Targeted Mechanistic Evidence Synthesis to Inform Evidence Integration Decisions on the Potential Human Carcinogenicity of Naphthalene Exposure

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Background

Naphthalene has been demonstrated to cause respiratory tumors in rats and mice, but the few available epidemiologic studies are inadequate to evaluate the potential for naphthalene to cause cancer in humans. In lieu of human studies, mechanistic information may be used to inform the potential carcinogenicity of naphthalene for human health risk assessment.

Multiple modes of action (MOAs) for naphthalene-induced carcinogenesis have been proposed based on animal and in vitro studies, including genotoxicity, cytotoxicity, and sustained regenerative cell proliferation. While these proposed MOAs may differ in specific key events, the formation of toxic naphthalene metabolites and the biological relevance of these toxic metabolites to humans has emerged as a key component in answering the question of applicability of carcinogenic risk to humans. There is a great deal of similarity between the rodent and human naphthalene metabolic pathways; however, the activity of the enzymes involved in naphthalene metabolism and therefore the number of metabolites and stereoisomers of the produced metabolites may differ between rodents to humans.

Here, concurrent with a broad systematic review of health effects related to naphthalene exposure, animal and in vitro studies of the available mechanistic evidence was analyzed to (1) integrate the available evidence in vitro models on the formation and toxicity of each of the key toxic metabolites of naphthalene and (2) determine the biological plausibility that each of these key metabolites could be generated in human tissue and increase human oncogenic risk.

Methods

Literature Search and Tagging: Mechanistic studies were identified by tagging studies during screening of the broad literature search focused on the potential human health impacts associated with napthalene exposure.

Study evaluation: Studies tagged as mechanistic were evaluated using the SciRAP web tool (www.scirap.org) for either in vivo or in vitro study evaluation for factors rated to reporting quality, methodological quality, and relevance. SciRAP was selected for this evaluation because it has both in vivo and in vitro study evaluation tools available.

Evidence synthesis: For the specific question of metabolic relevance, we used the metabolic pathway for napthalene (developed from rodent models) as a scaffold and then evaluated studies that addressed the applicability of this metabolic pathway to humans, focusing on three key napthalene metabolites (Figure 1): 1S,2R-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone. Studies that had deficiencies in reporting critically important study details (e.g., missing experimental exposure details) were excluded.

The evidence regarding the formation, toxicity, and human relevance of these three key naphthalene metabolites was integrated in a tabular format describing the formation and toxicity of each metabolite, factors that increase strength of evidence, and factors that decrease strength of evidence (Table 1).

