

## Long-term Health Effects of Exposure to Naphthalene

### ***Background and status of Naphthalene as a Toxic Air Contaminant and Potential Carcinogen***

Naphthalene (CAS Registry Number: 91-20-3) is a natural constituent of coal tar, comprising approximately 11% of that material by weight (HSDB, 2003). It is present in gasoline and diesel fuels. All pesticide registrations of naphthalene, including use as a moth repellent, were cancelled in California in 1991 due to data gap inadequacies. However, naphthalene is included on a list of "inert" or "other ingredients" found in registered pesticide products (U.S. EPA, 2004). It is used in the manufacture of a wide variety of industrial products. A profile dated 1987 showed proportionate naphthalene use for various products as: Phthalic anhydride, 60%; 1-naphthyl methyl carbamate insecticide and related products (tetralin and 1-naphthol), 10%; dispersant chemicals, 10%; moth repellent, 6%; synthetic tanning agents, 5%; miscellaneous uses, 5%; exports, 4% (HSDB, 2003). Other products listed as derived from naphthalene include phthalic and anthranilic acids, naphthols, naphthylamines, naphthalene sulfonates, synthetic resins, celluloid, lampblack, smokeless powder, anthraquinone, indigo, perylene, and hydronaphthalenes (NTP, 1992; HSDB, 2003).

Naphthalene enters the atmosphere both from emissions from industrial facilities and other localized sources, and from mobile sources. Vehicle exhaust contains naphthalene both due to its presence in fuel oil and gasoline, and its formation as a combustion by-product. In addition, there are discharges of naphthalene on land and into water from spills during the storage, transport and disposal of fuel oil, coal tar, etc. Naphthalene is emitted when wood is burned (IARC, 2002). Naphthalene is a component of environmental tobacco smoke and a number of consumer products, resulting in its presence as a contaminant of indoor air. The statewide annual emissions from facilities reporting under the Air Toxics Hot Spots Act in California, were estimated to be 164,459 pounds of naphthalene (CARB, 1999). U.S. EPA's Toxics Release Inventory most recent database (U.S. EPA, 2001) listed 2,603,377 pounds total on- and off-site releases of naphthalene in the United States, of which 2,002,901 pounds were air emissions and the rest divided among land, surface water and underground emissions.

In polluted urban areas, the primary route of atmospheric transformation for naphthalene is daytime reaction with the OH radical. For a 12-hr average OH radical concentration of  $2.0 \times 10^6$  molecule  $\text{cm}^{-3}$ , the calculated lifetime of naphthalene is 6 hr (Atkinson and Arey, 1994; Arey and Atkinson, 2003). Nighttime reaction of naphthalene with  $\text{NO}_3$  radicals can be important in the formation of nitronaphthalenes (Atkinson and Arey, 1994; Arey and Atkinson, 2003). Observed products of naphthalene reaction with the OH and/or  $\text{NO}_3$  radicals include 2-formylcinnamaldehyde, 1-naphthol, 2-naphthol, 1-nitronaphthalene, 2-nitronaphthalene, 1-hydroxy-2-nitronaphthalene, and 1,4-naphthoquinone (Sasaki *et al.*, 1997).

The statewide emissions of naphthalene from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory available, were estimated

to be 360,000 pounds (CARB, 1997). In addition to emissions from facilities and other localized sources, mobile sources also contribute to the ambient levels of naphthalene in California air. Atkinson (1995) measured 12-hour average ambient concentrations of naphthalene in Redlands, California in October 1994. The levels observed ranged from 348 to 715 ng/m<sup>3</sup>.

Naphthalene is individually identified under the section 112(b)(1) of the U.S. Clean Air Act amendment of 1990 as a Hazardous Air Pollutant (HAP). This followed the U.S. EPA's determination that it is known to have, or may have, adverse effects on human health or the environment. On April 8, 1993, the California Air Resources Board (ARB) identified, by regulation, all 189 of the then listed HAPs as Toxic Air Contaminants (TACs). This was in response to the requirement of Health and Safety Code Section 39657(b). In addition, naphthalene meets the U.S. Clean Air Act definition of Polycyclic Organic Matter (POM; "Includes organic compounds with more than one benzene ring, and which have a boiling point greater than or equal to 100°C"), so its identification as a California TAC could be regarded as arising either from its individual listing as a HAP, or from the listing of POM. The health effects summary (OEHHA, 1993) prepared for benzo[a]pyrene included cancer risk estimates for a number of other polycyclic aromatic hydrocarbons and related derivatives which fall under the general classification of POM. However, estimates for naphthalene were not included in that document, since at the time unequivocal evidence for the carcinogenicity of naphthalene was not available.

