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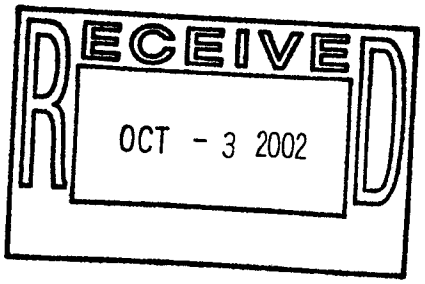

October 2, 2002

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
Draft Report on Carcinogens Background Document for
Naphthalene, 26 August, 2002

Dear Dr. Jameson:

The Naphthalene Panel (Panel) of the American Chemistry Council submits these comments on the National Toxicology Program's (NTP) *Draft Report on Carcinogens Background Document for Naphthalene*, dated August 26, 2002, posted on NTP's web site (http://ntp-server.niehs.nih.gov/NewHomeRoc/roc11Bkgrnd.html). NTP prepared the draft background document in preparation for the Board of Scientific Counselors Report on Carcinogens (*RoC*) Subcommittee meeting scheduled for November 19-20, 2002, at which nominations for the Eleventh Edition of the *RoC* will be reviewed[1].

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,

Signature

[1] Federal Register July 24, 2001 (Vol. 66, No. 142) pages 38430-38432; Federal Register: March 28, 2002 (Vol. 67, No. 60) page 14957; Federal Register: May 24, 2002 (Vol. 67, No. 101) pages 36621-36622; Federal Register: September 20, 2002 (Vol. 67, No. 183) pages 59301 – 59303.

Comments of the Naphthalene Panel of the American Chemistry Council on *Draft Report on Carcinogens Background Document for Naphthalene* (August 26, 2002)

Executive Summary

The Naphthalene Panel (Panel) of the American Chemistry Council submits these comments on the *Draft Report on Carcinogens Background Document for Naphthalene* (Aug. 26, 2002) (Draft Background Document), prepared by the National Toxicology Program (NTP) to assist in the review of naphthalene for possible listing in the Eleventh Edition of the *Report on Carcinogens* (*RoC*). The Panel requests that these comments be placed in the record for the review of naphthalene for possible listing and that copies be provided to the appropriate reviewers for consideration during the NTP Executive Committee Working Group for the *RoC* (RG2) review scheduled for November 19-20, 2002. 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 NTP bioassays on rats and on mice. The Draft Background Document contains a number of deficiencies that should be corrected, including a failure to address the factors indicating that naphthalene does not meet the criteria for listing in the *RoC*. These deficiencies may be summarized as follows:

- The Draft Background Document contains outdated and incorrect information on production, exposure, use, and environmental fate of naphthalene.

- The toxicology section of the Draft Background Document should include a discussion of the information and factors that demonstrate that naphthalene does or does not meet the criteria for listing in the *RoC*.

- The Draft Background Document should supplement and integrate the toxicology data on several additional matters and should make overall conclusions on those matters.

    - The document should incorporate additional genotoxicity data and should state that the available relevant data strongly support the conclusion that naphthalene is not genotoxic.

    - The document should better integrate the toxicology data, including data demonstrating that observed toxic and metabolic effects relevant to the tumorigenic process are species-specific, and

data regarding the relevance of laboratory animal responses for extrapolating to humans.

- The document should explain that the compensatory cell proliferation following cell toxicity is a potentially important mechanism for the development of nasal tumors in rats exposed to naphthalene by inhalation.

- The Draft Background Document should be corrected, updated, and supplemented with regard to naphthalene production, exposure, use, environmental fate, and toxicology in accordance with the additional specific comments set forth in these comments.

Comments of the Naphthalene Panel of the American Chemistry Council on *Draft Report on Carcinogens Background Document for Naphthalene*, (August 26, 2002)

INTRODUCTION

The Naphthalene Panel (Panel) of the American Chemistry Council submits these comments on *Draft Report on Carcinogens Background Document for Naphthalene* (Aug. 26, 2002) (Draft Background Document), prepared by the National Toxicology Program (NTP) to assist in the review of naphthalene for possible listing in the Eleventh Edition of the *Report on Carcinogens* (*RoC*). The Panel requests that these comments be placed in the record for the review of naphthalene for possible listing and that copies be provided to the appropriate reviewers for consideration during the NTP Executive Committee Working Group for the *RoC* (RG2) review. The Panel is comprised of the major domestic producers and importers of naphthalene.[1]

I. THE DRAFT BACKGROUND DOCUMENT CONTAINS OUTDATED AND INCORRECT INFORMATION ON PRODUCTION, EXPOSURE, USE AND ENVIRONMENTAL FATE OF NAPHTHALENE

Discussions of production, exposure and use in the Draft Background Document are outdated and incorrect. Further, discussions of the data on the environmental fate of naphthalene are cursory. NTP should make every effort to be comprehensive because NTP's documents are often cited as authoritative in many different places. In this case, NTP's efforts fall short. Two examples of the Draft Background Document's reliance on outdated and inaccurate information, discussed in detail in comments that follow, are NTP's continued reliance (NTP, 1992, 2000, 2002) on 30-year-old occupational exposure reports that have been found to be of doubtful relevance by other review bodies (cited below) and the repeated citation of a 1983 study of suspect quality as a source of numbers of occupationally exposed workers.

The Panel urges NTP to revise the Draft Background Document to caveat appropriately its use of old data sources and incomplete analysis of more recent and more accurate data. The Draft Background Document should be revised to reflect current naphthalene usage in the U.S. as well as to reflect the current state of knowledge about the environmental fate of naphthalene.

The Panel also urges NTP to discontinue its reliance on poorly documented and designed, three-decade-old East German occupational exposure reports that have been evaluated and found to be of doubtful relevance by the U.S. Environmental Protection Agency (EPA), the United Kingdom (UK) Health and Safety Executive (HSE), and the German *Bundesanstalt für*

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[1] Panel members are the International Tar Association, Koppers Industries, Inc. and Recochem, Inc.

Arbeitsschutz und Arbeitsmedizin (BAuA). To assist NTP in this matter, Attachments A and B are English translations of the German occupational exposure reports (Wolf, 1976, 1978).

The Panel was approached by the National Institute for Occupational Safety and Health (NIOSH) about the possibility of identifying a meaningful cohort for studying naphthalene exposure in manufacturing workers. The Panel responded to NIOSH in a letter dated May 4, 2001, included here as Attachment C.

II. THE TOXICOLOGY SECTION OF THE DRAFT BACKGROUND DOCUMENT SHOULD INCLUDE A DISCUSSION OF INFORMATION THAT DEMONSTRATES THAT NAPHTHALENE DOES NOT MEET THE CRITERIA FOR LISTING IN THE *RoC*

The Panel submitted comments in response to NTP's nomination of naphthalene for possible listing in the *RoC* (NTP, 2001). These comments (included here as Attachment D) address the proposed listing of naphthalene.

Naphthalene was 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 (NTP, 2000) and an NTP bioassay on mice that reported some evidence of carcinogenicity in female mice (NTP, 1992). For the reasons discussed in detail in Attachment D, 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, should not be considered 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 corroborative evidence that would support a listing in the *RoC* is lacking insufficient.

These points are explained fully in the Panel's previous comments (Attachment D).

III. THE DRAFT BACKGROUND DOCUMENT SHOULD INTEGRATE THE TOXICOLOGY DATA ON SEVERAL ADDITIONAL MATTERS AND SHOULD MAKE OVERALL CONCLUSIONS ON THOSE MATTERS

The toxicology data in the Draft Background Document (NTP, 2002) is reasonably comprehensive as a catalogue of studies, but NTP does not integrate the data in any meaningful way. We urge NTP to revise the document in the manner described below.

A. The Draft Background Document Should Incorporate Additional Genotoxicity Data and Should State That the Available Data Strongly Support the Conclusion That Naphthalene Is Not Genotoxic

The presented results for genotoxicity strongly support a conclusion that naphthalene is not genotoxic. This conclusion is further supported by data reported in Schreiner (in press), included here as Attachment E. The Draft Background Document should include these additional data and this conclusion in the discussion of genotoxicity.

B. The Draft Background Document Should Better Integrate the Toxicology Data, Including Data Demonstrating That Observed Toxic and Metabolic Effects Relevant to the Tumorigenic Process Are Species-Specific, and Data Regarding the Relevance of Laboratory Animal Responses for Extrapolating to Humans.

Although the species specificity issue is mentioned in the Draft Background Document, the discussion does not portray what the data show. The data show that effects on the rat nasal epithelium occur regardless of route of exposure. Similarly, lung effects in mice have been seen with IP dosing. These findings strongly correlate with the species-specific arguments advanced by Buckpitt and others that these are species-specific effects related to metabolism (Buckpitt *et al.*, 1992; Schultz *et al.*, 2001; West *et al.*, 2001; Buckpitt *et al.*, 2002).

The species-specific metabolism data are presented in the Draft Background Document but no conclusion regarding the relationship to the tumorigenic process is made. The International Agency for Research on Cancer (IARC) reviewed these data in its final analysis and recognized that monkey and/or human metabolic data may play a role in future risk assessments. The IARC monograph is expected to be published in early 2003; NTP may be able to request a pre-print copy, however, to assist in its *RoC* evaluation. We urge NTP to contact IARC on this matter.

It appears that NTP did not consider the relevance of the observed tumors in mice and rats to humans.

C. The Draft Background Document Should Explain That Cell Proliferation Is a Potentially Important Mechanism for the Development of Nasal Tumors in Rats Exposed to Naphthalene by Inhalation

The issue of cell proliferation and its relationship to the carcinogenic process is mentioned but essentially dismissed (section 4.2.1). Cell proliferation may, however, be a potentially important mechanism in the development of nasal tumors in rats exposed to naphthalene by inhalation. This is discussed in the pathology review report, prepared by Dr. J. Harkema, included here as Attachment F. The Draft Background Document should include a discussion of the potential importance of the cell proliferation mechanism.

IV. In Accordance with the Specific Comments Below, the Draft Background Document Should be Corrected, Updated and Supplemented to Accurately Represent Naphthalene Production, Exposure, Use, Environmental Fate and Toxicology

EXECUTIVE SUMMARY (and corresponding discussions in document text)

USE – Occupational exposure, page v:

In the year 2002, the following products were not manufactured in the U.S.A.:

- Beta Naphthol
- Celluloid
- Dye Chemicals
- Smokeless Powder.

In addition, the following are not direct uses for naphthalene in the US:

- Tannery workers (we know of no tannery using naphthalene directly)
- Textile chemical workers (we know of no textile industry using naphthalene directly) and
- To our knowledge, naphthalene is not used in the production of toilet bowl deodorizers.

PRODUCTION page v –

In 2000, Koppers Industries, Inc. produced 135 million pounds. Allied Signal production was between 42 and 45 million pounds from tar. Production from coal tar distillation for 2000 was approximately 180 million pounds. In March 2000, Recochem, Inc. based in Montreal, QC, Canada purchased Allied Signal's facility in Ohio that was operational until December 2000 and ceased production thereafter.

Most naphthalene manufactured in the US is produced from coal tar, not petroleum.

OCCUPATIONAL EXPOSURE page v:

NTP cites the National Occupational Exposure Survey (NOES) (NIOSH, 1983), conducted from 1981 to 1983, as the source of the estimate "*that 112,702 workers potentially were exposed to naphthalene.*" EPA (1999) has said, "*Now over 15 years old, the NOES data have become progressively dated, and as a consequence, less representative of current exposure situations.*"

The current total employee population in Koppers Industries' tar distillation and wood preserving plants and phthalic anhydride production facilities with a potential naphthalene exposure was 1,020 in 2000 and 1,000 in 2001 and will average 990 in 2002. Koppers Industries is the largest employer in the tar distillation and wood preservation industries in the US. The number of exposed employees given in the background document appears excessive even for 1983.

Fewer than 50 workers with potential naphthalene exposure are employed in the moth repellent industry on a direct basis.

The estimate of over 112,000 workers exposed to naphthalene thus seems highly unlikely. Although naphthalene is used in applications in Europe that are not applicable in the US, the Panel believes the occupational exposure assessment discussed in the EU's Naphthalene Risk Assessment Report (EU, 2002) to be more representative of exposure in the US than the discussion in NTP's draft background document. The EU Risk Assessment Report (EU, 2002; Section 4.1.1.1.2) contains the following estimates of workers exposed during naphthalene manufacturing in the EU:

> It is unrealistic to attempt to estimate the total number of workers exposed to naphthalene. The number exposed during naphthalene manufacture and subsequent use is estimated to be 250 to 500 in the UK and 1500 to 2000 in the EU (this does not include operators handling creosote treated timber or brush applicators or users of tar paints/membranes). The number exposed as a result of incomplete combustion of organic materials is likely to be significantly higher than these figures.

The Panel, whose members are naphthalene manufacturers, has no information about the "numbers exposed as a result of incomplete combustion of organic materials."

HUMAN CANCER STUDIES, page vi

Recent publications about naphthalene by the NTP (*e.g.*, NTP 1992, 2000) have cited three-decade old East German reports of health effects observed in tar distillation workers. The production methods described in these studies have not been in use in North America in over 30 years.

The health effects information contained within the reports themselves has been evaluated recently by German and UK authorities. Translations and evaluations of the reports are included here as Attachments A and B. Both the UK HSE Risk Assessment Report for Naphthalene (EU, 2002) and the German BAuA (BAuA, 1998) conclude that no conclusions can be drawn about the carcinogenicity of naphthalene from the limited information available in humans.

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.

As noted above, the Panel discussed the possibility of identifying a meaningful cohort for studying naphthalene exposure in manufacturing workers with NIOSH (see Attachment C). To date, NIOSH has not responded to the Panel's letter of May 2001, and has presumably concluded that a meaningful cohort of workers exposed to naphthalene is unlikely to be identified in the US. The Panel understands that naphthalene manufacturers in the EU were similarly contacted about identifying a cohort during development of the EU Risk Assessment, with a similar outcome. The Risk Assessment Report (EU, 2002; 4.1.1.1.4) provides the

following estimates of the number of occupationally exposed workers during the manufacture of naphthalene:

> It is understood that about 10 persons (including maintenance operators) are exposed to naphthalene vapour during distillation of the coal tar to produce the naphthalene oil at each of the two UK tar distillation plants. The total exposed during the distillation of coal tar to produce the naphthalene oil throughout the EU was not established. However, it is estimated that it is in the region of 100 to 200 employees.
>
> During subsequent purification of the naphthalene by either distillation or crystallisation there are an estimated further 4 exposed in the UK, with a further 50 to 60 throughout the EU.

1. INTRODUCTION

Paragraph 1, Page 1

Coal tar contains up to only 10% naphthalene, therefore, although naphthalene may or may not be the "most abundant" single constituent, coal tar cannot be described as predominantly naphthalene.

2. HUMAN EXPOSURE

2.1 USES, page 5

In the year 2002, the following products were not manufactured in the US:

- Beta Naphthol
- Celluloid
- Dye Chemicals
- Smokeless Powder.

In addition, the following are not uses for naphthalene in the US:

- Tannery workers (we know of no tannery using naphthalene directly)
- Textile chemical workers (we know of no textile industry using naphthalene directly) and
- To our knowledge, naphthalene is not used in the production of toilet bowl deodorizers.

To our knowledge, naphthalene sulphonates are not used in the paint and dye industries and are also not used as toilet bowl deodorizers. Naphthalene itself is definitely not used in the production of toilet bowl deodorizers.

2.2. PRODUCTION

"Hot pressing" was discontinued over 30 years ago. The product is purified by further distillation.

In the first paragraph of page 6 of the Draft Background Document it is stated, "Naphthalene content in crude oil is as follows: 100 to 2,800 mg/kg in oil from coal; 402 to 900 mg/kg in oil from petroleum and 203 to 1,390 mg/kg in oil from shale..." It is not clear what "oil from coal may refer to, however, if it is meant to refer to coal tar, and we accept that coal tar contains up to 10% naphthalene (as stated in the Introduction), then the naphthalene content of coal tar cannot have an upper range of 2,800 mg/kg.

In the second paragraph of page 6 of the Draft Background Document it is stated, "Since 1960, the most common commercial production process in the United

States has been recovery of naphthalene from petroleum." This is inconsistent with per production figures given in Table 2-2.

The discussion of production in the U.S. in the third and fourth paragraphs of page 6 is internally inconsistent and is also inconsistent with the production figures provided in Table 2-2.

2.4.2 ENVIRONMENTAL OCCURRENCE - WATER

Treated effluent waters from wood preserving and tar distillation operations go to local privately-owned treatment works (POTW) facility. Spills and leaks are promptly cleaned up to minimize any naphthalene release to water or soil.

If petroleum facilities are in fact the largest producers of naphthalene, is there a reason that fact is not noted in this section, as well as in sections 2.4.1 and 2.4.3?

2.5 ENVIRONMENTAL FATE

The rich scientific literature on the environmental fate of naphthalene is only cursorily addressed in the Draft Background Document. For one example, there is a wealth of information about the photolysis of naphthalene in air. The EU Risk Assessment Report (EU, 2002, p. 41) contains the following summary:

Atmospheric oxidation

Atmospheric oxidation of naphthalene occurs by reaction with the hydroxyl radical and reactions may also occur with ozone and nitrogen oxides. The rate constants for the gas phase reactions of hydroxyl radicals and ozone with naphthalene were determined under atmospheric conditions at 294±1 K (Atkinson et al., 1984). The rate constant for the reaction of naphthalene with hydroxyl radicals was (2.42±0.19) x 10^{-11} cm^3 $molecule^{-1}$ s^{-1}. Assuming an average daytime atmospheric hydroxyl radical concentration of approximately 1 x 10^6 molecule cm^{-3}, the lifetime of naphthalene due to reaction with hydroxyl radicals can be estimated to be approximately 1 day. The rate constant for reaction of naphthalene with ozone was measured and no decay of naphthalene by ozone was observed in the dark. Naphthalene was also found to react with NO_3 radicals which indicates that this may be an additional sink for naphthalene during nighttime hours in a polluted urban atmosphere.

Klöpffer et al. (1986) measured the rate of reaction of naphthalene with OH radicals in a smog chamber. At 300 K and 1.05 x 10^5 Pa the rate constant was 2 x 10^{-11} cm^3 $molec^{-1}$ sec^{-1}.

Masclet and Mouvier (1988) also measured the reaction rate of naphthalene with hydroxyl radicals. The rate constant was measured as 2.4 x 10^{-11} cm^3 $molecule^{-1}sec^{-1}$ and, based on a hydroxyl radical concentration of 1 x 10^6 molecule/cm^3, the lifetime was calculated to be 12 hours.

