CASE STUDY – Rhomberg, LR; Bailey, LA; Goodman, JE. 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.

See attached paper.

SUPPLEMENT TO CASE STUDY – Application of the HBWoE Evaluation to Dose-response Modeling for Naphthalene Carcinogenesis

The HBWoE evaluation of naphthalene, as presented in our paper (Rhomberg *et al*. 2010), integrated data from all realms of evidence for naphthalene (epidemiology, animal toxicology, mechanistic, and toxicokinetic), tracing and comparing the logic within and across realms of evidence for how the data bear on the individual and competing overarching hypotheses for a carcinogenic mode of action (*i.e.*, initiating genotoxic mode of action at less than cytotoxic doses *vs*. cytotoxic or dual cytotoxic/genotoxic mode of action where genotoxicity does not occur until cytotoxic doses or higher), considering uncertainties and inconsistencies in the data set, and any *ad hoc* assumptions required to support each hypothesis. The outcome of our evaluation was that the current data more strongly support a mode of action (MoA) that is either cytotoxic or dual cytotoxic/genotoxic and that there is a much larger degree of *ad hoc* argument in the hypothesis that accounts for the data as supporting an initiating genotoxic mode of action.

Guided by the results of the HBWoE evaluation, we are now conducting a dose-response evaluation of naphthalene exposure and neoplastic and non-neoplastic lesions, with the ultimate goal of deriving naphthalene toxicity values applicable to human health risk assessment that are consistent with an integrated evaluation of all realms of evidence for naphthalene.

The challenge we are faced with, one that is typical in human health risk assessment, is that the available information on a possible dose-response association between naphthalene exposure and respiratory carcinogenicity in humans is indirect. That is, inhalation of naphthalene causes olfactory epithelial nasal tumors in rats (but not mice) and benign lung adenomas in mice (but not rats) (NTP, 1992, 2000), and there are no other animal carcinogenesis studies for inhaled naphthalene. In addition, the bioassays are limited in that they were only conducted at high doses (10-60 ppm naphthalene) that were also highly cytotoxic, providing no information on incidence of non-neoplastic lesions at lower doses.

Further, there is an evident lack of a tumor effect in occupationally exposed people and for people in the general population. Although nasal tumors are rare in humans, lung tumors are not. Studies in humans have not identified naphthalene exposure as associated with lower respiratory tract cancer risk, although this has not been explicitly examined. Therefore, the question of naphthalene's respiratory carcinogenicity in humans bears entirely on experimental evidence in rats and mice.

Our approach is to consider how applicable the rat nasal tumors are to serve as a basis for estimation of potential human respiratory-tract cancer risk. This is done by considering the potential MoA underlying the effects seen in animal bioassays (*i.e.*, the MoA that is best supported by the available data in the HBWoE evaluation). Key aspects of this consideration

include evaluation of the metabolic activation and detoxification of inhaled naphthalene as they depend on air concentration, as well as the nature, tissue locations, and dependence on tissue-dose of key precursor non-neoplastic responses (*i.e.*, chronic inflammation, epithelial hyperplasia, degeneration, and necrosis).

The animal bioassay and mechanistic data provide several important observations that need to be considered in the dose-response evaluation:

1. The tumors are confined to very particular epithelial tissues of the respiratory tract that are directly exposed to naphthalene vapors (localized adenomas in mouse bronchioles and similar localized tumors in olfactory and respiratory epithelial tissue in rats), suggesting very specific and local metabolic activation;
2. In both rats and mice, the tissues in which tumors occur are subject (at tumorigenic exposure levels) to widespread cytotoxicity and inflammation;
3. Respiratory tissues subject to toxicity in rodents are at sites of concentrated and localized metabolic activity toward naphthalene that is mediated by CYP450, and inhibition of this metabolism extinguishes cytotoxicity in these tissues; and
4. Exposure to naphthalene by intraperitoneal (IP) injection results in the same pattern of metabolic activation and cytotoxicity in the same respiratory tract epithelia, showing that the localization of effects in rodents is attributable to localized high metabolic activity rather than to the direct inhalation exposure of the tissues.

