

CCSG

COAL CHEMICALS SECTOR GROUP

Attn. Dr. S. R. Vink
The Health Council of The Netherlands,
Subcommittee on the Classification of Carcinogenic Substances
PO Box 16052

NL - 2500 BB The Hague
The Netherlands

Actv.	246
Nr.	1315
ING.	13 AUG 2012
V	V RGO
VV	.
AS	HB
S	
s	
Archief	
Bijlagen	

August 7, 2012

Comments on Draft Report of DECOS/NL on Naphthalene

Dear Dr. Vink,

European naphthalene producers organised in Cefic CCSG would like to contribute to the latest draft DECOS report on naphthalene following the invitation published on 25 May 2012.

We therefore send to your consideration our comments and conclusions.

Should you require any further clarification, please do not hesitate to contact us.

Yours faithfully,



Walter Cremers
Manager CCSG
wcr@cefic.be



Chemistry making a world of difference

European Chemical Industry Council
Avenue E. van Nieuwenhuysse 4 B - 1160 Brussels Belgium
Tel: +32 2 676 72 11 Fax: +32 2 676 73 01 mail@cefic.be www.cefic.org



1. Summary of Conclusions Drawn by DECOS (Committee)

The Committee concludes “naphthalene acts by a non-genotoxic mode of action” (4.4, p. 16), but states (4.2, p 16): “It is currently not known whether the bioactivation, which occurs in rodents, also plays a significant role in humans.” Overall, they believe “that the relevance for humans of the exposure conditions, under which the nasal tumours have been observed in rats, is currently unclear”.

In the final **Evaluation of data on carcinogenicity and genotoxicity (5.1, p. 17)**, the Committee summarises their position based on the following aspects:

“The number of available human studies on naphthalene is very limited, and does not provide any specific information on naphthalene. Therefore, the Committee considers the human data to be inadequate for drawing a conclusion on the carcinogenic properties of naphthalene.”

Furthermore: “The Committee questions whether the exposure conditions at which the carcinogenic effects occurred in rats, *i.e.*, relatively high exposure levels of naphthalene leading to pronounced local cytotoxicity, are relevant for humans. However, as the formation of reactive metabolites and subsequent toxicity at high exposure levels of naphthalene cannot be excluded, the Committee considers the carcinogenic effects of naphthalene observed in the rat as relevant for humans.”

The Committee arrives at the following **recommendation (5.2, p. 17)**:

“Based on the available information, the Committee is of the opinion that naphthalene is suspected to be carcinogenic to man, and proposes to classify the compound in category 2.” That means: The compound is suspected to be carcinogenic to man (Appendix G, p. 32).

2. Opinion and Comments

2.1 General

The Report lacks relevant literature published within the last eight to 10 years. The Committee seems to rely mainly upon the status of the IARC Monograph of 2002, with a few exceptions published later. The set of literature discussed and provided can be updated as follows:

Recent reviews that focus on the mode of action are

Piccirillo, V.J.; Bird, M.G.; Lewis, R.J.; Bover, W.J. 2012: Preliminary evaluation of the human relevance of respiratory tumors observed in rodents exposed to naphthalene. *Regul. Toxicol. Pharmacol.* 62, 433-440

Rhomberg, L.R.; Bailey, L.A.; Goodman, J.E. 2010: Hypothesis-based weight of evidence: A tool for evaluating and communicating uncertainties and inconsistencies in the large body of evidence in proposing a carcinogenic mode of action—naphthalene as an example. *Crit. Rev. Toxicol.* 40: 671-696

Note: *Specific publications which are useful for an understanding of naphthalene’s mode of action and its relevance for humans will be used and referred to to a greater extent within the scope of a more detailed discussion on this topic in the subsequent section 2.2.*

The key message from the review by Lewis (2012) - although cited - has been ignored, namely that there had never been any naphthalene-associated evidence for cancer, in particular, nasal and lung cancer among exposed populations.

Reference:

Lewis, R.J. 2012: Naphthalene animal carcinogenicity and human relevancy: overview of industries with naphthalene-containing streams. *Regul. Toxicol. Pharmacol.* 62, 7 131-137.

Note: This reference has erroneously been cited with "Jeffrey" as author, however surname is "Lewis".

2.2 Discussion of the Mode of Action

2.2.1 Mode of action of naphthalene toxicity in rodents

In two 2-year inhalation studies, naphthalene induced dose-related increases of tumours in rats and mice (NTP 2000; NTP 1992) increases in adenomas and malign neuroblastomas were statistically significant in the respiratory epithelium of male rats at 10 ppm (52 mg/m³) and in the olfactory epithelium of female rats, respectively, at 60 ppm (314 mg/m³), while there was a significant increase of pulmonary bronchial adenomas in female mice at 30 ppm (157 mg/m³).

The tumour types induced by naphthalene in rodents are very particular and strictly localised to epithelial tissues of the respiratory tract: in mice, adenomas develop in the bronchioles, while in rats similar tumours are localised in the olfactory and respiratory epithelium of the nasal cavity but not in the lung area. This suggests a very site-specific and particular mode of action.

Tumorigenicity has only been observed in tissues that are affected by marked cellular toxicity. This indicates that chronic cytotoxicity precedes the formation of cancer. This included atypical hyperplasia in the olfactory region of almost all exposed rats at all exposure concentrations, but not described in the lung of mice.