Biermann et al. (1985) measured the rate constant for reaction of naphthalene with hydroxyl radicals to be 2.35 x 10^{-11} cm^3 $molecule^{-1}sec^{-1}$ and calculated the half life to be approximately 12 hours assuming a hydroxyl radical concentration of 1 x 10^6 molecule/cm^3.

The major products of the reaction of naphthalene with hydroxyl radicals were found to be 1- and 2-naphthols and 1- and 2-nitronaphthalenes (Atkinson et al., 1987). The reaction products and kinetics of the reaction of naphthalene with N_2O_5 were also studied. The rate constant was determined to be (1.4±0.2) x 10^{-17} cm^3 $molecule^{-1}$ s^{-1} and the major products

were 1- and 2- nitronaphthalene. The atmospheric lifetime of naphthalene due to reaction with N_2O_5 was calculated to be approximately 80 days for an estimated ambient N_2O_5 concentration of 2×10^6 molecules cm^{-3} during 12 hour night-time periods.

2.6.2 ENVIRONMENTAL EXPOSURE - WATER

Contamination in groundwater is a risk factor for human exposure only if there is an exposure pathway. At sites where contamination (*e.g.*, from legacy operations) is known to exist, exposure would likely be reduced/minimized by deed restrictions or local ordinances. Migration of contamination should be controlled by remediation. The Panel believes that current anthropogenic groundwater contamination would be primarily related to historic activities/releases, not to current operations.

Indeed, EPA (2002) recently reported the following about the occurrence of naphthalene in Public Water Systems (PWSs):

> EPA also finds that naphthalene has a very low occurrence in PWSs. Naphthalene at ≤½ health reference level (HRL) was found at approximately 0.01% of public water supplies surveyed in Round 1 and Round 2 cross section samples, affecting less than 0.007% of the population served. Because naphthalene has such a low occurrence level, EPA finds that the regulation of naphthalene in drinking water does not present a meaningful opportunity for health risk reduction for persons served by PWSs.

2.7. OCCUPATIONAL EXPOSURE

As noted in the comment on Section 2.1, many of the uses listed are not relevant in the US in 2002, and thus, occupational exposures related to those uses are likewise not relevant in the US.

NTP cites the National Occupational Exposure Survey (NOES) (NIOSH, 1983), conducted from 1981 to 1983, as the source of the estimate "*that 112,702 workers potentially were exposed to naphthalene.*" EPA (1999) has said, "*Now over 15 years old, the NOES data have become progressively dated, and as a consequence, less representative of current exposure situations.*"

The current total employee population in Koppers Industries' tar distillation and wood preserving plants and phthalic anhydride production facilities with a potential naphthalene exposure was 1,020 in 2000 and 1,000 in 2001 and will average 990 in 2002. Koppers Industries is the largest employer in the tar distillation and wood preservation industries in the US. The number of exposed employees given in the background document appears excessive even for 1983.

Fewer than 50 workers with potential naphthalene exposure are employed in the moth repellent industry on a direct basis.

The estimate of over 112,000 workers exposed to naphthalene thus seems highly unlikely. Although naphthalene is used in applications in Europe that are not applicable in the US, the Panel believes the occupational exposure assessment discussed in the EU's Naphthalene Risk Assessment Report (EU, 2002) to be more representative of exposure in the US than the discussion in NTP's draft

background document. The EU Risk Assessment Report (EU, 2002; Section 4.1.1.1.2) contains the following estimates of workers exposed during naphthalene manufacturing in the EU:

> It is unrealistic to attempt to estimate the total number of workers exposed to naphthalene. The number exposed during naphthalene manufacture and subsequent use is estimated to be 250 to 500 in the UK and 1500 to 2000 in the EU (this does not include operators handling creosote treated timber or brush applicators or users of tar paints/membranes). The number exposed as a result of incomplete combustion of organic materials is likely to be significantly higher than these figures.

The Panel, whose members are naphthalene manufacturers, has no information about the "numbers exposed as a result of incomplete combustion of organic materials."

3. HUMAN CANCER STUDIES

Recent publications about naphthalene by the NTP (*e.g.*, NTP 1992, 2000) have cited three-decade old East German reports of health effects observed in tar distillation workers, and used these reports as part of the rationale for conducting assays in rodents. The production methods described in these studies have not been in use in North America in over 30 years.

NTP has, in its publications about naphthalene, used information contained in the East German reports (Wolf, 1976, 1978) to introduce a calculation that the data indicate a "greater than 4000-fold" increase in the incidence of the laryngeal cancers (NTP, 2000). This "4000-fold" figure appears to result from the ratio of 4/15 (incidence in naphthalene workers) to 6.3/100,000 (incidence in general male population in 1970). The increase in incidence given by Wolf (1978) was a factor of 62. His derivation is not transparent on the basis of the presented data, but it is reasonable to assume that he may have taken the age-adjusted control group into account, as he states that this kind of tumor takes appreciable time to develop and that all individuals who developed the tumors in the "naphthalene worker" population were beyond the age of 60. In evaluating the East German reports, NTP should also take into consideration that, although Wolf (1978) suggested that tar fumes in combination with heat as causative, all four workers who developed laryngeal cancer were smokers, and the 15 workers in the study all were likely to have been exposed to many confounding factors in the workplace described by Wolf. The published statement by NTP (NTP, 2000, page 20) that the Wolf data indicate a "4000-fold" increase in tumor incidence is an example of an inaccuracy that should be corrected in the Draft Background Document.

NTP (2000) also refers to a publication by Kup (1978) as though it contains additional information about workers exposed to naphthalene in East Germany. However, the Kup publication seems to be a lecture or presentation, apparently before a group of medical scientists or physicians. Kup's report is far from comprehensive and the four cancer cases are not the sole topic of his lecture. They are just mentioned without reference to any cohort, but are clearly the cases discussed in detail by Wolf (1976, 1978). The Draft Background Document should include accurate discussions of the Wolf (1976, 1978) and Kup (1978)

publications to correct misimpressions resulting from discussions in previous NTP publications about naphthalene, such as TR 500 (NTP, 2000).

The health effects information contained within the Wolf (1976, 1978) reports has been evaluated recently by German and UK authorities. Translations of Wolf's reports are included here as Attachments A and B. The German BAuA (1998) notes that the cases referred to by Ajao *et al.* (1988) involved oral intake of "a concoction containing naphthalene." Both the UK HSE Risk Assessment Report for Naphthalene (EU, 2002) and the German BAuA (BAuA, 1998) conclude that no conclusions can be drawn about the carcinogenicity of naphthalene from the limited information available in humans.

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.

As noted above, the Panel discussed the possibility of identifying a meaningful cohort for studying naphthalene exposure in manufacturing workers with NIOSH (see Attachment C). To date, NIOSH has not responded to the Panel's letter of May 2001, and has presumably concluded that a meaningful cohort of workers exposed to naphthalene is unlikely to be identified in the US. The Panel understands that naphthalene manufacturers in the EU were similarly contacted about identifying a cohort during development of the EU Risk Assessment, with a similar outcome. The Risk Assessment Report (EU, 2002; 4.1.1.1.4) provides the following estimates of the number of occupationally exposed workers during the manufacture of naphthalene:

> It is understood that about 10 persons (including maintenance operators) are exposed to naphthalene vapour during distillation of the coal tar to produce the naphthalene oil at each of the two UK tar distillation plants. The total exposed during the distillation of coal tar to produce the naphthalene oil throughout the EU was not established. However, it is estimated that it is in the region of 100 to 200 employees.
>
> During subsequent purification of the naphthalene by either distillation or crystallisation there are an estimated further 4 exposed in the UK, with a further 50 to 60 throughout the EU.

4. STUDIES OF CANCER IN EXPERIMENTAL ANIMALS

The Panel has commented previously on NTP's studies of cancer in rodents, and includes those comments here as Attachment D.

5. GENOTOXICITY

In addition to the Schreiner (in press) manuscript included here as Attachment E, a number of genotoxicity studies are not included in the draft background document. In particular no standard *in vivo* study is mentioned.

The following studies are missing:

Tests in vitro	Result	References
Cytogenetic assay: pre-implantation mouse embryo cells	positive	Gollahon *et al.*, 1990
Cell transformation	negative	Purchase *et al.*, 1978
Cell transformation (gamma GT foci) ***[note: included in Chapter 4!]***	negative	Tsuda *et al.*, 1980
UDS in vitro (rat hepatocytes)	negative	Barfknecht, 1985
Tests in vivo	**Result**	**References**
UDS assay on rat hepatocytes after in-vivo treatment	negative	RTC, 1999
Micronucleus assay (mouse, oral)	negative	Harper *et al.*, 1984
Micronucleus assay (mouse, i.p.)	negative	Sorg *et al.*, 1985,

6. OTHER RELEVANT DATA

p. 44/45, upper §, 6.2.1: The indices 1, 2, and 3 of the GSH conjugates are not clear because they are not depicted in the referenced Fig 6-1 (p. 45).

p. 58/59, Chapter 6.5:
Table 6-4 appears to be of little relevance for the elucidation of naphthalene properties, as genotoxicity and carcinogenicity of the amine derivatives are sure to share a mechanism different from that of naphthalene activation.

References

Ajao, O.G., Adunuga, M.O. and Ladipo, J.K. (1988). Colorectal carcinoma in patients under the age of 30 years: a review of 11 cases. *J. R. Coll. Surg. Edinb.* 33: 277-279.

Barfknecht, T.R. 1985. Rat hepatocyte primary cultures/DNA repair test: Naphthalene. EPA Regis. No. 62766-1 (1991). Pharmakon Research International, Inc., Waverly, PA.

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Attachment A

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Cancer Morbidity amongst Chemical Workers from a Former Naphthalene Cleaning Plant

Author: O. Wolf

Summary

Despite the large number of chemical substances and compounds with a carcinogenic effect used in the working environment, very few cases of suspected occupational cancer have been notified. Here we report on a cluster of 7 cases of morbidity amongst 15 former naphthalene cleaning workers. On the basis of the results of animal experiments, naphthalene and dichlordiethylether are suspected of being carcinogenic. Chronic irritations of the mucous membranes and the effects of heat are suspected of syncarcinogenesis. The chance nature of the discovery of such a relationship suggests the need for greater co-operation between clinical doctors, oncological centres and occupational doctors.

The Problem

The number of chemical substances and compounds which are carcinogenic in animal experiments has risen to more than 1000 and, of these, approximately 40 are recognised as being carcinogenic in humans (14). Many of these substances are also found in the working environment such as e.g. arsenic, asbestos, beryllium, nickel, chromium, tar, soot, bitumen, benzol and many other hydrocarbon compounds(1, 10, 11, 13). In the list of occupational diseases of 14.11.1957 No. 18 is listed as skin cancers caused by work-related carcinogenic effects, No. 19 as cancers of the urinary tract due to aromatic amines and No. 31 as cancers of the respiratory tract due to occupational carcinogenic effects (12).

The number of suspected occupational cancers which are notified each year is very small and this is surprising in view of the large number of known carcinogens which are to be found in the working environment. For example, in the German Democratic Republic only 26 cases of cancer were recognised as an occupational disease between 1960 and 1971 and 32 cases in 1972 (14). In 1973 the number of acknowledged new cases of occupational disease 31 increased to 77. This is due to a recording

phenomenon, since 63 of these new cases were due to asbestos (23).

One reason for the large number of unreported cases of occupational cancer is that too little has previously been known about the relationship between occupational exposure and malignant tumours. Secondly, it is difficult to identify a relationship because:

1. from the description of the occupation e.g. specialist chemical worker, it is not immediately possible to identify any possible harmful effects
2. a lot of workers and doctors are not aware of the possible harmful substances
3. because of the long latency periods, workers have often changed their jobs several times so that the causal relationships are obscured even further.

It is always necessary to think of the interaction between several harmful factors in the sense of syncarcinogenesis (1, 11).

Our Own Observations

A cluster of cancer cases, particularly of carcinomas of the larynx, amongst workers from a former naphthalene purification plant, were brought to our notice. This section was in existence between 1917 and 1968. Most of the employees had worked in the same job for several decades. The average period of exposure was 20 years with only 2 of the naphthalene cleaners having done this job for less than 10 years (cases 4 and 12).

In response to our enquiries, the factory gave us names of 15 workers who had worked in this section in the last 20 - 30 years of its existence. Five of these have died, four of them from cancers (Table 1). Two of them had died from cancer of the larynx (cases 1 and 4), one from a stomach cancer (case 3) and one from a caecum cancer (case 2). We were unable to ascertain the cause of death of one of the former naphthalene workers (case 5). Since the competent oncological centre had received no notification of him, cancer can probably be ruled out.

Table 1: Naphthalene cleaning workers who have already died

1. F. O.	7.12.1905 2.7.1970 (71)	Period of exposure 30 years. Smoker, 10 - 12 cigarettes per day. Dec. 1968 hoarseness, Feb. 1969 PE from a ruberous tumour of the whole right vocal chord. Histology: polymorphocellular, undiff. carcinoma, suspected metastatic HLC bds.
2. R. G.	4.6.1900 23.3.1971 (76)	Period of exposure more than 30 years. Non-smoker. 1920 lung TBC Section: manured tubo-alveolar adenocarcinoma of the caecum. Intrapulmonary metastasis. Chronic bronchitis.
3. Sch. O.	17.3.1901 25.6.1971 (75)	Period of exposure more than 30 years. Medical history unknown. Section: circular stenosing parvicellular scirrhoid solid carcinoma of the pylorus ventr. metastases.
4. R. P.	22.10.1901 15.7.1973 (75)	Period of exposure 7 years. Smoker, 2 - 3 packets of tobacco per week. Hoarseness from early age. Dyspnoea from 60 onwards. Tracheotomy and PE of a stenosing tumour of the larynx due to acute deterioration on 1.1.1973. Section: circular stenosing keratinizing squamous cell carcinoma of the entire larynx, paratracheal metastases.
5. H. W.	16.8.1997 ? (79)	Period of exposure 27 years. Smoking habits unknown. Clinical: chronic bronchitis, coronary insufficiency, cirrhosis of the liver.

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Table 2. Naphthalene workers treated for cancer

6. K. W.	7.5.1904 (72)	Period of exposure 16 years. Smoker, 10 cigarettes per day. Hoarseness since 1960, 1964 total laryngectomy because of cancer of the larynx.
7. A. O.	1.12.1908 (66)	Period of exposure 19 years. Smoker, 5 cigarettes per day. 1965 histologically confirmed Hodgkin's disease. Diabetes mellitus, hoarseness since 1946.
8. R. E.	7.10.1912 (66)	Period of exposure 18 years. Smoker, 5 cigarettes + per day. Hoarseness since 1972. 1973 total laryngectomy because of cancer of the larynx. Chronic pneumato-bronchitis, Lupus erythmatosus, left cheek.

Table 3. Free from cancer symptoms

9. St. E.	2.5.1900 (76)	Period of exposure 28 years. Occasional smoker. previous gastric complaints, chronic pharyngitis.
10. Th. F.	6.8.1902 (74)	Period of exposure 21 years. Non-smoker. 1924 otitis medius, renal and biliary colic.
11. S. A.	21.11.1909 (67)	Period of exposure 32 years. Smoker, 10 - 15 cigarettes per day. 1940 ear-radical-op, chronic laryngitis.
12. F. W.	24.11.1911 (65)	Period of exposure 2 years. Smoker, 1 packet of tobacco per week. 1960 lung section because of tuberculosis.

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13. J. J.	17.10.1915 (61)	Period of exposure 18 years. Smoker, two packets of tobacco per week. Hoarseness since 1950s, polyps on vocal chords removed twice, chronic laryngitis.
14. P. K.	22.12.1917 (54)	Period of exposure 20 years. Smoker, 10 - 15 cigarettes per day. Chronic rhino-pharyngitis-laryngitis sicca
15. K. H.	18.3.1929 (47)	Period of exposure 18 years. Smoker, 10 cigarettes per day. Chronic pharyngitis-laryngitis.

Of those who are still alive, three have been treated for a tumour or a systematic disease (Table 2).
Two were operated on for carcinoma of the larynx and have so far been recurrence-free for 11 and 2 years respectively (cases 6 and 8).
One has survived a histologically confirmed malignant granuloma [Hodgkin's disease] after treatment with endoxane and radio-therapy and has been symptom-free for 5 years (case 7).
As yet none of the remaining 7 workers from the former naphthalene cleaning plant (Table 3, cases 9 -15) is known to be suffering from any malignant disease. The control studies done on these 7 workers revealed five cases of chronic pharyngitis-laryngitis, which is recognised as one of the factors favouring carcinogenesis (5, 18).

The information which was obtained about smoking habits, where this was possible, cannot be regarded as very objective. A comparison between the cancer sufferers and those who had remained disease free did not reveal any striking differences between them.

Technology of Naphthalene Purification

The crude naphthalene which is produced from coal tar was bought in 20 - 30 kg. blocks. It was then melted at 80 - 85°C in underground, closable melting boilers on the open factory site. The melted product was then sucked out into 7.5 t cleaning reactors and heated to 130°C and the remaining water content distilled off. Sodium metal was then added whilst stirring to remove the sulphur. After a reaction time of a few hours, some of the naphthalene was then driven off by increasing the temperature to 180°C. The residue was filled up with crude naphthalene. This was again followed by desiccation and desulphurisation with subsequent distillation. These cycles were repeated up to eight times. The residue was then sucked into a vessel containing water (separating funnel). After sodium sulphide and sodium metal had been scrubbed out, the tar residue was distilled once again. After it had cooled down to approximately 140°C, the remaining naphthalene hard pitch was poured into moulds in an open hall. The technical naphthalene produced by the multiple distillation processes was subject to certain purity requirements in accordance with the terms of TGL 2758. The maximum sulphur content was 0.5 % and the maximum tar content 0.14 %.

Potential for Harm

The total naphthalene purification workers comprised 10 - 12 people. Since the work was done on a 4-shift system with an average of 2 -3 people per shift, all carrying out all of the necessary work, all of the employees were exposed to the same harmful substances.
Most of the naphthalene cleaning process took place in a closed vacuum system. Due to the technology, the only possibility of contact with tar products was during filling of the melting boilers with crude naphthalene, emptying the distillation residue, the so-called naphthalene hard pitch, and also during repair of the apparatus, pipework and such like when there was a possibility of contamination. The crude naphthalene which was delivered in wagons, was intermediately stored in the factory yard and the melting boilers had to be refilled several times a day, 1 - 2 times per shift, using wheel-barrows. When the boilers were opened, tar-laden naphthalene sublimate rose out. The colourless crystal flakes with the

characteristic moth-ball smell would shimmer in the sunlight. Each crude naphthalene recharging procedure lasted approximately 2 hours.
The MAK[1] value for naphthalene is set at 20 mg/m^3 with admissible peak concentrations of up to 50 mg/m^3 (10, 13). Since, at that time, there was no way of determining the MAK value for naphthalene, workplace concentrations were not determined in the former naphthalene cleaning plant. There are no MAK values for tar and pitch vapours.

