4. Naphthalene

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General description

Naphthalene (CAS Registry Number 91-20-3; molecular formula $C_{10}H_8$) is a white crystalline powder with a characteristic odour (of mothballs). It is a two-ring aromatic hydrocarbon isolated from coal tar. Synonyms used are antimite, naphthalin, naphthaline, naphthene and tar camphor. Naphthalene is the most volatile polycyclic aromatic hydrocarbon (PAH) with a gas-phase part of 90–100%, and has a relatively short half-life of 3–8 hours in the atmosphere. Its physicochemical properties are as follows *(1–7)*: molecular weight 128.17 g/mol; melting point 80.2 °C; boiling point 218 °C; relative vapour density 4.42g/cm^3 at 20 °C and 1 atm; vapour pressure 10 Pa at 25 °C; and diffusion coefficient 7.20×10^{-2} cm^2/s at 298 K. It is soluble in alcohol and acetate but not in water.

Conversion factors

At 760 mmHg and 20 °C, 1 ppm = 5.331 mg/m^3 and 1 mg/m^3 = 0.188 ppm; at 25 °C, 1 ppm = 5.241 mg/m^3 and 1 mg/m^3 = 0.191 ppm.

Sources and pathways of exposure

Naphthalene is produced from coal tar fractions by distillation and crystallization. It is used as feedstock in the manufacture of phthalic anhydride for the synthesis of phthalate plasticizers and synthetic resins. It is also used as feedstock for naphthalene sulfonic acids often used in the production of plasticizers for concrete, as ingredients for plasterboards, as dispersants in synthetic and natural rubbers and as tanning agents in the leather industry. Naphthalene is also used in paints and in the production of the insecticide carbaryl, used in home yards and gardens. Still predominant in the exposure of consumers worldwide is the production and use of crystalline (pure) naphthalene as a moth repellent and disinfectant. Its use as a solid block deodorizer for toilets is also reported. Wood smoke, fuel oil and gasoline also contain naphthalene. The major constituent of creosote, used for timber impregnation, is naphthalene and its alkyl homologues.

Outdoor naphthalene sources mainly originate from fugitive emissions and motor vehicle exhaust. Spills to land and water during the storage, transport and disposal of fuel oil and coal tar are released to the atmosphere by volatilization,

photolysis, adsorption and biodegradation. Usual indoor sources of naphthalene are unvented kerosene heaters and tobacco smoke *(8)*.

Outdoor sources can contribute to low levels of indoor naphthalene. The highest indoor concentrations, however, usually orders of magnitude above the outdoor air levels, come from consumer products such as multipurpose solvents, lubricants, herbicides, charcoal lighters and hair sprays, unvented kerosene heaters, tobacco smoke, rubber materials and – most importantly – naphthalene insect repellents (mothballs) used to protect textiles stored indoors in closets (although this use has decreased, mainly in western Europe).

It is assessed that the primary route of exposure is inhalation, especially in the vicinity of heavy traffic, petrol stations and oil refineries. Although inhalation is the major route of the total human exposure to naphthalene, dermal exposure is not to be neglected. Preuss et al. *(9)* assessed the total daily naphthalene intake from air, food and house dust (including soil) at 1.127, 0.237 and 0.235 μg/kg per day, respectively, for a 70-kg adult. Since people spend most of their time indoors, inhalation of indoor air plays the major role in human total exposure to naphthalene.

Indoor concentrations and exposures

There is limited information available in the literature on indoor air concentrations and personal exposure levels of naphthalene. In Europe, two large-scale population-based studies, EXPOLIS *(10)* and the German Environmental Survey (GerES) *(11)*, provide useful data on indoor air exposure and outdoor air concentrations of naphthalene. Some other studies have been reviewed in the course of the INDEX project *(12,13)*. Results from some studies carried out in and outside Europe are summarized in Table 4.1 and are discussed below.

In Europe, indoor concentrations and personal exposures are usually low, typically below 1–2 μg/m^3 *(14)*. In a large-scale study representative of the Federal Republic of Germany before reunification (n = 479), a mean naphthalene concentration of 2.0 μg/m^3 in residential indoor air within a range of individual samples from 0.7 to 14 μg/m^3 was reported *(9)*. In a follow-up study, 555 dwellings in 150 cities were monitored between May 2003 and May 2006 (child's bedroom; passive sampling for one week) *(15)*. The indoor concentration of naphthalene was below the detection limit (1 μg/m^3) in 93% of the houses. The median concentration and 90th percentile were below the detection limit, whereas the 95th percentile and maximum value were respectively 1.2 and 4.9 μg/m^3.

In contrast to this, naphthalene exposures in Athens were found to be remarkably higher. Here, the USEPA's 2006 inhalation reference concentration of 3 μg/m^3 *(16)* and the INDEX project's long-term guideline value of 10 μg/m^3 *(13)* were exceeded in every personal exposure, and the mean and median concentrations were 54.0 and 22.6 μg/m^3, respectively. In Athens, there were five

participants whose personal exposures were considerably higher than the rest of the population and ranged from 74 to 469 μg/m³. Indoor concentrations were even higher, ranging from 114 to 989 μg/m³, respectively *(14)*.

Few data could be found on naphthalene concentrations in public spaces, transport and schools, and these are summarized in Table 4.1. Only two European studies carried out in Germany deal with schools and hospitality venues, respectively. In addition, some non-European studies were reviewed.

In Schleswig-Holstein, 285 classrooms from 105 schools and day-care centres were investigated for VOCs (active sampling) between July 2005 and February 2007 *(17)*. In 216 classrooms (76%), the naphthalene concentration was below the detection limit of 1 μg/m³. The median concentration, 90th and 95th percentiles and maximum value were respectively <1.0, 1.0, 3.7 and 22 μg/m³. Naphthalene was not measured in a previous campaign carried out in Schleswig-Holstein in 1990–1993, so no comparison can be provided.

Active sampling of indoor air was conducted for 4 hours during the main opening hours in 28 hospitality venues in the cities of Augsburg and Munich, from April 2005 to May 2006 at a time when smoking was allowed *(18)*. Median levels of naphthalene were 80.0 μg/m³ in restaurants and cafés (n = 11), 59.0 μg/m³ in pubs and bars (n = 7) and 98.5 μg/m³ in discotheques (n = 10).

In Table 4.1, the naphthalene concentrations vary widely between 0.036 and 143.9 μg/m³. Although it would be more appropriate to differentiate between the data measured in different ways, such a differentiation is not reflected in Table 4.1.

In the studies reviewed in the European INDEX project, residential indoor concentrations were elsewhere low, typically averaging below 2 μg/m³, whereas in Athens clearly higher indoor levels were measured, being on average 90 μg/m³ *(10)*. Personal exposures to naphthalene elsewhere ranged from 1 to 3 μg/m³ *(10,11)*, whereas in Athens the average exposure was 46 μg/m³. In general, we can conclude that exposures to naphthalene are usually low in Europe, but in Athens (and presumably also other countries in eastern and southern Europe) remarkably higher indoor levels of naphthalene were present.

Maroni et al. *(19)* reported typical median and 90th percentile naphthalene concentrations in indoor air in Italy of 2 μg/m³ and 5 μg/m³, respectively. Kostiainen et al. *(20)* detected slightly lower indoor concentrations in Helsinki, 0.44 μg/m³ and 1.63 μg/m³ being the mean and maximum concentrations. Bituminous materials commonly used in the United Kingdom for damp-proofing floors emit naphthalene *(21)*. Naphthalene concentrations up to 970 μg/m³ were found in homes having an objectionable smell, where a damp-proof membrane had been applied, compared with less than 300 μg/m³ for control homes *(22)*. Rubber flooring may also emit naphthalene in odorous amounts. In an Italian study, the average indoor naphthalene concentration was 11 μg/m³ and the maximum level 70 μg/m³ *(23)*.

In tropical areas, indoor naphthalene concentrations seem to be generally higher. Mean values in Burundi and Taiwan, China were about 30 μg/m³ *(9)*. Zuraimi et al. *(24)* compared the characteristics of VOCs and the associated factors affecting them in office buildings in Europe (EU) and in Singapore. They found that concentrations of naphthalene were significantly higher (mean and maximum 144 μg/m³ and 745 μg/m³, respectively) in Singapore buildings compared to the EU buildings (mean and maximum 6.5 μg/m³ and 68.5 μg/m³, respectively, see Table 4.1).

Area-specific emission rates of naphthalene were also significantly higher and ventilation rates significantly lower in Singapore buildings. Higher levels of naphthalene in ETS-free Singapore buildings were associated with human activity.

Jia et al. *(25)* measured VOCs in indoor and outdoor environments in Michigan, United States to assess their health risk drivers. Monitoring was conducted during two seasons inside and outside 159 residences in industrial, urban and suburban cities. Outdoor concentrations were elevated in winter in the suburban community and were highest in the industrial community. Indoor concentrations were higher in the summer. Seasonal changes were small or inconsistent. Indoor levels of naphthalene exceeded the inhalation reference concentration of 3 mg/m³ in 12% of residences. The highest level measured was 91.7 μg/m³.

Yu *(26)* pointed out that indoor naphthalene pollution may also be an issue in Chinese archives. The Chinese Government banned the production and sale of mothballs in 1993, but the use of mothballs in archives and libraries is still permitted for the protection of documents and specimens. It was estimated that up to 10–12 mothballs per m² were used in a typical Chinese archive, but unfortunately no measurements have been reported for such an environment.