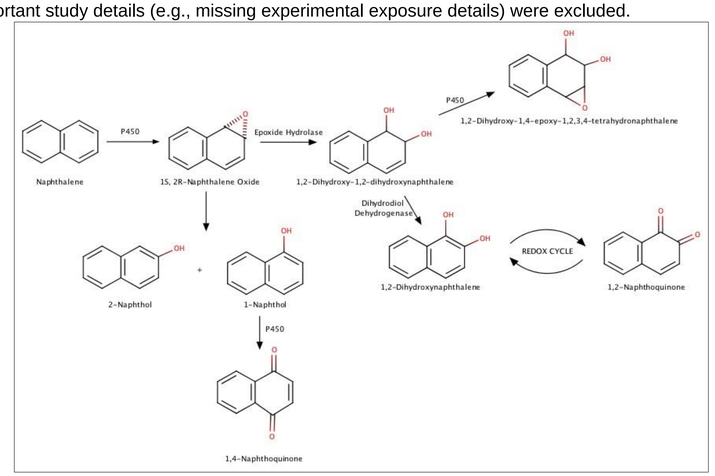

Figure 1. Naphthalene Metabolic Pathway

SciRAP Study Evaluation Results

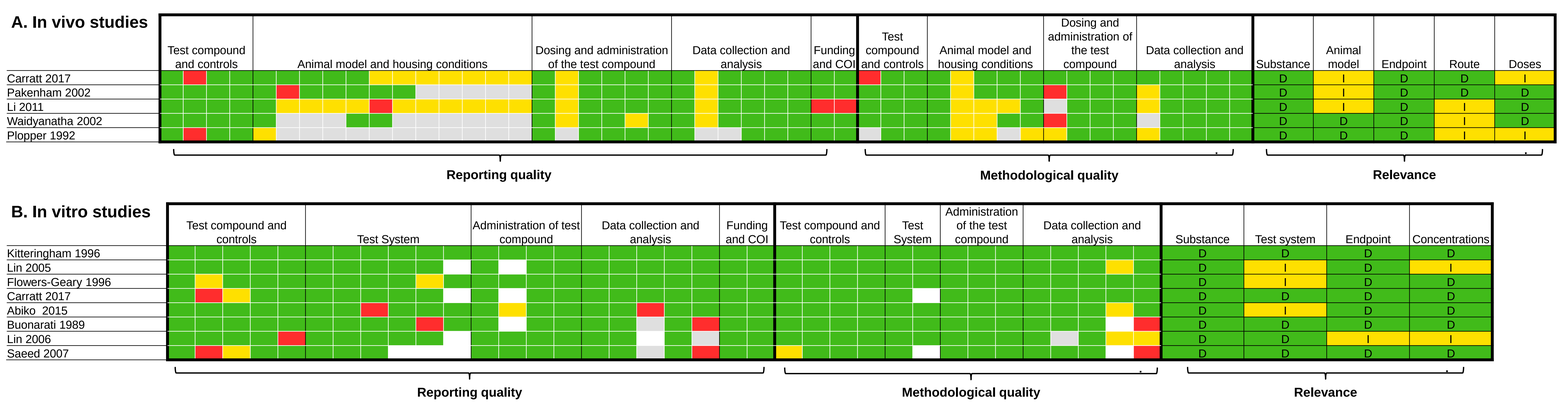

Figure 2. Representative study evaluation results. Representative studies examining three key naphthalene metabolites of interest (see Figure 1) were evaluated using SciRAP tool (n= 5 in vivo studies, n= 8 in vitro studies). For reporting and methodological quality criteria, green = fulfilled, yellow = partially fulfilled, red = not fulfilled, gray = not determined, and white = not applicable. For relevance categories, green (D) indicates that the study design was directly relevant to human health, and yellow indicates that the study design was indirectly relevant to human health.

Evidence Synthesis

*Identification number in EPA's Health & Environmental Research Online (HERO) database