However, naphthalene-related cytotoxicity of the target tissues is not a biological event that necessarily ends in carcinogenicity: for instance, the mice showed high metabolic activity in the nasal tract including the olfactory mucosa (Buckpitt et al. 2002), associated with pronounced inflammation of the local epithelium, however without resulting in secondary development of tumours (NTP 1992). On the other hand, under similar exposure conditions, tumours developed in the nasal epithelium of the rat, following manifestation of severe inflammation (NTP 2000).

Inflammatory irritation of the olfactory epithelium sets on very early in rats exposed to naphthalene concentrations as low as 1 ppm (5 mg/m³) after single exposure of 6 hours (Dodd et al. 2010). The lesions included incipient but distinct necrotic changes in both genders. The respiratory epithelium proved to be less sensitive with necrosis observed at 10 ppm (52 mg/m³) in all animals. On the other hand, in the rat lung no histological tissue lesion could be detected even after high levels of naphthalene (100 ppm, 4h) (West et al. 2001).

It was shown that this was due to very low metabolic activity in the Clara cells in rat lung (Cruzan et al. 2009). However, in mice, following exposure to low concentrations (1 – 3 ppm), the epithelium of the total respiratory tract including lung tissue was affected, with the Clara cells showing the highest lesions, the site of tumorigenesis (West et al. 2001).

In this context, it is interesting to note that - on the basis of special inhalation studies under steady-state conditions over 1 hour - the total uptake of inhaled naphthalene in the upper respiratory tract of rats has been calculated to range from about 3 to 70 nmol/min per animal exposed to concentrations from 1 to 30 ppm at air flow rates of 150 mL/min and 300 mL/min.

However, the maximum total metabolic capacity of the olfactory mucosa has been estimated to amount to approximately 20 nmol/min. This means that saturation or even capacity exceedance occurred: At around 10 ppm, uptake and metabolic rate may still be balanced, whereas exposure to 30 ppm results in clear overload (Morris and Buckpitt 2009).

Based on these results, the NTP inhalation studies were conducted under metabolic overload conditions, in particular when taking into account a possibly accumulating effect during long-term exposure.

Parenteral routes of exposure, in particular after intraperitoneal injection, result in the same pattern of cytotoxicity in mice and rats in the corresponding epithelia of the respiratory tract of rodents (Plopper et al. 1992). This underlines that irritation and inflammation in the target tissues is dependent on strictly localised metabolic activation rather than on direct inhalation contact to naphthalene.

It is characteristic that dramatic glutathione (GSH) depletion precedes visible histological lesions in the target tissues (Phimister et al. 2004): With the loss of GSH, an essential defence mechanism of the living cell against accumulation of reactive intermediate products becomes blocked. Besides naphthalene, other examples such as bromobenzene, acetaminophen and 3-hydroxyacetanilide indicate that only at doses causing significant GSH depletion covalent binding to proteins and toxicity will become evident (see Buckpitt et al. 1992).

The following parts focus on naphthalene metabolism in rodents and primates and the mode of action for naphthalene carcinogenesis. A flow sheet illustrating central and partly hypothetical metabolic pathways is included in 2.2.4 (Figure 1).

Naphthalene is activated to the 1,2-epoxide by action of CYP2F2 (mouse) and CYP2F4 (rat). After inhalation exposure, the 1R,2S- is more prevalent than the 1S,2R-stereoisomer in mice, while in rats the latter tends to dominate.

Levels of CYP2F expression in the pulmonary and nasal tissues were found to be greater in mice and rats than in rhesus macaques, the expression levels in rodents largely correlating with the susceptibility to naphthalene-induced cytotoxicity (Baldwin et al. 2004). Hence, terminal bronchioles and tracheae of mice had 30- and 40-fold higher levels of CYP2F than corresponding areas in rats.

The olfactory area contained the highest amount of CYP2F protein (Baldwin et al. 2004). Upon inhalation of naphthalene for 4 h, the site of severe nasal injury (the olfactory area) in rats corresponded to the site of highest rate of naphthalene metabolism to the epoxide (Lee et al. 2005).

Naphthalene uptake and overall metabolism in the upper respiratory tract of rats could be blocked by cytochrome-P450 inhibitor 5-phenyl-1-pentyne by about 80 %, thus giving additional evidence that CYP2F likely catalyses the primary step in naphthalene metabolism to the 1,2-epoxide (Morris and Buckpitt 2009). Likewise, cytotoxicity in nasal tissue of mice has been prevented by pretreatment of the animals with the same CYP inhibitor due to blockage of metabolism of naphthalene (Genter et al. 2006).

High specific monooxygenase activity has been found in lung microsomal fractions of rats and hamsters at least two orders of magnitude higher than in humans, but about 1000-fold higher in the mouse than in humans (Lorenz et al. 1984).

In the subsequent phase-II reactions, the fate of the naphthalene-1,2 epoxide depends on the species- and tissue-specific balance of various downstream enzyme activities: These are in particular the cytosolic glutathione-S-transferase (GST), the microsomal epoxide hydrolase (EH), and the dihydrodiol dehydrogenase (DD), an enzyme of the aldo-keto reductase family (AKR). However, chemical rearrangement of the 1,2-epoxide to 1-naphthol may also take place.

