxicity in olfactory epithelium would occur in humans under conditions of naphthalene use.

Differences in the rate of metabolism and the character of the metabolites of naphthalene in rats and humans also support the hypothesis that the NTP rat bioassay results are not relevant to humans. Of all mammalian species, the human has the greatest capacity for the detoxification of naphthalene epoxide, the initial metabolite of naphthalene. This epoxide is a reactive and short-lived intermediary metabolite, which is thought to be the proximate carcinogen in the rat causing the neuroblastoma. Humans metabolize naphthalene epoxide at a rate 6-fold greater than rats, providing a protective mechanism from naphthalene effects. As explained by Kitteringham, *et al.* (1996), ". . . both rodent species [(rat and mouse)] showed consistently low (epoxide hydrolase) activity which, coupled with the possibility of differences in substrate specificity, cautions against the choice of rodent species for toxicity testing of compounds for which epoxide intermediates are suspected metabolites."[24]

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21 Frederick, C.B., Morris, J.B., Kimbell, J.S., Morgan, K.T., Scherer, P.W. (1994). "Comparison of four biologically based dosimetry models for the deposition of rapidly metabolized vapors in the rodent nasal cavity." *Inh. Toxicol.* 6(suppl.):135-157.

22 Draft EU Risk Assessment, at Section 4.1.2.8.3.

23 SCOEL (Scientific Committee on Occupational Exposure Limits) (2001). "Recommendation from Scientific Committee on Occupational Exposure Limits for Naphthalene." SCOEL/SUM/90 final, June, 2001.

24 Kitteringham, N.R., Davis, C., Howard, N., Pirmohamed, M., Park, B.K., (1996). "Interindividual and interspecies variation in hepatic microsomal epoxide hydrolase activity: studies with cis-stilbene oxide, carbamazepine 10, 11-epoxide and naphthalene." *J. Pharmacol. Exp. Ther.* 278(3):1018-1-27.

In light of the foregoing anatomical, physiological, and metabolic considerations, there is sufficient question about the relevance of the rat nasal tumors to humans to preclude a finding that naphthalene is "reasonably anticipated to be a human carcinogen," under conditions of use.[25]

IV. THE WEIGHT-OF-THE-EVIDENCE INDICATES THAT NAPHTHALENE IS NOT GENOTOXIC, AND OTHER CORROBORATIVE EVIDENCE THAT WOULD SUPPORT A LISTING IN THE *RoC* IS LACKING

The Panel concurs with the conclusion of the Draft EU Risk Assessment that the weight-of-evidence indicates that naphthalene is not genotoxic.[26] The NTP Technical Report for the rat bioassay also appears to concur with this conclusion, indicating that "[t]here is little evidence for mutagenic potential of naphthalene in the most widely used genotoxicity bioassays."[27] The Panel refers NTP to the discussion of mutagencity data in the Draft EU Risk Assessment[28] and concurs with the following summary of the mutagenicity data in that document:

> Naphthalene has given reproducible negative results in bacterial mutation assays, and was negative in an *in vitro* UDS [unscheduled DNA synthesis] assay. It was however found to be clastogenic in CHO cells in the presence but not the absence of S9. Two *in vitro* studies using CHO cells and human peripheral lymphocytes were negative for induction of SCE. Naphthalene was found to be negative in two *in vivo* bone-marrow micronucleus tests and an *in vivo* rat liver UDS study. Overall, the balance of evidence indicates that naphthalene is not genotoxic.[29]

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25 While the Draft EU Risk Assessment states that there is some uncertainty as to the relevance of the rat nasal effects to human health, it concludes that: there is currently insufficient evidence to rule out the relevance to humans. Draft HSE Risk Assessment, at Section 4.1.2.8.3. Based on the foregoing consideration, the Panel believes that the available data and information adequately support the conclusion that the rat nasal tumors are highly unlikely to be relevant to human risk and therefore that it would be inappropriate to determine naphthalene to be "reasonably anticipated to be a human carcinogen."

26 Draft EU Risk Assessment, at Section 4.1.2.7.4.

27 NTP Rat Bioassay at 20.

28 Draft EU Risk Assessment, at Section 4.1.2.7.

29 *Id.* at Section 4.1.2.7.4.

Finally, naphthalene, an unsubstituted bicyclic compound, is structurally dissimilar to larger multiple-fused ring or substituted compounds (such as polycyclic aromatic hydrocarbon compounds or naphthylamine) listed by NTP in the *RoC* as cartcinogenic. Because of this difference, naphthalene does not belong to a well-defined, structurally related class of substances whose members whose members are listed in a previous *RoC*.

