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Review

A novel application of the Margin of Exposure approach: Segregation of tobacco smoke toxicants

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ABSTRACT

This paper presents a rationale for utilising a Margin of Exposure (MOE) approach to the segregation of tobacco smoke toxicants for risk assessment and management purposes. Future regulatory frameworks and product modifications aimed at tobacco harm reduction could utilise data that segregate toxicants using associations with specific diseases caused by cigarette smoking together with an indication of their relative contribution to that disease. Compounds with MOEs >10,000 accompanied by appropriate narrative are considered "low priority for risk management actions". This paper applies the MOE model to representative examples of tobacco smoke toxicants associated with respiratory tract carcinogenesis and other respiratory diseases. A multiplicity of published dose response data on individual toxicants has been used to determine the range of possible MOE values, thus demonstrating the consistency of the relationships. Acetaldehyde, acrolein, acrylonitrile, cadmium, ethylene oxide, formaldehyde and isoprene all segregate with MOEs <10,000 and should be considered as high priority for exposure reduction research whereas benzo(a)pyrene and vinyl chloride segregate with an MOE >10,000 and therefore may be considered as a low priority. 1,3-Butadiene, m-/p-cresols, NNK and NNN are assumed to segregate with high priority although additional data would be required to complete a full MOE assessment.

Contents

Abbreviations: AIC, Akaike's information criteria; ALARA, as low as reasonably achievable; B(a)P, benzo(a)pyrene; BMD, benchmark dose; BMDL and $BMDL_{10}$, benchmark dose lower confidence limit; BMDS, Benchmark Dose Software; BMR, benchmark response; CalEPA, Californian Environmental Protection Agency; CAS, Chemical Abstract Service; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; EFSA, European Food Safety Authority; IARC, International Agency for Research on Cancer; ILSI, International Life Sciences Institute; IPCS, International Program on Chemical Safety; ISO, International Organization for Standardization; LOAEL, Lowest Observed Adverse Effect Level; MOA, mode of action; MOE, Margin of Exposure; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-Nitrosonornicotine; NOAEL, No Observed Adverse Effect Level; NTP, National Toxicology Program; OEHHA, Office of Environmental Health Hazard Assessment; PBPK, physiologically based pharmacokinetic; POD, point of departure; UK, United Kingdom; USEPA, United States Environmental Protection Agency; WHO, World Health Organization.

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1. Introduction

In this paper we present the rationale, with examples, for utilising a Margin of Exposure (MOE) approach to the segregation of tobacco smoke toxicants for risk management purposes. The MOE approach to evaluate chemicals has gained advocacy over the last decade as a suitable method to highlight the comparative risks to human health from exposure to different chemicals, provided that a consistent approach is adopted (EFSA, 2005). In particular, the MOE approach has been debated and refined within those scientific communities that are responsible for the risk assessment of compounds in foods that are both genotoxic and carcinogenic (EFSA, 2006; O'Brien et al., 2006). The MOE approach is considered to be pragmatic and lends itself to the narrative presentation of underlying scientific assumptions. It makes use of all available data and can be used to compare and rank substances and to set targets for risk reduction strategies (EFSA, 2005). The application of the MOE approach, however, does not exclude the use of risk management approaches such as the 'as low as reasonably achievable' (ALARA) or the threshold for toxicological concern (TTC) (Barlow et al., 2006). The International Life Sciences Institute (ILSI) considers that appropriately derived MOEs, accompanied by a suitable narrative can be used as a prioritisation tool to compare different chemicals, to inform risk managers on the level of potential health concerns and to identify knowledge gaps and uncertainties (ILSI, 2009).

Previously, the MOE methodology has been used to compare the margin between the dose of or exposure to a chemical that is known to cause cancer in animals (or humans) and the estimated human exposure. Crucially, the MOE approach uses a reference point, or point of departure (POD), calculated from available toxicology data corresponding to a daily dose that causes a low but detectable increase in tumour incidence. This dose is then divided by an estimate of human exposure to yield a dimensionless ratio known as the MOE (ILSI, 2009).

The recommended POD for deriving a MOE is a benchmark dose (BMD), as defined by Crump (1984). Consensus has been reached that a benchmark response (BMR) corresponding to a 10% increase in incidence of the effect in animal studies above control values is an appropriate reference point for chemicals that are both genotoxic and carcinogenic (EFSA, 2005). Specifically, a BMD lower limit (BMDL) can be derived corresponding to the lower limit of a one-sided 95% confidence interval on the BMD (defined as $BMDL_{10}$).