Non-cancer health effects of naphthalene have been recognized for some time, and these were the basis for a Chronic Inhalation Reference Exposure Level (cREL) developed by OEHHA (2000) for use in the Air Toxics Hot Spots (AB2588) program. The cREL adopted was 9 µg/m<sup>3</sup> (2 ppb), based on respiratory effects (nasal inflammation, olfactory epithelial metaplasia, respiratory epithelial hyperplasia) in mice.

### *Summary of Health Effects of Naphthalene*

#### *Non-cancer Health Effects*

Hemolysis has been reported in infants exposed to high doses of naphthalene (Siegel and Wason, 1986; U.S. EPA, 1998). The effect appears to be caused by the metabolites (1- and 2- naphthol and naphthoquinones), which produce methemoglobinemia. Infants appear to be more sensitive than adults, due to their lower capacity for methemoglobin reduction. However, even in infants the doses at which this effect occurs are high. A few cases related to high (but unquantified) inhalation exposure have been reported, but the incidents generally involved absorption by the dermal route (contact with substantial amounts of solid naphthalene), or ingestion of several grams of naphthalene as a solid or dissolved in oils. Individuals with glucose-6-phosphate dehydrogenase deficiency are hypersensitive to this effect.

Several studies in animals report damage to the respiratory tract as a result of exposure to naphthalene. In a study by the National Toxicology Program (NTP, 1992), male and female B6C3F<sub>1</sub> mice were exposed to naphthalene (>99% pure) vapor for 6 hours per

day, 5 days per week over 104 weeks. Concentrations used were 0 (150 mice), 10 (150 mice), or 30 ppm (300 mice) naphthalene. Lesions were observed in the nose and lungs of exposed mice, including increased incidences of chronic nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia.

Naphthalene causes damage to both ciliated and Clara cells of the bronchiolar epithelium in mice (Van Winkle *et al.*, 1995; Plopper *et al.*, 1992a,b). Neonatal mice were more sensitive to this damage than adult mice (Fanucchi *et al.*, 1997). Swiss Webster Mice at post-natal day (PND) 7 or 14, or adults, received 25, 50 or 100 mg/kg naphthalene by intraperitoneal injection, and the lungs were prepared for histological examination. Both observational and morphometric evaluation showed dose-dependent damage to the bronchiolar epithelium. There was loss of both ciliated and non-ciliated (Clara) cells, as indicated by changes in total epithelial thickness and in volume fractions of the various cell types, and appearance of vacuolated (injured) cells. Effects were similar in adults and young mice, but whereas the adult mice showed a LOAEL of 100 mg/kg for most effects, the 7 and 14-day old mice showed LOAELs of 25-50 mg/kg. Although the doses in this experiment were given intraperitoneally, the effects appear to depend on metabolism of naphthalene in the target tissues and are therefore anticipated to occur regardless of the dose route. Mice, which have high cytochrome P-450 activity in the bronchiolar epithelium, are more sensitive to naphthalene than rats or hamsters where this activity is lower (Plopper *et al.*, 1992b).

There are a number of case reports of human exposures to naphthalene, generally involving acute poisonings or other situations with high exposures. Toxic effects seen generally include respiratory or hematological effects similar to those described above, nausea, vomiting, and ocular effects such as cataracts and retinal damage. These reports, and additional studies in animals, are further described in the chronic toxicity summary in support of the cREL derivation (OEHHA, 2000).

### Carcinogenicity

Early carcinogenicity studies (by various routes) of naphthalene had mostly equivocal or non-positive results, although those studies were of low power (Adkins *et al.*, 1986; Kennaway, 1930; Schmahl, 1955). Genetic toxicology results were mixed: *Salmonella* reverse mutation assays were generally negative, but some test results with eukaryotic systems *in vivo* or *in vitro* were positive (NTP, 2000). Inhalation studies in mice by the National Toxicology Program (NTP, 1992) were generally considered at the time to provide only equivocal evidence of carcinogenic activity, when considered in conjunction with the earlier studies. However, the observation of possible tumor responses in the mice prompted the National Toxicology program to undertake inhalation studies in rats, the results of which became available more recently (NTP, 2000). These studies found clear evidence of carcinogenic activity in rats, based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose in both sexes. These additional findings prompted IARC to re-evaluate naphthalene, which was re-classified as Group 2B: possibly carcinogenic to humans (IARC, 2002). The State of California's Proposition 65 program listed naphthalene as a substance known to the State