In rodents, the epoxide is supposed to be preferentially conjugated with GSH: In microsomal incubations of the olfactory and non-olfactory region of rats in the presence of optimal amounts of cytosolic GSH and GST, the majority of metabolites proved to be GSH conjugates of naphthalene 1,2-epoxide (4 diastereomers), about 72% – 78% accounting for the 1R-hydroxy-2R-glutathionyl-1,2-dihydronaphthalene. Only a small portion (about 3 – 7%) was transformed into the 1,2-dihydrodiol by microsomal epoxide hydrolase. The catalytic activity in the olfactory mucosa exceeded that in the respiratory epithelium by at least 40-fold (Lee et al .2005). This indicates that in rodents the reactive naphthalene 1,2-epoxide is likely to be readily captured by GST, thus readily detoxified and finally after various biotransformations excreted as mercapturic acid.

The competitive action of EH predominantly results in the 1R,2R-isomer of 1,2-dihydroxy-1,2-dihydronaphthalene. After enzymatic dehydrogenation by dihydrodiol dehydrogenase, the 1,2-dihydroxynaphthalene is formed. This is at chemical equilibrium with 1,2-naphthoquinone via a redox cycle. In rodents, the formation of the 1,2-dihydrodiol and 1,2-diol occurs at a high rate and appears to determine the intensity of cytotoxicity in the target tissues (Buckpitt et al. 2002). It should be noted that on the dihydrodiol level a secondary epoxidation in 3,4-position may occur resulting in the reactive tetrahydrodiol epoxide (“diol epoxide”) [1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene].

However, the 1,2-naphthoquinone has been considered to be the main cytotoxic agent or precursor of reactive follow-up intermediates that may interfere with essential functional cell proteins or with DNA resulting in cell death or possibly in cell transformation (Buckpitt et al. 2002). Covalent binding of reactive metabolites and cell toxicity correlated with doses of naphthalene that also caused significant GSH depletion (Warren et al 1982; Buckpitt and Warren 1983). The redox cycle mentioned above may be a potential driving force for the generation of reactive oxygen species (ROS) which contribute to cell damage (e.g. Penning et al. 1999).

The depletion of the target tissue from cell protective glutathione is the detrimental key process that initiates cellular dysfunction and destruction or possibly cell transformation which may cause cancer.

The 4-position of 1,2-naphthoquinone, a reactive electrophile, may directly react with functional groups (e.g. HS- or H₂N-) of proteins, amino acids or of glutathione but in principle also with bases of DNA (e.g. adenine or guanine).

The reaction with amino-groups of amino acids, chemically a Michael addition, may result in the formation of highly reactive 1,4-benzoquinone imine structures: Since recently, it has been postulated (but not yet proven) that a glutamine or asparagine conjugate of 1,2-naphthoquinone be hydrolysed by a species- and site-specific arylamidase, thus forming the quinone imino structure by introducing an amino group into the 4-position of 1,2-naphthoquinone. This still hypothetical naphthoquinone imine is assumed to be the ultimate carcinogenic agent to explain species- and site-specific naphthalene carcinogenesis (Piccirillo et al. 2012).

Conclusions on the mode of action of naphthalene in rodents:

The formation of lung tumours in mice and the nasal tumours in rats very likely share the same mode of action.

Formation of the naphthalene-1,2-epoxide by CYP2F followed by GSH depletion is the first and critical step in naphthalene-induced tissue injury.

Glutathione depletion is the decisive determinant to initiate subsequent detrimental processes, but the potential outcome will vary depending on the extent of activities of the downstream enzymes in different target tissues.

That means: Simple presence and absence of CYP2F is not the only critical element of metabolism of naphthalene, and GSH depletion is not sufficient to predetermine the inevitable route to cancer. The absence of tumorigenesis in the murine nose before the background of marked cytotoxicity suggests that for cancer to occur an additional event is

required that is expressed in the rat nose and mouse lung but not in rat lung and the mouse nose.

Unfortunately, this cannot be fully explained on the basis of current knowledge, and hence the understanding of the mode of action remains incomplete for the time being. The current state of the arylamidase concept, although an attractive and realistic approach, does not appear to be sufficiently robust as to offer the missing link in rodent metabolism of naphthalene towards tumorigenesis.

Despite these shortcomings, the question whether naphthalene has genotoxic/mutagenic potential and whether carcinogenesis is driven by genotoxic events may be plausibly answered by weight of evidence:

The absence of tumours in the rat lung may potentially be explained by the low level of CYP2F4. However, the absence of tumours in the mouse nose cannot simply be explained by low CYP2F2 activity, because of marked cytotoxicity without subsequent appearance of tumours, although primary bioactivation is somewhat higher than in the rat olfactory mucosa. If naphthalene had a genotoxic/mutagenic potential, we would have expected tumours in the mouse olfactory epithelium under the test conditions of the NTP study (1992). But this is not the case.

The most relevant secondary intermediate that is potentially genotoxic is probably 1,2-naphthoquinone: It was shown to be mutagenic in the Ames test (Flowers-Geary et al. 1996) and in the SCE assay (Wilson et al. 1996).

In-vitro mutation test data suggest that occasional genotoxicity of naphthalene goes via 1,2-naphthoquinone concurrent with or subsequent to cytotoxicity, and therefore is not an initiating event.

Neither mutagenicity testing nor observations in animals provide evidence that naphthalene has a primary mutagenic potential and that naphthalene-induced genotoxicity occurs prior to cytotoxicity as a cancer initiating event.