Naphthalene, $C_{10}H_8$, is hardly water soluble but is readily fat-soluble. It is incorporated into the human organism by inhalation or swallowing of dust particles and causes irritation of the mucous membranes. The incorporation of high concentrations leads to headaches, vertigo, optic neuritis and haematuria (10, 13, 20).
As yet there is no information available about the carcinogenic effect of naphthalene in humans (1, 9), although, in experiments with rats and mice, the substance displayed slight carcinogenic effects (21).

When the tar was drained off, 2 -3 times per week, the hard pitch was poured into moulds in the open hall at approximately 140°C. During this process the room was practically fogged up. The tar vapours irritated the mucous membranes, irritated the throat and caused fits of coughing. The process lasted approximately 1 hour. Tar and its derivatives are the earliest known occupational carcinogens. In 1975 Pott described "chimney sweeps' cancer" and in 1975 Volkmann described the skin cancer of the tar worker (1, 9).
The endangered workers are those who come into contact with tar, soot, pitch, paraffin, asphalt, naphthalene, tar oil and similar (11). This can include chimney sweeps, coal-tar workers, pitch workers, roofers, workers cleaning distillation apparatus, pitch crushers (11).

The carcinogenity of the tar is determined by its 3 - 4 benzpyrene content (1, 8).
Mostly the effects are via the skin or the respiratory tract and consequently the main organs which are affected are the skin, the larynx and the lungs (1, 11), but the carcinogenic hydrocarbons are capable of inducing cancer

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1 MAK = Maximale Arbeitsplatz-Konzentration
Maximum workplace concentration

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in any organism and in any organ (1). The average latency is given as 20 - 24 years (1 - 50 years) (11).

In accordance with the technology, the naphthalene hard pitch, the residue from naphthalene /purification was drained off at 130 -140°C. However, sometimes they did not wait for it to cool and the hard pitch was drained off at higher temperatures (approximately 200°C), so that the naphthalene workers could also be exposed to the effects of heat at this time and this is regarded as a determining factor in the mechanism of syncarcinogenesis (1, 6, 13, 18).

Between 1958 and 1968 aroxane distillation was also done in the same section. Aroxane is a dixylenyldiethylether which is made from dimethylphenol and dichlordiethylether and was used as a plasticiser for paints. It was processed in a closed system. It was only as the distillation residue was being drained off that there was brief contact with vapours, which are said to be caustic. This work, which had to be done once a week, lasted for half an hour and was generally very unpopular because of the extremely unpleasant smell. Interestingly, of the raw materials used in aroxane distillation, dichlordiethylether is also known to be an effective carcinogen from animal experiments, even though there are as yet no observations for humans available for this substance either.

Conclusions

Because of the cluster of 7 malignant diseases in this small group of 15 people, who were exposed to the same occupational hazards, we must suspect a relationship between the influence of occupational noxae and the occurrence of cancer and this caused us to notify the carcinomas of the larynx as suspected cases of occupational disease 31 and also to notify our suspicion of the other tumours as an occupational disease. The assessment of these cases is still outstanding.

The chronic irritations of the mucous membrane (caused by naphthalene sublimate during refilling of the crude naphthalene, by residual vapours from aroxane distillation and pitch vapours during emptying of the naphthalene residue) are also under suspicion as syncarcinogens as are the tar-vapours as a known carcinogen and the effects of heat.

Because of the complex process of carcinogenesis in occupational cancer with long affection and latency periods, because of the multiplicity of chemical and physical noxae with cumulative and synergetic effects, it is always difficult to identify a possible relationship between occupation and the occurrence of cancer in an isolated case. Thus, even in the seven cases of malignant disease described amongst the naphthalene workers, such a relationship was not suspected in each individual case. It was not until one of the patients with a carcinoma of the larynx mentioned that three of his colleagues were already suffering from throat cancer that we decided to look at the question of a relationship and discovered the other cases of cancer. The chance nature of the discovery of such a relationship is worrying and leads us to consider how the co-operation between clinical doctors, oncological centres and works doctors can be improved in order to uncover the large number of unreported cases of occupationally-induced cancers.

One possible approach would be if:

1. for each cancer patient the possibility of an occupationally related tumour is considered and the occupational history is thoroughly searched for any possibility of harmful substances.
2. more details of possible occupational effects are given on Form II for the notification of a notifiable cancer. Particularly if the patients have had jobs in which they have been exposed to chemical and physical hazards for more than 10 years, even if (or particularly if) this was many years ago.
3. if there is the slightest suspicion of an occupational cancer, the works doctors who are aware of the industrial production processes should be informed so that they can give an opinion regarding the hazard, thus making it possible to record epidemiological and statistical data so that any clustering of cancer cases in circumscribed working collectives can be identified.
4. works doctors register any workers whose work involves particularly high levels of exposure so that, in addition to the routine occupational examinations, preventative medical examinations can also be carried out.

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Attachment B

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Carcinoma of the larynx in workers engaged in the purification of naphthalene

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Summary

Exogenous factors are primarily involved in the genesis of cancer of the larynx. Nevertheless, the number of notifications of occupational disease no.31 (occupationally-induced cancer of the respiratory tract) is small. This paper reports on four cases of laryngeal cancer in a group of 15 persons who had been engaged in the purification of naphthalene; this incidence is highly significant. Chronic irritation of the mucous membranes and the effects of heat, coal-tar fumes and cigarette smoke have been indicted as syncarcinogenic factors. The possibility of occupationally-induced tumours should always be borne in mind in the case of workers exposed to these carcinogens.

Introduction

Exogenous causes are prime suspects in the causal pathogenesis of cancer of the larynx. Harmful chemicals are particularly important in addition to physical hazards in the form of mechanical, thermal and actinic irritation.

The number of persons occupationally exposed to the effects of carcinogenic substances is steadily increasing as a result of technical progress and the increasing use of chemicals. Although the number of occupational carcinogens used in industry has greatly increased, the number of suspected cases of occupationally-induced cancer which are reported annually remains small (see Table 1). The number of confirmed cases of occupationally-

induced cancer has risen slightly in the last few years but the number of unreported cases is still large.

In the period 1957–1967, 20 (19 bronchial carcinomas and 1 laryngeal carcinoma [7]) out of 56 notifications of suspected occupationally-induced cancer were recognised as being due to occupational disease no.31 (occupationally-induced cancer of the respiratory tract).

Table 1: The number of new notifications of occupational disease no.31 in relation to the total notifications of occupational diseases and the new notifications of malignant diseases in men [9]

	1967	1968	1969	1970	1971	1972	1973	1974
Total occupational diseases Occupational disease no.31	11,177 3	11,904 Ø	11,460 2	11,077 5	11,539 5	11,934 24	11,800 77	11,789 42
Larynx (161 Trachea, bronchus, lung (162) Total malignant tumours in men	475 6,249 25,347	524 6,647 27,132	483 6,583 26,965		495 6,726 27,490	517 6,496 27,322		

Author's observations

The author was aware of four cases of laryngeal cancer among a group of 15 workers who had been previously engaged in the purification of naphthalene [6]. This represents a 62-fold higher incidence of laryngeal cancer in this group of men when compared with the incidence of laryngeal cancer in the German Democratic Republic (GDR) for 1970 (6.3 per 100,000 males). Calculation of the significance of this difference with the t-test (Student's distribution) showed that the probability of error was 1% and that there thus was a highly significant difference between the incidence of laryngeal cancer in naphthalene workers and that in the remaining male population.

Since all workers in the group had been engaged in the same type of work, they had had the same opportunity for occupational exposure. Naphthalene purification was carried out from 1917 to 1968 and involved the processing of crude naphthalene, a product of coal-tar, to produce technical-grade naphthalene by repeated distillation processes. The distillation of Aroxan was also carried out in the same department from 1958 to 1968. Aroxan is a dixylenyldiethyl ether, prepared from dimethylphenol and dichlorodiethyl ether, used in industry as a plasticiser for varnishes.

Tar and its derivatives have long been known to be occupational carcinogens [1]. Naphthalene, dimethylphenol and dichlorodiethyl ether have also been shown to be carcinogenic agents in animal experiments [5].

Chronic irritation of the mucous membranes occurred during the production process due to the sublimed naphthalene produced while charging the still with crude naphthalene, the residual vapour produced during the distillation of Aroxan, and the fumes of pitch produced when removing the residual naphthalene. In the case of workers in naphthalene purification, the actual carcinogenic factors are thought to be the fumes of coal-tar and the effects of heat [6].

Case histories

Patient 1. K.W., date of birth: 07.05.1904

Period of exposure 16 years. Latent period 16 years. Smokes 10 cigarettes/day and cigars. Repeated attacks of hoarseness; renewed hoarseness and breathlessness for 3–4 weeks before the first ENT consultation on 5th October 1964. Total laryngectomy on 23rd October 1964. Free from recurrences. At operation the tumour was found to involve the left vestibular ligament, the left vocal cord and arytenoid cartilage, the anterior commissure, the laryngeal surface of the epiglottis, the anterior third of the right vocal cord, and the left sub-glottal area. No metastases.
Histology: a solid immature squamous-cell carcinoma.

Patient 2. F.O., date of birth: 07.12.1905, date of death 02.07.1970

Period of exposure 31 years. Latent period 32 years. Smoked 10–12 cigarettes/day. Bronchitis since 1967. Hoarseness in mid-December 1968. First ENT consultation on 31st January 1969.
Biopsy and histological diagnosis of tumour on 4th February 1969. The tumour spread during in-patient treatment. An extensive bosselated tumour of the right vocal cord. Bilateral enlarged lymph nodes in neck.
Histology: polymorphocellular undifferentiated carcinoma.

Patient 3. R.P., date of birth: 22.10.1901, date of death: 15.07.1973

Period of exposure 7 years. Latent period 13 years. Smoked 2–3 packets of tobacco per week. Hoarse since childhood. Dyspnoea since the early 1960s. Exploratory operation advised in 1971 due to a bosselated tumour in the centre of the right vocal cord. Acute deterioration on 1st January 1973; emergency tracheostomy, exploratory operation. The circular and stenosing tumour involved the whole of the larynx.
Histology: horny squamous-cell carcinoma.

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Patient 4. E.E., date of birth: 07.10.1912

Period of exposure: 15 years. Latent period: 28 years. Smokes 15–20 cigarettes per day. Hoarseness since April 1972. First ENT consultation on 12th May 1972. Operation on 10th May 1973 with intra-operative histological examination. Total laryngectomy. Free from recurrences.

At operation the tumour involved the right vestibular ligament and the base of the epiglottis. Spread to the left vocal cord and prelaryngeal space, right ventricle and right vocal cord.

Histology: non-horny squamous-cell carcinoma.

Discussion

All these laryngeal carcinomas observed in naphthalene purification workers appeared after the age of 60. According to the GDR statistics [9], the general age-distribution of laryngeal cancer shows a 70% excess after 60 years of age with the mean age for occupationally-induced cancer of the respiratory tract being 62.5 years for the year 1975 and a mean exposure period of 18 years.

In the case of my patients, the mean period of exposure to mucosal irritants and carcinogenic substances was 17.5 years and the mean latent period was 21 years. Hueper [3] quotes the mean latency for cancer caused by tar as 20 to 40 years (range 1 to 50 years). In the case of malignant disease in industry, Bittersohl [2] found the latent period to be less that 10 years in 13.5% of cases, from 10 to 30 years in 43.5% of cases and upwards of 30 years in 43% of cases.

All my patients were smokers of more than 10 cigarettes per day or 2 to 3 packets of tobacco per week. Along with occupational carcinogens, cigarette smoke is regarded as a causal factor in the genesis of laryngeal cancer [4].

The histological findings did not reveal any particular features suggestive of occupational cancer. Squamous-cell carcinomas typical for the larynx were predominant; these carcinomas were present in 95% of the cases.

The spread of the tumours was very extensive by the time the disease was recognised and in two cases the enlargement of the lymph nodes in the neck was suggestive of the presence of metastases. For this reason, one of these patients with an increased surgical risk due to pulmonary emphysema, cor pulmonale and mitral valve defect was not

operated upon, while the other patient refused operation. Two of the patients were operated upon in the University ENT Department in Halle and are free from recurrence after 12 and 13 years respectively.*

In the case of two patients, the time from confirmation of the diagnosis of cancer to starting treatment was less than three weeks. Patient 3 declined an exploratory operation which had been advised two years previously and also refused a later operation. In the case of Patient 4, five biopsies were performed within one year due to a suspected tumour but none of these produced any evidence of malignancy. Nevertheless, an operation was performed owing to continuing clinical suspicion of a tumour and the intra-operative histological examination confirmed the diagnosis of a tumour which had already spread.

The expert medical assessment of my four patients, who were suspected of suffering from an occupational disease, led to the recognition of their malignant disease as occupational disease 31.

The possibility of a neoplasm caused by occupation should always be borne in mind with every tumour patient, the more so, if the patient has been exposed for more than 10 years to harmful chemical or physical agents in the workplace.

A review of substances which can act as carcinogens in humans or animals has been compiled by Teichmann and Schramm [5].

ORIGINAL

References

[1] Bauer, K.H.: Das Krebsproblem (The problem of cancer). Berlin/Heidelberg/New York: Springer-Verlag 1963.

[2] Bittersohl, G.: Arch. Geschwulstforsch. 1971, 38, 198.

[3] Hueper, W.C.: Berufskrebs (Occupational cancer). Dresden and Leipzig: Verlag Theodor Steinkopff 1964.

[4] Leicher, H.: Bösartige Geschwülste des Kehlkopfes und Hypopharynx (Malignant tumours of the larynx and hypopharynx). In: Handbuch der HNO-Heilkunde II/2, edited by: Berendes, J., Link, R. and Zöllner, F., Stuttgart: Georg Thieme Verlag 1963.

*I thank Prof. Dr H. Jakobi, Director of the University ENT Department in Halle, for providing the medical records of these patients.

[5] Teichmann, B. and Schramm, T.: Substanzen mit kanzerogener Wirkung (Substances with carcinogenic effects), 2nd edition, Akademie der Wissenschaften der DDR, Zentralinstitut für Krebsforschung, Berlin-Buch.

[6] Wolf, O.: Dt. Gesundh.-Wesen, 1976, 31, 996.

[7] Zenk, H.: Z. Laryng. Rhinol., 1970, 49, 100.

[8] Zipfel, L.: Z. Laryng. Rhinol., 1974, 53, 909.

[9] Jahrbuch: Das Gesundheitswesen DDR. 1970, 1972, 1973, 1974, 1975. Akademie für Ärztliche Fortbildung der DDR. Berlin.

Author's address

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DDR-4502 Dessau-Alten.

ORIGINAL

Attachment C

Letter to Ms. Virginia O'Neill (NIOSH) from Courtney M. Price (ACC) on behalf of the Naphthalene Panel, dated May 4, 2001.

May 4, 2001

Via E-mail and US Mail

Ms. Virginia O'Neill
National Institute for Occupational Safety and Health (NIOSH)
4676 Columbia Parkway
Cincinnati, Ohio 45226-1998

e-mail: vlr1@cdc.gov

Dear Ms. O'Neill:

The Naphthalene Panel of the American Chemistry Council submits this letter in response to conversations with you in which you indicated that NIOSH is interested in obtaining cohort information about worker exposure to naphthalene. The Panel is comprised of the major domestic producers and importers of naphthalene.

In principle the Naphthalene Panel would be pleased to assist NIOSH in obtaining relevant information in this regard. However, NIOSH should be aware that locating a meaningful cohort for studying naphthalene exposure to manufacturing workers is likely to be difficult, if not impossible. This is because the size of the domestic work force is extremely small -- approximately 200 workers -- and has remained constant at this size over the past 15 years.

Even if a large enough manufacturing worker cohort could be located for study, the Panel questions the basis for NIOSH's apparent investment in worker exposure to naphthalene at this time because the reported exposure levels are well below regulatory levels of concern.

Because the membership of the Naphthalene Panel consists of companies engaged in the manufacture of naphthalene, the Panel does not have direct access to information regarding non-manufacturing exposures to naphthalene, such as ambient naphthalene resulting from the combustion of fuel. As you are no doubt aware, however, the U.S. Environmental Protection Agency (EPA) has included naphthalene in its recent final rule addressing mobile source air toxics (MSAT) emissions from motor vehicles and their fuels, and some pertinent information is available for that source.[1] The MSAT rule requires refiners to maintain the current toxic emissions performance standards for reformulated and conventional gasoline. EPA declined to set any additional vehicle-based air toxics controls in this rule.

Inclusion in the MSAT rule does not represent "a determination by EPA that emissions of the compound in fact present a risk to public health or welfare, or that it is appropriate to adopt controls to limit the emissions of such a compound from motor vehicles or their fuels."[2] Rather, in EPA's words, "[t]he purpose of the list is to provide a screening tool that identifies those compounds emitted from motor vehicles or their fuels for which further evaluation of emissions controls is appropriate."[3]

EPA's Technical Support Document for the rule identifies naphthalene as both a fuel component and an exhaust component.[4] As EPA explains in the rule,

> The majority of gaseous MSATs are hydrocarbons that are primarily the result of incomplete combustion of petroleum fuels. Since a small amount of raw fuel passes through the engine unburned, MSATs present in the fuel are also emitted in the exhaust.[5]

The Technical Support Documents states: "Naphthalene is found in small quantities in gasoline and diesel fuels."[6] It also states: "Naphthalene emissions have been

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1 66 Fed. Reg. 17230 (Mar. 29, 2001).

2 66 Fed. Reg. at 17234.

3 *Id.*

4 EPA, Air and Radiation, "Technical Support Document: Control of Emissions of Hazardous Air Pollutants from Motor Vehicles and Motor Vehicle Fuels," 420-R-00-023 (Dec. 2000) at 142 (Technical Support Document).

5 66 Fed. Reg. at 17242.

6 Technical Support Document at 75.

measured in larger quantities in both gasoline and diesel exhaust and evaporative emissions from mobile sources."[7] EPA also states, however, that it does not have baseline inventory data for naphthalene emissions.[8] As noted above, EPA has determined that no additional regulatory controls are necessary at this time for naphthalene and the other MSAT compounds.

In summary, based on the small number workers exposed in the US during naphthalene manufacturing and the fact that reported naphthalene exposures of these workers are well below regulatory levels of concern, the Panel believes that it would be ill advised and unreasonable for NIOSH to expend its limited resources on studying such workers. Information on non-manufacturing exposures to naphthalene may be available from other sources, such as EPA's MSAT rule.