	References [HERO ID*]	Factors that increase strength	Factors that decrease strength	Summary of evidence
1S,2R-Naphthalene oxide	**In vivo** • Plopper, 1992 [1469611] • Waidyanatha, 2002 [1469054] • Li, 2011 [1005231] **In vitro** • Buonarati,1989 [94674] • Buckpitt, 1992 [067441] • Lanza, 1999 [1489430] • Wilson, 1995	• No serious reporting or methodological quality limitations • Metabolite formation and cytotoxicity observed in models with greater directness (nonhuman primates and humanized mice) [Buckpitt, 1992; Li, 2011]	• Indirectness in some studies (studies in isolated rodent primary hepatocytes; route of in vivo exposure i.p. [Plopper, 1992] • Inconsistency (potential lack of metabolite formation and cytotoxicity in vitro) [Lanza, 1999; Wilson, 1995]	• CYP450 activity varies across species and determines severity of cytotoxicity produced by 1,2-naphthalene oxide [Buonarati, 1989; Plopper, 1992] • 1,2-naphthalene oxide is produced as two isomers: 1S,2R- (predominant human form) and 1R,2S. Animal studies suggest the 1S,2R isomer's cytotoxicity is > the 1R, 2S isomer [Buckpitt, 1992]. Conversely, in vitro assays in lymphoblastoid cells showed that napthalene oxide was not genotoxic in a sister chromatid exchange (SCE) assay [Wilson, 1995]. • Human CYP2A13 and 2F1, which catalyze the formation of 1,2-naphthalene oxide, were demonstrated to bioactivate naphthalene and induce toxicity in humanized transgenic mice [Li 2011] at occupationally relevant exposure levels. Conversely, microsomal assays found that recombinant human CYP2F1 had <0.1% the rats of metabolism observed with the mouse orthologue [Lanza, 1999].
1,2-Naphthoquinone	**In vivo** • Waidyanatha, 2002 [1469054] • Carratt, 2017 [345264] **In vitro** • Abiko, 2015 [4331236] • Carratt, 2017 [345264] • Flowers-Geary, 1996 [1012266] • Kitteringham, 1996 [1469475] • Saeed, 2007 [517040] • Wilson, 1996 [081049]	• No serious reporting or methodological quality limitations • Multiple positive mutagenicity assays including salmonella and SCE assays [Flowers-Geary, 1996]. • Cytotoxicity observed [Carrat, 2017; Kitterhangham 1996;]	• Indirectness in in vitro studies that observed effects (direct incubation with DNA and/or in vitro studies; mutagenicity assays were all tested in conditions that did not have an exogenous metabolic system) [Wilson, 1996; Saeed, 2007] • Mutagenesis assay information all came from a single source [Flowers-Geary 1996]	• 1,2-naphthoquinone produces cytotoxicity and increased formation of reactive oxygen species [Carratt, 2017; Kitteringham, 1996]. • 1,2-naphthoquinone forms adducts with proteins and DNA adducts that are linked to mutagenicity, chromosome aberrations, tumor promotion, and cancer [Abiko, 2015, Waidyanatha, 2002; Saeed, 2007; Flowers-Geary, 1996]. • In addition, 1,4-Naphthoquinone produced a dose dependent increase in SCE in vitro [Wilson, 1996]
1,4-Naphthoquinone	**In vivo** • Waidyanatha, 2002 [1469054] **In vitro** • Abiko, 2015 [4331236] • Lin, 2005 [148718] • Lin, 2006 [1468615] • Destephano-Shields, 2010 [1467694] • Wilson, 1996 [081049]	• No serious reporting or methodological quality limitations • Directness in the study by DeStephano-Shields, 2010 adducts formed in non-human primates after in situ exposure	• Indirectness in some studies that observed effects (direct incubation with DNA in vitro; proteomics study; route of exposure in vivo) [Lin, 2005; Lin, 2006].	• 1,4-naphthoquinone leads to protein and DNA adduct formation that are linked to chromosome aberrations, tumor promotion, and cancer [Abiko 2015; Lin 2005, Lin 2006, Waidyanatha, 2002] • In addition, 1,4-Naphthoquinone produced a dose dependent increase in SCE in vitro [Wilson, 1996]

Table 1 Evidence profile table describing a summary of the toxicological evidence for each of the known naphthalene metabolites

- The available evidence showed that 1S,2R-naphthalene oxide (the prevalent naphthalene metabolite in humans) is a highly reactive metabolite that is more toxic and metabolized more slowly than the 1R,2S enantiomer more commonly observed in mice, which may allow it more time to produce cytotoxicity.
- 1S,2R-naphthalene oxide can be metabolized to 1,2-naphthoquinone or 1,4-naphthoquinone (Figure 1), which have been shown to elicit cytotoxicity. These quinone metabolites can bind to proteins and have been demonstrated in situ and across species (including non-human primate tissue) to form protein adducts. In addition, these quinones may also undergo protein adduction and disrupt normal cellular function by binding to CYP450 enzymes and to proteins involved in cell signaling and transduction.
- The electrophilic nature of 1,2- and 1,4-napthoquinone cause these metabolites to undergo 1,4-Michael addition and covalently bind to DNA, forming depurinating N3Ade and N7Gua adducts as well as stable adducts. Therefore, it is biologically plausible for the reactive naphthalene metabolites 1,2- and 1,4-naphthoquinone to form depurinating and stable DNA adducts.