V. IF FOLLOWING THE RG1 REVIEW NTP CONCLUDES THAT NAPHTHALENE WARRANTS FURTHER CONSIDERATION FOR LISTING, IT SHOULD DEFER ANY SUCH FURTHER CONSIDERATION BY THE RG2 COMMITTEE UNTIL AFTER IARC ISSUES A MONOGRAPH FOLLOWING ITS UPCOMING REVIEW OF NAPHTHALENE

The International Agency for Research on Cancer (IARC) has announced that naphthalene will be reviewed under the IARC Monograph Programme in February 2002.[30] If following the RG1 Review NTP concludes, despite all of the reasons stated above, that naphthalene warrants further consideration for listing, NTP should defer any such further consideration by the RG2 Committee until after IARC issues a monograph following its review of naphthalene. The Panel believes that such a modest deferral of NTP's further consideration of naphthalene, until IARC issues its monograph on naphthalene, would be appropriate and would avoid unnecessary duplication of efforts, especially as NTP is taking a lead role in the IARC review.

CONCLUSION

For the reasons discussed above, the Panel believes that the available studies and data do not establish that naphthalene is "reasonably anticipated to be a human carcinogen" and therefore that NTP should determine that listing of naphthalene in the *RoC* would not be appropriate. If NTP nevertheless determines after the RG1 level review that further review of naphthalene is warranted, that further review at the RG2 level should be deferred until completion of the upcoming IARC review of the chemical.

30 *See* http://193.51.164.11/past&future/agentsfuture.html.

NAPHTHALENE NASAL TUMORS IN RATS – RELEVANCE TO HUMANS

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February 1, 2001

Naphthalene Nasal Tumors in Rats – Relevance to Humans

I. PURPOSE

The carcinogenic potential of naphthalene upon chronic inhalation exposure was evaluated in B6C3F1 mice and F344 rats. The results of these studies demonstrated increased incidences of benign and malignant tumors of the nasal epithelium in male and female rats but not in mice. The purpose of this paper is to evaluate the relationship between the metabolism of naphthalene and the differential tumorigenic responses of the nasal cavity seen in mice and rats. Further, the metabolism of naphthalene in the human is discussed in relationship to potential for nasal tumor development. Finally, physiological differences between rats and humans are discussed as these differences reduce the likelihood that humans incur the same risk as rats.

II. ANATOMY AND PHYSIOLOGY OF THE NASAL CAVITY

Across species, the surface of the nasal cavity is composed of squamous, transitional, respiratory, and olfactory epithelium. Histologic evaluations show that human respiratory and olfactory epithelia are histologically similar to the rodent respiratory and olfactory epithelia. However, marked differences in anatomy, mucociliary clearance, airflow dynamics and regional distribution of xenobiotics make correlation between rodent effects and the potential risks to human difficult (Monticello, 1994).

The rat is an obligatory nose breather and must rely on olfaction for survival. The olfactory mucosa of rodents is a highly developed system of cellular structures that performs complicated integration of olfaction and air humidification. The vast majority (approximately 50% of the total surface area) of the posterior region of the rat nasal cavity is comprised of the olfactory epithelium (Gross, 1982, Uriah, 1990). Inhaled vapors need traverse only a few millimeters past the resistant respiratory epithelium to reach the sensitive olfactory tissue.

By contrast to the rat, the human olfactory system is poorly developed. The olfactory epithelium comprises about 10% of the human nasal cavity and is confined to the posterior dorsal region of the nasal cavity (Frederick, 1994). The ciliated respiratory epithelium is the major lining of the human nasal cavity. In humans, inhaled vapors traverse several centimeters through the ciliated respiratory epithelium before reaching the olfactory epithelium. Via mucociliary actions, the respiratory epithelium provides a protective system for the olfactory epithelium and other respiratory tissues.

III. EFFECT OF NAPHTHALENE ON THE NASAL EPITHELIUM IN THE RAT

In a two-year inhalation study conducted for NTP, F344N rats (49/sex/group) were exposed to 0, 10, 30, or 60 ppm naphthalene, 6 hr/d, 5 d/wk, for 105 weeks. The results of this study clearly showed that naphthalene was toxic to the olfactory epithelium as well as respiratory and glandular tissues of rats. Within the olfactory epithelium, naphthalene effects were cell type specific. The major components of the olfactory epithelium are the basal cells, the long ducts of Bowman's glands, sensory cells, and the sustentacular or support cells. In the olfactory epithelium specifically, histopathological examination of rats from the NTP study revealed atypical (basal cell) hyperplasia, atrophy, chronic inflammation, and hyaline degeneration. In the respiratory epithelium, hyperplasia, squamous metaplasia, hyaline degeneration and goblet cell hyperplasia was observed, as well as glandular hyperplasia and squamous metaplasia (NTP, 2000). The severity of these lesions corresponded to increasing naphthalene concentration.