The Panel hopes that the information that it has provided has been helpful. Please direct any questions to Anne P. LeHuray, Ph.D., Manager of the Naphthalene Panel, at (703) 741-5630.

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

cc: Naphthalene Panel

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7 *Id.*

8 66 Fed. Reg. at 17238.

Attachment D

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*

September 24, 2001

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council *Good Chemistry Makes It Possible*

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)	)

Courtney M. Price
Vice President
CHEMSTAR

David F. Zoll, Esquire
Vice President and
General Counsel

Anne P. LeHuray, Ph.D
Manager, Naphthalene Panel

Theodore R. Waugh, Esquire
Counsel, CHEMSTAR

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Lynn L. Bergeson, Esquire
Lisa M. Campbell, Esquire
Richard P. Bozof, Esquire
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Washington, D.C. 20036-2401

September 24, 2001

AMERICAN CHEMISTRY COUNCIL
1300 Wilson Boulevard
Arlington, VA 22209
(703) 741-5000

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.

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

NAPHTHALENE NASAL TUMORS IN RATS – RELEVANCE TO HUMANS

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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 (6^{th} 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 naphtha lene 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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Attachment E

Genetic Toxicity of Naphthalene: A Review

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Genetic Toxicity of Naphthalene: A Review

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ABSTRACT

Results of five previously unpublished studies of the genotoxicity of naphthalene are presented and extensively discussed in relation to the large database that exists in the published literature. According to the published literature, naphthalene has not induced gene mutations in bacterial assays or in a metabolically competent human cell line. However naphthalene has caused cytotoxicity in some cell lines, and induced clastogenicity in CHO cells, in a human lymphoblastoid cell line and in preimplantation mouse embryos. Some naphthalene metabolites were cytotoxic but only naphthoquinones produced chromosomal damage *in vitro*. No chromosomal damage was observed *in vivo* in bone marrow erythrocytes from treated mice; however a positive response reported in a *Drosophila* assay for wing somatic mutation and recombination. The five unpublished studies of naphthalene genotoxicity include three studies *in vitro* (2 Ames bacterial assays, and an in vitro unscheduled DNA synthesis assay) and two *in vivo* (mouse micronucleus and in vivo unscheduled DNA synthesis). Naphthalene was inactive in all 5 studies, in agreement with reports in the published literature.

Chronic inhalation of naphthalene over 2 years induced an increased incidence of benign alveolar/bronchial adenomas, in female mice, and nasal epithelial tumors in both sexes of rats. Inflammation, tissue damage, and subsequent regenerative hyperplasia at target organ sites occurred in both species. Results of standard genetic toxicity assays suggest that naphthalene is not likely to be genotoxic *in vivo*. Since the *in vitro* results come primarily from assays utilizing liver-mediated activation systems, and the *in vivo* results come from rodent organs that are not targets for tumors, tests using naphthalene -sensitive rodent tissues would determine the applicability of current data in addressing the mechanisms of these species and site-specific cancers. The standard assays reported here may be useful in predicting potential health hazard in other species, or in humans, in whom there are few reported instances of naphthalene-induced cancer, especially as more data on species-specific differences in naphthalene metabolism become available. Despite present data limitations, a threshold mechanism for tumorigenesis can be proposed. The absence of naphthalene-induced gene mutation and the presence of cytotoxicity and some chromosomal events *in vitro* are consistent with a threshold-related mechanism of tumor induction driven by cytotoxicity and cell regeneration, followed by genetic events.

INTRODUCTION

Naphthalene ($C_{10}H_8$; CAS #91-20-3) is an aromatic hydrocarbon composed of two fused benzene rings with a molecular weight of 128.16. It is a white solid with a characteristic odor of mothballs (vapor pressure 0.087 mmHg), with a maximum achievable vapor concentration (without aerosol production) of approximately 80ppm at ambient temperature (NTP, 2000), and sublimes slowly at room temperature. It is very slightly soluble in water (approx. 0.03g/l) but is appreciably soluble in organic solvents such as alcohol and benzene. Naphthalene is produced by the distillation and fractionation of petroleum or coal tar. Its principal use is as an intermediate in the production of phthalic anhydride for the manufacture of plasticizers, leather tanning agents and the insecticide, carbaryl (Sevin®), and is a constituent of creosote. It is also a moth repellant, an air freshener, a deodorizer for diaper pails and toilets, and is present in cigarette smoke as a pyrolysis product. In past medical practices, naphthalene was used as an antiseptic, anthelminthic, and dusting powder in treatment of skin diseases. (IRIS 1998; UK HSE, 2001).

The purpose of this review is to summarize the genetic toxicology information on naphthalene, including results of data from several unpublished studies that contribute valuable information to the overall database. Summary tables present 16 bacterial assays, 9 cytogenetic assays (7 *in vitro*, 2 *in vivo*) and 13 assays from other systems, including 6 cell transformation assays, 3 unscheduled DNA synthesis assays, two alkaline elution assays, one *Drosophila* assay and a human cell gene mutation assay. Naphthalene did not induce positive responses in 30 *in vitro* assays with non-mammalian and mammalian cells and gave negative results in all 4 assays in which mammals were exposed. Positive results were reported only in the NTP *in vitro* chromosome aberration assay, an *in vitro* micronucleus assay in a human lymphoblastoid cell line, an *in vitro* mouse embryo chromosome assay and the *Drosophila* assay.

The unpublished studies presented in detail here include two Ames *Salmonella* assays, an *in vivo* Micronucleus assay and an *in vitro* Unscheduled DNA Synthesis assay in primary rat hepatocytes performed by Pharmakon Research International, Waverly, PA. These study results have been submitted to the EPA and were cited as unpublished studies in the 1998 IRIS Toxicology profile and other regulatory dossiers. The *in vivo/in vitro* UDS assay is a recent study monitored by RÜTGERS VFT AG, Germany, sponsored by the International Tar Association and performed at Research Toxicology Center (RTC), Rome, Italy.

Naphthalene can be absorbed orally, dermally and by inhalation. Health hazards from excessive exposure include hemolytic anemia accompanied by jaundice, headache, confusion, nausea and vomiting, cataracts, and toxicity to the respiratory tract. Children and infants exposed to naphthalene vapor or dermal contact from clothing or bedding stored in mothballs may also develop neurological symptoms characterized by lethargy and decreased crying, which may be secondary to decreased oxygen carrying capacity of blood. Although exposure of neonates can result in death, cessation of exposure usually allows recovery from symptoms and toxic effects.

It is recognized that toxicity of naphthalene is metabolically mediated. The first step in mammalian naphthalene metabolism is oxidation, catalyzed by cytochrome P450 oxygenases, to its electrophilic arene epoxide intermediate, naphthalene-1,2-epoxide; both enantiomers may be formed. The epoxide has a very short half-life of 3.6 minutes (Buonarati et al., 1989) and spontaneously rearranges to form naphthols (primarily 1-naphthol), leading eventually to the formation of naphthalene diols and naphthoquinones. The epoxide can be enzymatically conjugated with glutathione by glutathione S-transferases to form a variety of glutathione conjugates. These are excreted as n-acetylcysteine conjugates in the urine. 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- hydrodiol by catechol reductase, thence oxidized to naphthoquinone. (IRIS, 1998). Naphthols may undergo further hydroxylation, catalyzed by O_2/$NADPH_2$-dependent monooxygenases to result in naphthalene diols, thence to 1,2- and 1,4-naphthoquinones through enzymatic and autocatalytic oxidation (fig.1).

The National Toxicology Program (NTP 1992, 2000) has performed 2-year cancer bioassays of naphthalene in mice and rats. In the 1992 study in which male and female B6C3F1 mice were exposed by whole body inhalation to naphthalene vapors at concentrations of 0, 10 or 30 ppm for two years, a statistically significant increase in alveolar/bronchiolar adenomas in the high dose females (28/135 mice) and one high dose female with an alveolar/bronchiolar carcinoma were reported. The combined incidence of alveolar/bronchiolar adenomas and carcinomas (22%) in high dose females was above those for control mice in NTP feed, water and inhalation studies (7.8%, range of 0-16%) and was attributed to naphthalene exposure. The incidence of adenomas in male mice increased with dose but did not reach statistical

significance. In both sexes, naphthalene exposure was associated with chronic inflammation, metaplasia of olfactory epithelium and hyperplasia of respiratory epithelium in the nose, and chronic inflammation in the lung. The NTP Peer Review Panel evaluated this study as indicative of "some" evidence of carcinogenicity in female mice and "no evidence" in male mice. In the NTP bioassay reported in 2000, in which male and female Fischer 344/N rats were exposed to naphthalene at 0, 10, 30 and 60 ppm for 2 years (105 wks), a significant, dose-related increase in adenomas of the respiratory epithelium of the nose occurred in males in all exposed groups with a maximum incidence of 31% at the highest dose, and in females at 30 (8%) and 60 ppm (4%) groups. Neuroblastomas of the olfactory epithelium occurred with positive trends in both sexes; in females at all doses with a maximum incidence of 24% at the highest dose and in males at 30 (8%) and 60 ppm (6%). Non-neoplastic inflammatory changes were also present in nasal epithelium. Since these neoplasms are rare and were not seen in concurrent chamber controls or in historical chamber control rats from NTP 2 year inhalation studies(0/1048 males; 0/1044 females), results were considered to constitute clear evidence of carcinogenic activity of naphthalene in F344N rats, under conditions of this assay. In both the mouse and rat inhalation studies, repeated exposure to naphthalene produced extensive cytotoxicity, chronic inflammation, and regenerative hyperplasia to the nasal epithelial cells at all exposure concentrations.

SUMMARY OF PREVIOUSLY PUBLISHED STUDIES

Bacterial assays: Table 1

Published studies, in general, indicate that naphthalene, tested at maximum non-toxic doses does not induce gene mutation in *Salmonella typhimurium* standard testing strains without or with metabolic activation from rat or hamster liver homogenate (S9). Carrier solvents employed in these systems included dimethyl sulfoxide, acetone, ethanol, and incorporation of naphthalene without carrier into culture medium. The absence of mutagenic response was similar in all cases. In one study, Narbonne et al. (1987) reported a small increase in revertant colonies in TA1535 at naphthalene concentrations of 5 and 10 ug/plate but not at higher concentrations, yielding an overall negative finding. Metabolites of naphthalene, 1-naphthol (McCann et al., 1975, Narbonne et al., 1987), and naphthoquinone (Sakai et al., 1985) also did not induce mutation in *Salmonella*. Naphthalene also was negative in the *Salmonella* TM677 8-

azaguanine resistant assay, *Escherichia coli* rec and pol assays and did not induce SOS responses in the *Salmonella* TA1535 uMuC-lacZ system or *E. coli* K12 induct test or SOS Chromotest with *E. coli* PQ37.

Mammalian assays: Table 2

Naphthalene did not induce chromosome damage in the *in vivo* studies; however three positive *in vitro* findings have been reported. Naphthoquinone, a naphthalene metabolite tested separately, induced sister chromatid exchange and micronuclei in two *in vitro* assays. The National Toxicology Program reported that naphthalene (99% pure) induced sister chromatid exchange with and without metabolic activation from rat liver S9 (±S9) and chromosome aberrations with S9 only in Chinese hamster ovary cells. Sister chromatid exchange is the transfer of like-segments of genetic material between sister strands. A question of biological relevance of the SCE results from this NTP assay has been raised because the effect was seen only in the second of two trials and the statistical significance of the increased relative SCE/chromosome ratio appeared dependent on lower control values in the second trial. The UK Health and Safety Executive (HSE) when considering this assay for the EEC human health risk assessment report on naphthalene (draft April 2001), cited the fact that the number of SCE was at most increased by only 50% compared to solvent controls and considered the overall result to be negative. Studies evaluating naphthalene (0.01-0.10mM) and isolated metabolites in human peripheral mononuclear leukocytes (MNL) stimulated with phytohemagglutinin (Tingle at al., 1993; Wilson et al., 1995, 1996) demonstrated that naphthalene did not induce sister chromatid exchanges with or without metabolic activation by human liver microsomes, but naphthalene and 1-naphthol covalently bound to protein, and were cytotoxic to MNL in the presence of human liver microsomes. In contrast, benzo(a)pyrene and aflatoxin B, known mutagens/carcinogens, showed positive genotoxic responses under similar test conditions, in these assays. The cytotoxicity and covalent protein-binding of naphthalene were significantly higher with phenobarbital induced mouse liver microsomes than with human microsomes; the major stable metabolite with human microsomes was naphthalene 1,2 dihydrodiol and with mouse microsomes, 1-naphthol. When activated by human or rat liver microsomes, 1-naphthol was more cytotoxic than naphthalene, suggesting that the toxicity of naphthalene appeared dependent on the bioactivation of 1-naphthol. Liver microsomes, CYP2E1-enriched, from acetone-induced

rats significantly enhanced the metabolism and cytotoxicity of naphthalene and the metabolism of 1-naphthol over that observed with control rat liver microsomes but did not induce SCE formation by naphthalene. Naphthalene 1,2 epoxide and naphthalene 1,2-dihydrodiol did not induce SCE and were not cytotoxic in MNL cells (Wilson et al., 1996). Although naphthoquinone was reported as non-mutagenic to *Salmonella* (Sakai et al., 1985), SCE were induced in this study by the 1,2 and 1,4-naphthoquinones without metabolic activation. An *in vitro* micronucleus test performed using the CREST modified technique, which employs antibody staining of chromosomal kinetichords to distinguish between micronuclei formed by chromosome loss or chromosome breakage, demonstrated that naphthalene (30ug/ml) induced primarily chromosome breakage-type micronuclei, and 1,4-naphthoquinone (0.10μg/ml) induced chromosome loss-type micronuclei in the metabolically competent human lymphoblastoid cell line, MCL-5 (Sasaki et al., 1997). The chromosome aberration study in CHO cells reported by NTP (1992), was a well-conducted assay in which naphthalene induced a statistically significant incidence of chromosome aberrations but only in the presence of rat liver microsomal fraction. Chromosomal damage was also reported with exposure to naphthalene at 0.16mM, close to maximum water solubility in cells of preimplantation mouse embryos cultured with and without rat liver S9 (Gollahon et al, 1990).

Although there are instances of positive cytogenetic results *in vitro,* the *in vivo* results are consistently negative when animals are exposed to naphthalene. Negative results from Harper et al. (1984) from oral exposure of ICR-1 Swiss mice to naphthalene at doses of 50, 250 and 500 mg/kg and a single sacrifice time of 24 hrs are complemented by the study by Sorg (1985) reported fully here in which naphthalene administered intraperitoneally at 250 mg/kg did not induce an increased incidence of micronucleated polychromatic erythrocytes in bone marrow cells of animals sacrificed at 30, 48 and 72 hrs to evaluate all cell cycle stages.

Table 3: Other Systems

When tested in rodent cell types and human lung fibroblasts in culture, naphthalene did not induce transformed foci. Transformation assays are the only *in vitro* tests which, when positive, demonstrate a phenotypic expression (transformed cell foci) of carcinogenicity-related events. Transformed foci, injected into immunosuppressed mice will grow into tumors. Positive results in these assays correlate with the carcinogenic potential of polycyclic aromatic

hydrocarbons. Further, partially hepatectomized F344 rats given naphthalene in a single oral dose (100 mg/kg in corn oil) did not show neoplastic transformation expressed as gamma-glutamyl transpeptidase (GGTP) foci, in liver cells. In contrast, benzo(a)pyrene, given in a single oral gavage dose of 200 mg/kg induced a significant increase in number, area and size of GGTP foci (Tsuda et al., 1980).

Unscheduled DNA synthesis (UDS), a process measuring DNA perturbation and subsequent excision-repair, was not induced in rat hepatocytes treated in culture with naphthalene (Barfknecht, 1985) or when treated with 1-naphthol or 2-naphthol (Probst et al., 1981) at doses approaching toxicity. UDS was also not induced in hepatocytes from rats treated orally with naphthalene at a highest dose representing approximately 80% of the maximum tolerated dose (RTC, 1999), however, the liver is not a target organ for carcinogenesis in rats. DNA single strand breaks measured by alkaline elution did not occur in rat hepatocyte cells treated *in vitro* (Sina et al., 1983) or in hepatocytes from rats treated orally twice with naphthalene at a high dose of 1/5 LD_{50} [359 mg/kg] (Kitchin et al., 1992, 1994). However, the sensitivity of the *in vivo* alkaline elution assay may be limited since neither benzo(a)pyrene or aflatoxin, known mutagens and carcinogens, produced an increase in DNA single strand breaks. Naphthalene did not induce DNA damage either *in vitro* or *in vivo* in these test systems. Naphthalene and 1,4-naphthoquinone did not induce mutation at the hemizygous hprt locus or heterozygous thymidine kinase (tk) locus in the metabolically competent human B-lymphoblastoid cell line MCL-5 (Sasaki et al., 1997).

Naphthalene has been reported to show some genetic activity in a few non-mammalian species: the bioluminescent marine bacterium, *Vibrio fischeri* in the Mutatox® test (Arfsten et al., 1994) and in *Drosophila melanogaster* in the wing somatic mutation and recombination test (Delgado-Rodriguez et al., 1995). The relevance of these results to *in vivo* situations in mammals has yet to be determined.

MATERIALS AND METHODS

Salmonella assays: Both of these studies were performed in the Pharmakon laboratory in 1985 and 1987, according to standard Ames plate incorporation procedures (Maron and Ames, 1983). They employed *Salmonella* strains TA1535, TA1537, TA1538, TA98, TA100 with and without

metabolic activation. Naphthalene, diluted in dimethyl sulfoxide (DMSO) was administered at doses of 0, 3, 10, 30, 100, and 300 μg/plate (3 plates/dose group± S9). Metabolic activation system was derived from livers of Aroclor induced male Sprague Dawley rats and used at a concentration of 0.08ml S9 homogenate in 1 ml S-9 mix. Positive control compounds not requiring S9 activation were: sodium azide for TA1535, TA100; 9-aminoacridine for TA1537, and 2-nitrofluorene for TA1538, TA98; 2-aminoanthracene was the positive control requiring S9 activation for all strains. Only one trial was performed in each study.