Lu et al. *(27)* modelled the regional distributions and human exposures to naphthalene in southern California. Petrol and diesel engine exhaust, with related vaporization from fuels, were found to contribute roughly half of the daily total naphthalene burden in southern California. Based on their analysis, the mean hourly naphthalene exposure of the population was 0.27 μg/m³ in the summer and 0.43 μg/m³ in the winter. Higher exposures are experienced by a fraction of the population. More than one million people were exposed to naphthalene levels greater than 1 μg/m³ during wintertime and nearly 100 000 were exposed to average concentration exceeding 2 μg/m³.

Lu et al. *(28)* reported the results of a PAH pollution survey in the air in public places in Hangzhou, China. The most serious PAH pollution was found in indoor air in shopping centres and the least in railway stations. The highest naphthalene concentration (23.5 μg/m³) was measured in a shopping centre (see Table 4.1). The authors concluded that emissions of 2–4-ring PAHs occurred from indoor sources in shopping centres and supermarkets, whereas 5–6-ring PAHs originated predominantly from outdoor air. In temples, PAHs in indoor air

mainly originated from incense burning. Naphthalene was the largest contributor (62.4%) to the total health risk when risks associated with the inhalation of PAHs were assessed.

To understand PAH generation in kitchens, Zhu & Wang *(29)* surveyed six representative homes and four commercial kitchens in Hangzhou, China. The highest naphthalene concentrations in a commercial kitchen, in a domestic kitchen of a non-smoking family and in a kitchen of a smoking family were 3.0, 2.7 and 9.9 μg/m^3, respectively. Naphthalene was identified as the most predominant PAH, mostly resulting from the evaporation of mothballs used to protect clothes.

Liu et al. *(30)* measured PAHs simultaneously in the indoor and outdoor air of eight homes in Hangzhou, China. Of the 12 PAHs, naphthalene was the most abundant in both indoor (0.122–26.9 μg/m^3) and outdoor air (0.072–25.1 μg/m^3). Both in summer and in autumn, it contributed more than 60% to the sum of PAHs.

Using standard methods, Lin et al. *(31)* studied the role of incense burning on human exposure to 21 PAHs and total suspended particulates (TSP) in and around a temple in Taiwan, China. Indoor mean total PAH, particle-bound PAH and TSP concentrations were 6.26 μg/m^3, 490 μg/g and 1.32 μg/m^3, respectively. Values for outdoor readings were 0.23 μg/m^3, 245 μg/g and 73 μg/m^3, respectively. With respect to concentrations of individual PAHs (particulate + gas phase), the naphthalene concentration was the second highest at 1.26 μg/m^3. The median indoor : outdoor ratio for naphthalene was 8.6. Median values for indoor : outdoor ratios of individual PAHs ranged from 5.7 to 388, which implied that the temple was a significant PAH source. Moreover, the PAH content of the tested stick incense and ash was low. PAH levels inside the temple were much higher than those measured in the vicinity and inside residential houses, and were in fact close to levels measured at a nearby traffic intersection.

Li & Ro *(32)* measured 15 PAHs simultaneously in the indoor and outdoor air of 14 homes in the Taipei urban area during the summer and winter seasons. They reported that indoor PAH concentrations generally exceeded the corresponding outdoor PAH concentrations. In homes using incense, PAHs could be attributed mainly to incense burning. The most abundant PAH found indoors was naphthalene.

In Australia, several studies have been conducted to detect naphthalene but so far no direct indoor naphthalene concentration data have been forthcoming. The only two indoor studies on indoor naphthalene are summarized below.

Zou et al. *(33)* investigated PAH profiles from the combustion of different Australian firewood species in a domestic wood heater in a laboratory. The 16 PAH emission rates obtained varied between 5965 and 11 508 μg/kg for four firewood species and they were mainly emitted in the gaseous phase (91–98.8%). Overall, gaseous naphthalene accounted for up to 69% of total PAHs in the air.

Table 4.1. Naphthalene concentrations in air reported in the reviewed scientific literature

Reference	Country/city	Period	Environment
Residential settings, European studies			
Jantunen et al. *(10)*	Athens	1996–1997	Residences, indoors
	Basel	1996–1997	Residences, indoors
	Helsinki	1996–1997	Residences, indoors
	Milan	1996–1997	Residences, indoors
	Oxford	1998–2000	Residences, indoors
	Prague	1996–1997	Residences, indoors
Jantunen et al. *(10)*	Athens	1996–1997	Personal exposure
	Basel	1996–1997	Personal exposure
	Helsinki	1996–1997	Personal exposure
	Oxford	1998–2000	Personal exposure
	Prague	1996–1997	Personal exposure
Hoffman et al. *(11)*	German survey	1990–1992	Personal exposure
KUS *(15)*	German survey	2003–2006	Residences. indoors
Non-European studies			
Jia et al. *(25)*	Michigan, USA	2004–2005	Residences, indoors Residences, outdoors
Zhu & Wang *(29)*	Hangzhou, China	1999–2000	Domestic kitchen, non-smoking Domestic kitchen, smoking Commercial kitchen
Ohura et al. *(35)*	Shimizu, Japan	2000 2001	Residences, indoors, summer Residences, indoors, winter
Public spaces			
Lu et al. *(28)*	Hangzhou, China	2006	Railway station, indoors Shopping centre, indoors Supermarket, indoors Supermarket, indoors/outdoors Hotel, indoors Temple, indoors Temple, indoors/outdoors
Zuraimi et al. *(24)*	Singapore Europe	2006 2006	Office buildings Office buildings
Heinzow & Ostendorp *(17)*	Germany	2005–2007	Schools
Bolte et al. *(18)*	Germany	2005–2006	Hospitality venues
Lin et al. *(31)*	Taiwan, China	1996	Temple, indoors Temple, outdoors

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Averaging time	No. of samples	Concentration (µg/m³) [a]			
		AM	SD	GM	Max
30 hours	42	83.5	197		
30 hours	47	0.7	0.3		
30 hours	188	0.6	0.5		
30 hours	41	21.0	81.6		
30 hours	40	1.3	1.5		
30 hours	46	2.0	1.9		
48 hours	46	47.1	78.0		
48 hours	50	0.8	0.6		
48 hours	193	0.7	0.2		
48 hours	42	0.8	0.5		
48 hours	49	2.4	2.8		
1 week	113	2.3		2.1	
1 week		< 1			4.9
3–4 days	226 samples	3.5			91.8
3–4 days	252 samples	0.3			4.7
12 hours	3 kitchens	1.8			2.7
12 hours	3 kitchens	5.3			9.9
12 hours	4 kitchens	2.3			3.0
24 hours	25 houses	1.1			
24 hours	22 houses	1.0			
12 hours	2 samples	2.7			
12 hours	2 samples	23.5			
12 hours	2 samples	19.7			
12 hours	20 samples	2.38	0.59		3.5
12 hours	2 samples	16.3			
9 hours	2 samples	16.1			
9 hours	16 samples	4.14	1.98		7.1
–	8 buildings	143.9	93.0		745
–	50 buildings	6.5	4.3		68.5
	105 schools	< 1			22
4 hours	28 venues				
	Restaurants & cafés (11)	80.0			
	Pubs and bars (7)	59.0			
	Discotheques (10)	98.5			
8 hours	6 samples			1.22	
24 hours	6 samples			0.16	

Duigu et al. *(34)* examined PAH composition on the surface films from the glass windows of 18 residential buildings. The results indicated an average naphthalene concentration on the films of 33.7 ± 44.2 ng/m^2.

Comparison of indoor with outdoor concentrations

Average outdoor naphthalene concentrations are low in Europe, ranging typically from 1 to 4 μg/m^3 *(10)*. Even lower outdoor levels, below 1 μg/m^3, have been reported in Taiwan, China and the United States (see Table 4.1). The outdoor concentration of naphthalene in air is generally lower in rural than in urban areas.

The indoor mean concentration of naphthalene is reported to range up to a maximum of 143.9 μg/m^3, although the majority of studies report average naphthalene indoor levels below 10 μg/m^3.

Table 4.1 shows the naphthalene air concentrations reported in a number of scientific publications. However, several different sampling techniques were used in these studies. For example, Ohura et al. *(35)* employed glass fibre filters and XAD-2 resin for particulate and gaseous naphthalene sampling, respectively, while the EXPOLIS project utilized only a Tenax TA tube to collect both phases of naphthalene. It was also reported by Lin et al. *(31)* that polyurethane foam had been used to sample gas-phase naphthalene with other vapour PAHs.

Biomarkers of human exposure to naphthalene

Urinary 1- and 2-naphthol are well-established human biological exposure indices to evaluate the exposure to naphthalene of workers as well as the general population. Median 1-naphthol concentrations found in non-smokers without known occupational exposure range from 1 to 5 μg/l urine and median 2-naphthol concentrations from 1 to 3.6 μg/l *(36)*. Smokers show significantly higher naphthol concentrations *(9,36)*.

Both 1- and 2-naphthol were used to check the impact of genetic polymorphisms on naphthalene metabolism *(37–39)*. Urinary 2-naphthol concentrations were higher in smokers with the CYP2E1 genotypes c1/c2 or c2/c2 than in smokers with the more common c1/c1 genotype. Higher concentrations of 1- and 2-naphthol were found in the urine of smokers deficient in glutathione S-transferase M1. In recent studies, 2-naphthol was used as a biomarker to evaluate polymorphisms in patients with lung cancer or oral squamous cell carcinoma *(40,41)*.