A significant increase in the incidence of malignant neuroblastoma of the nasal epithelium was observed in male rats exposed at 30 and 60 ppm (4/48, and 3/48, respectively, as compared to 0/49 and 0/49 for the respective control and 10 ppm males). In females, the incidence of this tumor was increased at all exposure levels (0/49, 2/49, 3/49, and 12/49). Benign adenoma of the nasal respiratory epithelium also was increased in both sexes with the following incidences: 0/49, 6/49, 8/48, 15/48 for males and 0/49, 0/49, 4/49, 2/49 for females. No other neoplasms were reported to occur at higher incidences than experimental or historical controls in this study (NTP, 2000).

From the results of this study, NTP concluded that naphthalene shows clear evidence of carcinogenic activity in male and female F344N rats. This conclusion was drawn because 1) the incidence of neuroblastoma of the nasal epithelium was increased in both sexes, 2) this tumor is considered rare and did not occur in the study or historical controls, 3) this tumor also occurs in humans, 4) the incidence of nasal respiratory epithelial adenoma also was increased in both sexes at the two higher dose levels, and 5) the tumor response, particularly for respiratory epithelial adenoma in males, showed a positive dose-response.

As degeneration, inflammation, hyperplasia and metaplasia also were reported in the NTP study, a relationship between this significant irritation and the neoplasic responses cannot be ruled out and, at the very least, is an obvious confounding variable.

In contrast to the rat, a chronic inhalation study in B6C3F1 mice was conducted (NTP 1992) in which mice were exposed (6 hr/d, 5 d/wk for 103 weeks) to atmospheres containing 0, 10, or 30 ppm naphthalene. Pulmonary alveolar/bronchiolar adenomas were increased only in females at 30 ppm (28/134 or 28% vs. control incidence of 5/68 or 7%). As a result of this study, NTP concluded that there was some evidence for the carcinogenicity of naphthalene in female but not male mice based on an increase in adenomas. In describing this mouse study, NTP (2000) also reported: "Additionally, naphthalene caused exposure-related increases in the incidences of chronic inflammation,

metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium of the nose as well as exposure-related increases in the incidences of chronic inflammation of the lung in male and female mice."

The TLV Documentation (6th Ed) reviewed another chronic mouse study (1996). In it, mice inhaled naphthalene at 30 ppm 6 hr/d, 5 d/wk, for 6 months (ACGIH, 1996). An increase in the number of tumors per mouse was detected although the number of mice with tumors apparently was not increased. Mouse skin painting and subcutaneous injection studies, reviewed by NTP (2000), were largely negative. Neoplasia has not been reported in other animal species.

IV. COMPARATIVE MAMMALIAN METABOLISM OF NAPHTHALENE

The initial step in the metabolism of naphthalene in mammals is the formation of a naphthalene epoxide. This formation is a "Phase 1" cytochrome P450 reaction in which oxygen is added to the naphthalene molecule. Experimental evidence indicates that this epoxide may occur in two stereoisomeric forms, each of which may be formed by a distinct P450 isoform. Once formed, the epoxide may 1) be hydrolyzed by epoxide hydrolase, through addition of a water molecule, to a dihydrodiol; 2) be conjugated with glutathione by glutathione transferase, ultimately to form the mercapturic acid, 3) spontaneously isomerize to naphthol, it's hydroxy metabolite, or 4) react with nucleophilic cellular constituents such as proteins or nuclear material (Franklin, 1987, Klaassen, 1996). The first two pathways generally are considered to detoxify the epoxide. The third pathway, formation of the hydroxy-metabolite, naphthol, may continue with conjugation to sulfate or glucuronide in a "Phase 2" reaction, for ultimate excretion. It is also possible that naphthol and other stable metabolites may be further re-circulated through the P450 system, leading to the formation of other metabolites. Finally, the forth pathway indicates possible reactions with sensitive cellular constituents that may lead to carcinogenesis (ibid).