to cause cancer on April 19, 2002. In view of these new data and conclusions, it is appropriate to provide a cancer risk estimate for naphthalene for use in the Toxic Air Contaminants program, in addition to the Reference Exposure Level already available for the chronic non-cancer effects. The following summary (to be included as an addendum to the *Air Toxics Hot Spots Program Risk Assessment Guidelines: Part II, Technical Support Document for describing available Cancer Potency Factors*) provides an analysis of the carcinogenicity data for naphthalene, and derives a cancer potency factor and unit risk factor for use in risk assessment of inhalation exposures to naphthalene.

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**NAPHTHALENE**

CAS No: 91-20-3

**I. PHYSICAL AND CHEMICAL PROPERTIES**

(From HSDB, 2003 except as noted)

Molecular weight	128.2
Boiling point	218°C
Melting point	80.5 °C
Vapor pressure	0.078 Torr @ 25°C (Sonnenfeld <i>et al.</i> , 1983); 0.10 Torr @ 27°C (CRC, 1994)
Air concentration conversion	1 ppm = 5.24 mg/m <sup>3</sup> (NIOSH, 2004)

**II. HEALTH ASSESSMENT VALUES**

Unit Risk Factor:	$3.4 \times 10^{-5} (\mu\text{g}/\text{m}^3)^{-1}$
Slope Factor:	$1.2 \times 10^{-1} (\text{mg}/\text{kg}\text{-day})^{-1}$

[Male rat nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma incidence data (NTP, 2000), linearized multistage procedure (OEHHA, 2002).]

**III. CARCINOGENIC EFFECTS**Human Studies

Although a number of reports exist which describe non-cancer health effects in humans (OEHHA, 2000), no studies of carcinogenic effects in humans were identified.

Animal Studies

The National Toxicology Program (NTP) conducted inhalation cancer studies of naphthalene using male and female B6C3F<sub>1</sub> mice (NTP, 1992). Animals were exposed to 0 (70 males, 69 females), 10 (69 males, 65 females) or 30 ppm naphthalene (135 males, 135 females) for 6 hours/day, 5 days/week for 104 weeks.

The survival rates of exposed female mice were similar to that of controls (86%, 88% and 76% for controls, 10 and 30 ppm exposure groups, respectively). However, survival of male control mice was significantly less than that of exposed male mice (37%, 75% and 89% for controls, 10 and 30 ppm exposure groups, respectively). NTP stated that the reduced control survival was due to wound trauma and secondary infections due to fighting among the group-housed mice.

Almost all of the male and female mice in the NTP 1992 mouse inhalation studies demonstrated an increased incidence of nasal respiratory epithelium hyperplasia and olfactory epithelium metaplasia (Table 1).

Table 1. Incidence of nonneoplastic nasal lesions in male and female B6C3F<sub>1</sub> exposed to naphthalene by inhalation for 104 weeks (NTP, 1992).

Lesion type	Sex	Naphthalene concentration		
		0 ppm	10 ppm	30 ppm
respiratory epithelium hyperplasia	male			
overall rate		0/70 (0%)	66/69 (96%)	134/135 (99%)
average severity grade <sup>a</sup>		0	2.6	2.8
olfactory epithelium metaplasia	male			
overall rate		0/70 (0%)	66/69 (96%)	134/135 (99%)
average severity grade <sup>a</sup>		0	2.5	2.6
respiratory epithelium hyperplasia	female			
overall rate		0/69 (0%)	65/65 (100%)	135/135 (100%)
average severity grade <sup>a</sup>		0	2.5	2.7
olfactory epithelium metaplasia	female			
overall rate		0/69 (0%)	65/65 (100%)	135/135 (100%)
average severity grade <sup>a</sup>		0	2.5	2.4

a: Average severity grade based on 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