Given the observations in the high-dose animal bioassays, and the results of the HBWoE evaluation, the questions we are faced with for the dose-response evaluation for humans, and how we plan to address them, are as follows:

How can we use the results of HBWoE evaluation (i.e., insights on mode of action) to inform the dose response?

The HBWoE evaluation suggests a mode of action for naphthalene carcinogenesis in rodents that is not likely to involve genotoxicity as a tumor initiating event at low subcytotoxic doses. The MoA more likely involves high-dose cytotoxicity (including chronic inflammation and regenerative hyperplasia), and possible high-dose genotoxicity from downstream naphthalene metabolites but only at doses that are also cytotoxic. That is, the data support a mode of action that involves a threshold, *i.e.* a dose below which tumors are not expected to occur. The dose-response evaluation, therefore, needs to attempt to identify this threshold in rodent tissues, and to identify the lowest doses associated with non-neoplastic lesions that are likely precursors to tumors (*e.g.*, cytotoxicity, inflammation, and regenerative hyperplasia).

Since the NTP data are the result of high, cytotoxic doses, how can we better understand what is happening in rat nasal tissues at low doses?

Dodd *et al*. (2012) conducted a 90-day low-dose (0.1, 1, 10, 30 ppm naphthalene) inhalation study in rats to explore the exposure-response relationship and possible threshold for nasal epithelial effects in rats. The study identified doses at which non-neoplastic lesions did not occur,

thereby suggesting a naphthalene inhalation threshold exposure concentration in rats for nasal effects. Our approach is based on the presumption that a no-effect level for these apparent precursor lesions in rats will also be a no-effect level for tumors.

We will also be conducting a dose-response evaluation based on analysis of gene expression changes in olfactory and respiratory epithelium in rat nasal tissue in response to exposures to low concentrations of naphthalene (0.1-10 ppm) (Clewell *et al*. in preparation). The gene expression results will be considered in the context of our understanding of the MoA (based on the outcome of the HBWoE evaluation) and the dose-response relationship for non-neoplastic and neoplastic lesions to see what gene expression profiles align with key events in the proposed MoA (*i.e*., cytotoxicity, chronic inflammation, regenerative hyperplasia, degeneration, and necrosis), particularly looking at gene expression profiles at low exposure concentrations.

Further, a recent rat/human PBPK model that is linked to computational fluid dynamic models of airflow in the nose (Campbell *et al*., in preparation) has allowed for an understanding of tissue doses in rats in locations of nasal lesions, and also the prediction of metabolically activated doses that are associated with non-neoplastic effects in the rat nose at low doses.

In view of the apparent dependence on metabolic activation and noncancer toxicity precursors, what is likely to be responsible for rat nasal tumors and what of that process might be relevant to humans for either nasal tissues or for other places in the respiratory tract?

Our dose-response evaluation attempts to align exposure-response relationships of key precursor non-neoplastic lesions and gene expression responses to exposure-response relationships for tumors in an attempt to develop a sequence of key events for tumor formation in the rat nose that is consistent with the tissue dose-response and the biologically plausible mode of action supported by the HBWoE evaluation. Part of our evaluation involves examining what non-neoplastic lesions occur in the individual rats with tumors and then trying to understand the dose-response relationship for those precursors compared to the dose-response for tumors, and the tissue concentrations associated with those precursor effects.

The low dose evaluation in the recent 90-day rat bioassay by Dodd *et al*. (2012), in addition to the recent rat/human Computational Fluid Dynamic (CFD)-PBPK model (Campbell *et al*., in preparation), has allowed for incorporation of species differences in tissue dosimetry to evaluate whether parallel tissues, or other tissues in the respiratory tract, in humans will be subject to tissue doses that could prompt the key events of the apparent mode of action and associated adverse effects in humans at typical human exposures.