A glucose-6-phosphate dehydrogenase deficiency has been suggested to lead to an increased susceptibility to haemolytic anaemia in children and newborn infants exposed to naphthalene, but exposure levels of naphthalene were not estimated in most reports. Haemolytic anaemia observed in neonates could also be explained by a lower ability to metabolize naphthalene and eliminate naphthalene metabolites. In a Nigerian study, five neonates presenting with jaundice or

tetanus showed very high urinary 1-naphthol concentrations *(42)*. Three of them were deficient in glucose-6-phosphate dehydrogenase. In this group, the 1-naphthol concentrations ranged from 1140 to 11 690 μg/l urine, similar to those of the non-deficient newborn infants (750–9550 μg/l). Such high naphthol concentrations have been reported in occupational settings but not in humans *(9)*.

It is recommended that 1- and 2-naphthol be measured simultaneously, since both metabolites correlate. An elevated level of 1-naphthol alone may be an indicator of an additional exposure, such as to the biocide 1-naphthyl methylcarbamate (carbaryl) or to some hair dyes *(43)*. A study in the Republic of Korea on non-smoking municipal middle-school students showed significant correlations between urinary 2-naphthol concentrations and the daily mean total suspended particulate level estimated for 1–2 days before and for the day of the survey *(44)*.

In a recent study, a method was developed for measuring urinary 1,2- and 1,4-dihydroxynaphthalene *(45)*. Strong correlations were observed among these naphthadiols and both naphthols in urine. Further, the urinary concentrations of 1,2-dihydroxynaphthalene were significantly correlated with the serum concentrations of 1,2-naphthoquinone albumin adducts.

Kinetics and metabolism

Kinetics

There are no published studies that document the precise bioavailability of naphthalene after oral, dermal or inhalation exposure. It is clear from human poisoning cases *(46)*, the exposure of air force personnel to jet fuel containing naphthalene *(47,48)* and numerous animal studies *(49)* that naphthalene can be absorbed by all three routes. In exposed human volunteers, dermal administration of naphthalene resulted in relatively rapid uptake of the parent compound, with peak levels observed in approximately 60 minutes. Calculated partition coefficients demonstrate high partitioning of naphthalene in the fat, while toxicokinetic studies in mice after inhalation exposure and in rats after both inhalation and intravenous administration *(49)* demonstrate rapid clearance from the blood. Very little naphthalene is eliminated unchanged in expired breath, a finding consistent with the results of the physiologically based toxicokinetic analysis suggesting that 88–98% of inhaled naphthalene is eliminated as metabolic by-products.

Very recently, work has been taken up to better understand gender and species differences in upper respiratory tract uptake and in situ metabolism of naphthalene *(50)*. At a flow of 150 ml/minute, upper respiratory tract uptake in female F344 rats exposed to naphthalene concentrations of 5, 21, 53 or 181 mg/m^3 was concentration-dependent, with rates of 56%, 40%, 35% and 28%, respectively. These rates were similar to the uptake observed in male rats (57%, 49%, 37% and 36%, respectively). The concentration dependence of naphthalene uptake in the upper respiratory tract is probably due to nasal metabolism of

naphthalene. A significant reduction of naphthalene uptake was observed after pre-treatment with the inhibitor 5-phenyl-1-pentyne.

Metabolism

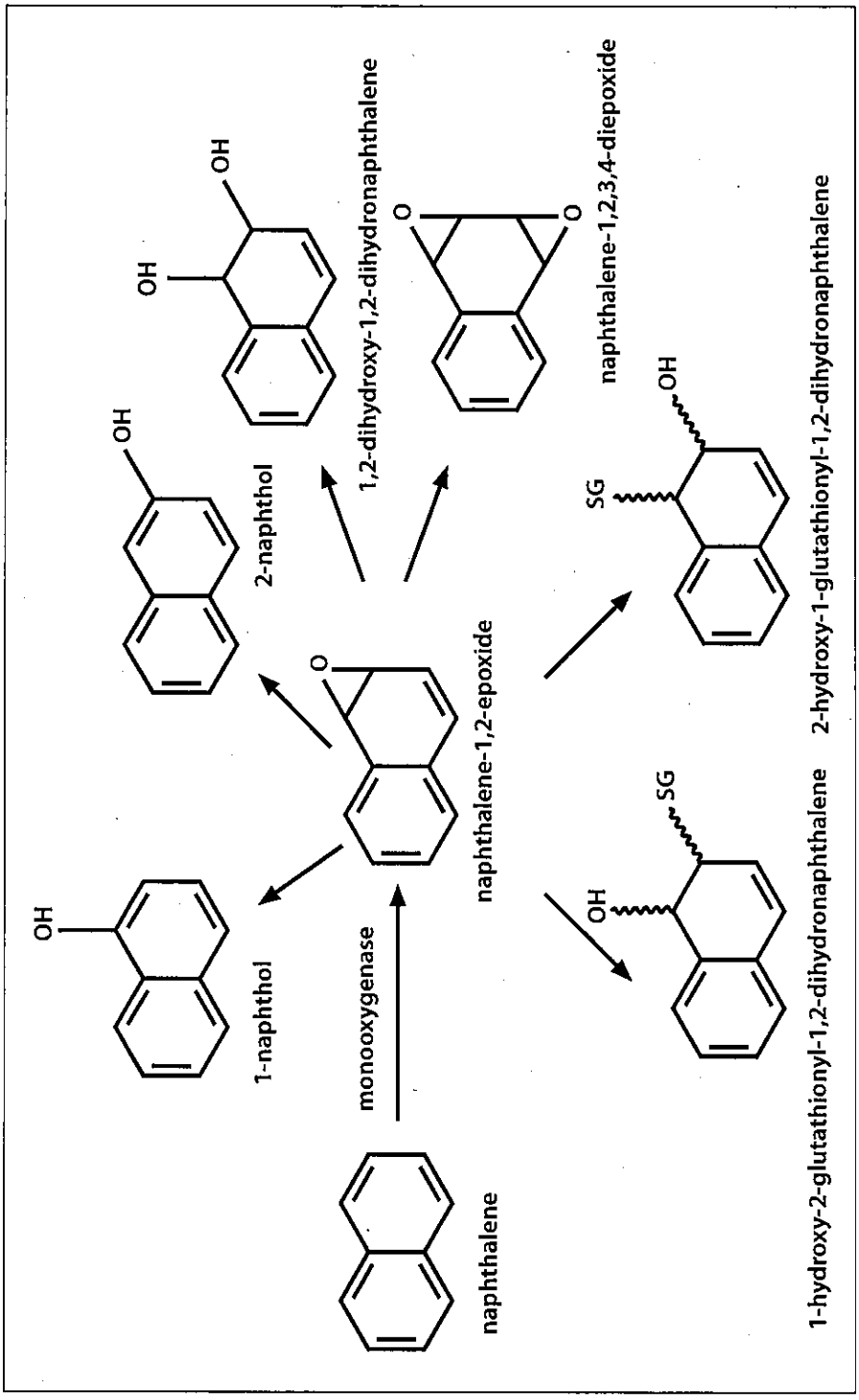
The metabolism of naphthalene to metabolites that can be excreted by mammals occurs as a multi-step process involving both initial oxygenation reactions and subsequent conjugation. The first step in metabolism involves the formation of an unstable 1,2-epoxide (Fig. 4.1) that can be catalysed by several cytochrome P450 monooxygenases. Several (e.g. 2A13, 2E1, 2F1 and 2F2) can oxidize naphthalene to naphthalene-1,2-epoxide and further to 1,2,3,4-diepoxide. Naphthalene-1,2-epoxide can also be rearranged to 1- or 2-naphthol or be transformed by epoxide hydrolases to dihydroxy-dihydro-naphthalene or by glutathione transferases to glutathionyl derivates.

These monooxygenases are discussed in detail below, since the initial formation of naphthalene oxide is a key step in the downstream toxicological activities associated with naphthalene exposure. A number of further metabolites can be generated directly from the epoxide by both enzymatic and non-enzymatic processes. The cytochrome P450 monooxygenases can biotransform naphthalene to a putative diepoxide or diolepoxide *(51,52)*, microsomal epoxide hydrolases generate a trans-dihydrodiol *(53)* and the glutathione transferases form diastereomeric glutathione conjugates *(54)*, which are eliminated primarily as mercapturic acids *(51,52)*. In water, 1- (major) and 2-naphthol (minor) arise from non-enzymatic rearrangement of the epoxide *(55)*. In human liver microsomal incubations, the calculated V_{max} for the formation of 1-naphthol, 2-naphthol and 1,2-dihydroxy-1,2-dihydronaphthalene were 268, 22 and 2860 pmoles/min per mg protein, respectively *(56)*. Each of these secondary metabolites can undergo further biotransformation and with two of these metabolites (1-naphthol and 1,2-dihydroxy-1,2-dihydronaphthalene), more reactive chemical entities can result. The suspected reactive, toxicologically active metabolites include naphthalene epoxide, naphthalene diepoxide (or diol epoxide), 1,2-naphthoquinone and 1,4-naphthoquinone; the formation and disposition of these will be discussed individually. The primary urinary metabolite eliminated in exposed human populations is 1-naphthol glucuronide *(37,57–59)*. In recent surveys in the United States, this metabolite could be detected in the urine of all 2748 individuals sampled, thus establishing widespread exposure of human populations *(60)*.

Naphthalene-1,2-epoxide

The stability of various aromatic and aliphatic hydrocarbon epoxides varies considerably, which in turn affects the interactions with key cellular macromolecules and overall downstream impact *(61)*. In contrast to aflatoxin epoxide, which has an estimated half life of 1 second in water, naphthalene epoxide has a half-life of 2–3 minutes in water and 11 minutes in solutions of albumin *(62)*. Thus, naph-

Fig. 4.1. The first steps in the metabolism of naphthalene



thalene oxide is sufficiently stable to circulate from organs able to rapidly generate this metabolite to those with lower metabolic rates. While there is some evidence that circulating naphthalene oxide can produce injury in the lung *(62)*, there is a strong possibility that such circulating metabolites may enhance the susceptibility of tissues such as the lung to injury by depleting protective thiols such as glutathione *(63)*.