Increased incidences of alveolar/bronchiolar adenomas and carcinomas were observed in male B6C3F<sub>1</sub> mice. Alveolar/bronchiolar adenoma or carcinoma incidences in the male mice as cited by NTP were 7/70, 17/69 and 31/135 for controls, and the 10 and 30 ppm exposure groups, respectively. The increased tumor incidences observed for the 10 and 30 ppm groups were significant when a pairwise comparison to control was performed using the Fisher exact test ( $p = 0.019$  and  $0.016$  for the 10 and 30 ppm groups, respectively). However, NTP noted that an evaluation of the dose-response trend ( $p = 0.530$ ) and pairwise comparisons between the controls and exposure groups ( $p = 0.212$  and  $0.394$  for the 10 and 30 ppm exposure groups, respectively) using a logistic regression test indicated a lack of statistical significance. This was explained by NTP as being the result of the early control mortality due to fighting which lowered considerably the number of control animals at risk of developing lung tumors. NTP also noted that the alveolar/bronchiolar adenoma and carcinoma incidence (adjusted rate 26% in the high dose group) was within the historical control range for male B6C3F<sub>1</sub> mice (total incidence 19.7%, range 10-30%). NTP therefore concluded that the marginally increased alveolar/bronchiolar adenoma and carcinoma incidence in the male mice was more likely to be related to survival difference between exposed and control groups, than directly related to naphthalene exposure.

Increased incidences of alveolar/bronchiolar adenomas and carcinomas were also observed in female B6C3F<sub>1</sub> mice. The incidences of alveolar/bronchiolar adenoma or carcinoma, combined, in the female mice as cited by NTP were 5/69, 2/65 and 29/135 for controls, and the 10 and 30 ppm exposure groups, respectively. The tumors were primarily adenomas; one carcinoma was observed in high dose female mice. The

increased tumor incidence in the 30 ppm exposure group females was statistically significant when compared to controls.

These results were generally considered at the time to provide only equivocal evidence of carcinogenic activity, when considered in conjunction with earlier studies by various routes, which, although of lower power, also had nonpositive or equivocal results (Adkins *et al.*, 1986; Kennaway, 1930; Schmahl, 1955). However, the observation of possible tumor responses in the mice prompted the National Toxicology Program to undertake naphthalene inhalation cancer studies in rats.

NTP (2000) exposed groups of 49 male and female Fischer 344N (F344) rats to naphthalene by inhalation at concentrations of 0, 10, 30 or 60 ppm for 6.2 hours/day, five days/week for 105 weeks. Survival of the male and female exposure groups were similar to that of controls.

These studies found clear evidence of carcinogenic activity in male and female rats, based on increased incidences of rare tumors, respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose, in both sexes. Respiratory epithelial adenoma incidence occurred with a positive dose-response trend in male rats and was significantly increased in all exposed male rat groups. Male rat respiratory epithelial adenoma incidence as cited by NTP was 0/49, 6/49, 8/48 and 15/48 for controls, and the 10, 30 and 60 ppm exposure groups, respectively. Respiratory epithelial adenoma incidences in female rats exposed to 30 or 60 ppm were also increased, but the increase in the 60 ppm animals was not significant, and the increase in the 30 ppm animals was of borderline significance ( $p = 0.053$  by Poly-3 test). Female rat respiratory epithelial adenoma incidence as cited by NTP was 0/49, 0/49, 4/49 and 2/49 for controls, and the 10, 30 and 60 ppm exposure groups, respectively.

Olfactory epithelial neuroblastomas occurred in males exposed to 30 and 60 ppm naphthalene and in all dose groups of naphthalene-exposed females. Neuroblastoma incidences occurred with positive dose-response trends in males and females. The incidence in females exposed to 60 ppm was significantly greater ( $p < 0.001$  by Poly-3 test) than that in controls. Male rat olfactory epithelial neuroblastoma incidence as cited by NTP was 0/49, 0/49, 4/48 and 3/48 for controls, and the 10, 30 and 60 ppm exposure groups, respectively. Female rat olfactory epithelium neuroblastoma incidence as cited by NTP was 0/49, 2/49, 3/49 and 12/49 for controls, and the 10, 30 and 60 ppm exposure groups, respectively.

NTP also noted that nasal olfactory epithelial neuroblastomas and nasal respiratory epithelial adenomas have not been observed in male or female control rats in the NTP historical control database for animals fed NIH-07 feed in 2-year inhalation studies or in the more recent, smaller database for control rats fed NTP-2000 feed. Additionally, almost all of the male and female mice in the NTP 1992 inhalation studies demonstrated increased nasal respiratory epithelium hyperplasia and olfactory epithelium metaplasia (Table 1). These tissue types correspond to the tumor sites observed in rats exposed to naphthalene by inhalation.