Micronucleus assay: This *in vivo* assay was performed in accordance with OECD guideline 474, (adopted 12 May 1981). Naphthalene diluted in corn oil was administered intraperitoneally to CD-1 mice (5M, 5F/group/sacrifice time) in a single dose of 250 mg/kg. The dose was selected based on results of a preliminary range-finding trial using single intraperitoneal doses of 250, 500, 1666, 3000 and 5000 mg/kg. All doses greater than 250 mg/kg produced death within 24 hours. Some animals in the 250mg/kg group demonstrated decreased body tone and activity, abnormal gait and lacrimation occurring 4-72 hours post dose but all animals survived treatment. In the full study, test groups were sacrificed and femoral bone marrow harvested at 30, 48 and 72 hours after dosing. The positive control compound, triethylenemelamine was administered intraperitoneally at a dose of 0.5 mg/kg and mice were sacrificed at 30 hours post-dose. Corn oil control mice were sacrificed at 48 hours post-dose. Slides of bone marrow erythrocytes were prepared and stained with Giemsa. One thousand polychromatic erythrocytes per mouse were evaluated for the presence of micronuclei (MN). To evaluate the impact of the test material on erythrocyte maturation cycle, the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was determined by counting 1000 cells. Data was reported for males and females individually. Statistical evaluation of increased incidence of micronucleated PCEs employed pair-wise comparisons of treatment groups with negative controls using a one-tailed t-test; PCE/NCE ratio comparison used pair-wise t-tests after arc sin transformation of the data.

Unscheduled DNA Synthesis assays: Two assays were performed in two different laboratories in 1985 and 1999. In this assay, the amount of radioactivity from ^{3}H-thymidine incorporated into the nucleus of the exposed cells compared to that in unexposed cells determines the extent of DNA repair by excision and removal of the damaged segment and incorporation of the tritium

labeled base. In the 1985 Pharmakon *in vitro* study, hepatocytes were harvested from the perfused liver of a single male Fischer 344 rat, yielding $2.63x10^6$ cells/ml medium with 86% viability. The protocol employed the method of Williams (1978) and modifications by Kornbrust and Barfknecht (1984). Triplicate cultures ($1x10^5$ viable cells/culture) were prepared on plastic coverslips in Williams medium supplemented with 10% calf serum, allowed to attach for 2 hours, and then were treated with 10μCi/ml tritiated thymidine, and naphthalene diluted with DMSO to concentrations of 0, 0.16, 0.5, 1.6, 5.0, 16, 50, 166, 500, 1666, and 5000 μg/ml medium. The positive control compound was 2-acetamidofluorene (10^{-5}M). Due to toxicity, UDS was evaluated only in cultures containing 0.16-16μg/ml. Cultures were incubated at 37^0C for 18-20 hours, then washed with phosphate buffered saline, swelled with 1% sodium citrate and fixed in 100% ethanol/glacial acetic acid (3:1). After air drying, coverslips were mounted cell surface side up on glass slides, dipped in Kodak NTB-2 photographic emulsion in the dark, dried overnight and stored at 4^0C in a light-proof box. After 7 days of exposure, these autoradiographs were developed and stained with Harris alum hematoxylin. Unscheduled DNA repair synthesis was quantified by a net nuclear increase in black silver grains (≥+5/nucleus) above negative controls for 20 cells/coverslip, 3 coverslips/dose group. The value was determined by subtracting the highest count of 3 adjacent cytoplasmic areas similar in size to areas of the nucleus counts, from the nuclear count.

The 1999 Research Toxicology Center (RTC) DNA repair assay involved treatment of rats with naphthalene prior to isolation of hepatocytes for labeling and evaluation. This assay was performed in accordance with OECD guideline #486 (adopted 21 July 1997) and reflects the complex uptake, distribution, metabolism, detoxification and excretion mechanisms that occur in the whole animal. Naphthalene (99.8% pure) diluted in 0.5% methylcellulose, was administered once by oral gavage to Sprague Dawley male rats (4 rats/group; 7-10 wks old) at doses of 0, 600, 1000 and 1600mg/kg. Maximum dose of 1600 mg/kg was approximately 80% of the oral LD50 >2000 mg/kg; lower doses were approximately 50% and 30% respectively. Two independent experiments were performed, one with a sacrifice time of 14 hours post-dose and 2-acetylaminofluorene as positive control, the other with a sacrifice time of 2 hour post-dose and methyl nitrosourea as positive control. Liver perfusions were performed on 3 rats/group. The fourth rat was kept in reserve and sacrificed unused at study end, when not needed. Hepatocytes were harvested, plated at a concentration of $0.45x10^6$ viable cells/culture in wells containing

plastic coverslips and incubated 90 minutes to allow cells to attach. After aspirating off the unattached cells, cells were exposed to tritiated thymidine (10μCi/ml) for approximately 4 hours at 37^0C, then cells were washed and incubated overnight with unlabelled thymidine. The following day, cultures were checked for sterility, washed and fixed in 1:3 acetic acid/ethanol solution and stored for 30 minutes at 4^0C. After additional washings, coverslips were air-dried and mounted with cell surface side up on microscope slides. Slides were dipped in Kodak NTB2 radiographic emulsion in the dark and stored in light tight boxes at -20^0C for 10 days, then developed. Slides were stained with haemotoxylin/eosin solution and evaluated for nuclear grain counts. Fifty cells were scored from each of two slides per rat to obtain 100 cells/rat. Since no cytotoxic effects were observed in any hepatocyte preparation at any dose level, only the 1000 and 1600 mg/kg groups were evaluated. Background grain counts were estimated from three areas of cytoplasm the size of the nucleus and subtracted from the nucleus count to give net grains/nucleus. Percentage of cells in repair (cells with net grain count ≥+5) was calculated for each rat.

RESULTS

Naphthalene did not induce gene mutations in either *Salmonella* assay with or without metabolic activation from a rat liver S-9 metabolic activation system (Table 4, Godek, 1985; Stankowski, 1987). In the 1987 assay, some inhibition of growth in background lawn was reported for all strains at the highest dose (300μg/plate), somewhat more severe in plates without S9 than with S9. Lawn growth inhibition and the occurrence of fewer revertant colonies at highest doses in some strains indicate that the test material was available to bacteria and the maximum tolerated dose was reached.

In the micronucleus test, naphthalene given at a single intraperitoneal dose of 250 mg/kg did not produce a statistically significant increase in the number of micronucleated polychromatic erythrocytes (MN-PCE) in any mouse at any sacrifice time (Table 5, Sorg, 1985). Indeed, at the 30 hr sacrifice, the combined sex average MN-PCE was statistically significantly lower than vehicle controls. A statistically significant depression in the PCE/NCE ratio was observed in treated animals sacrificed at 72 hours. A time related trend in lowering of this ratio

was also noted at 30 and 48 hour sacrifices. Depression of the PCE/NCE ratio generally confirms bioavailability of the test material to target cells.

Results of the Unscheduled DNA Synthesis assays are presented in Tables 6 (Barfknecht, 1985) and 7 (RTC, 1999). Administration of naphthalene to rat hepatocyte primary cultures over a range of non-toxic doses from 0.16-16 ug/ml did not increase net nuclear grain counts above those of the solvent controls at any dose level. In the study employing administration of naphthalene to male rats in single oral doses of 600, 1000 and 1600 mg/kg, isolation of hepatocytes was performed at 14 hours±30 minutes or 2 hours±15minutes after treatment to determine if different stages in metabolism of naphthalene would alter the UDS profile. Oral treatment with naphthalene did not produce any increase in mean net grains per nucleus in hepatocyte cultures from any treated rat at either sacrifice time. In the 14-hour sacrifice test, no negative control or naphthalene treated rats had any cells in repair. In the 2-hour sacrifice test, one negative control rat had 4% and one high dose rat had 1% cells in repair. No naphthalene treated rats had the 20% cells in repair considered indicative of a positive response. Clinical signs observed in treated animals after dosage were reduced activity in all rats in the 1600 and 1000 mg/kg groups, and some in the 600 mg/kg group, and piloerection in all 1600 mg/kg rats.

Both unscheduled DNA synthesis studies demonstrated that naphthalene does not cause damage to DNA in rat hepatocytes that results in excision repair either to cells in culture or following *in vivo* treatment.

DISCUSSION

The data from five previously unpublished studies presented here support the body of evidence from standard genetic toxicology assays, that naphthalene is not genotoxic in bacterial systems and does not induce genetic events in mammals at selected organ sites. The absence of a positive response in bacterial systems developed to identify point mutations, suggests that naphthalene and its metabolites do not induce gene mutations without or with metabolic activation supplied by P450-rich liver microsomes. This supposition is supported by a recent study of Sasaki et al (1997) using the metabolically-competent human B-lymphoblastoid cell line, MCL-5, which expresses several transfected P450 and epoxide hydrolase genes. In this system, neither naphthalene nor 1,4 naphthoquinone induced mutation at the hemizygous hprt

locus which measures intragenic events, or at the heterozygous tk locus for chromosome-type events. (Table 3). Both naphthalene and 1,4 naphthoquinone induced chromosome breakage and chromosome loss type micronucleus formation in the CREST assay using the same human cell line (Table 2). Naphthalene required a dose 300-fold higher than 1,4-naphthoquinone to induce similar overall micronucleus formation, indicating that metabolism to naphthoquinone is the likely route for clastogenic activity. Identification of mutagenesis at the hprt and tk loci is dependent on survival of cells for cloning, the absence of mutagenesis may suggest that severe cell damage, if it occurred, may not allow survival of viable mutants for expression in a clonogenic assay. Benzo(a)pyrene, tested in the same assays, induced gene mutation but not chromosome damage, demonstrating differences between naphthalene activity and that of a recognized genotoxic, carcinogenic hydrocarbon. Metabolic activation of naphthalene by rat liver microsomes was adequate to produce chromosome aberrations in CHO cells (NTP, 1992). From results of studies testing naphthalene and isolated samples of its metabolites for SCE induction, cytotoxicity and protein binding in human lymphocytes, Wilson et al. (1996) have suggested that cytotoxic and potential genotoxic effects of naphthalene are associated with formation of quinones from 1-naphthol metabolism rather than from the initial metabolite, naphthalene- 1,2 epoxide. The role of naphthoquinones has been supported by investigations into the nature of sulfur-protein adducts with naphthalene metabolites in murine non-ciliated bronchial epithelial (Clara) cells found to contain covalently bound naphthoquinones (Zheng et al., 1997). Naphthalene did not induce transformation of target cells in metabolically competent cell lines or in human and hamster cell lines employing exogenous metabolic activation. These results also suggest that the ability of naphthalene and its metabolites (e.g., arene epoxides and quinones) to induce cancer may involve a different mode of action (e.g., cytoxicity and promotional events) from other carcinogenic aromatic hydrocarbons.

Positive cytogenetic results observed *in vitro* were not observed in two mouse bone marrow erythrocyte micronucleus assays following oral or intraperitoneal administration of naphthalene. Intraperitoneal administration of naphthalene has been demonstrated to induce cellular damage in the Clara cells of mice (O'Brien et al, 1985, Buckpitt et al., 1995) and in the olfactory epithelium in rats at one-half the dose required to produce similar toxicity in mice (Plopper et al, 1992). Naphthalene is also readily absorbed when administered orally (Bakke et al., 1985, NTP, 2000). It is probable that naphthalene and its intermediates transported to bone

marrow are either not converted in situ to genotoxic metabolites or are detoxified by conjugation before reaching target cells. Other organs, such as mouse lung, could activate naphthalene more readily to toxic metabolites or may not be as efficient in detoxifying naphthalene and its metabolites, rendering these cells more sensitive to potential clastogenic activity. Unscheduled DNA synthesis in rat hepatocytes was not induced by naphthalene administered *in vitro or in vivo*. Although the liver is a major site of naphthalene metabolism, the absence of DNA perturbation should be demonstrated in mouse lung or rat nasal epithelium to be fully relevant to site-specific tumor induction in rodents.

The NTP 2 year cancer bioassays on naphthalene resulted in benign alveolar/bronchiolar adenomas and one carcinoma in female mice, and adenomas of the respiratory epithelium of the nose and neuroblastomas of olfactory epithelium in rats. Wilson et al. (1996) theorize that the naphthoquinones, derived from 1-naphthol metabolism, which induce SCE *in vitro,* contribute to the rodent carcinogenicity induced by naphthalene. The pathway to naphthols and quinones appears favored in cases where detoxification via alternative routes (e.g., reduced glutathione [GSH] conjugation) is hampered by events such as metabolic overload, or inhibition or lack of epoxide hydrolase. The variation in affected sites between rodent species: lung in mice, nasal tissue in rats, appears linked to differences in naphthalene metabolism. 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 than in rat tissue. The 1-naphthol:1, 2-dihydrodiol ratio 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, each depending on the initial substrate naphthalene concentration, while lung microsomal preparations from rat, hamster and monkey yielded only ratios of 0.48, 0.61, and 0.12, respectively. Subsequent investigation of the role of cytochrome P450 (CYP) monooxygenases in the mouse lung, demonstrated that

CYP 2F2 catalyzes the stereoselectivity of naphthalene metabolism to 1R,2S-oxide in non-ciliated 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 naphthalene with high levels of 1R,2S epoxide over 2 years could stimulate increased expression of these tumors. In the rat, an obligate nose breather, nasal tumors develop only at sites where atrophy, hyperplasia and metaplasia occurred. Results of extensive studies of genotoxicity by standard methods demonstrate that naphthalene and naphthoquinone do not induce point mutations *in vitro* in bacterial cells with or without exogenous metabolic activation or in a human cell line with inherent metabolic capabilities, suggesting that a single hit, linear model of carcinogenesis is unlikely. However, because of the organ selectivity of naphthalene-induced cancer in rodents and the importance of CYP 2F2 in naphthalene metabolism in mouse lung, data from mutagenesis testing with rodent cancer-target tissues would be valuable. Naphthalene, 1-naphthol and naphthoquinone induce cytotoxicity *in vitro*, consistent with the cell damage observed in lung and nasal passages in rodent bioassays; naphthalene and naphthoquinone have also induced chromosome damage *in vitro*. From these results, naphthalene does not appear to induce viable discrete mutations, but rather induces multi-step events resulting in cell toxicity and potential cytogenetic effects. *In vivo*, naphthalene induced recombination in *Drosophila* but did not induce genetic damage in mammals, albeit in organs which were not cancer targets in rodents. Evaluation of cytogenentic effects in rodent cancer-target organs would be valuable to fully determine whether metabolism is sufficient to allow expression of *in vitro* clastogenicity at tumor sites *in vivo*. Tumors in mice and rats appear at sites where cytotoxicity, inflammation and regenerative hyperplasia occur. Significant cell damage, followed by cell proliferation and repair, frequently include mutational events, secondary to the induced toxicity. Chromosomal alterations demonstrated by naphthalene and naphthoquinone *in vitro* may be part of this process. The significance of rodent cancer studies to human health is difficult to assess. Naphthalene primarily induces hemolytic anemia in humans. Limited reports of laryngeal cancer with naphthalene exposure were confounded by smoking and concurrent exposure to various carcinogenic hydrocarbons (Wolf, 1976; Kup, 1979). Non-human primates appear to metabolize naphthalene in the lung at a much lower rate than either rats or mice (Buckpitt, 1992). The major stable metabolites produced *in vitro* with hepatic microsomes also differ: in human cells, the

non-cytotoxic 1, 2 dihydrodiol; in mice, the cytotoxic 1-napthol (Tingle et al., 1993). The differences in pulmonary metabolism between species and the susceptibility of mice to spontaneously develop lung adenomas suggest that the results of the mouse bioassay are unlikely to be relevant to human health, as discussed by the HSE/UK in the EEC naphthalene human health risk assessment draft (2001). Taken together, results of available genetic toxicity assays and the association between cell damage and tumors at target sites, suggest that naphthalene carcinogenesis involves cytotoxicity rather than mutagenesis as the primary event with tissue regeneration and possible chromosomal changes occurring thereafter, consistent with a threshold-related model of action. Genetic studies employing cancer-target tissue from rodents and further mechanistic studies will contribute to filling data gaps for metabolism in humans and primates relative to rodent carcinogenesis. The results of standard genetic toxicity tests may be useful in predicting the potential health hazard of naphthalene in other species and in humans, considering the limited reports of tumorigenesis in humans, and that naphthalene did not induce gene mutation *in vitro* or chromosome damage *in vivo* and was not mutagenic in a battery of other genetic assays. Despite present data limitations, a threshold mechanism for tumorigenesis based on cytotoxicity can be proposed.

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TABLE 1. Naphthalene Genetic Toxicology: Bacterial Systems

Assay Type	Organism	Doses[a]	Results	Reference
Bacterial Mutation	Sal. typhimurium plate incorp. ±rat S9 TA1535, TA1537, TA100, TA98	Naphthalene 100µg/plate	Negative <70 revertants/plate	McCann et al., 1975
		1-naphthol 1000µg/plate	Negative <70 revertants/plate	
	Sal. typhimurium Plate incorp. ±rat and hamster S9 TA1535, TA1537, TA100, TA98	0.3-100µg/plate	Negative toxic at max dose	Mortelmans et al. 1986
	Sal. typhimurium Plate incorp. ±rat and hamster S9 TA1535, TA1537, TA100, TA98	0.3-100µg/plate	Negative toxic at max dose	NTP, 1992
	Sal. typhimurium Plate incorp. ±rat S9 TA1537, TA1538	10-200µg/plate	Negative; toxic above 100µg/plate	Gatehouse, 1980
	Sal. typhimurium Taped plate assays for volatiles ±rat S9 TA100, TA98	10-50µg/plate	Negative	Bos et al, 1988
	Sal. typhimurium. Plate incorp. ±rat S9 TA1535, TA1537, TA100, TA98	0.03-30µmole/plate toxic >3µmole/plate	Negative	Florin et al., 1980
	Sal. typhimurium. ±rat S9 TA1535, TA1537, TA100, TA98	250µg/plate Naphthalene, Naphthoquinone	Negative	Sakai et al, 1995
	Sal. typhimurium. ±rat S9 TA1535, TA1537, TA1538, TA100, TA98	3-300µg/plate	Negative, toxic above 300µg/plate	Godek, 1985 Stankowski, 1987 (details in Table 4)

TABLE 1 (cont)

Assay	Organism	Doses[a]	Results	Reference
Bacterial Mutation	Sal. typhimurium TM677 (8-azaguanine resistant) ±rat S9	1-2mM	Negative	Kaden et al., 1979
	Sal. typhimurium TA98, TA 1535 ±rat S9	Naphthalene, 1-naphthol: 5- 1000 µg /plate	Naphthalene and 1-naphthol negative at 1000µg /plate in both strains. Naphthalene weakly positive in TA1535 at 5, 10µg /plate, no dose response	Narbonne et al, 1987
	Sal. typhimurium UTH8414, 8413 TA100, TA98. ±rat S9	100-2000µg/plate	Negative	Conner et al., 1985
SOS Response	Sal typhimurium TA1535/p5K1002 (uMuC-lacZ) ±rat S9	83µg/ml	Negative	Nakamura et al., 1987
	E. coli K12 inductest (λ lysogen GY5027; uvrB$^-$,envA$^-$) quantitative plate test ±rat S9	2000µg/plate	Negative	Mamber et al, 1984
SOS Chromotest	E. coli PQ37 (sfiA::lacZ fusion). ±rat S9 (50%standard mix)	0.156 –10.0µg/assay	Negative	Mersch-Sundermann et al, 1993
E. coli rec assay	WP2/WP100 (uvrA$^-$, recA$^-$) suspension assay ±rat S9	2000µg/ml	Negative	Mamber et al., 1983
E. coli pol assay	WP2/WP67 (uvrA$^-$, polA$^-$) ±rat S9	none given	Negative	Mamber et al., 1983
	WP2/WP3478 (polA$^-$) ±rat S9	none given	Negative	

a- Doses identifies dose range or highest inactive dose.