The importance of regiochemistry and stereochemistry in the biological effects of epoxides and diol epoxides of larger PAHs is well-established. Many PAH-specific P450s show remarkable regioselectivity and stereoselectivity in the metabolites they produce. Similarly, several of the P450s show a high degree of stereoselectivity in naphthalene metabolism. By using N-acetylcysteine to trap reactive naphthalene epoxides, van Bladeren et al. *(64,65)* were able to show that cytochrome P450 2B shows a slight preference for the formation of the (1*S*,2*R*)-naphthalene epoxide (74%) whereas cytochrome P450 1A1 preferentially generates (1*R*,2*S*)-epoxide (73–95%). Studies showing marked differences in the ratio of glutathione conjugates formed in microsomal incubations from mouse lung vs liver demonstrated substantial differences in the stereoselectivity of naphthalene epoxide in target (lung) compared to non-target (liver) tissues *(54)*. Approximately equal rates of formation of the (1*R*,2*S*)- and (1*S*,2*R*)-epoxide were observed in liver microsomes, whereas 10 : 1 ratios of the (1*R*,2*S*)- to (1*S*,2*R*)-epoxide were made in the lung. Similarly, subsequent work using dissected airways from susceptible mice and non-susceptible rats showed the same pattern of stereoselectivity. Metabolism in target regions of the respiratory tract of the mouse resulted in highly selective formation of the (1*R*,2*S*)-epoxide whereas approximately equal proportions of the epoxide enantiomers were made by rat lung airways *(66)*.

This high degree of selectivity in the formation of a single stereoisomer of naphthalene oxide was consistent with 60 : 1 ratios in isomer generation catalysed by cDNA-directed expression of cytochrome P450 2F2 in baculovirus-infected SF-21 cells and with immunolocalization experiments showing that that airway epithelial cells were highly stained. Thus, while it appears that cytochrome P450 2F2 is responsible for the rapid and stereoselective formation of (1*R*,2*S*)-naphthalene oxide, it is not at all clear that the stereoselectivity of this process is relevant to the cytotoxicity associated with naphthalene in the respiratory tract. Although it is possible that the toxicological potency of the naphthalene epoxide enantiomers differ, it seems far more likely that the differential susceptibility of rat and mouse airways and mouse lung and liver are due to the rates of initial substrate turnover. Published data in isolated mouse hepatocytes have shown that the intracellular residence time of the epoxide isomers may differ because of different rates of glutathione conjugation or hydrolysis by epoxide hydrolase, and that this does indeed translate into differential toxicity of these two epoxides *(67)*. However, definitive analysis of the importance of the stereochemistry of

epoxidation of naphthalene in the lung is problematic because of the instability of the epoxide. Short incubations of racemic naphthalene epoxide with dissected airways of both the rat and mouse and in proximal vs distal airways showed very little difference in the rates of formation of glutathione conjugates or in the diastereomers produced. Likewise, there were no discernable differences in the rates of dihydrodiol production between rat and mouse airways that appeared to relate to the species differences in response to naphthalene *(66)*.

1-Naphthol

One of the primary metabolites generated from naphthalene oxide in aqueous solutions is 1-naphthol. The ratios of this rearrangement product to the 1,2-dihydroxy-1,2-dihydronaphthalene (dihydrodiol) are dependent on the rates of formation of the epoxide and the activities of microsomal epoxide hydrolase which are, in turn, species-dependent. 1-Naphthol can be metabolized to protein-reactive metabolites both in vitro *(68,69)* and in vivo *(70)*. Conjugation with sulfate and UDP glucuronic acid results in derivatives that, in many species, constitute major urinary metabolites (see above). 1-Naphthol is a precursor to the formation of 1,4-naphthoquinone, a potential cytotoxic metabolite *(56,71)*. The 1,4-naphthoquinone can stimulate the redox cycle *(72)* and binds covalently to proteins in vitro *(73,74)* and in vivo *(75–77)*.

1,2-Dihydroxy-1,2-dihydronaphthalene

The dihydrodiol, generated through metabolism of the epoxide by epoxide hydrolase, is converted by a dihydrodiol dehydrogenase (aldose reductase) *(78–80)* to the 1,2-dihydroxynaphthalene, which auto-oxidizes to a 1,2-quinone. The 1,2-quinone can bind covalently to protein both in vitro and in vivo *(75–77,81)* and forms depurinating adducts on DNA in vitro *(82)*.

1,2-Naphthalene diepoxide (diolepoxide)

Indirect evidence for the formation of a diepoxide/diolepoxide comes from the isolation of the 1,2,3,4-tetrahydroxytetrahydronaphthalene from urine of naphthalene-treated rats *(83)*.

Glutathione is depleted in murine tissues capable of metabolizing naphthalene in a dose/concentration-dependent fashion after either intraperitoneal administration *(84)* or inhalation *(85)*. Glutathione adducts are generated at both the allylic and benzylic carbons of naphthalene *(54)*. Although the initial studies resolved only three diasteromers, with improved techniques a fourth, minor conjugate has been identified. These glutathione conjugates are eliminated primarily as mercapturic acids and account for 25–35% of a dose of naphthalene administered intraperitoneally to either mice or rats. No species differences were noted in the percentage of dose eliminated as mercapturate *(86)*. In mice, exposure to 319 mg/m^3 resulted in levels of mercapturate in the urine that were similar to

those observed after intraperitoneal administration of 50 mg/kg. It is interesting to note that there appears to be a significant species difference in the amounts of naphthalene eliminated as mercapturates in rodents and non-human primates. In both the chimpanzee *(87)* and the Rhesus monkey *(88)*, an increase in urinary thioether elimination, measured after conjugate hydrolysis with the Ellman assay, was not observed in response to orally administered naphthalene. In comparison, diethylmaleate administration resulted in dose-dependent increases in thioether elimination in both species.

The primary products eliminated in the urine of mice following the intravenous administration of naphthalene glutathione conjugates were mercapturic acids, and accounted for 40–85% of the administered dose *(89)*. Small amounts of cysteine conjugate were measured in the urine. There was a significant difference noted in the metabolic disposition of the benzylic compared to the allylic adducts. Some 15–20% of the administered dose of the 1R-glutathionyl-2R-hydroxydihydronaphthalene was excreted as a thiopyruvic acid derivative.

Enzymes involved in naphthalene metabolism

There is considerable experimental evidence showing that metabolism of naphthalene is required for any of the downstream toxicities associated with this compound in animal models. Thus, a substantial amount of effort has been focused on species comparisons in the rates of formation of naphthalene oxide, as well as on understanding the importance of specific pulmonary cytochrome P450 monooxygenases in the metabolic activation of this agent. The contribution of each of these P450 proteins to the conversion of naphthalene to more biologically active derivatives is dependent not only on the amounts of protein present but also on the catalytic activities of each of the proteins. Unfortunately, quantifying the amounts of each of the cytochrome P450 isoforms present in various subcompartments of the lung is difficult and in only a few cases has purified protein been available as standard *(90)*. More information is available on the catalytic properties of some of the P450 monooxygenases through the use of recombinant proteins. Since the environmental levels of naphthalene are quite low, data on the catalytic efficiencies (K_m) of the individual P450 monooxygenases is also a key to assessments designed to determine whether low-level, long-term exposures are a potential risk to human health. Accordingly, the following sections discuss what is known about the overall rates of metabolism of naphthalene in target and non-target tissues of rodents and primates, along with a discussion of P450s known to metabolically activate this substrate.

Comparative metabolism studies in rodents and primates

There are 50–100-fold differences in the rates of naphthalene metabolism to water-soluble metabolites in microsomal incubations prepared from target and non-target rodent tissues and corresponding tissues of the Rhesus monkey and

human *(91,92)*. In general, the rates of metabolism correlate well with the tissue susceptibility to toxicity. At saturating substrate concentrations, mouse lung (target tissue) microsomal naphthalene metabolism occurs at rates of 15 nmoles/mg microsomal protein per minute, compared to less than 2 nmoles/mg per minute in rat lung (non-susceptible). Likewise, the rates of microsomal naphthalene metabolism in rat olfactory epithelial tissues (highly susceptible to naphthalene) are approximately 16 nmoles/mg per minute *(93)*. In comparison, Rhesus monkey lung microsomes metabolize naphthalene at a rate of 0.15 nmoles/mg per minute. Similar rodent-to-primate differences were observed using more specific approaches, where metabolism was measured in target subcompartments *(66,94)*.

Enzymology of naphthalene epoxide formation

CYP2F. Nagata and co-workers *(95)* purified a cytochrome P450 monooxygenase from mouse liver that metabolized naphthalene rapidly and with high stereoselectivity. The gene was cloned and sequenced *(96)* and had 82% sequence homology to a cDNA that had been cloned earlier from human lung *(97)*.

CYP2F2 (mouse). Naphthalene is metabolized with a high degree of stereoselectivity by recombinant mouse CYP2F2 expressed in either yeast *(96)* or in SF-21 insect cells *(98)*. A very high V_{max} (107 nmoles product/nmole P450 per minute) and low K_m (3 μM) for the metabolism of naphthalene by recombinant CYP2F2 are consistent with the importance of this protein in the metabolic activation and toxicity of naphthalene in mouse lung. The low K_m observed is well below the range of expected tissue concentrations in the lung after inhalation exposure at the 53-mg/m^3 level. N-terminally truncated recombinant human keratinocyte growth factor (DeltaN23_KGF) lowers the expression of CYP2F2 in mice, thus reducing the airway injury of naphthalene *(99)*.