2.2.2 Naphthalene metabolism in humans

The dominant metabolic pathway in rodents that results in the formation of 1,2-naphthoquinone at high exposure to naphthalene is potentially present in human nasal and lung tissue. However, the amount and catalytic activity of the enzymes involved appear to be much less than in the mouse and rat:

- The initial cytochrome-P450 monooxygenase type, CYP2F1, is likely expressed in human lung and nasal turbinates to a lesser extent than in rodents. CYP2F1 mRNA has been identified in human respiratory tissue, but resulted in much lower expression than in rats (Bogen et al. 2008) (Ding and Kaminsky 2003, cited in Rhomberg et al. 2010). Reliable direct evidence of CYP2F1 expression is obviously not available for humans (Baldwin et al. 2005).

However, immunohistochemical studies in pulmonary and nasal biopsies of rhesus macaques, a nonhuman primate, confirmed the absence of any CYP2F isozyme in the lung and only low presence of CYP2F in the ethmoturbinates of the nose (including the olfactory epithelium) as compared to rat and mice (Baldwin et al. 2004).

- There are structural differences between CYP2F1 as compared to CYP2F2 and 2F4 in rodents which result in lower bioactivation of naphthalene in the human tissue (Lewis et al. 2009) (see below). This CYP variant prevalently catalyses the formation of 1S,2R-epoxide, the chiral stereoisomer of that one primarily formed in rodents.
- In the human target tissues, the cytochrome-P450 types of the CYP2A family may play a more dominant role than CYP2F types that prevail in rodents: The highest

concentration/activity of CYP2A13, the central monooxygenase, was found in the human nasal mucosa, followed by lung and trachea (Su et al. 2000; Fukima et al. 2008). Reaction products of this oxidation step were mainly 1- and 2-naphthol. These are normally detoxified by conjugation with sulfate or glucuronic acid without consumption of GSH. The toxicological role of CYP2A13 and of naphthols is unclear, but no epoxide appears to be formed and probably only little if any 1,2-naphthoquinone from naphthols. Further oxidation of 1-naphthol may also result in 1,4-naphthoquinone, a potentially celltoxic intermediate, too.

The most likely and toxicologically most relevant metabolic route leads via primary epoxidation by CYP2F1. The initial naphthalene 1,2-epoxide which is formed in rodent at a high rate is only slowly generated in human tissue: Comparative in vitro enzyme studies revealed that the specific monooxygenase activity in lung microsomal fractions was at least two orders of magnitude lower in humans than in rat and hamster; about 1000-fold lower activity levels were found in the mouse lung microsomes (Lorenz et al. 1984).

The overall turnover rate of naphthalene to the naphthalene-1,2-diol is low in the human target tissues. For instance, in human lung microsomes the metabolic rate for naphthalene to the 1,2-dihydrodiol was just about 3% of that observed in rodents (Buckpitt and Bahnson 1986). Metabolic studies using tissue sections of the respiratory tract demonstrated that the formation rate in monkey of 1,2-dihydrodiol from naphthalene was only about 1 % as compared to that in rodents (Boland et al. 2004). Similar metabolic rates were found with lung microsomes, about 100-fold lower in monkey than in mice, thus confirming the observations with human microsomes (Buckpitt et al. 1992).

2.2.3 Overall conclusion

The proposed mode of action of naphthalene-induced carcinogenesis in rodents comprises the following necessary key events:

1. Metabolic activation to the 1,2-epoxide (or other intermediates) by CYP2F (and/or other CYPs);
2. Cytotoxicity including GSH depletion in affected tissue;
3. Chronic inflammation and regenerative hyperplasia.

Although plausible, these steps are not sufficient to explain the absence of nasal tumours in mice; at least the development of these very tumours would have to be expected in analogy to the rat on the basis of this sequential series of processes.

As this is not the case, a cancer-initiating factor that is obviously present in the rat nasal mucosa is absent in the respective murine tissue. With regard to genotoxicity in the sense of an early critical event, there is no evidence to suggest that this is a key factor in naphthalene tumorigenesis. If so, we could expect to see tumours in the absence of (marked) concurrent cytotoxicity in various tissues, for instance also in the mouse nose, where naphthalene metabolism takes place.

All data so far provides no evidence of tumorigenesis in the absence of cytotoxicity, but only in its presence.

This indicates that carcinogenesis is a secondary late process in cellular metabolism. It is only the GSH depletion that levels of reactive metabolites accumulate high enough to produce cell toxicity and tumours secondary to such toxicity.

Lacking detailed knowledge about mechanisms that trigger cell transformation, it is assumed that 1,2-naphthoquinone plays a central role in this scenario, since this intermediate has been shown to be mutagenic and cytotoxic in vitro, forming adducts with proteins and also

DNA. Another potentially cytotoxic and mutagenic candidate is e.g. 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, the diol epoxide.

Irrespective of the exact identification of the causal agent, potential reactions with DNA are likely to occur concurrent with or subsequent to cytotoxicity, since the 1,2-naphthoquinone and the diol epoxide would not likely form until GSH has been depleted and cytotoxicity has already occurred.

Whether there is a contribution by genotoxic effects in vivo is not proven. However, this would be at issue only at high doses which cause depletion of the GSH pool.

Under this premise, it becomes evident that the mode of action in rodents is very unlikely to be operative in humans, because they have insufficient metabolic activation to deplete GSH or to generate sufficient levels of reactive metabolites.

Moreover, by weight of evidence it can be concluded that initiating genotoxicity is not the key event but that it is a non-genotoxic/cytotoxic or a dual cytotoxic/ genotoxic event in the mode of action for naphthalene-induced carcinogenesis (Rhomberg et al 2010).