#### IV. DERIVATION OF CANCER POTENCY

##### Basis for Cancer Potency

Unit risk values for naphthalene were calculated based on data in female mice, male rats and female rats from the studies of NTP (1992, 2000). The mouse lung alveolar/bronchiolar adenoma or carcinoma incidence data, rat nasal respiratory epithelial adenoma data and nasal olfactory epithelial neuroblastoma data used to calculate unit risk values are listed in Tables 2, 3 and 4, respectively.

Table 2. Incidence of lung alveolar/bronchiolar adenoma or carcinoma in female B6C3F<sub>1</sub> mice exposed to naphthalene via inhalation (from NTP, 1992)

Chamber Concentration (ppm)	Average Concentration <sup>a</sup> (mg/m <sup>3</sup> )	Tumor Incidence <sup>b</sup> (%)	Statistical Significance <sup>c</sup>
0	0	5/67 (7)	$p < 0.001$
10	9.36	2/61 (3)	$p = 1$
30	28.1	29/129 <sup>d</sup> (22)	$p < 0.01$

- Average concentration calculated by multiplying chamber concentration by six hours/24 hours, 5 days/7 days and 5.24 mg/m<sup>3</sup>/ppm.
- Effective rate. Animals that died before the first occurrence of tumor (day 471) were removed from the denominator.
- The  $p$ -value listed next to dose groups is the result of pairwise comparison with controls using the Fisher exact test. The  $p$ -value listed next to the control group is the result of trend tests conducted by NTP (1992) using the logistic regression, life table, and Cochran-Armitage methods (all three methods produced the same result).
- One carcinoma was observed in the high dose group.

Table 3. Incidence of nasal respiratory epithelial adenoma in male F344/N rats exposed to naphthalene via inhalation (from NTP, 2000)

Chamber Concentration (ppm)	Average Concentration <sup>a</sup> (mg/m <sup>3</sup> )	Tumor Incidence <sup>b</sup> (%)	Statistical Significance <sup>c</sup>
0	0	0/44 (0)	$p < 0.001$
10	9.67	6/42 (14)	$p < 0.05$
30	29.0	8/44 (18)	$p < 0.01$
60	58.0	15/41 (37)	$p < 0.001$

a. Average concentration calculated by multiplying chamber concentration by 6.2 hours/24 hours, 5 days/7 days, and 5.24 mg/m<sup>3</sup>/ppm.

b. Effective rate. Animals that died before the first occurrence of tumor (day 552) were removed from the denominator.

c. The  $p$ -value listed next to dose groups is the result of pairwise comparison with controls using the Fisher exact test. The  $p$ -value listed next to the control group is the result of the Poly-3 trend test, as reported by NTP (2000).

Table 4. Incidence of nasal olfactory epithelial neuroblastoma in F344/N rats exposed to naphthalene via inhalation (from NTP, 2000)

Chamber Concentration (ppm)	Average Concentration <sup>a</sup> (mg/m <sup>3</sup> )	Tumor Incidence <sup>b</sup> (%)	Statistical Significance <sup>c</sup>
<i>Males</i>			
0	0	0/49 (0)	$p = 0.027$
10	9.67	0/48 (0)	$p = 1$
30	29.0	4/48 (8)	$p = 0.056$
60	58.0	3/48 (6)	$p = 0.117$
<i>Females</i>			
0	0	0/49 (0)	$p < 0.001$
10	9.67	2/49 (4)	$p = 0.247$
30	29.0	3/49 (6)	$p = 0.121$
60	58.0	12/48 (25)	$p < 0.001$

a. Average concentration calculated by multiplying chamber concentration by 6.2 hours/24 hours, 5 days/7days, and 5.24 mg/m<sup>3</sup>/ppm.

b. Effective rate. Animals that died before the first occurrence of tumor (males, day 399; females, day 429) were removed from the denominator.

c. The  $p$ -value listed next to dose groups is the result of pairwise comparison with controls using the Fisher Exact test. The  $p$ -value listed next to the control group is the result of the Poly-3 trend test, as reported by NTP (2000).

### **Methodology**

The default approach, as originally delineated by CDHS (1985), is based on a linearized form of the multistage model of carcinogenesis (Armitage and Doll, 1954). Cancer potency is estimated from the upper 95% confidence bound,  $q_1^*$ , on the linear coefficient  $q_1$  in a model relating lifetime probability of cancer ( $p$ ) to dose ( $d$ ):

$$p = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \dots)] \quad (1)$$

The parameter  $q_1^*$  is estimated by fitting the above model to dose response data using MSTAGE (Crouch, 1992).