The metabolism of naphthalene by the mouse is different from that of other species. It appears that the naphthalene epoxide stereoisomer formed by the mouse is different from that of the rat (Buckpitt et.al., 1992). It has been postulated that this stereoisomer may not have the carcinogenic potential of that produced in the rat. The rate of metabolism and detoxification of naphthalene in mice is greater than rats. Both glutathione conjugation and formation of dihydrodiol exceeds that of the rat. The importance of these metabolic reactions may relate to differential responses seen in the nasal epithelium of these species. In mice, the respiratory epithelium is more sensitive while the olfactory epithelium is more sensitive in the rats. It should be further noted that the neuroblastomas in the rats arise from the olfactory epithelium. Collectively, the rates of naphthalene metabolism and excretion and the character of the metabolites may account for the lack of nasal tumors in mice (Quick and Shuler, 1999).

The stereoisomer configuration of naphthalene epoxide in humans is not known but the literature suggests that naphthalene metabolism in humans is similar to the rat. The rate of metabolism of the epoxide in humans exceeds that of all other species (Kitteringham et al., 1996). As noted previously, the formation of naphthalene dihydrodiol from the epoxide (by epoxide hydrolase) is a detoxification mechanism. In in vitro studies with liver tissue, humans were shown to have the highest rate of naphthalene dihydrodiol formation, followed in order by, rabbit, dog, hamster, mouse, and, finally, rat (Kitteringham et al., 1996). The overall rate was up to six-fold higher for humans as compared to rats. If an epoxide mediates the tumorigenic response in rats, the greater detoxification capacity of humans argues against extrapolating results from rats (or mice) to humans. The difference in human and rodent metabolism of xenobiotics may be qualitative as well as quantitative. Rates and efficiencies of metabolism may depend upon the "tightness" of coupling between enzymes responsible for phase one and phase two reactions, as in the analogy of a train track. The efficiency of oxidation for a xenobiotic may depend as much upon the tight coupling of P450 with epoxide hydrolase as well as upon the levels of the latter enzyme or amount of glutathione available for mercapturate formation. The Kitteringham study may not have measured this coupling efficiency in liver microsomal preparations and, consequently, may have underestimated the greater efficiency of humans compared with rodents.

V. CONCLUSIONS

The tumorigenic responses seen in the nasal epithelium of the rat raises a concern regarding the potential for naphthalene to induce tumors in humans. In considering the relevance of this rat study for human carcinogen risk characterization, the differences in the anatomy and physiology of the nasal cavity and the metabolic capacities of the species must be considered. Human nasal physiology is vastly different from that of rodents. A primary site of action in rats is the olfactory epithelium, which comprises a significant portion of the total nasal cavity. By comparison, the total surface area for chemical exposure is much less in humans (by a factor of five) since human nasal turbinates are much less convoluted than in the rodent. As a result, the efficiency of extracting chemicals from air inhaled through the nose is much less in humans than in rodents, which rely heavily on their sense of smell to locate food. Consequently, the resulting dose deposited to the human olfactory epithelium from inspired air is far less than for rodents for any given naphthalene concentration in air.

Irritation occurred in the nasal olfactory and respiratory epithelium in both the mouse and rat studies. It is likely that irritation may play a central, facilitating role in the induction of nasal tumors seen in the rat. This hypothesis is supported by the fact that naphthalene is largely negative in genotoxicity studies. It appears that the EU Scientific Committee on Occupational Exposure Limits concurs with this premise. This committee states in a report for naphthalene: ". .it seems plausible to speculate that the tumours produced in

rodents arose from a background of chronic cytotoxicity, and that controlling exposure to avoid such cytotoxicity would also prevent carcinogenicity." (SCOEL, 2000).

From the metabolism standpoint, comparison of mice and rats permits a hypothesis that differences in the rate of metabolism and the character of the metabolites results in a tumorigenic response in the rat nasal cavity while only an inflammatory response in mice. The regional distribution of the response also supports differential metabolism by those tissues. Of all mammalian species, the human has the greatest capacity for the detoxification of naphthalene epoxide, the initial metabolite of naphthalene. This epoxide is a reactive and short-lived intermediary metabolite, which is thought to be the proximate carcinogen in the rat causing neuroblastoma. Humans metabolize naphthalene epoxide at a rate 6-fold greater than rats providing a protective mechanism from naphthalene effects. Kitteringham et al. (1996) state: ". . . both rodent species (rat and mouse) showed consistently low (epoxide hydrolase) activity which, coupled with the possibility of differences in substrate specificity, cautions against the choice of rodent species for toxicity testing of compounds for which epoxide intermediates are suspected metabolites."

In conclusion, the physiologic and metabolic differences between human and rats suggest that naphthalene should not pose an unreasonable carcinogenic risk for humans under conditions of use.

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