CYP2F4 (rat). Immunocytochemistry with antibodies generated to the mouse 2F *(66)* and northern blot analysis initially failed to demonstrate the presence of a P4502F orthologue in the rat. More detailed investigations uncovered a transcript that had 94% similarity in the deduced amino acid sequence to CYP2F2 *(100)*. cDNA-directed expression of CYP2F in SF-21 insect cells yielded a protein with nearly identical catalytic activities to the mouse orthologue. Thus, the substantial differences in susceptibility of mouse compared to rat lung was not likely to be due to differences in the catalytic differences in metabolism by CYP2F, but rather appears to be related to the amounts of protein present as assessed by immunoblot analysis *(101)*.

CYP2F1 (human)/CYP2F5 (monkey). CYP2F1 has been expressed in a number of different recombinant protein expression systems. Although substantial pro-

tein is produced in the baculovirus-infected SF-21 cells, a P450 spectrum could not be obtained. Similiarly, cDNA-directed expression of CYP2F5 from the Rhesus monkey resulted in protein but no haem incorporation. Both proteins were catalytically inactive *(100)*. Expression of CYP2F1 in lymphoblastoid cells *(102)* resulted in the production of a protein with very low rates of naphthalene turnover (~0.035 nmoles conjugate/min per nmole P450). This rate is less than 0.1% the rate of metabolism observed with the mouse orthologue. The recombinant human CYP2F1 showed slight stereopreference in the generation of (1*S*,2*R*)-naphthalene epoxide.

Other cytochrome P450 monooxygenases. While it is likely that CYP2F is primarily responsible for the metabolic activation of naphthalene in mice, other P450 monooxygenases may play an important role in catalysing the turnover of this substrate in humans. Cho et al. *(56)* have published a very thorough investigation of the catalytic activity of various commercially available cytochrome P450 monooxygenases with naphthalene. Cytochrome P450 2E1 has the lowest K_m of any of the proteins tested (10 μM) with a V_{max} that is 8 pmoles/min per pmole P450 for the formation of 1-naphthol. This is 10-fold lower than the V_{max} for CYP2F2. Cytochrome P450 2E1 has been reported in human lungs based on both immunoblotting and activity assays *(103,104)*. Recent work showing high catalytic activities of CYP2A13 *(105)*, a protein reported in human respiratory tissue *(106)*, suggests that this protein may be important in human metabolism of naphthalene. The K_m and K_{cat} for the formation of 1-naphthol were 36 μM and 143 min^{-1}, respectively. Aryl hydrocarbon receptor-mediated enzymes do not contribute significantly to naphthalene bioactivation in mice *(107)*.

Formation and possible importance of protein-bound metabolites

The concept that reactive metabolite formation can, but does not always, lead to cellular necrosis has been well-established with a number of hepatic, renal and pulmonary toxicants. Early studies with naphthalene showed that reactive metabolites become bound covalently to cellular proteins both in vivo and in vitro in a dose/concentration-dependent manner *(84)*. The irreversible binding of reactive metabolites occurs prior to any signs of cellular degradation, and prior treatment with inhibitors of cytochrome P450 or with glutathione depletors alters the extent and severity of cytotoxicity in concert with the amounts of reactive metabolite bound *(108)*. The binding levels generally correlate with target tissue susceptibility. Although considerable progress has been made in identifying proteins that are adducted by a variety of reactive metabolites, including naphthalene *(109–111)*, it has not been demonstrated that a particular protein adduct (or adducts) results in toxicity. What is clear is that there are commonalities in proteins that are adducted by reactive naphthalene metabolites across species, and the 50–100-fold differences in rates of water-soluble metabolite formation

between rodents and primates are not observed when total reactive metabolite binding is compared. Incubations of dissected airways of Rhesus monkeys with naphthalene resulted in levels of covalent adduct varying from 0.3 nmoles/mg protein in the trachea to 1.2 and 1.4 nmoles/mg protein in the distal airway and parenchyma, respectively *(94)*. Under similar conditions, the rates of formation of reactive metabolites that become bound covalently in dissected airways of mouse lung varied from 0.8 to 3.8 nmoles adduct per mg protein from trachea to distal airway *(112)*. Recent comparisons between rat nasal olfactory epithelium, which is highly susceptible to naphthalene *(93)*, and ethmoid tissues from the Rhesus monkey show nearly identical levels of reactive metabolite formation in in vitro incubations *(111)*.

It is important to note that several naphthalene metabolites are protein-reactive, including the epoxide *(67)* and both the 1,2- and the 1,4-naphthoquinones *(113,114)*. Which (if any) of these metabolites are essential to the steps leading to cytotoxic injury is not clear, nor are the relative contributions of each metabolite to the overall levels of adducts measured. At least in rats and mice, Waidyanatha & Rappaport *(77)* have shown that naphthalene oxide is the primary metabolite that adducts albumin and haemoglobin in both species.

Health effects

Most of the data available on the toxic effects of naphthalene have been derived from animal studies conducted either in vivo or with in vitro preparations *(22,46,115)*. There are reports of acute poisoning through unintentional or suicidal naphthalene exposures in humans but, as described below, the epidemiological data are very scarce regarding dose–response relationships for human health effects with acute, subchronic or chronic exposure by any route. The effects in humans are now discussed, followed by a description of animal data. In some cases, reference will be made to literature in humans, which, based on mechanistic data derived from animals, would be consistent with adverse health effects of naphthalene. It is important to note that the associations and consistency with mechanisms does not constitute proof of health effects and the data may be explained in many other ways. This is especially true when exposure occurred not solely to naphthalene but to mixtures containing naphthalene such as PAH.

Identification of studies

Published studies on health effects of exposure to naphthalene were identified by hand searching references in former reviews by IARC *(115)*, ECB *(22)* and ATSDR *(46)* and completed by electronic search in February and September 2009 in PubMed, using the descriptors "naphthalene" and "health effects", "toxicity", "lung", "epidemiology", "susceptibility", "cancer", "mothballs" or "poisoning". Following the last review, only a few new epidemiological studies and about two

dozen toxicity studies in mammals or in vitro studies were found. We excluded studies that referred to PAHs but were lacking a sufficient description of exposure to naphthalene.

Effects on humans

Acute effects

Many of the case reports of human exposure to naphthalene involve ingestion of mothballs. The most serious effects are reported in individuals with glucose 6-phosphate dehydrogenase deficiency, where haemolytic anaemia is the primary adverse effect. Many of these involve poisoning in paediatric patients *(116,117)*. In a recent survey of 24 paediatric patients admitted to hospital with acute haemolysis, nearly 60% were found to have been associated with naphthalene-containing mothballs *(118)*. In one case report, involving accidental prenatal exposure to mothballs, both the mother and, following birth, her preterm infant presented with haemolytic anaemia and methaemoglobinaemia *(119)*. Follow-up of both mother and child a year later revealed nothing remarkable. The effortless availability and widespread domestic use of naphthalene-containing mothballs may further lead to acute naphthalene poisoning, including the non-accidental ingestion of mothballs *(120)*.

Chronic effects

Very few cases have been documented of chronic naphthalene exposure in humans. Two of the reports purportedly showing a link between laryngeal *(121)* or colon cancer *(122)* with naphthalene exposure have been judged by both the US National Toxicology Program (NTP) *(123)* and IARC *(115)* as being sufficiently poorly controlled to be unreliable. In a population-based case-control study among women in New York State, the increase in risk of non-Hodgkin's lymphoma diagnosed between October 1995 and September 1998 was significantly associated with the household use of mothballs *(124)*. The lack of a dose–response among users, the unknown chemical constituent(s) of the mothballs used (naphthalene or para-dichlorobenzene), and selection and recall bias limit the drawing of firm conclusions.

There is an early report of human cataractogenesis induced by naphthalene in a dye manufacturing facility, which is consistent with subsequent work in animal models (discussed below) *(125)*. Some studies suggest an association between exposure to biomass fuel smoke and cataracts or lens opacity *(126,127)*, but exposure levels of naphthalene associated with these effects have not been estimated. The final case is of a middle-aged woman who had been sniffing mothballs containing naphthalene for more than 30 years *(128)*. The patient presented with signs of peripheral neuropathy and renal failure. Naphthalene was thought to be a possible contributing factor, but these symptoms were also likely to be related to diabetes, hypertension and obesity.

Odour perception

Naphthalene has a mothball-like odour. Published odour thresholds of naphthalene range from 0.0075 to 0.42 mg/m^3 *(129,130)*.