For a given chemical, the model is fit to one or more data sets. The default approach is to select the data for the most sensitive species and sex. For carcinogens that induce tumors at multiple sites in a particular species and sex, cancer potency is taken to be the sum of potencies from the different sites. Because of the statistical uncertainty in individual estimates of potency, the terms are summed statistically as follows. A distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term ( $q_1$ ) of the multistage model is generated for each treatment-related tumor site in a given species and sex using the computer program MSTAGE (Crouch, 1992), modified to tabulate percentile values. (Distributional values stem from the assumption that twice the log likelihood function is  $\chi^2$  distributed). The discretized distributions were used to obtain a distribution of the sum of  $q_1$ s for each site affected by the chemical using Monte Carlo simulation (100,000 trials; Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound on the summed  $q_1$ s is taken as  $q_1^*$  for the combined tumor sites.

To estimate animal potency,  $q_{\text{animal}}$ , the parameter  $q_1^*$  is adjusted to account for short duration of an experiment by assuming that the lifetime incidence of cancer increases with the third power of age. However, the durations of the studies examined here (NTP, 1992; 2000) were at least the standard lifespan of the test animals (104 weeks for rodents), so this correction was not required. Thus, for the calculations based on the NTP (1992; 2000) studies,  $q_1^*$  is equivalent to  $q_{\text{animal}}$ .

Interspecies extrapolation from experimental animals to humans is normally based on the following relationship (Anderson *et al.*, 1983), where  $bw_h$  and  $bw_a$  are human and animal body weights, respectively, and potency (e.g.,  $q_{\text{animal}}$ ) is expressed on a dose per body weight basis:

$$q_{\text{human}} = q_{\text{animal}} \times \left( \frac{bw_h}{bw_a} \right)^{\frac{1}{3}} \quad (2)$$

Alternatively, when performing calculations based on applied dose in terms of air concentrations, the assumption has sometimes been made that air concentration values are equivalent between species (CDHS, 1985). However, using the interspecies scaling

factor shown above is preferred because it is assumed to account not only for pharmacokinetic differences (*e.g.*, breathing rate, metabolism), but also for pharmacodynamic considerations. Therefore, lifetime average doses in mg/kg-day were determined (details provided below) and used in the calculation of  $q_{\text{animal}}$  in  $(\text{mg/kg-day})^{-1}$ . The interspecies scaling factor was applied to  $q_{\text{animal}}$  to obtain  $q_{\text{human}}$  in  $(\text{mg/kg-day})^{-1}$ . Unit risk in  $(\text{mg/m}^3)^{-1}$  was determined from  $q_{\text{human}}$  in  $(\text{mg/kg-day})^{-1}$  by applying a conversion factor (the ratio of human breathing rate [20 m<sup>3</sup>/day] to human body weight [70 kg]).

Male and female rats (NTP, 2000) were exposed 6.2 hours/day, five days/week for 105 weeks. Female mice (NTP 1992) were exposed six hours/day, five days/week for 104 weeks. Average concentrations during the dosing period were calculated by multiplying the reported chamber concentrations by 6 or 6.2 hours/24 hours, five days/seven days and 5.24 mg/m<sup>3</sup>/ppm. The average body weight of female mice was estimated to be approximately 0.029 kg based on data for controls reported by NTP (1992). The average body weights of male and female rats were calculated to be 0.445 kg and 0.258 kg, respectively, based on data for controls reported by NTP (2000). Inhalation rates (I) in m<sup>3</sup>/day for mice and rats were calculated based on Anderson *et al.* (1983):

$$I_{\text{mice}} = 0.0345 \times (\text{bw}_{\text{mice}}/0.025)^{2/3} \quad (3)$$

$$I_{\text{rats}} = 0.105 \times (\text{bw}_{\text{rats}}/0.113)^{2/3} \quad (4)$$

Breathing rates were calculated to be 0.038 m<sup>3</sup>/day for female mice, 0.262 m<sup>3</sup>/day for male rats, and 0.182 m<sup>3</sup>/day for female rats. Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the appropriate animal breathing rate divided by the corresponding animal body weight.