TABLE 2. Naphthalene Genetic Toxicology: Cytogenetic Systems

Assay Type	Test Method	Organism	Doses[a]	Results	Reference
Cytogenetics in vitro	Sister chromatid exchange (SCE) (Litton Bionetics, Inc.)	Chinese hamster ovary cells (CHO) ± rat S9 (Aroclor 1254 induced)	-S9: 9-90μg/ml; 26 hr exposure +S9: 2.7-27μg/ml; 2-hr exposure (2 trials)	Positive (2nd trial only) - S9 at 27-90μg/ml; +S9, at 15, 27μg/ml	NTP, 1992 (results considered negative by UK HSE)
	SCE (Univ. Liverpool, Dept Pharmacol. & Therapeutics)	Human peripheral mononuclear leukocytes (MNL) ± human liver microsomes	100μM (13μg), 2 hr exposure; 72 hr harvest	Negative for SCE, mitotic and proliferative indices ± human microsomes; cytotoxic+ microsomes	Tingle et al., 1993 Wilson et al, 1995
	SCE (Univ. Liverpool, Dept. Pharmacol. & Therapeutics)	Human peripheral mononuclear leucocytes (MNL) ± human liver microsomes	10- 100μM (1.3 –13ug), 2hr exposure; 72 hr harvest naphthalene-1,2-dihydrodiol naphthalene epoxide 1-naphthol 1,2 and 1,4-naphthoquinone	1,2 –dihyrodiol and epoxide negative for SCE and cytotoxicity; 1-naphthol - cytotoxic + microsomes; naphthoquinones- positive for SCE –microsomes and cytotoxic	Wilson et al, 1996
	Micronucleus (MN): CREST assay (Univ. Calif., Riverside, CA	Human B-lymphoblastoid cells MCL-5	Naphthalene 40ug/ml 1,4 naphthoquinone 0.1ug/ml	Positive: chromosome breakage-type MN Positive: chromosome loss-type MN.	Sasaki et al, 1997
	Chromosome aberrations (Litton Bionetics)	Chinese hamster ovary cells (CHO) ± rat S9 (Aroclor 1254 induced)	-S9, 15-75 (8-10hr exposure; 10.1 & 20.5 hr harvest; +S9, 30-67.5μg/ml (2 hr exposure; ~20.5 hr harvest)	Positive +S9 at 30-67.5μg/ml; cell cycle delay	NTP, 1992

TABLE 2 (cont)

Assay Type	Test Method	Organism	Doses[a]	Results	Reference
	Chromosome aberrations (Texas A&M Univ., Vet Anatomy Dept & TEES Engin Toxicol. Div.)	Preimplantation whole mouse embryos (72 hr post-conception) ± rat S9	0.16mM	Positive; 10 fold inc. –S9; 30 fold inc. +S9, slightly embryotoxic	Gollahon et al, 1990 (abstract only)
Cytogenetics in vivo	Micronucleus assay	ICR-1 Swiss mice, male	50, 250, 500mg/kg single oral gavage	Negative at 24 hr sacrifice	Harper et al, 1984
	Micronucleus assay (Pharmakon Res. Intern'l)	CD-1 mice, male and female	250mg/kg single intraperitoneal	Negative at 30, 48, 72 hr sacrifices, toxic>250mg/kg	Sorg, 1985 (see details in Table 5)

a- Doses identifies dose range, highest soluble dose or highest inactive dose.

TABLE 3. Naphthalene Genetic Toxicology: Other Systems

Assay Type	Organism	Doses[a]	Results	Reference
In vitro cell transformation	High passage Fischer rat embryo cells, F1706P96	0.1, 0.5μg/ml	Negative	Freeman et al., 1973
	Syrian baby hamster kidney cells (BHK-21C13) + rat S9 (Aroclor induced)	0.08-250μg/ml	Negative	Purchase et al., 1978
	Human diploid fibroblasts (WI-38) + rat S9 (Aroclor-induced)	0.08-250μg/ml	Negative	Purchase et al., 1978
	Mouse (BALB/c) whole mammary gland cultures	0.001-1.0μg/gland	Negative cytotoxic above 0.1μg based on gland regression and absence/paucity of alveolar buds	Tonelli et al., 1979
	BALB/c-3T3 mouse cell culture	15-150μg/ml; max. conc. based on 10-20% cell survival	Negative toxic at highest dose	Rundell et al., 1983
In vivo neoplastic transformation	F344 partially hepatectomized rats (sex not specified)	100mg/kg in corn oil, single oral dose	Negative for gamma glutamyl transpeptidase foci	Tsuda et al., 1980
Gene mutation in human cells	Human B- lymphoblastoid cell line MCL-5 (hprt and tk loci)	Naphthalene 40μg/ml 1,4-naphthoquinone 0.1μg/ml	Negative	Sasaki et al, 1997

TABLE 3 (cont)

Assay Type	Organism	Doses[a]	Results	Reference
Unscheduled DNA Synthesis (UDS)	Primary rat hepatocytes in vitro	0.5-1000nM/ml; 1-naphthol 2-naphthol, only	Both negative at 100nM/ml, highest non-toxic dose	Probst et al., 1981
	Primary rat hepatocytes in vitro	0.16-5000µg/ml	Negative Toxic above 16µg/ml	Barfknecht, 1985 (details in Table 6)
	Primary hepatocyte cultures from rats treated in vivo	600, 1000, 1600mg/kg single oral gavage dose	Negative No toxicity	RTC, 1999 (details in Table 7)
Alkaline Elution	Rat hepatocytes in vitro	3mM; 3 hr exposure	Negative for increased incidence of DNA single strand breaks	Sina et al., 1983
	Hepatocytes from treated female Sprague Dawley rats	359mg/kg oral (1/5 LD50) at 21 and 4 hrs prior to sacrifice	Negative for DNA single strand breaks in hepatocytes; dose inhibited liver GSH	Kitchin et al, 1992, 1994
Drosophila melanogaster	Somatic mutation and recombination (SMART assay)	1, 5, 10mM in feed of larva for 48 hrs until pupation	Positive dose dependent loss of heterozygosity of 2 recessive wing genes (mwh, flr)	Delgado-Rodriguez et al., 1995

a- Doses identifies dose range, highest soluble dose or highest inactive dose.

TABLE 4. Naphthalene Bacterial Mutagenesis Assays in *Salmonella typhimurium*

Test Article	Dose µg/ plate	S9	*Salmonella* Strains (Revertants /plate[a])									
			TA1535		TA1537		TA1538		TA98		TA100	
			1985	1987	1985	1987	1985	1987	1985	1987	1985	1987
DMSO	0	-	17	13	12	9	19	12	31	39	172	106
Naphthalene	3	-	16	10	8	12	12	11	30	51	156	100
	10	-	13	9	11	8	16	16	33	49	161	107
	30	-	14	12	6	9	19	17	33	46	150	98
	100	-	14	16	8	11	18	15	35	48	156	101
	300	-	15	8	8	5	18	8	27	22	156	70
Pos. Control	b	-	1152	1192	1644	820	565	897	579	561	1329	1631
DMSO	0	+	10	17	10	11	24	32	44	43	129	114
Naphthalene	3	+	15	18	6	12	28	29	48	59	151	114
	10	+	9	17	6	13	29	35	45	59	157	112
	30	+	13	11	6	15	33	24	42	56	155	118
	100	+	8	10	13	10	23	33	38	44	175	113
	300	+	10	13	7	8	24	25	44	38	134	74
Pos. Control	2.5	+	217	221	259	596	1072	2405	1682	2468	1635	2756

a. Average based on 3 plates/dose level

b. Positive controls –S9: TA 1535 & TA100 – sodium azide (10µg); TA1537 –9-aminoacridine (150µg); TA1538 & TA98 –2 nitrofluorene (5 µg); +S9 all strains 2- anthramine.

Microsomal mix (S9) derived from livers of Aroclor treated rats.

Reference: Godek, 1985; Stankowski, 1987, Pharmakon Research International, Inc.

TABLE 5. Naphthalene Micronucleus Assay Results from Mice Treated *In Vivo*

Animal No./Sex	Controls				Naphthalene 250mg/kg ip					
	Corn Oil 10ml/kg		TEM 0.5mg/kg ip		30 hr		48 hr		72 hr	
	MN-PCE[a]	PCE/NCE[b]	MN-PCE[a]	PCE/NCE[b]	MN-PCE[a]	PCE/NCE[b]	MN-PCE[a]	PCE/NCE[b]	MN-PCE[a]	PCE/NCE[b]
1M	0	1.268	60	0.724	1	1.101	2	0.786	2	1.288
2M	1	1.558	63	0.953	0	1.033	0	1.165	1	1.041
3M	3	0.890	65	1.560	1	0.855	1	0.739	1	0.869
4M	1	1.242	55	0.748	1	1.278	0	0.864	1	0.377
5M	2	1.320	72	1.058	0	1.028	0	0.805	1	0.706
6F	0	2.175	55	0.391	1	1.137	1	0.776	0	0.742
7F	2	0.613	56	0.412	0	1.119	0	0.862	0	0.618
8F	0	1.198	38	0.520	1	0.842	1	1.577	1	0.992
9F	2	0.976	75	0.634	0	0.887	2	0.661	1	0.873
10F	2	0.890	73	0.481	1	0.736	0	1.101	0	0.377
Mean ± std. dev	1.30±1.06	1.21±0.43	61.2 ±11.09*	0.65 ±0.22*	0.60 ±0.52*	1.00±0.17	0.70±0.82	0.93±0.28	0.80±0.63	0.79 ±0.29*

M = Male; F = Female

a. MN-PCE = micronucleated polychromatic erythrocytes/1000 polychromatic erythrocytes/mouse

b. PCE/NCE ratio = ratio of polychromatic erythrocytes to normochromatic erythrocytes in 1000 erythrocytes/mouse

* $p \leq 0.05$

Reference: Sorg, 1985, Pharmakon Research International, Inc.

TABLE 6. Naphthalene UDS repair in rat hepatocytes *in vitro*

Test Article	Concentration µg/ml	Net Nuclear Grains ±S.D. (avg. triplicate cultures)
DMSO (vehicle control)	0	0.4 ± 1.0
Untreated control	0	0.2 ± 0.6
2 acetamidofluorene (Pos. control)	$1x10^{-7}$M final conc. in media	23.9 ± 6.9[a]
Naphthalene	0.16	0.0 ± 0.0
	0.50	0.2 ± 0.8
	1.6	0.0 ± 0.3
	5.0	0.0 ± 0.0
	16.0	0.0 ± 0.1
	50.0	cytotoxic
	166.0	cytotoxic
	500.0	cytotoxic
	1666.0	cytotoxic
	5000.0	cytotoxic

Positive result. Mean net nuclear grain count of 5 or greater than vehicle control.

Reference: Barfknecht, 1985 Pharmakon Research International, Inc.

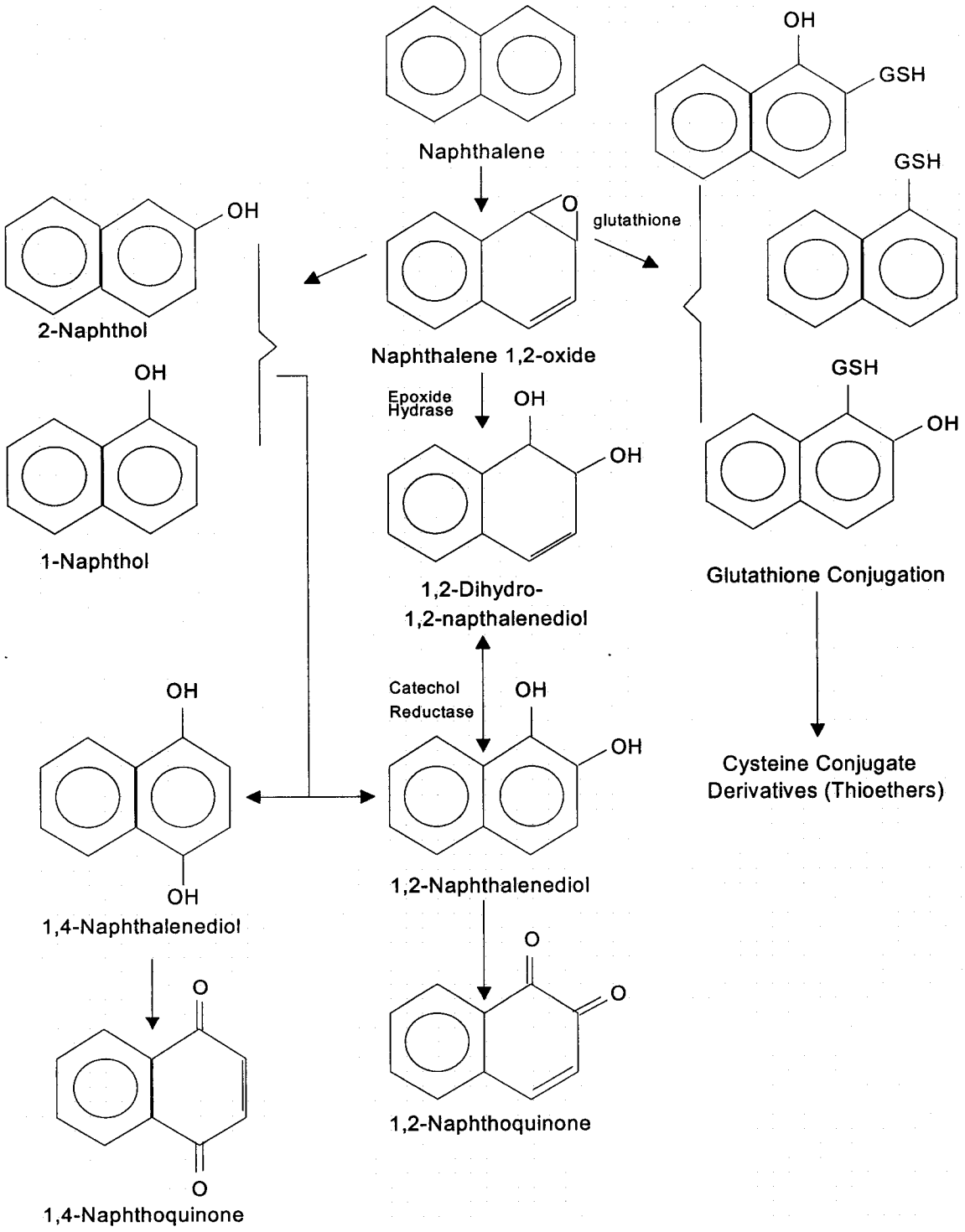
TABLE 7. Naphthalene UDS repair in rat hepatocytes after *in vivo* treatment

Male Rat No.	Dose mg/kg	Sample time	Net Grains ± S.D. (mean/rat)	Net Grain ± S.E. (mean/dose group)
2	0 (vehicle)	14	-2.91 ± 2.16	-3.67 ± 0.46
4			-3.61 ± 2.09	
6			-4.50 ± 2.41	
18	1000	14	-3.70 ± 2.20	-3.85 ± 0.19
20			-3.62 ± 2.01	
22			-4.23 ± 2.92	
26	1600	14	-3.35 ± 2.30	-4.30 ± 0.80
28			-3.67 ± 2.50	
30			-5.89 ± 2.66	
34	2-AAF 100	14	+3.78 ± 6.27	+7.22 ± 2.16
36	(Pos. control)		+11.20 ± 8.23	
38			+6.69 ± 5.18	
42	0 (vehicle)	2	-2.92 ± 2.88	-3.14 ± 0.16
44			-3.44 ± 1.90	
46			-3.05 ± 2.33	
58	1000	2	-3.80 ± 2.55	-3.16 ± 0.32
60			-2.85 ± 2.58	
62			-2.83 ± 2.85	
66	1600	2	-2.30 ± 2.22	-2.35 ± 0.08
68			-2.51 ± 1.66	
70			-2.23 ± 1.83	
74	MNU 80	2	+6.26 ± 4.91	+6.98 ± 0.50
76	(Pos. control)		+7.93 ± 4.41	
78			+6.75 ± 5.44	

Positive controls are 2-acetylaminofluorene (AAF) and methyl nitrosourea (MNU).
Negative control is methyl cellulose, the naphthalene vehicle.

Reference: Research Toxicology Center, Rome, Italy, 1999.

Figure 1. Proposed pathways for Naphthalene metabolism
(from ATSDR report, update 1995)


Naphthalene
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GSH
GSH
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glutathione
OH
2-Naphthol
Naphthalene 1,2-oxide
GSH
OH
Epoxide
Hydrase
OH
OH
1-Naphthol
1,2-Dihydro-
1,2-napthalenediol
Glutathione Conjugation
Catechol
Reductase
OH
OH
OH
OH
1,2-Naphthalenediol
Cysteine Conjugate
Derivatives (Thioethers)
1,4-Naphthalenediol
O
O
O
O
1,2-Naphthoquinone
1,4-Naphthoquinone

Attachment F

Pathology Review of NTP Chronic Study of Naphthalene in Rats
for the
American Chemistry Council

Report (dated January 29, 2001)
Presentation (dated September 7, 2001)

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Report: **Pathology Review of NTP Chronic Study of Naphthalene in Rats for the American Chemical Council**

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Introduction

On December 27, 2000, the American Chemistry Council (ACC) entered into an Agreement (HSP-4.0-MSU-NAP), on behalf of the Hydrogen Solvents Panel, to retain the services of Dr. Jack Harkema to conduct an independent pathology review of the National Toxicology Program's (NTP) chronic rat inhalation study of naphthalene. Dr. Harkema, the Contractor, agreed to conduct a microscopic review of the nasal tumor slides from approximately 59 laboratory animals used in the NTP study, in the manner described in the scope of work outlined in a letter from Dr. Harkema to Andrew Jacques, dated 11/22/00. The following is a report from Dr. Harkema describing his findings after reviewing the designated slides at the NTP Archives in Research Triangle Park, NC in late January 2001.