The PBPK model is linked to computational fluid dynamic models of human air flows and metabolic capacities so that the model can predict metabolically activated doses in humans in the nose and other locations in the respiratory tract. Since our approach is based on the presumption that a no-effect level for the apparent precursor lesions in rats will also be a no-effect level for tumors, we can use this assumption, in combination with an understanding about what tissue doses are not likely to lead to precursor lesions, to answer the question about the possibility of human responses in other tissues besides nose (*e.g.*, lung). We apply the PBPK model to ask

whether humans have sufficient metabolic activation in nasal or non-nasal tissues to be near levels needed to produce the non-neoplastic lesions seen in the rat nose.

Overall, our approach is to look at the dose-response for different components of the apparent MoA in the rat nose (considering gene expression and non-neoplastic lesion responses) as they depend on tissue delivered dose, and then through application of the CFD-PBPK model try to understand the relevance of those associations to adverse effects in nasal and non-nasal human tissues.

Specifics of the Dose-Response Evaluation

We have evaluated a variety of cancer and non-cancer endpoints for naphthalene using a number of quantitative inhalation dose-response assessment approaches. These approaches include mathematical modeling of dose-response functions to derive points of departure (PODs) based on: incidence of neoplastic and non-neoplastic lesions; severity of non-neoplastic lesions; and the temporal nature of the development of neoplastic lesions using a time-to-tumor model. Dose-response estimates have been derived based on both the inhaled dose and the metabolized dose. Responses based on the metabolized dose were derived through application of the recent PBPK model (Campbell *et al*., in preparation) that incorporates metabolic rate constants for naphthalene from rat and rhesus monkey nasal and lung tissue.

Application of US EPA Bench Mark Dose Software (BMDS) Package allows us to estimate the fraction or percent of individuals that exhibit a specific non-neoplastic lesion, using various models (*e.g.*, logistic, probit, Weibulll, *etc.*) that consider the incidence of the lesions over a concentration continuum from zero to any exposure concentration. We also use the US EPA CatReg software to estimate PODs based on the probability of a non-neoplastic lesion of a defined severity (*e.g.,* minimal, mild, moderate, or marked). We apply a categorical regression approach in order to better understand the severity level of a particular lesion that is required to be a precursor to tumorigenic effects, and also to better interpret the occurrence of mild lesions in the controls.

We are also using multiple modeling approaches to quantitatively estimate low-dose human cancer risk based on tumor incidence. We apply the US EPA BMDS software as a multistage model of carcinogenesis to serve as the basis for one estimation of inhalation cancer potency. We have also evaluated the temporal aspect of both tumor appearance and animal survival over the entire length of the NTP naphthalene animal bioassay using a Multistage Weibull (MSW) time to tumor model. In comparison to quantal models (*e.g.,* BMDS Multistage Cancer Model), this model provides a robust estimate of cancer risk by accounting for differential mortality between animals and dose groups.

When conducting a quantitative dose-response assessment to estimate a single point on a dose-response continuum (*e.g.*, a BMD_{10}), it is important to evaluate how the choice of both the endpoint and the quantitative dose-response model will influence the estimate. The use of multiple modeling approaches provides an opportunity to evaluate if the POD estimated from a certain dose-response model is a reliable estimate of a biological response at an unknown datum (*i.e.*, dose or concentration), in comparison to an artifactual response based on a model "forcing"

a fit between two known data points. The use of additional data in estimating a POD (*i.e.*, lesion severity, or time of observation) will often provide a more statistically robust and precise estimate of the risk at a certain dose when comparing these dose-response assessments across studies/chemicals/endpoints. For example, by including a temporal component, the MSW model allows for consideration of factors such as early mortality, or time to lesion formation. As toxicity is dependent upon both exposure concentration and duration, this approach may yield a more accurate prediction of the true physiology of that lesion, and therefore a better estimate of human health risk.

References

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