In vitro studies

There are a number of studies indicating that human cells are susceptible to naphthalene metabolites in vitro. Tingle et al. *(69)* used human liver microsomes to generate reactive metabolites from naphthalene, which were subsequently tested for cytotoxicity using peripheral blood mononuclear leukocytes. Cell death was dependent on the presence of NADPH. Inhibition of epoxide hydrolase with trichloropropylene oxide enhanced toxicity at all three concentrations of naphthalene studied (1, 10 and 100 μM). Interestingly, no effects were noted in sister chromatid exchange (SCE) frequency in cells incubated with human liver microsomes, with or without NADPH. An increase in SCE frequency was observed with the positive control, aflatoxin B_1. Later studies that tested the toxicity of naphthalene, 1-naphthol, 1,2- and 1,4-naphthoquinone and naphthalene oxide on human mononuclear leucocytes and lymphocytes showed that both quinones resulted in concentration-dependent cytotoxicity and that 1-naphthol required the presence of an activating system to generate metabolites that were cytotoxic *(131)*. The dihydrodiol was not cytotoxic at concentrations up to 100 μM. Similarly, both quinones resulted in increased numbers of SCEs. More recent work with cord blood showed that naphthalene at high concentrations (500 μM) increased the expression of several antiapoptotic proteins, including BCl-2 *(132)*. Similarly, three naphthalene metabolites (1- and 2-naphthol and 1,4-naphthoquinone) produce concentration-dependent decreases in the clonogenicity of colony-forming units, granulocyte-macrophage (CFU-GM) in cord blood from both male and female donors. Ranked IC_{50} (concentrations required to decrease clonogenicity by 50%) values for these metabolites were 2-naphthol > 1-naphthol > 1,4-naphthoquinone *(133)*. The reported IC_{50} for the quinone was 0.5–1.9 μM. Naphthalene was inactive at concentrations as high as 5 mM.

Overall, these studies indicate that human liver microsomes are capable of metabolically activating naphthalene to derivatives that are cytotoxic to human cells, and that the known metabolites of naphthalene are capable of producing cytotoxicity when added to cells. With some of these metabolites, cytotoxicity is observed at relatively low levels.

Effects on experimental animals and in vitro test systems

Animal studies in vivo

Acute/subacute studies. Toxicity to the respiratory tract is the most notable lesion associated with naphthalene exposure in animals but the subcompartments of the respiratory tract targeted by this compound depend highly on the species, the age and sex of the animals and the route of administration (Table 4.2). Ocular

injury has also been observed in a number of species and the mechanisms for this appear to be well-established.

Work examining the acute toxicity of naphthalene administered by inhalation has recently been completed *(134)*. Four-hour exposures to concentrations as low as 11 mg/m^3 resulted in detectable Clara cell injury in the proximal airways of adult male mice. Injury was concentration-dependent and proceeded from the proximal, most sensitive airways to distal and less sensitive airways. As the concentration increased, injury became more severe in the proximal airways and extended down into more distal portions of the lung. At 53 mg/m^3, significant cell disruption was noted at all airway levels in mice. In contrast, airway epithelial injury was not observed at any exposure concentration up to the highest concentration tested (585 mg/m^3). Substantial injury of nasal olfactory epithelium in Sprague-Dawley rats was observed following naphthalene inhalation at low exposure concentrations (18 mg/m^3 for four hours) *(93)*. More recently, olfactory epithelium necrosis occurred in SD and F344 rats after a single six-hour whole-body exposure to 5 mg/m^3 naphthalene *(135)*. Lesions of the respiratory and olfactory epithelium were observed at the 53- and 160-mg/m^3 exposure concentrations in male and female F344 and SD rats. The preliminary report indicates that SD rats appear to be more sensitive and that the threshold for injury may be much lower – in the 0.5–1.6-mg/m^3 range. In a subacute study that was not published but reviewed by the European Chemicals Bureau *(22)* and retained as valuable information in the INDEX project *(13)*, male and female Sprague-Dawley rats were exposed nose-only to 0, 5, 17, 55, 153 or 372 mg/m^3 vaporized naphthalene (D. W. Coombs, unpublished data, 1993). In the nasal olfactory epithelium, local lesions with signs of proliferative repair were observed at all doses down to 5 mg/m^3. The findings were similar to those from a subchronic study (see below).

The olfactory region of the nose is also sensitive to naphthalene after intraperitoneal administration in both the mouse and the rat *(136)*. The rat nasal olfactory epithelium is more sensitive than the mouse epithelium: significant necrosis was observed in the rat at intraperitoneal doses of 200 mg/kg, whereas injury in the mouse was not observed until 400 mg/kg. Finally, more recent studies investigating the sex and strain differences in susceptibility to naphthalene toxicity indicate that female Swiss Webster mice are more susceptible to the cytotoxicity of naphthalene than males *(137)*. These differences were detected primarily by differences in uptake of vital dye and consisted of earlier and more extensive injury following a 200-mg/kg dose. Few differences were noted in the extent of initial injury in different mouse strains *(138)*. As discussed below, the chronic bioassay investigating the possible neoplastic effects of naphthalene showed a sex difference in susceptibility: female mice showed a slight increase in bronchioloalveolar neoplasms over the control, whereas in males there was no effect.

In addition to the lesions observed in the nose, the pulmonary toxicity of naphthalene has been studied extensively by a number of laboratories

(136,137,139,140). More recently, naphthalene has been used as a selective Clara cell toxicant to evaluate progenitor cells involved in the repair of the airway epithelium *(141–143)* and to determine whether co-exposures to pulmonary toxicants alter either the initial response or the later repair of the injury *(144,145)*. Pulmonary regenerative response to naphthalene-induced lung injury in mice depends on gender, showing a significantly greater cell proliferation in female compared to male mice *(146)*. Clara cells lining the airway epithelium of the mouse are the primary target cells for naphthalene toxicity, irrespective of the route of administration. After parenteral administration of low doses of the compound, the only tissue affected is the respiratory tract (Table 4.2). Hepatic necrosis is not observed at any dose of naphthalene tested, while proximal tubular cells of the kidney are injured only in some mouse strains and only at very high doses (400 and 600 mg/kg) *(147)*. Swelling of Clara cells in terminal airways is detected in mice at intraperitoneal doses as low as 50 mg/kg. In contrast, in rats even at LD_{50} intraperitoneal doses (1600 mg/kg) airway Clara cells appear normal. Slight swelling of Clara cells in the hamster is observed at the LD_{50} intraperitoneal dose (800 mg/kg) *(136,140)*. In all of the species tested, no injury to the alveolar type I or II cells has been observed. Recently, naphthoquinone was shown to enhance an antigen-related airway inflammation with goblet cell hyperplasia in mice *(148)*. Following an intratracheal application of naphthoquinone to ICR mice for six weeks, airway hyperresponsiveness was enhanced by naphthoquinone in the presence or absence of an antigen *(149)*.

In contrast to the Clara cell toxicity observed after single doses of naphthalene, multiple daily treatments with naphthalene by either the intraperitoneal or inhalation routes result in tolerance to high challenge doses of the compound *(150–152)*. Although acute 200-mg/kg doses intraperitoneally result in substantial injury to Clara cells of mice, treatment for seven days at this same dose caused slight hyperplasia of the epithelium but no frank necrosis or vacuolation. Seven daily treatments with 200 mg/kg naphthalene markedly attenuated the toxicity observed following a 300-mg/kg challenge dose given 24 hours after the last 200-mg/kg dose in comparison to corn-oil-treated controls challenged with 300 mg/kg naphthalene (Table 4.2). As the time between the last 200-mg/kg dose and the challenge dose was extended from 24 to 96 hours, the lungs regained a portion of their sensitivity to the 300-mg/kg challenge dose. Later studies using inhalation exposures at 80 mg/m^3 showed similar effects *(152)*. Tolerance to repeated naphthalene exposures does not appear to be related to changes in the metabolic activation of naphthalene but rather to faster turnover of glutathione associated with upregulation of γ-glutamylcysteine synthase *(153,154)*. These data, showing that the lung becomes tolerant to multiple doses of naphthalene at dose levels that produced substantial toxicity in airway epithelial cells after single administration, are consistent with the 14- and 90-day oral gavage studies. This work demonstrated no significant alterations in serum enzyme levels, body weight,

Table 4.2. Species, tissue and regional differences in naphthalene toxicity

Species	Dose	Lung	
		Trachea/lobar bronchus	Terminal bronchiole
Mouse, adult, LD_{50} = 380 mg/kg	50 mg/kg	0	+
	100 mg/kg	0	++
	200 mg/kg	+/0	+++
	300 mg/kg	++	++++
	400 mg/kg	+++	++++
	11–27 mg/m³	+/0	0
	45–61 mg/m³	+	+/0
	133–165 mg/m³	++	+
	383–410 mg/m³	+++	+++
	511–591 mg/m³	+++	+++
Mouse, adult, tolerance	200 mg/kg x 7	ND	0
	200 mg/kg x 7 + 300 (24 hours)	ND	0
	200 mg/kg x 7 + 300 (48 hours)	ND	+
	200 mg/kg x 7 + 300 (96 hours)	ND	+++
	200 mg/kg x 7 + 300 (144 hours)	ND	++++
Rat, adult, LD_{50} = 1600 mg/kg	100 mg/kg	ND	ND
	200 mg/kg	0	0
	400 mg/kg	0	0
	800 mg/kg	0	0
	1600 mg/kg	0	0
	585 mg/m³	0	0
	0.5–1.6 mg/m³ x 6 hours	ND	ND
	18 mg/m³	ND	ND
	127 mg/m³	ND	ND
Hamster, adult, LD_{50} = 800 mg/kg	200 mg/kg	0	0
	400 mg/kg	0	0
	800 mg/kg	+	0

[a] ND = not determined.

organ weight or various indices of immune function in CD-1 mice treated daily with doses up to 267 mg/kg (14 days) or 133 mg/kg (90 days) *(155)*.