Background

Dr. Harkema is a professor of pathology in the College of Veterinary Medicine at Michigan State University (MSU). He is currently the Director of the Laboratory for Experimental and Toxicologic Pathology and Co-Director of the *Collaborative Air Research Effort (CARE)* between MSU and the University of Michigan. He is a Diplomate (by examination, 1980) of the American College of Veterinary Pathologists. Dr. Harkema is an internationally recognized expert in the area of nasal toxicology and pathology of laboratory animals. He has written numerous peer-reviewed publications and book chapters in this scientific field and continues to maintain a well-funded research program on the effects of air pollutants on the nasal and pulmonary airways of laboratory rodents. Dr. Harkema has served on several pathology peer-review working committees for government (e.g., NIEHS, USEPA) and industry.

Review of Nasal Histopathology

Dr. Harkema traveled to the NTP Archives at the Environmental Pathology Laboratories (EPL) in Durham, NC on January 22, 2001. At EPL, he conducted a microscopic review of the glass slides of 58 nasal tumors from 56 rats in the NTP Chronic Naphthalene study. Dr. Harkema finished his review on the morning of January 23, 2001. Dr. Melvin Hamlin II, Laboratory Director at EPL had the appropriate slides pulled from the archives prior to Dr. Harkema's

arrival. Dr. Arlene Medeiros, Senior Toxicologist at ExxonMobil, monitored Dr. Harkema's work at EPL on the 22nd of January.

Dr. Harkema conducted his microscopic examination without knowledge of the individual animal neoplastic and non-neoplastic diagnoses that were made by the NTP Study Pathologist. Only after the completion of Dr. Harkema's review were his findings compared to those documented in the NTP pathology report.

Concurrence with NTP Report. In general the histopathological findings of Dr. Harkema agreed remarkably well with those reported by the NTP. Dr. Harkema concurs with both the number and the morphologic character of the nasal tumors identified by the NTP Study Pathologist. There were only minor, inconsequential, differences in terminology between the descriptions of Dr. Harkema and the NTP report. Both found two distinct types of nasal tumors. One type of nasal tumors was located in the proximal nasal cavity of naphthalene-exposed rats. These tumors were classified as Transitional (polypoid) Adenomas or Carcinomas by Dr. Harkema, and as Adenomas, Respiratory Epithelium in the NTP report. Dr. Harkema's description is based on the terminology recently described by Morgan and Harkema in *Monographs on Pathology of Laboratory Animals: Respiratory System*, Eds. Jones, Dungworth and Mohr (1996). This terminology suggests that these nasal tumors arose from the transitional nasal epithelium that lines the lateral meatus (airway) in the proximal nasal cavity. Most of these neoplasms were small polyploid tumors protruding from the epithelial surface (exophytic) lining the lateral aspect of the nasoturbinate, maxilloturbinate or lateral wall. A few were classified as transitional carcinomas by Dr. Harkema because they were large polyploid tumors that partially effaced the naso- or maxillo-turbinate and obstructed a large portion of the lateral meatus.

The second type of nasal tumor was located in the distal aspect of the nasal airway at the level of the ethmoturbinates. These tumors were classified as Neuroblastomas, Olfactory epithelium in the NTP report and as Neuroepithelial Carcinomas, Olfactory epithelium, possible Basal cell origin (Neuroblastoma) by Dr. Harkema. Again, the latter terminology is based on the classification of nasal tumors of rats and mice as described by Morgan and Harkema in *Monographs on Pathology of Laboratory Animals: Respiratory System*, Eds. Jones, Dungworth and Mohr (1996). Many of these tumors arising from the olfactory epithelium lining the ethmoturbinates or the dorsal aspect of the distal septum were highly undifferentiated and therefore diagnostically difficult to assign a specific cell of origin. Feron et al. (Environ. Health Perspect. 85:305-315, 1990) have suggested that most chemical-induced olfactory epithelial neoplasms are derived from basal cells and should be classified as neuroepitheliomas.

Possible Pathogenesis (Hypotheses): Concurrent with the neoplastic nasal lesions there was often chronic active inflammation, epithelial degeneration (cytotoxicity), epithelial hyperplasia, and glandular hyperplasia of varying severity in the examined tissues. This close association of non-neoplastic epithelial and inflammatory lesions with the nasal neoplasms, suggests important roles for cytotoxicity and ongoing reparative proliferation of surface epithelium in the pathogenesis of these nasal tumors, especially the neuroepithelial carcinomas. Since both the olfactory and transitional epithelium contain cells with high levels of cytochrome-P450 metabolizing enzymes, it is probable that a toxic metabolite of naphthalene may be inducing epithelial degeneration and necrosis with subsequent reparative basal cell proliferation.

In addition, the site-specific intranasal location of these non-neoplastic and neoplastic lesions corresponds with known areas of high airflow in the nasal passages and therefore possible "hot spots" for surface epithelial deposition of the inhaled chemical. The ongoing metabolism with cytoxicity and chronic proliferation of the transitional epithelium and olfactory epithelium at these intranasal "hot spots" may be critical for the process of nasal carcinogenesis. Interestingly, though exposed rats may not have had a neuroepithelial carcinoma or a transitional adenoma/carcinoma at these "hot spots", the severity of the non-neoplastic lesions (e.g., basal cell hyperplasia) was often the highest in these focal intranasal sites.

Carefully designed studies need to be conducted to better understand the intranasal dosimetry of inhaled naphthalene and the possible role of epithelial proliferation, nasal metabolism and chronic secondary inflammation in the pathogenesis of these naphthalene-induced nasal neoplasms. There may also be important differences in the nasal toxicity of naphthalene among mammalian species that would be critical in assessing the potential risk of this chemical to human health.

Respectively submitted,

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Contractor

Pathology Review of NTP Chronic Study of Naphthalene in Rats for the American Chemical Council

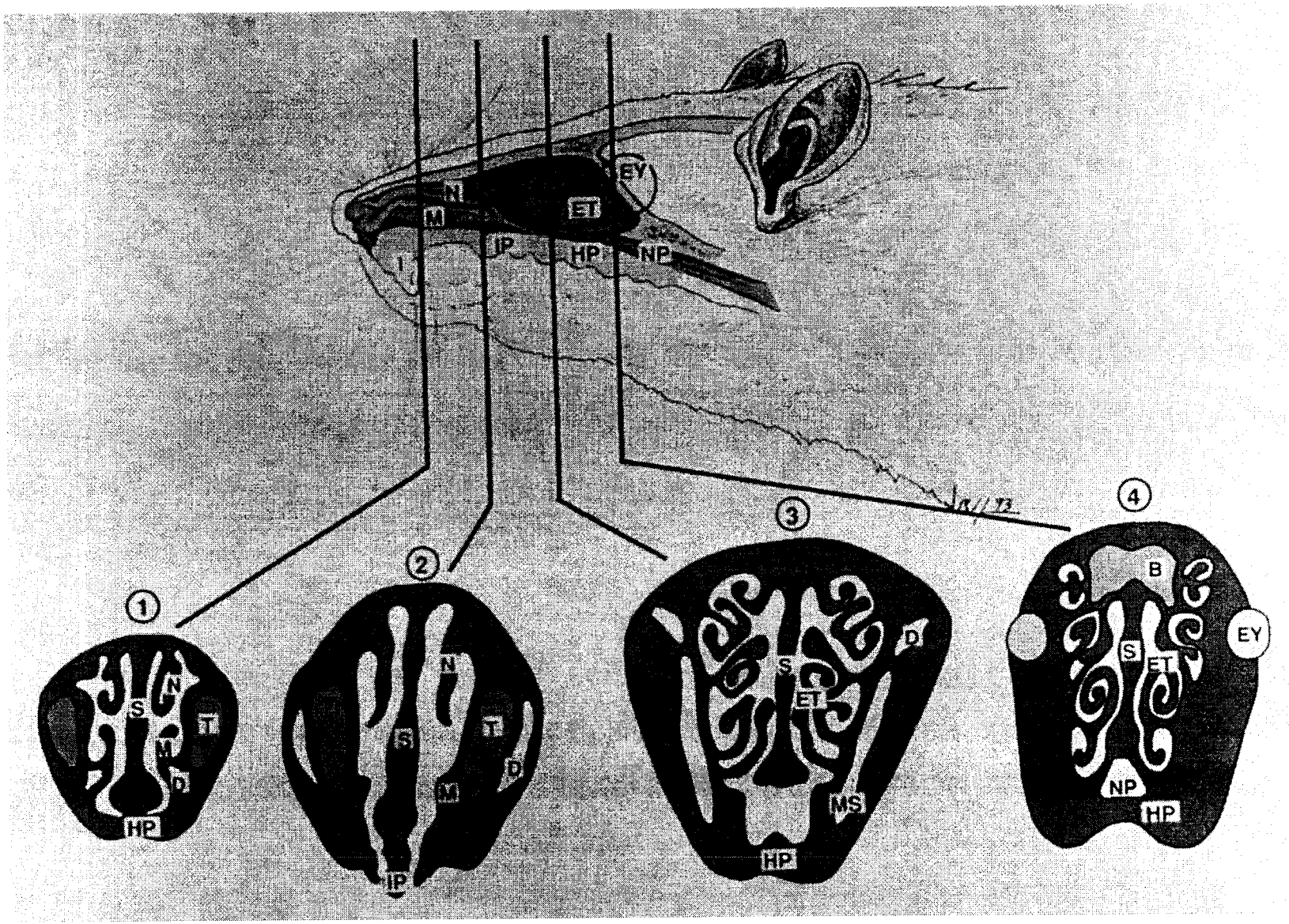
Jack R. Harkema, DVM, PhD, DACVP
Professor of Comparative Pathology
Michigan State University

ACC Meeting 9/7/01
CIIT, RTP, NC

Review of Histopathology

- Light microscopic examination of the glass slides of 58 nasal tumors from 56 rats in the NTP Chronic Naphthalene study

- H&E-stained transverse sections of the nasal airway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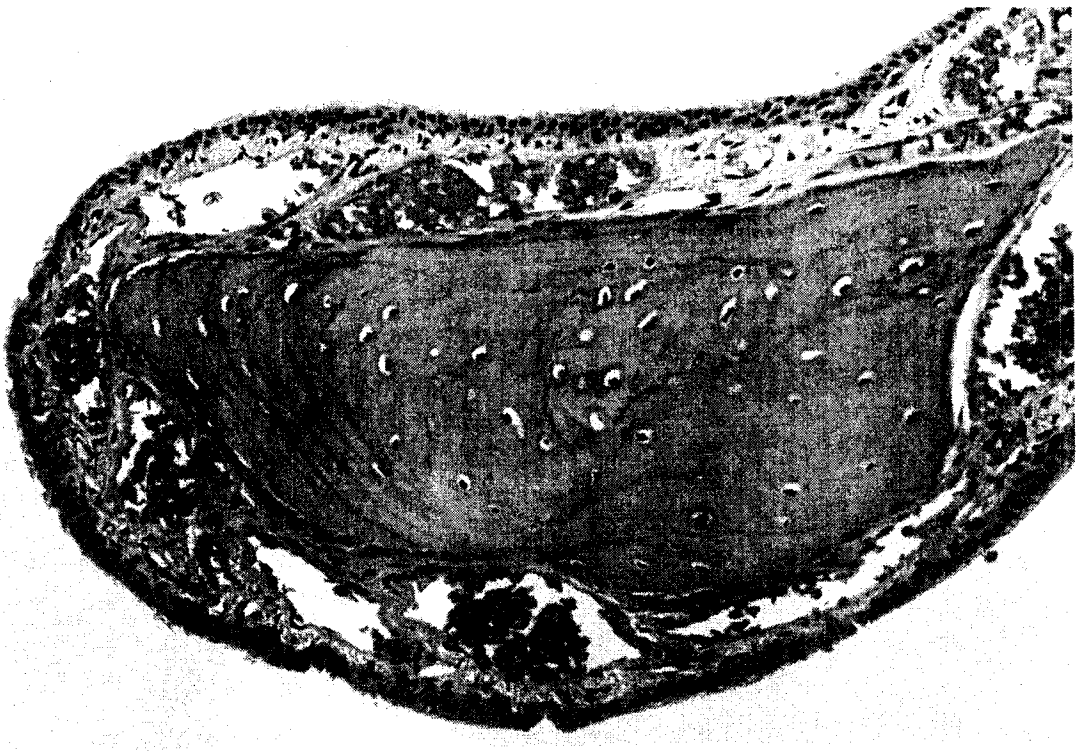