2.2.4 Flow Sheet on Naphthalene Biotransformation in Rodents and Humans

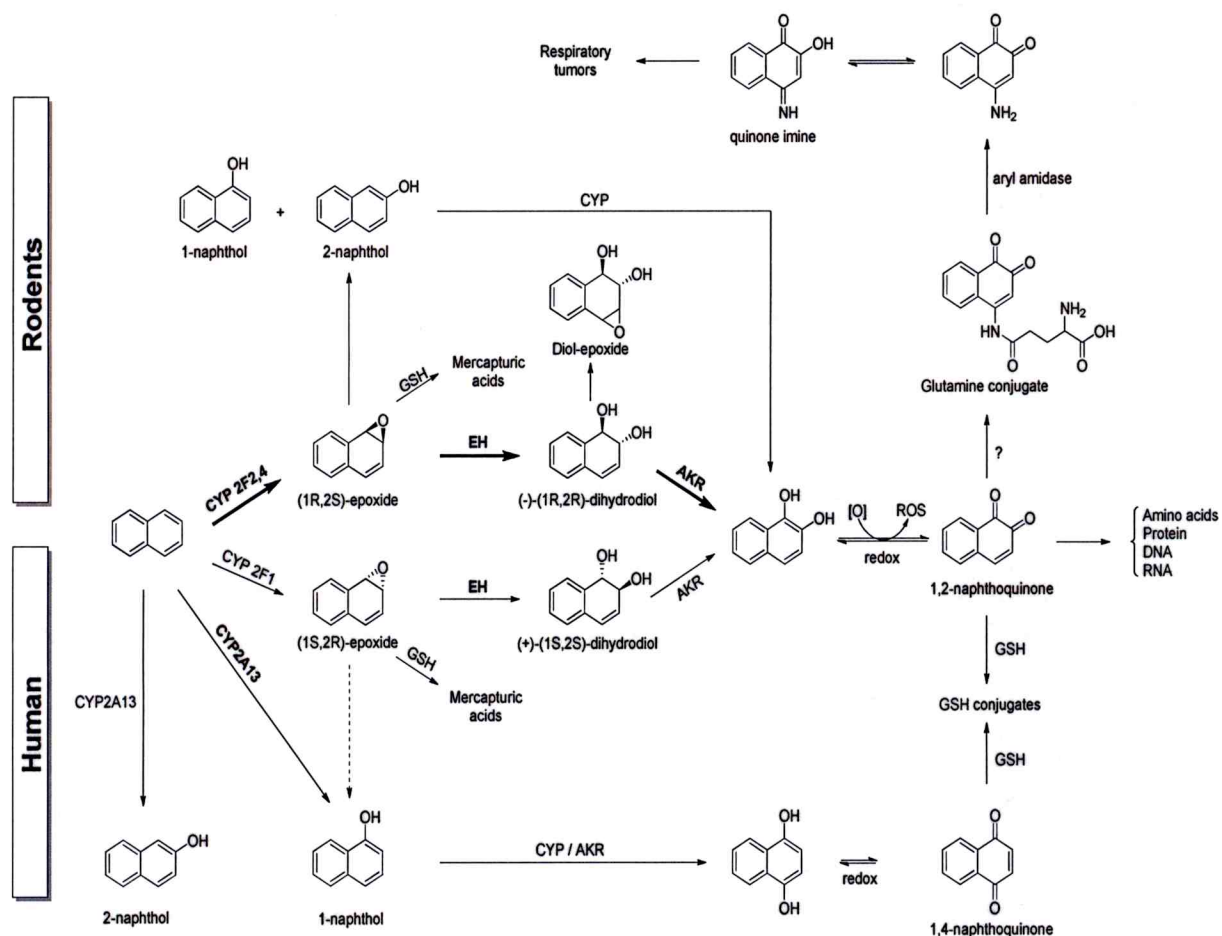


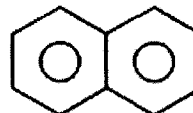
Figure 1: Proposed metabolism of naphthalene in the respiratory and nasal epithelia in rodents and humans including hypothetical pathways (according to Piccirillo et al. 2012, with modification)