An alternative dose description approach, using pharmacokinetic analyses based on models described in the literature (Willems *et al.*, 2001; Quick and Shuler, 1999; Sweeney *et al.*, 1996; Frederick *et al.*, 1998, 2001; NTP, 2000) was evaluated. Although no data were available on the metabolism of naphthalene by rodent nasal tissues, simulations were conducted using parameters for rats and mice assuming either lung-like or liver-like scaling. The model predictions evaluated included amounts of naphthalene metabolized in each of the seven nasal compartments and their sum and the areas under the concentration  $\times$  time curves (AUCs) for the olfactory and ventral respiratory compartments. Since all of these metrics appeared linear and in relative proportion to the applied doses, they did not indicate any substantial difference from the default potency analysis. If the assumptions used are correct, the concentrations used in the NTP studies were below those at which saturation of metabolism or other pharmacokinetic effects become important in the nasal and lung regions.

Application of an uptake rate for naphthalene was also considered. NTP (2000) estimated inhalation uptakes of 22 to 31 percent for rats and 65 to 73 percent for mice based on pharmacokinetic data and PBPK modeling. However, in the subsequent publication of NTP's PBPK modeling of inhaled naphthalene, uptakes are estimated to be

85 to 94 percent in rats and 92 to 96 percent in mice (Table 3, Willems *et al.*, 2001). Until more reliable estimates become available we assume there are no significant differences in uptake between mice and rats used in the NTP bioassay. Also we assume similar uptake in humans exposed to low levels of naphthalene.

Table 5 provides the  $q_{\text{animal}}$ ,  $q_{\text{human}}$  and unit risk values, calculated using the linearized multistage procedure as described above, based on data for female mice (NTP, 1992) and male and female rats (NTP, 2000).

Table 5. Cancer potency and unit risk values for naphthalene derived using the linearized multistage procedure based on data from NTP (1992) and NTP (2000).

Sex, Species	Site, Tumor Type	$q_{\text{animal}}$  (mg/kg-day) <sup>-1</sup>	$q_{\text{human}}^{\text{a}}$  (mg/kg-day) <sup>-1</sup>	Human Unit Risk Value <sup>b</sup>  (mg/m <sup>3</sup> ) <sup>-1</sup>	Goodness- of-Fit Test <sup>c</sup>
Female mice	Lung alveolar/ bronchiolar adenoma/ carcinoma	0.004382	0.059	0.017	$p = 0.1428$
Male rats	Nasal respiratory epithelial adenoma	0.01919	0.10	0.030	$p = 0.4192$
	Nasal olfactory epithelial neuroblastoma	0.004651	0.025	0.0072	$p = 0.4224$
	Nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma combined	0.02219	0.12	0.034	NA <sup>d</sup>
Female rats	Nasal olfactory epithelial neuroblastoma	0.007636	0.049	0.014	$p = 0.6342$

a. The interspecies extrapolation was applied to  $q_{\text{animal}}$  in (mg/kg-d)<sup>-1</sup> to determine  $q_{\text{human}}$  (mg/kg-day)<sup>-1</sup>, as described above.

b. Unit risk was determined by multiplying the human cancer potency in (mg/kg-day)<sup>-1</sup> by the human breathing rate divided by human body weight, as described above.

c. A  $p$ -value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.

d. Not applicable.

U.S. EPA (2003) and others (*e.g.* Gaylor *et al.*, 1994) have more recently advocated a benchmark dose method for estimating cancer risk. This involves fitting an arbitrary mathematical model to the dose-response data. A linear or multistage procedure is often used, although others may be chosen in particular cases, especially where mechanistic information is available which indicates that some other type of dose-response relationship is expected, or where another mathematical model form provides a better fit to the data. A point of departure on the fitted curve is defined: for animal carcinogenesis bioassays this is usually chosen as the lower 95% confidence bound on the dose predicted to cause a 10% increase in tumor incidence (LED<sub>10</sub>). Linear extrapolation from the point of departure to zero dose is used to estimate risk at low doses either when mutagenicity or other data imply that this is appropriate, or in the default case where no data on mechanism are available. The slope factor thus determined from the experimental data is corrected for experimental duration and interspecies extrapolation in the same way as the  $q_1^*$  adjustments described for the linearized multistage procedure. In the exceptional cases where data suggesting that some other form of low-dose extrapolation, such as the assumption of a threshold, is appropriate, a reference dose method with safety factors as required may be used instead.

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

Model fits, points of departure and unit risks calculated using the benchmark methodology and U.S. EPA's Benchmark Dose Software version 1.3 are shown in Table 6. In all three cases, the model used was either a multistage polynomial, or a quantal linear model, which is identical to the multistage procedure in cases where the higher terms are not significant.