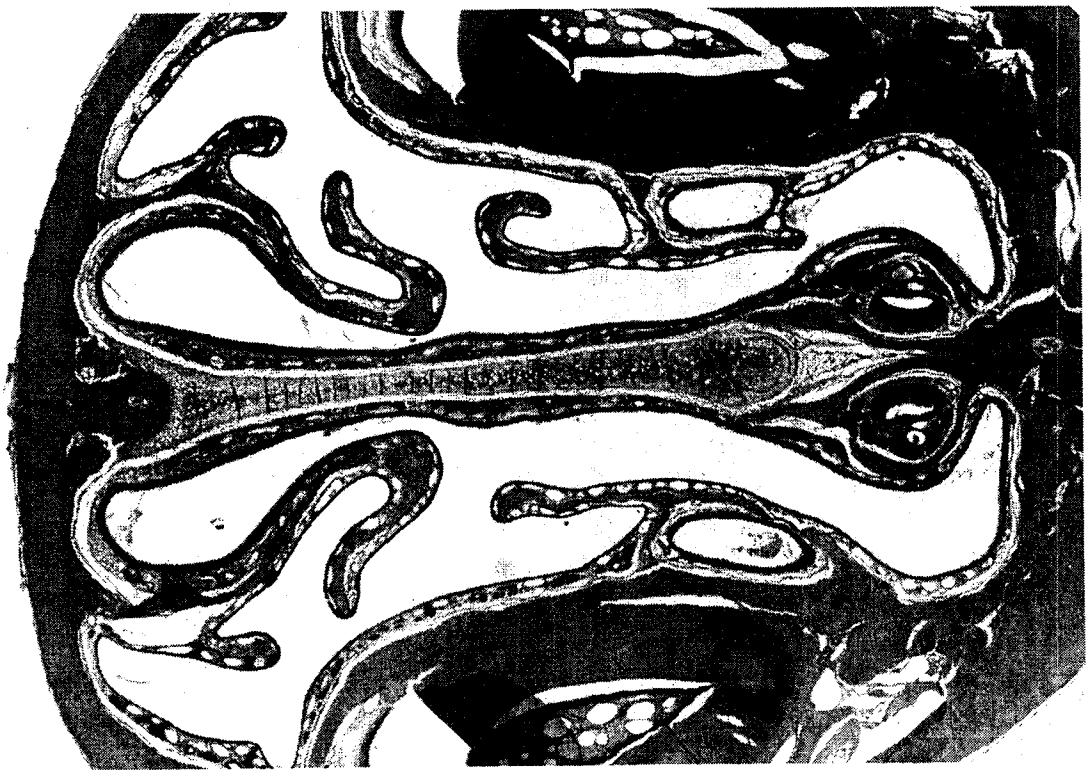

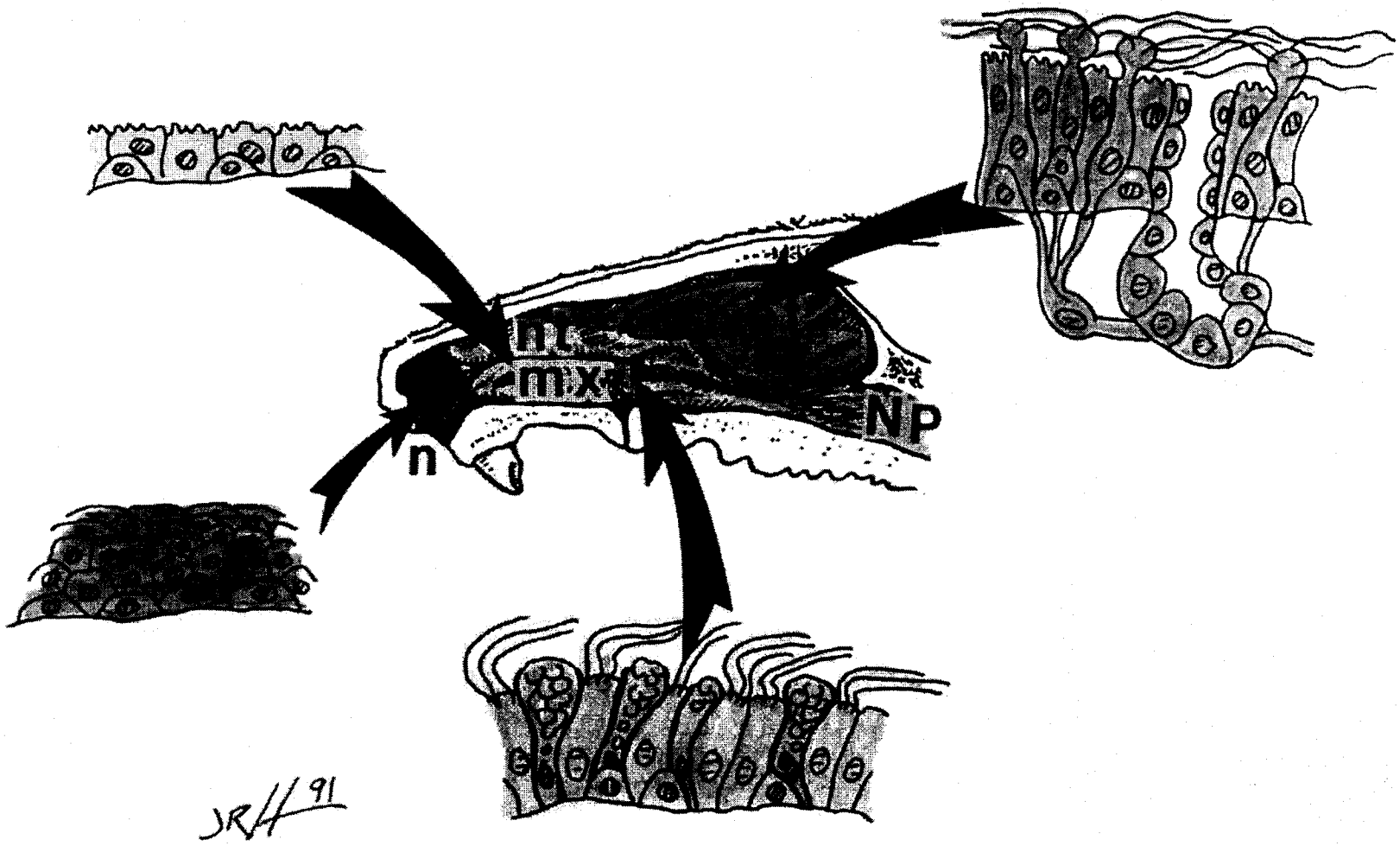
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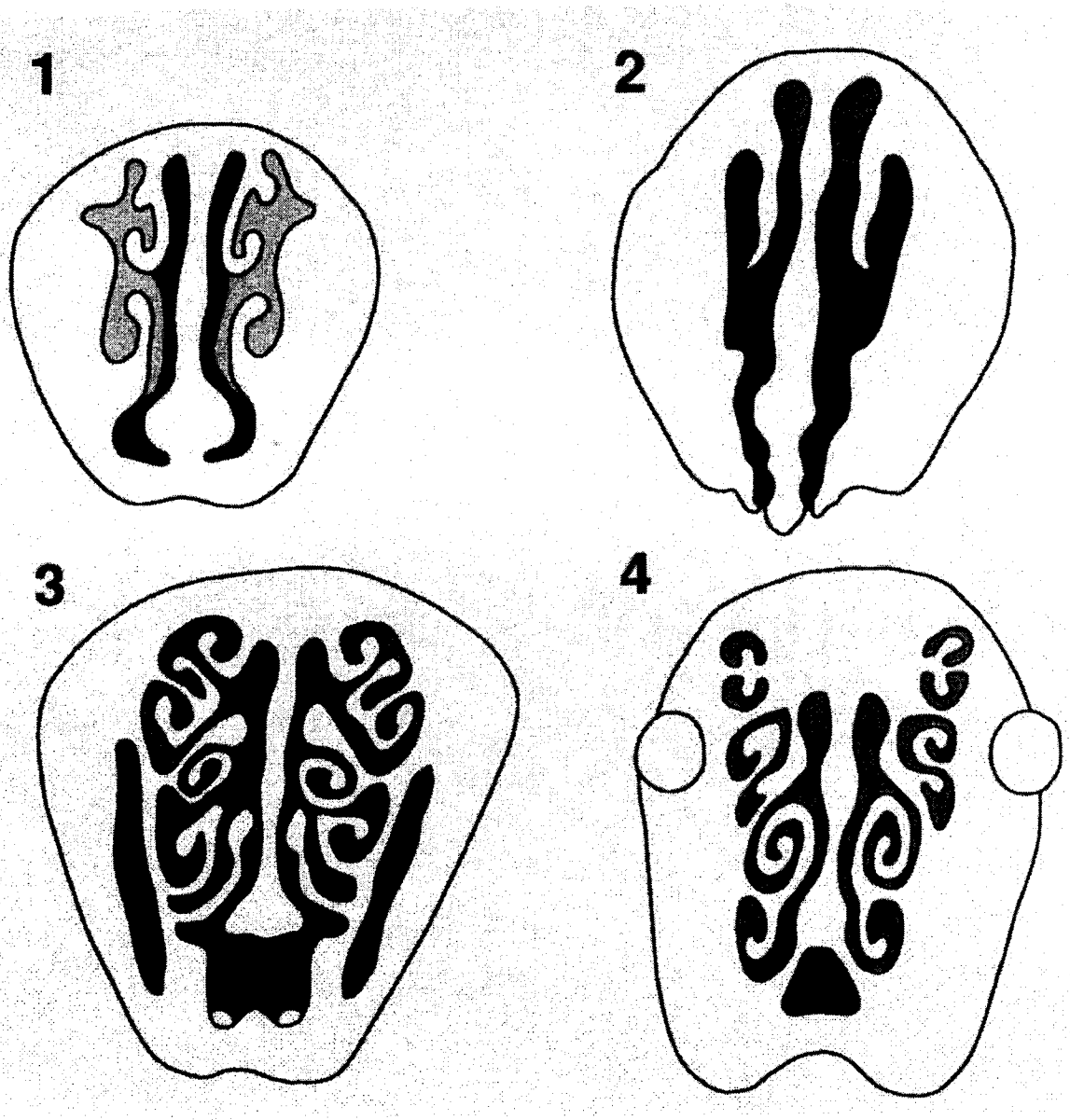
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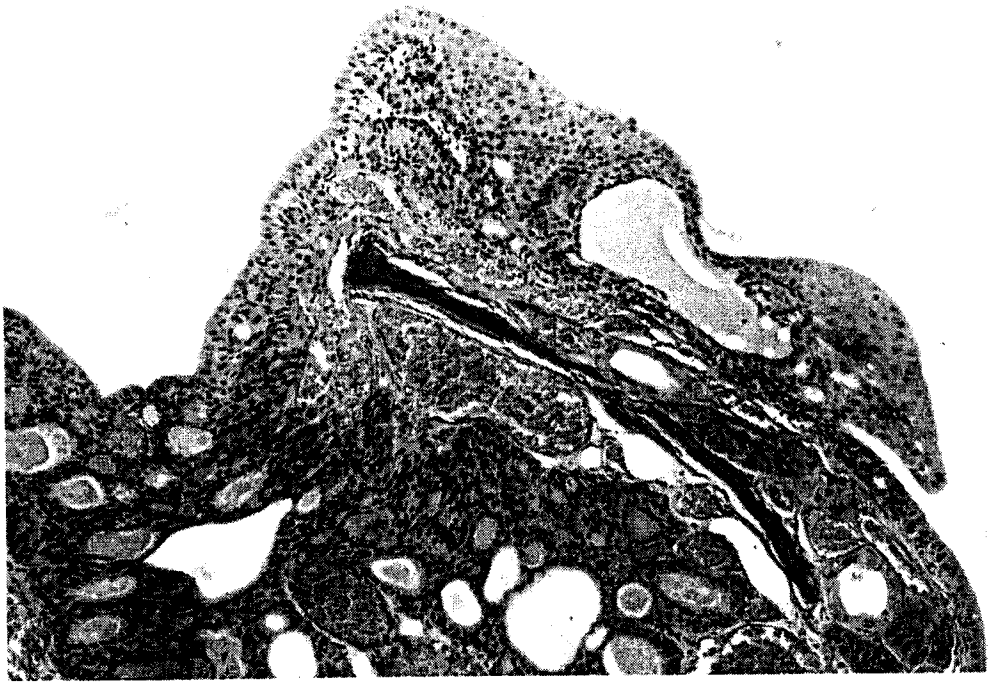
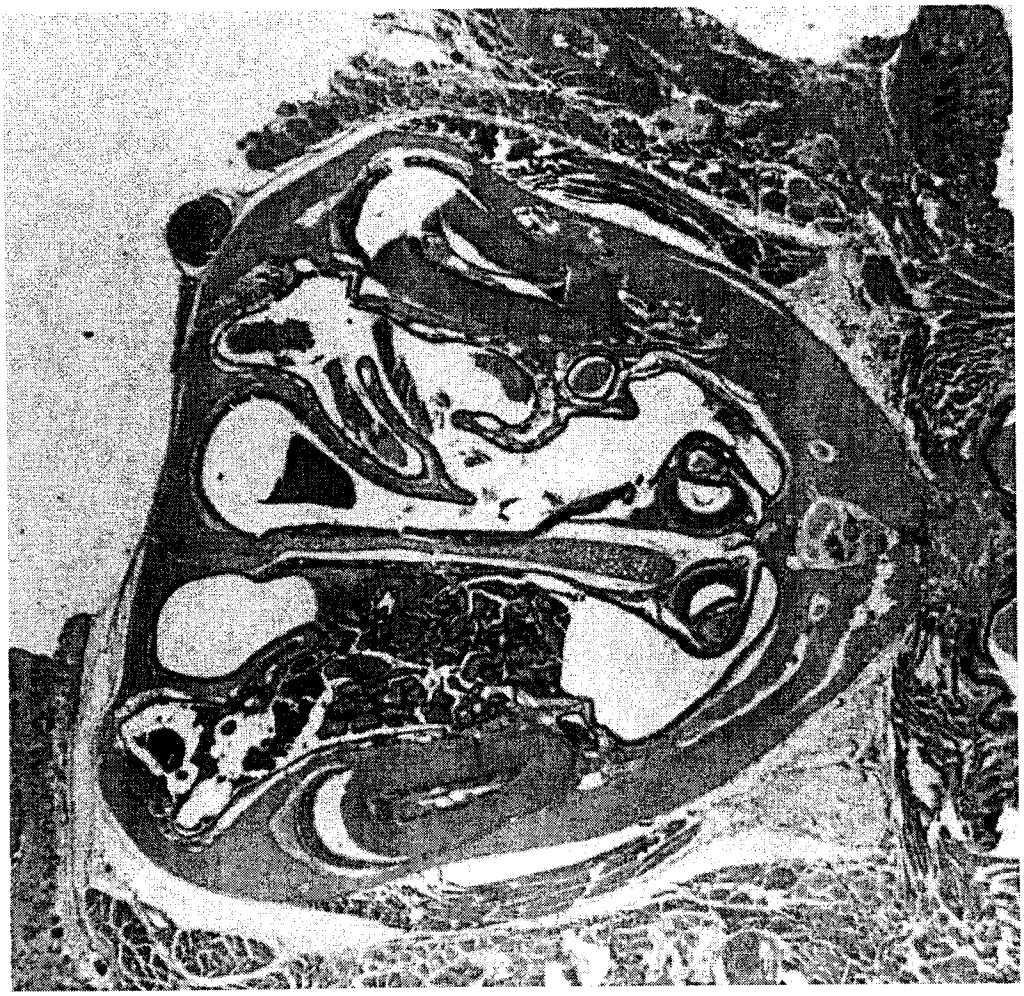
Naphthalene-Induced Non-Neoplastic Lesions

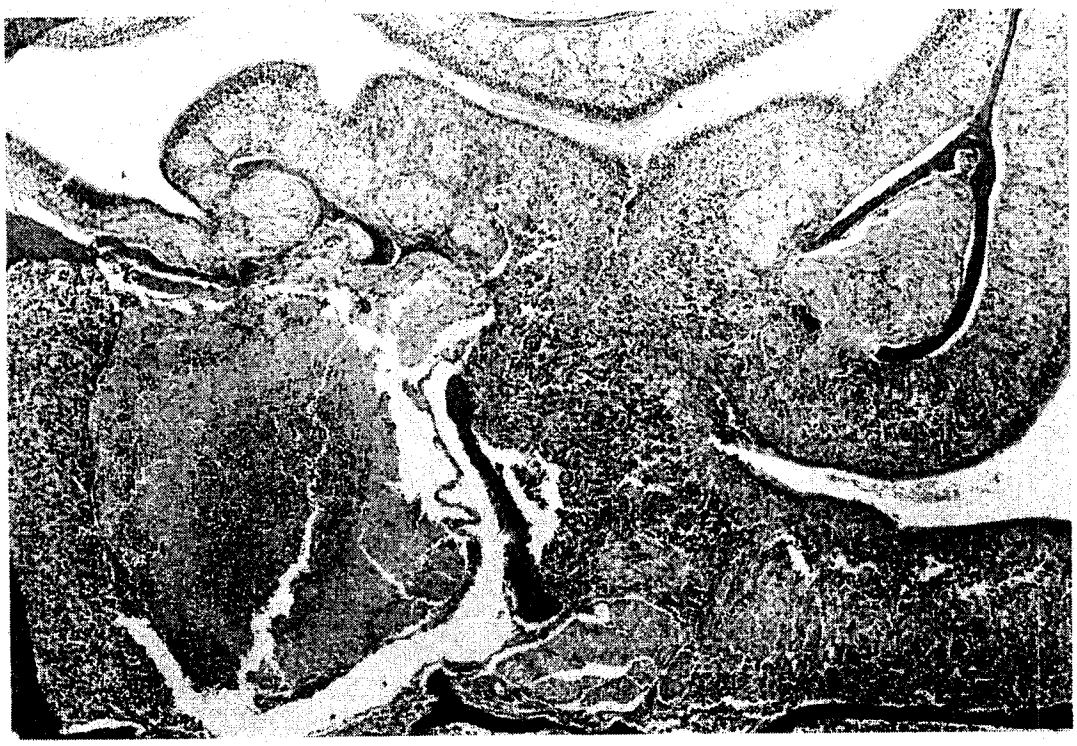
- Chronic active inflammation
- Epithelial degeneration (cytotoxicity)
- Epithelial hyperplasia, regenerative
- Glandular hyperplasia, regenerative

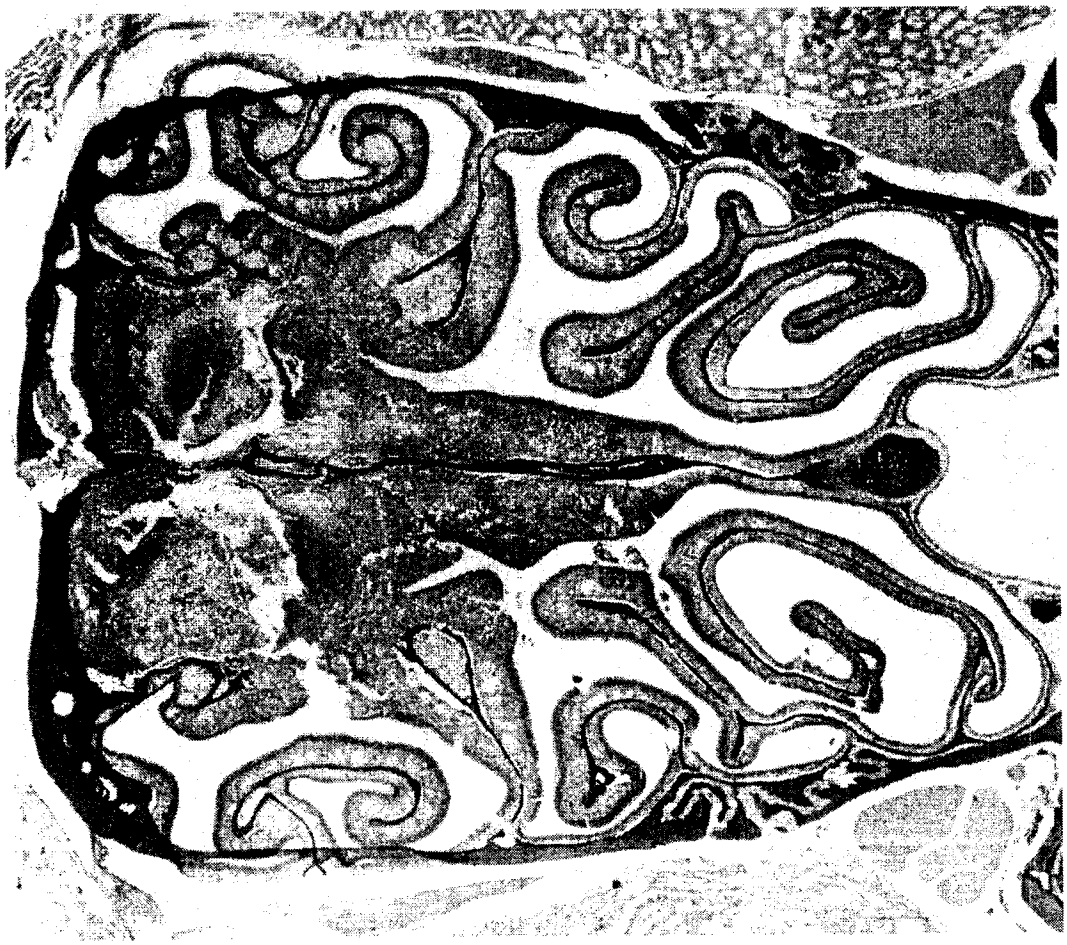
Naphthalene-Induced Nasal Neoplasms

- Adenoma, Polyploid, Solitary, Nasal Transitional Epithelium, Maxillo- and Naso-turbinates (30/58)
- Adenocarcinoma, Polyploid, Solitary, Nasal Transitional Epithelium, Maxillo- and Naso-turbinates (5/58)
- Neuroepithelial Carcinoma, Solitary, Olfactory Epithelium, Ethmoturbinates (23/58)

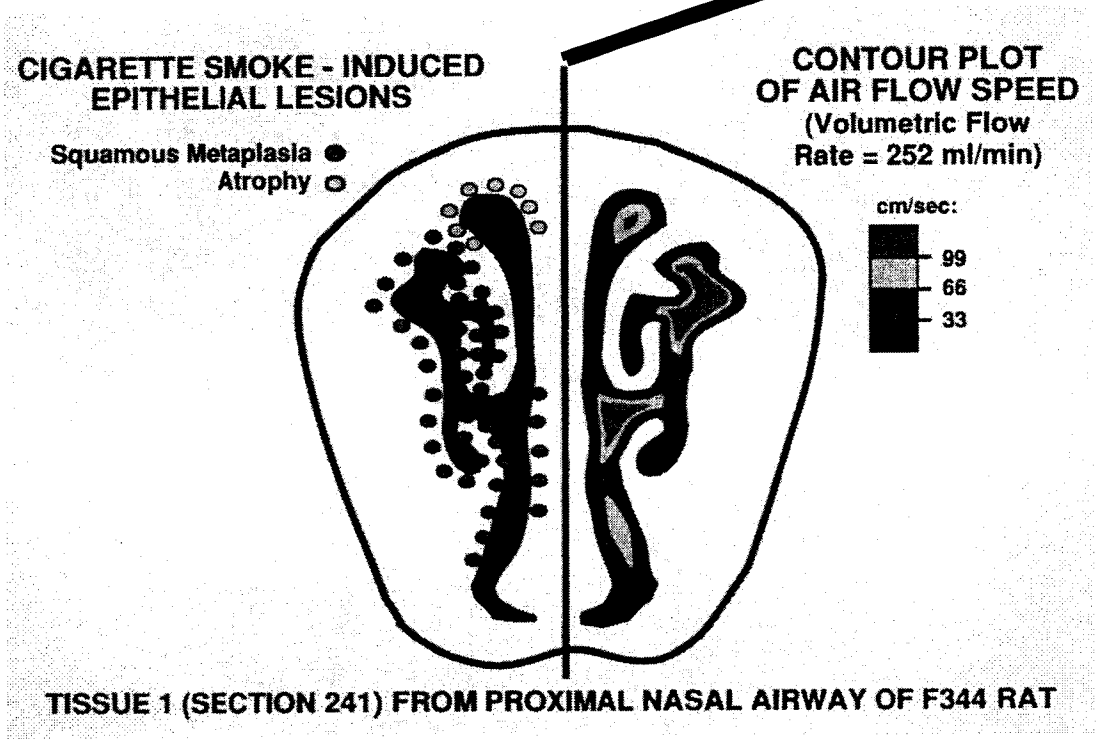








F344 Rat


CIGARETTE SMOKE - INDUCED
EPITHELIAL LESIONS
Squamous Metaplasia
Atrophy
CONTOUR PLOT
OF AIR FLOW SPEED
(Volumetric Flow
Rate = 252 ml/min)
cm/sec:
99
66
33
TISSUE 1 (SECTION 241) FROM PROXIMAL NASAL AIRWAY OF F344 RAT