Long-term exposure and carcinogenesis studies. In a subchronic study that was not published but reviewed by the European Chemicals Bureau *(22)* and evaluated in the INDEX project *(13)*, groups of 10 male and 10 female Sprague-Dawley rats were exposed snout-only to 0, 11, 51 or 306 mg/m³ vaporized naphthalene (D. W. Coombs et al., unpublished data, 1993). Gross pathological examinations on a wide range of tissues revealed no significant changes. There were also no toxicologically relevant haematological or clinical chemistry findings. Microscopic pathology revealed treatment-related effects in the nasal passages at all

Nasal epithelium			Comments
Parenchyma	Olfactory	Respiratory	
0	0	0	No toxicity noted in liver or kidney
0	0	0	of male SW mice; ICR mice showed
0	0	0	lesions of proximal tubule at
0	ND[a]	ND	highest doses (400 and 600 mg/kg)
0	+++	0	*(136,140,147)*
0	ND	ND	West et al. *(134)*
0	ND	ND	
0	ND	ND	
0	ND	ND	
0	ND	ND	
ND	ND	ND	Areas of bronchiolar epithelial cell
ND	ND	ND	hyperplasia observed after 7 days
	ND	ND	*(150,151)*
	ND	ND	
	ND	ND	
ND	+	0	Plopper et al. *(136,140)*
0	+++	0	
0	+++	0	
0	+++	0	
0	+++	0	
0	ND	ND	
ND	+/0	0	Dodd et al. *(135)*
ND	++	0	Lee et al. *(93)*
ND	+++	0	
0	0	0	
0	+++	0	
0	+++	0	

dose levels. Degenerative changes seen in the olfactory epithelium included slight disorganization, atrophy and erosion, loss of subepithelial Bowman's glands and signs of proliferative lesions of the olfactory epithelium. Changes were generally dose-related in that the more severe lesions and the more severe grades of all lesions occurred in the intermediate- and high-dose groups. At the lowest dose, no relevant treatment-related changes were observed in the nasal respiratory epithelium or in the lung.

Chronic exposure of B6C3F1 mice to naphthalene (53- or 160-mg/m^3) resulted in inflammation in the nose, metaplasia of the olfactory epithelium and hyperplasia of the respiratory epithelium *(156,157)*. The incidence of these lesions was 100% at both the 53- and 160-mg/m^3 exposure levels in both males and

females. The target sites for hyperplasia and metaplasia were identical to those susceptible to necrosis following acute exposures (see above). Alveolar/bronchiolar adenomas occurred in exposed male mice but the incidence did not achieve a level of statistical significance. Likewise, a small incidence of alveolar/bronchiolar carcinomas occurred in males but exposed animals did not differ statistically from unexposed. In contrast, a statistically significant though small increase in alveolar/bronchiolar adenomas was noted in high-dose (160 mg/m^3) females. Inflammation was observed in the lung of both males and females that was dose-dependent and occurred in approximately 40% of the animals in the high-dose group. There were no male/female differences in the incidence of chronic inflammation.

In similar chronic exposure studies in F 344/N rats, animals were exposed to vapour concentrations of 0, 53, 160 and 319 mg/m^3 for 105 weeks. The nasal epithelium was found to be a primary target for these exposures *(157–159)*. A dose-dependent increase in adenoma of the respiratory epithelium of the nose was noted in males, affecting 31% of the exposed population at the highest exposure levels. A much lower incidence of this lesion was observed in female rats and the incidence in exposed groups was not statistically different from that in controls. In females but not in males, there was a statistically significant increase in olfactory epithelial neuroblastomas. In several of the animals, nasal masses were observed, some of which had begun to invade the central nervous system *(159)*. A high incidence of non-neoplastic effects was observed in the nasal epithelium of both male and female rats. In the olfactory epithelium, the incidence of hyperplasia and chronic inflammation was nearly 100%, even at the lowest concentration tested (53 mg/m^3). In contrast, the respiratory epithelium was less sensitive, with 40–60% incidence for hyperplasia and inflammation in exposed animals. No differences were noted between males and females. These targets correlate well with the susceptibility of the nasal olfactory region to acute naphthalene-induced cytotoxicity and with the ability of those regions of the nasal epithelium to activate the parent substrate *(93)*.

Cataract formation. Sensitive animal models for studying naphthalene cataractogenesis have been established in rabbits *(160)*, rats *(161)* and mice *(162)*, and several in vitro methods have been used to more clearly define the mechanisms associated with the biological effects of naphthalene on the eye *(161,163)*. Doses required to produce the lesions are high: 1 g/kg per day in rabbits (number of days not specified), 1 g/kg per day for 14 days in rats and 750 mg/kg (single dose) in mice to produce a high incidence of cataracts. Van Heyningen & Pirie *(160)* presented evidence for the formation of 1,2-naphthoquinone and its involvement in cataract formation. The 1,2-quinone was thought to arise from metabolism of the parent hydrocarbon in the liver, with further processing of metabolites in the eye. Later work in mice *(162)* appears to implicate either the 1,2- or the 1,4-naph-

thoquinone. This conclusion is supported by the finding that (*a*) trichloropropylene oxide, an epoxide hydrolase inhibitor, does not alter the incidence of cataract formation and (*b*) 1-naphthol is intermediate in potency between naphthalene and the naphthoquinones, which are equipotent. Studies in rat lens cultures showed that 1,2-dihydroxy-1,2-dihydronaphthalene produced lesions similar to those observed when naphthalene was given to rats in vivo. This observation, along with the finding that an aldose reductase inhibitor blocked the lens opacity induced by the dihydrodiol, supports the importance of 1,2-naphthoquinone in mediating cataractogenesis in rats.

As indicated above, the doses used to produce cataracts in animal models are high. Lower doses, such as those reported in the subchronic oral naphthalene studies in mice (as high as 267 mg/kg per day for 14 days or 133 mg/kg per day for 90 days) apparently did not result in untoward effects in the eye *(155)*. Likewise, the chronic inhalation cancer bioassays in mice or rats did not report lesions in the eye *(123,164)*. Overall, whether these finding are relevant to humans is uncertain, since there are no reliable data on cataract formation in humans following naphthalene exposure.

Haemolytic anaemia. This principal toxicological effect of naphthalene observed in humans has not been seen in experimental animal studies with rats, mice or rabbits. The reason for this is not known. Therefore, for this end-point, there are no relevant data for extrapolation from experimental animal studies to human exposure.

Animal cells/explants/perfused tissues in vitro

As discussed in the kinetics and metabolism module, naphthalene is metabolized to several reactive metabolites that have the potential to produce the toxicities associated with the parent compound and, as discussed above, these metabolites can produce cellular injury to human cells in vitro. It is clear that naphthalene requires metabolism by the cytochrome P450 monooxygenases for lung toxicity *(84)* and that glutathione plays a major role in protecting the cells from injury *(85,165)*. There is some evidence that metabolites generated in the liver can enter the bloodstream, causing downstream toxicities in extrahepatic tissues either directly or through depletion of glutathione, with increased susceptibilities to metabolites generated in situ in the respiratory system *(108,166)*. Studies in isolated murine Clara cells *(167)* and in isolated perfused murine lung *(62,168)* demonstrated that target tissues were capable of generating sufficient metabolite from the parent compound to produce cytotoxicity in the airway epithelium. When tested in isolated Clara cells, naphthalene oxide and 1,4-naphthoquinone produced similar losses in cell viability at both 2 and 4 hours. The remaining metabolites were either less potent or did not cause a loss of cellular integrity (1-naphthol or dihydrodiol) at either point in time. Interestingly, preincubation

of cells with the cytochrome P450 monooxygenase inhibitor piperonyl butoxide inhibited the cytotoxic effects of naphthalene but not of naphthalene oxide *(167)*, a finding that suggests that metabolites downstream of the epoxide may not be keys to naphthalene toxicity. Likewise, naphthalene oxide produced selective injury to Clara cells in perfused lungs and the 1,2- and 1,4-quinones were approximately 10-fold less potent *(62)*. These studies need to be interpreted with caution, because isolated cells may or may not be a good model for the Clara cell in its normal microenvironment within the airway. Similarly, the toxicity of various metabolites in isolated perfused lungs would be strongly influenced by the amounts of these reaching the target cell from the perfusate, and there is no indication that the amounts of these were the same for the metabolites tested.

Short-term mutagenicity assays. Naphthalene and a number of naphthalene metabolites have been tested in a variety of mutagenicity assays. These have been reviewed thoroughly by IARC *(115)* and Schreiner *(169)* and will be addressed only briefly here. In all of the Ames assays using various *Salmonella typhimurium* strains, with and without activating enzyme, naphthalene is negative. As stated above, both 1,2- and 1,4-naphthoquinone were found to be positive in SCE assay *(131)*. Other short-term tests evaluating neoplastic transformations with γ-glutamyltranspeptidase-positive liver foci and in vitro cell transformation assays were, likewise, negative. Micronucleus assays for chromosome breakage were positive, as were assessments of chromosome aberrations in Chinese hamster ovary cells. Overall, the preponderance of evidence suggests that naphthalene is not a genotoxic carcinogen and that any DNA damage associated with the compound may derive from the cytotoxic actions of the naphthalene metabolites *(170)*.

The cytotoxicity associated with naphthalene exposure may play an important role in the overall effects observed in the chronic bioassay. 1,2-Naphthoquinone has been shown to bind to DNA, forming adducts at the N3 position of adenine and the N7 position of guanine that depurinate *(82)*. Recent work has disclosed formation of depurinating DNA adducts following a four-hour dermal exposure of female SENCAR mice to naphthalene, 1-naphthol, 1,2-DDN, 1,2-DHN or 1,2-NQ *(171)*. The relevance of these data is unknown, since markers of DNA reactivity associated with naphthalene in target tissues of animals and of biomarkers for evaluating these processes in humans have still to be developed *(172)*.