Table 6: Unit risk and human cancer potency values for naphthalene based on NTP (1992) and NTP (2000), derived using benchmark methodology.

Sex, Species	Site, Tumor Type	Model Fit: <sup>a</sup>	LED <sub>10</sub> [ED <sub>10</sub> ]  (mg/m <sup>3</sup> )	Animal Unit Risk Value <sup>b</sup>  (mg/m <sup>3</sup> ) <sup>-1</sup>	Human Unit Risk Value <sup>c</sup>  (mg/m <sup>3</sup> ) <sup>-1</sup>  [Human Cancer Potency] <sup>c</sup> (mg/kg-d) <sup>-1</sup>
Female mice	Lung alveolar/ bronchiolar adenoma/ carcinoma	$\chi^2 = 1.42$ $p = 0.23$	17.1 [22.8]	0.0058	0.017 [0.059]
Male rats	Nasal respiratory epithelial adenoma	$\chi^2 = 2.82$ $p = 0.42$	9.3 [12.5]	0.0108	0.028 [0.099]
	Nasal olfactory epithelial neuroblastoma	$\chi^2 = 2.82$ $p = 0.42$	38.5 [67.6]	0.0026	0.0068 [0.024]
	Nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma, combined	NA	8.1 <sup>d</sup> [10.6]	0.012	0.031 [0.11]
Female rats	Nasal olfactory epithelial neuroblastoma	$\chi^2 = 1.73$ $p = 0.63$	18.1 [26.4]	0.0055	0.014 [0.050]

- a. A  $p$ -value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.
- b. Animal unit risk was calculated using the relationship  $0.1/\text{LED}_{10}$ .
- c. The interspecies extrapolation from rodent unit risks to human unit risks was based on the (mg/kg-d)<sup>-1</sup> equivalents of the animal unit risks, as described above. The following parameters were used to derive the (mg/kg-day)<sup>-1</sup> equivalents of the animal unit risks:  $\text{bw}_{\text{animal}} = 0.029$  kg for female mice, 0.445 kg for male rats, and 0.258 kg for female rats;  $I_{\text{animal}} = 0.038$  m<sup>3</sup>/d for female mice, 0.262 m<sup>3</sup>/d for male rats and 0.182 m<sup>3</sup>/d for female rats. Human cancer potency was derived by applying the interspecies scaling factor to the (mg/kg-day)<sup>-1</sup> equivalents of the animal unit risks. The interspecies scaling factor is  $(\text{bw}_{\text{human}}/\text{bw}_{\text{animal}})^{1/3}$ , or 13.4 for female mice, 5.4 for male rats, and 6.5 for female rats. Human unit risks were then derived by multiplying human cancer potency by the human breathing rate (20 m<sup>3</sup>/day) divided by the human body weight (70 kg).
- d.. The LED<sub>10</sub> in mg/m<sup>3</sup> for the combined site analysis in rats was calculated by assuming a linear dose response relationship:  $\text{LED}_{10} = -\ln(0.9)/(\text{q}_{\text{animal}} \times [I_{\text{animal}}/\text{bw}_{\text{animal}}])$ . By inspection, this assumption appears reasonable for the dose-response curves considered, in the ED<sub>10</sub> range. For the current case,  $\text{q}_{\text{animal}}$  is the cancer potency in rats (0.02219 [mg/kg-day]<sup>-1</sup>) generated using the multisite analysis described above,  $I_{\text{animal}}$  is the breathing rate in male rats (0.262 m<sup>3</sup>/day), and  $\text{bw}_{\text{animal}}$  is the male rat body weight (0.445 kg).

Using either of these methodologies, the 95% upper confidence bound on the unit risk value for purposes of calculating cancer risks associated with exposure to naphthalene is in the range  $0.014\text{-}0.034\text{ (mg/m}^3\text{)}^{-1}$ , based on the incidence data in female mice and male and female rats from the NTP (1992; 2000) studies.

### Conclusion

The male rat was the most sensitive sex and species tested by NTP (1992; 2000) in the inhalation carcinogenesis studies of naphthalene. NTP considered the increased incidences of nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma, which are rare tumors, to provide clear evidence of the carcinogenic activity of naphthalene. The unit risk value of  $0.034\text{ (mg/m}^3\text{)}^{-1}$ , or  $3.4 \times 10^{-5}\text{ (}\mu\text{g/m}^3\text{)}^{-1}$ , based on the tumor incidence data in male rats, is therefore considered the most appropriate for use in risk assessment.

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