2.2.5 References

- Baldwin RM, Jewell WT, Fanucci MV, Plopper C, Buckpitt A 2004: Comparison of pulmonary/nasal CYP2F expression levels in rodents and rhesus macaque. *J. Pharmacol. Exp. Ther.* 309: 127-136
- Bogen, K.T.; Benson, J.M.; Yost, G.S.; Morris, J.B.; et al. 2008: Naphthalene metabolism in relation to target tissue anatomy, physiology, cytotoxicity and tumorigen mechanism of action. *Regul. Toxicol. Pharmacol.* 51: S27-S36
- Boland B, Lin CY, Morin D, Miller L, Plopper C, Buckpitt A 2004: Site-specific metabolism of naphthalene and 1-nitronaphthalene in dissected airways of rhesus macaques. *J. Pharmacol. Exp. Ther.* 310: 546-554
- Buckpitt RA, Bahnson LS 1986: Naphthalene metabolism by human lung microsomal enzymes. *Toxicology* 41: 333-341
- Buckpitt AR, Warren DL 1983: Evidence for hepatic formation, export and covalent binding of reactive naphthalene metabolites in extrahepatic tissues in vivo. *J. Pharmacol. Exp. Ther.* 225: 8-16
- Buckpitt A, Boland B, Isbell M, Morin D, et al. 2002: Naphthalene-induced respiratory tract toxicity: Metabolic mechanisms of toxicity. *Drug Metab. Rev.* 34(4): 791-820
- Cruzan G, Bus J, Banton M, Gingell R, Carlson G. 2009: Mouse lung tumors from CYP2F2-mediated cytotoxic metabolism: an endpoint/toxic response where data from multiple chemicals converge to support a mode of action. *Regul. Toxicol. Pharmacol.* 55: 205-218
- Ding, X.; Kaminsky, L.S. 2003: Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemicals toxicity in the respiratory and gastrointestinal tracts. *Annu. Rev. Pharmacol. Toxicol.* 43: 149-173
- Dodd DE, Gross EA, Miller RA, Wong BA 2010: Nasal olfactory epithelial lesions in F344 and SD rats following 1- and 5-day inhalation exposure to naphthalene. *Int. J. Toxicol.* 29: 175-184
- Flowers-Geary L, Bleczinski W, Harvey RG, Penning TM 1996: Cytotoxicity and mutagenicity of polycyclic aromatic hydrocarbon ortho-quinones produced by dihydrodiol dehydrogenase. *Chem. Biol. Interact.* 99: 55-72
- Fukami T, Katoh M, Yamazaki H, Yokoi T, Nakajima M 2008: Human cytochrome P450 2A13 efficiently metabolizes chemicals in air pollutants: naphthalene, styrene, and toluene. *Chem. Res. Toxicol.* 21: 720-725
- Genter, MB, Marlowe J, Kevin KJ, et al. 2006: Naphthalene toxicity in mice and aryl hydrocarbon receptor-mediated CYPs *Biochem. Biophys. Res. Commun.* 348:120-123
- Heydens WF, Wilson AG, Kier LD, Lau H, Thake DC, Martens MA 1999: An evaluation of the carcinogenic potential of the herbicide alachlor to man. *Hum. Exp. Toxicol.* 18: 363-391
- Lee MG, Phimister A, Morin D, Buckpitt A, Plopper C 2005: In situ naphthalene bioactivation and nasal airflow cause region-specific injury patterns in the nasal mucosa of rats exposed to naphthalene by inhalation. *J. Pharmacol. Exp. Ther.* 314: 103-110
- Lorenz J, Glatt HR, Fleischmann R, Ferlinz R, Oesch F 1984: Drug metabolism in man and its relationship to that in three rodent species: monoxygenase, epoxide hydrolase, and glutathione S-transferase activities in subcellular fractions of lung and liver. *Biochem. Med.* 32: 43-56
- Lewis DFV, Ito Y, Lake BG 2009: Molecular modeling of CYP2F substrates: Comparison of naphthalene metabolism by human, rat and mouse CYP2F subfamily enzymes. *Drug Metabol Drug Interact* 20(2-4): 229-257.
- Morris JB, Buckpitt AR 2009: Upper respiratory tract uptake of naphthalene. *Toxicol. Sci.* 111: 383-391
- NTP 1992: National Toxicology Program: Toxicology and carcinogenesis studies of naphthalene (CAS no. 91-20-3) in B6C3F1 mice (inhalation studies). U.S. Department

- of Health and Human Services, National Institutes of Health, Rockville, MD. NTP Technical report no. 410, April 1992, NIH pub. No. 92-3141
- NTP 2000: National Toxicology Program: Toxicology and carcinogenesis studies of naphthalene (CAS no. 91-20-3) in F344/N rats (inhalation studies). U.S. Department of Health and Human Services, National Institutes of Health, Rockville, MD. NTP Technical report no. 500, December 2000, NIH pub. No. 01-4434
- Penning TM, Burczynski ME, Hung C-F, et al. 1999: Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: Generation of reactive and redox active o-quinones. *Chem. Res. Toxicol.* 12: 1-18
- Phimister AJ, Lee MG, Morin D, Buckpitt AR, Plopper CG 2004: Glutathione depletion is a major determinant of inhaled naphthalene respiratory toxicity and naphthalene metabolism in mice. *Toxicol. Sci.* 82: 268-278
- Piccirillo VJ, Bird MG, Lewis RJ, Bover WJ 2012: Preliminary evaluation of the human relevance of respiratory tumors observed in rodents exposed to naphthalene. *Regul. Toxicol. Pharmacol.* 62, 433-440
- Plopper CG, Suverkropp C, Morin D, Nishio SJ, Buckpitt AR 1992: Relationship of cytochrome P-450 activity to Clara cell cytotoxicity I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. *J. Pharmacol. Exp. Ther.* 261: 353-363
- Rhomberg, L.R.; Bailey, L.A.; Goodman, J.E. 2010: Hypothesis-based weight of evidence: A tool for evaluating and communicating uncertainties and inconsistencies in the large body of evidence in proposing a carcinogenic mode of action—naphthalene as an example. *Crit. Rev. Toxicol.* 40: 671-696
- Shultz MA, Choudary PV, Buckpitt AR 1999: Role of murine cytochrome P-450 2F2 in metabolic activation of naphthalene and metabolism of other xenobiotics. *J. Pharmacol. Exp. Ther.* 290: 281-288 (cited in Buckpitt et al. 2002)
- Su T, Bao Z, Zhang QY, Smith, TJ, Hong JY, Ding X 2000: Human cytochrome P450 CYP2A13: Predominant expression in the respiratory tract and its high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res.* 60: 5074-5079
- Warren DL, Brown D, Buckpitt A 1982: Evidence for cytochrome P450 mediated metabolism in the bronchiolar damage by naphthalene. *Chem. Biol. Interact.* 40: 287-303
- West JA, Pakenham G, Morin D, Fleschner CA, Buckpitt AR, Plopper CG. 2001: Inhaled naphthalene causes dose dependent Clara cell cytotoxicity in mice but not in rats. *Toxicol. Appl. Pharmacol.* 173:114-119
- Wilson AS, Davis CD, Williams DP, Buckpitt AR et al. 1996: Characterization of the toxic metabolite(s) of naphthalene. *Toxicology* 114, 233-242

NAPHTHALENE COUNCIL, INC.