Health risk evaluation

Critical health outcomes

The principal health concerns of exposure to naphthalene are respiratory tract lesions, including respiratory tract carcinogenicity demonstrated in animal studies and haemolytic anaemia in humans. Regarding cataract formation seen in experimental animals after high oral exposure to (but not after inhalation of)

naphthalene, there is only suggestive evidence of an association with exposure to naphthalene in humans, if at all.

Most of the reports on haemolytic anaemia in humans refer to dermal uptake of naphthalene from clothes treated with naphthalene mothballs or unintentional or suicidal ingestion of mothballs. Many of the cases were in infants. For this end-point, data on dose–response relationships are insufficient. Since experimental rodents or rabbits do not disclose haemolytic anaemia following exposure to naphthalene, there is no relevant information from animals to extrapolate to human exposures for this effect.

No reliable data in humans are available for long-term inhalation toxicity of naphthalene, and evaluation of the risk to health of inhaled naphthalene has to rely essentially on animal studies and in vitro results. Evidence is sufficient to infer that naphthalene is a respiratory toxicant in rats and mice following acute and chronic exposure to rather low concentrations. Epithelial cells in the proximal airways are the primary target cells for naphthalene toxicity. In rats, a pronounced susceptibility of the olfactory region of the nasal mucosa was confined to the high air flow area of the medial meatus *(50)*. With increasing naphthalene concentrations, the proximal airway lesions became more severe and proceeded to the distal airways.

In two rat strains, olfactory epithelium necrosis occurred at a single six-hour whole-body exposure to the lowest naphthalene concentration of 5 mg/m^3. In a recent brief communication by Dodd et al. *(135)*, exposure to 0.5–2 mg/m^3 revealed very weak effects in a few animals, indicating a NOAEL for acute inhalation exposure. In mice, Clara cell injury was seen following a four-hour exposure to 11 mg/m^3 *(134)*.

In two reports that were not peer reviewed but have been examined and found to be of good quality, mild lesions of the nasal olfactory epithelium with signs of proliferative repair were observed following subacute or subchronic exposure down to 5 or 11 mg/m^3, respectively. This was the LOAEL after subacute or subchronic inhalation exposure (D. W. Coombs et al., unpublished data, 1993).

Compared to the acute (some hours), subacute (4 weeks) or subchronic (13 weeks) exposure studies, long-term (104 weeks) inhalation studies were performed only with relatively high naphthalene concentrations *(164)*. Chronic exposure of mice to naphthalene at 53 or 159 mg/m^3 resulted in nasal inflammation, metaplasia of the olfactory epithelium and hyperplasia of the respiratory epithelium in almost all exposed male and female animals. Alveolar/bronchiolar adenomas were seen in both exposed males and females. A statistical significance of an elevated incidence of adenomas was achieved only in the female high-dose group. A small, statistically insignificant incidence of alveolar/bronchiolar carcinomas occurred in male mice. The LOAEL for chronic respiratory tract inflammation seen with almost all mice in this study was 53 mg/m^3.

Similar chronic inhalation studies were performed in rats exposed to naphthalene at 53, 159 or 318 mg/m^3 *(123)*. In the nasal olfactory epithelium, hyperplasia, chronic inflammation and hyaline degeneration were seen in almost all animals, even in the lowest-dose group. A statistically significant dose-dependent increase in olfactory epithelial neuroblastomas occurred in females. The nasal respiratory epithelium was less sensitive, about half of the cells showing signs of hyperplasia, inflammation and hyalinization in both exposed males and females. The incidence of nasal respiratory adenomas increased dose-dependently in male rats. Again, the LOAEL for severe lesions in the olfactory region and, less pronounced, respiratory epithelium of rats chronically exposed to naphthalene was 53 mg/m^3.

The mechanisms responsible for the toxicity and carcinogenicity of naphthalene in the rodent respiratory tract and the gender differences in these responses are not fully understood. Target site cytotoxicity associated with naphthalene exposure is assumed to play a crucial role in the development of tumours observed in the inhalation studies. Studies indicate that metabolism is necessary for naphthalene to develop its cytotoxic effects. In rats, naphthalene metabolism rates are approximately 40-fold higher in the olfactory than in the septal non-olfactory mucosa *(93)*. The neuroblastomas observed in the rat olfactory epithelium are highly malignant and should be considered of relevance to humans, since P450 isoenzymes able to metabolically activate naphthalene in the rodent nose are also present in humans.

In mice, the particular susceptibility to naphthalene injury of Clara cells of the distal bronchiolar epithelium does not seem of high relevance to humans owing to the special nature of metabolism in mice *(173)*.

The possible involvement of a genotoxic mechanism in tumour formation in rodents cannot be ruled out owing to the metabolic activation of naphthalene to an epoxide, which may also be generated in the olfactory and respiratory epithelia of the rodent respiratory tract. There have been positive results in some in vitro tests for mutagenicity, but results of in vivo tests are consistently negative *(115)*. Although depurinating naphthalene-DNA adducts were identified in mouse skin *(171)*, naphthalene is not carcinogenic in this tissue.

Overall, naphthalene is considered a non-genotoxic carcinogen in the rodent respiratory tract, chronic inflammation (eventually resulting in secondary genotoxicity) being the key action in the formation of tumours.

Health relevance of indoor exposure

Indoor air levels of naphthalene may exceed outdoor concentrations manyfold owing to a variety of potential indoor sources, including tobacco smoke, indoor combustion and consumer products. Indoor air levels vary from a few to tens of μg/m^3, with levels markedly higher when mothballs are used.

Conclusions of other reviews

Naphthalene has been classified by IARC in Group 2B as "possibly carcinogenic to humans" on the basis of sufficient evidence of its carcinogenicity in experimental animals and inadequate evidence of carcinogenicity in humans *(115)*. The classification of naphthalene into carcinogenicity group Carc. 2 by the EU *(174)* and into group C by the USEPA *(16)* are compatible with the IARC evaluation.

Guidelines

The principal health concerns of exposure to naphthalene are respiratory tract lesions, including tumours in the upper respiratory tract demonstrated in animal studies and haemolytic anaemia in humans.

Lesions in the nasal olfactory and, at higher concentrations, also in the respiratory epithelia of rats appear to be the critical non-neoplastic effect. At concentrations about 100-fold higher than the lowest lesion level, severe inflammation and tumours have been reported to occur at these sites.

Increased cell proliferation due to cytotoxicity (cell damage) is considered a key element in the development of airway tumours. The likely involvement of cytotoxic metabolites in the carcinogenic response and the apparent primary non-genotoxicity of naphthalene favour the assumption of the existence of a threshold. Therefore, the use of a LOAEL/NOAEL as a threshold, combined with safety factors, is considered to be an appropriate approach for setting indoor air guidelines to minimize the carcinogenic risk to the respiratory tract of naphthalene exposure.

Associated with repeated inhalation exposure of 6 hours/day, 5 days a week for 104 weeks, severe effects in terms of inflammation were observed in almost all rats exposed to the lowest, but still relatively high, naphthalene dose of 53 mg/m^3 *(123)*. In the absence of adequately published data in relation to less severe effects, this can be taken as a LOAEL, even though it is related to severe effects.

Taking this LOAEL as a starting point and adjusting for continuous exposure (dividing by a factor of 24/6 and 7/5), a value of about 10 mg/m^3 is obtained. Further, incorporating a factor of 10 for using a LOAEL rather than a NOAEL, a factor of 10 for interspecies variation and a factor of 10 for inter-individual variation, a guideline value of 0.01 mg/m^3 is established. This guideline value should be applied as an annual average.

Extensive use or misuse of naphthalene mothballs may lead to haemolytic anaemia. Knowledge of the impact of exposure to naphthalene on the risk of haemolytic anaemia in susceptible individuals (glucose 6-phosphate dehydrogenase deficiency) cannot be used to define a guideline owing to the lack of adequate exposure data.

In the absence of mothballs or other sources such as combustion of biomass, indoor air concentrations of naphthalene are just above the typical limit of detec-

tion of about 0.001 mg/m^3. Since the concentration of naphthalene in the residential environment increases up to 100-fold when mothballs are used, the most efficient way to prevent high exposures would be to abandon (ban) the use of naphthalene-containing mothballs.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome for guideline definition

Respiratory tract lesions leading to inflammation and malignancy in animal studies.

Source of exposure–effect evidence

Nasal inflammation and olfactory epithelial metaplasia in nearly all rats chronically exposed to 53 mg/m^3 was considered as the LOAEL, even though related to severe effects *(157–159)*. This was adjusted for continuous exposure (dividing by a factor of 24/6 and 7/5). Further, a factor of 10 for using a LOAEL instead of a NOAEL, a factor of 10 for interspecies variation and a factor of 10 for inter-individual variation were incorporated, leading to a guideline value of 0.01 mg/m^3.

Supporting evidence

- Dose-dependent respiratory tract cytotoxicity following acute to chronic exposure in rats *(123)*.
- Airway toxicity was seen in several strains of rats and mice over a wide range of concentrations *(93,134–146,148,156,157)*.
- Human cells are susceptible to naphthalene metabolites in vitro *(69,131–133)*.

Results of other reviews

- IARC: Group 2B (possibly carcinogenic to humans) *(115)*.
- EU: Group 2 (suspected human carcinogen) *(174)*.
- USEPA: Group C (possible human carcinogen) *(16)*.
- EC INDEX project: guideline 0.01 mg/m^3 (annual average concentration) *(12,13)*.

Guidelines

0.01 mg/m^3 (annual average concentration).

Comments

The long-term guideline is also assumed to prevent potential malignant effects in the airways. No reliable human data for long-term inhalation toxicity are available.

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