31 August 2012

Dr. S.R. Vink
The Health Council of The Netherlands
Subcommittee on the Classification of Carcinogenic Substances
PO Box 16052
2500 BB The Hague
The Netherlands

Via email: sr.vink@gr.nl

Dear Dr. Vink,

On 29 May 2012, the Health Council of The Netherlands published a draft report titled in English *Naphthalene: Evaluation of the Carcinogenicity and Genotoxicity* (here after referred to as the Draft Report) prepared by the Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council. In response to the Health Council's invitation to interested parties to comment on the Draft Report, the Naphthalene Council respectfully submits the information in this letter for DECOS' consideration.

The Draft Report contains a clear explanation of DECOS procedures. In its evaluation, the Subcommittee relies, as appropriate, on monographs of the International Agency for Research on Cancer (IARC). The most recent IARC monograph on naphthalene was published in 2002. DECOS also relies on toxicological reviews conducted by other expert bodies. In the case of naphthalene, DECOS appropriately cites relatively recent reviews including the European Union's (EU) Risk Assessment for naphthalene (published in 2003) and the United States (US) Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile of naphthalene (published in 2005). The Draft Report cites only two studies relevant to evaluation of naphthalene carcinogenicity and genotoxicity published after 2005. The two cited studies are Lewis (2012; incorrectly listed in Reference Number 3 as "Jeffery, LR" – the correct citation is "Lewis, RJ") and Stefano-Shields *et al.* (2010).

The Naphthalene Council is an industry association the members of which manufacture naphthalene and/or use naphthalene to manufacture other products. The Naphthalene Council participates along with other trade associations and individual companies in the Naphthalene Research Committee (NRC). The NRC sponsors research to help improve naphthalene risk assessments and ensure that quality data are generated and published in the peer-reviewed

Actv.	246
Nr.	1335
ING.	03 SEP 2012
V	V RGO
VV	.
AS	HB
S	SV
s	fs
Archief	
Bijlagen	

2308 Mount Vernon Avenue, Suite 134
Alexandria Virginia 22301

Phone: +1 (703) 299-8470
Fax: +1 (703) 842-8850
alehuray@naphthalene.org

Comments on the Draft Report

Naphthalene: Evaluation of the Carcinogenicity and Genotoxicity

31 August 2012

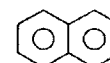
Page 2

scientific literature to reduce the use of default assumptions in the risk assessment for naphthalene. Research sponsored by the NRC is grounded in a symposium conducted to review naphthalene carcinogenicity and genotoxicity in California in 2006 called the *Naphthalene State-of-the-Science Symposium* (NS³). Proceedings of NS³ were published in a special issue of the peer-reviewed journal *Regulatory Toxicology and Pharmacology* in 2008. One procedural and six scientific papers, listed below, resulted from NS³. Each provides insight into what was known about naphthalene carcinogenicity and genotoxicity in 2006.

- Belzer, Richard B., James S. Bus, Ercole L. Cavalieri, Steven C. Lewis, D. Warner North and Richard C. Pleus (2008). The naphthalene state of the science symposium: Objectives, organization, structure, and charge. *Regul. Toxicol. Pharmacol.*, 51, S1-S5.
- Bogen, Kenneth T., Janet M. Benson, Garold S. Yost, John B. Morris, Alan R. Dahl, Harvey J. Clewell III, Kannan Krishnan and Curtis J. Omiecinski (2008). Naphthalene metabolism in relation to target tissue anatomy, physiology, cytotoxicity and tumorigenic mechanism of action. *Regul. Toxicol. Pharmacol.*, 51, S27-S36.
- Brusick, D. (2008), Critical assessment of the genetic toxicity of naphthalene, *Regul. Toxicol. Pharmacol.*, 51, S37-S42.
- Brusick, David, Mitchell S. Small, Ercole L. Cavalieri, Dhrubajyoti Chakravarti, Xinxin Ding, David G. Longfellow, Jun Nakamura, Eleanor C. Rogan and James A. Swenberg. (2008). Possible genotoxic modes of action for naphthalene, *Regul. Toxicol. Pharmacol.*, 51, S43-S50.
- Griego, F.Y., Bogen, K.T., Price, P.S., Weed, D.L. (2008). Exposure, epidemiology and human cancer incidence of naphthalene. *Regul. Toxicol. Pharmacol.* 51, S22-S26.
- North, D.W., Abdo, K.M., Benson, J.M., Dahl, A.R., Morris, J.B., Renne, R. and Witschi, H. (2008). A Review of Whole Animal Bioassays of the Carcinogenic Potential of Naphthalene, *Regulatory Toxicology and Pharmacology*, 51, S6-S14.
- Price, P.S. and Jayjock, M.A. (2008). Available data on naphthalene exposures: Strengths and limitations. *Regul. Toxicol. Pharmacol.*, 51, S15-S21.

In the seven years since 2005, many papers describing original scientific research concerning naphthalene have been published in refereed journals – many reporting research funded by the NRC. Recent publications reporting research focused on modes of action of naphthalene carcinogenicity include the following:

- Bogen, KT (2008). An Adjustment Factor for Mode-of-Action Uncertainty with Dual-Mode Carcinogens: The Case of Naphthalene-Induced Nasal Tumors in Rats. *Risk Analysis*, Vol. 28, No. 4, 1539-6924.
- Cruzan, G, Bus, J, Banton, M, Gingell, R and Carlson, G (2009). Mouse Specific Lung Tumors from CYP2F2-Mediated Cytotoxic Metabolism: An Endpoint/Toxic



Comments on the Draft Report

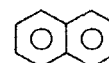
Naphthalene: Evaluation of the Carcinogenicity and Genotoxicity

31 August 2012

Page 3

-
- Response Where Data from Multiple Chemicals Converge to Support a Mode of Action. *Reg. Tox. Pharm.* 55(2), 205-218
- Dodd, DE, Gross, EA, Miller, RA and Wong, BA (2010). Nasal olfactory epithelial lesions in F344 and SD rats following 1- and 5-day inhalation exposure to naphthalene vapor. *International Journal of Toxicology* 29:175 - 184.
- Fasano, WJ and McDougal, JN (2008). *In vitro* dermal absorption rate testing of certain chemicals of interest to the Occupational Safety and Health Administration: Summary and evaluation of USEPA's mandated testing. *Regul. Toxicol. Pharmacol.*, 51:181-194.
- Lewis, DFV, Ito, Y, and Lake, BG (2009). Molecular Modelling of CYP2F Substrates: Comparison of Naphthalene Metabolism by Human, Rat and Mouse CYP2F Subfamily Enzymes. *Drug Metabolism & Drug Interactions* 24(2-4):229-237.
- Li, L, Wei, Y, Van Winkle, L, Zhang, Q-Y, Zhou, X, Hur, J, Xie, F, Kluetzman, K and Ding, S (2011). Generation and Characterization of a *Cyp2f2*-Null Mouse and Studies on the Role of CYP2F2 in Naphthalene-Induced Toxicity in the Lung and Nasal Olfactory Mucosa *J Pharmacol Exp Ther* October 2011 339:62-71;
- Magee, B., Samuelian, J., Haines, K., Chappel, M., Penn, I., Chin, D., Anders, D. and Hinz, J. (2010). Screening-level population risk assessment of nasal tumors in the US due to naphthalene exposure. *Regul. Toxicol. Pharmacol.* 57:168-180.
- Meng, F., Wang, Y., Myers, M., Wong, B., Gross, E., Clewell, H., Dodd, D., and Parsons, B. (2011). p53 codon 271 CGT to CAT mutant fraction does not increase in nasal respiratory and olfactory epithelia of rats exposed to inhaled naphthalene. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 721 (2011) 199-205
- Morris, JB and Buckpitt, AR (2009). Upper respiratory tract uptake of naphthalene. *Toxicological Sciences* 111(2), 383-391.
- Piccirillo, VJ, Bird, MG, Lewis, RJ and Bover, WJ (2012). Preliminary evaluation of the human relevance of respiratory tumors observed in rodents exposed to naphthalene. *Regul. Toxicol. Pharmacol.*, 62, 433-440.
- Recio L, Shepard KG, Hernandez, LG, Kedderis GL. 2012. Dose-Response Assessment of Naphthalene-Induced Genotoxicity and Glutathione Detoxication in Human TK6 Lymphoblasts. *Toxicol. Sci.* 126(2):405-412.
- Rhomberg, L and Bailey, L (2010). Hypothesis-Based Weight of Evidence – A Tool for Evaluating and Communicating Uncertainties and Inconsistencies in the Large Body of Evidence in Proposing a Potential Carcinogenic Mode of Action – Naphthalene as an Example. *Critical Reviews in Toxicology*. 40:8, 671-696. Web Address: <http://informahealthcare.com/doi/pdf/10.3109/10408444.2010.499504>

The Naphthalene Council is aware of additional publications that are in preparation that will be of relevance to consideration of naphthalene carcinogenicity. Manuscripts expected to be submitted for peer review in the coming months include papers detailing metabolic kinetics in



Comments on the Draft Report

Naphthalene: Evaluation of the Carcinogenicity and Genotoxicity

31 August 2012

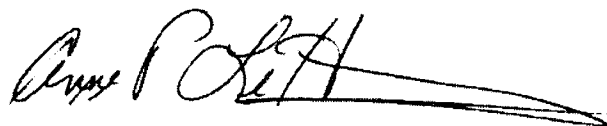
Page 4

rodent and primate species, a PBPK model of naphthalene in rodent and primate species, the results of a genomics study, a study of cytotoxicity in multiple organs of multiple cell lines exposed to naphthalene in vitro, and additional studies of upper respiratory tract dosimetry in additional species.

The Naphthalene Council hopes you will give full consideration to the literature identified above as DECOS finalizes its evaluation of naphthalene carcinogenicity and genotoxicity.

Please contact me by telephone (+1-703-299-8470) or email (alehuray@naphthalene.org) with questions or requests for additional information.

Yours truly,



Anne P. LeHuray, Ph.D.
Executive Director
Naphthalene Council