An additional facet of the MOE approach is that the use of consistent uncertainty factors (in the form of a critical value of 10,000), eliminates the application of arbitrary uncertainty factors. The value of 10,000, as given by EFSA, is derived by the multiplication of two key uncertainty factors. A 100-fold difference between the calculated reference point and human exposure is considered to account for species differences and human variability. An additional 100-fold difference is considered to account for inter-individual variability in cell cycle control and DNA repair and the fact that the reference point is not equivalent to a No Observed Adverse Effect Level (NOAEL), therefore effects can occur at lower doses. The view of EFSA is that an MOE of 10,000 or higher would be of low concern from a public health point of view and might reasonably be considered as a low priority for risk management actions (EFSA, 2005).

Whilst the MOE procedure was initially established for use with genotoxic and carcinogenic compounds in the area of food (EFSA, 2006; Benford et al., 2010), the procedure is also being applied within other areas of risk management and within other industries, such as agricultural application based on the risks associated with pesticide use (Boobis et al., 2008), as well as to non-cancer risks (Aylward et al., 2008; EC, 2009). Given the growth in literature, it seemed reasonable to consider the application of the MOE approach to tobacco smoke toxicants. Of particular interest is the potential of the MOE approach to contribute to a risk assessment strategy for the ranking of tobacco smoke toxicants for their impact on human health and also to contribute to a risk management strategy by determining priorities for the types of toxicants that should be targeted within risk reduction research programmes.

1.1. Tobacco smoke – The risk assessment challenges

Tobacco smoke is a complex mixture of over 5300 identified chemicals (Rodgman and Perfetti, 2009; Green, 2009), of which at least 150 are known to have specific toxicological properties and can be termed 'tobacco smoke toxicants'. There is significant interest in further characterising these (and additional) toxicants both from the perspective of future regulatory frameworks aimed at monitoring or lowering toxicant levels and from the perspective of tobacco product development focused on selective toxicant reduction as part of broader harm reduction initiatives. It is critical that the role of individual tobacco smoke toxicants in the development of diseases associated with cigarette smoking is further elucidated and that some consideration is given to the dose of individual toxicants to which the smoker may be exposed. Integrated together, these assessments may give guidance to tobacco product development in terms of research priorities for selective toxicant reduction. Additionally, they may also direct the development of associated biomarkers of exposure and ultimately biomarkers of biological effect that can be used within clinical studies to assess the efficacy of such products (Gregg et al., 2008; Lowe et al., 2009).

Attempts to associate individual smoke toxicants with specific diseases and to rank them for their potency are not new and there is existing literature identifying toxicants that may be responsible for the adverse health effects of cigarette smoking, focused on cancer, chronic obstructive pulmonary disease (COPD) and cardiovascular (CVD) effects (Stratton et al., 2001; Rodgman and Green, 2003). Many attempts have been made to list the important

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tobacco smoke toxicants including, the cysteine index (Leuchtenberger et al., 1974), the Herzfeld Index (Herzfeld, 1982), various lists by Hoffmann (Hoffmann and Hecht, 1990; Hoffmann and Hoffmann, 1997), the relative exposure index (Rickert and Kaiserman, 1998), relative toxicity (Smith and Hansch, 2000) and cancer risks (Hecht, 2006). From a regulatory perspective, recent lists have included "Emissions from tobacco products" (Department of Justice Canada, 2000). Scientific advisory committees have also generated such lists including the "Mandated lowering of toxicants in cigarette smoke" (Burns et al., 2008).

In 2003, Fowles and Dybing published a paper which was developed from a report for the New Zealand Ministry of Health (Fowles and Bates, 2000). These papers described the application of simple mathematical principles to generate two categories of hazard prioritisation lists for 158 chemical constituents of tobacco smoke. For carcinogenic endpoints, the calculations used a cancer potency value (obtained from the United States Environmental Protection Agency (USEPA) or the California Environmental Protection Agency (CalEPA) databases) multiplied by the machine smoked yield of the toxicant in tobacco smoke. For non-cancer diseases (categorised as both respiratory and cardiovascular effects), the calculation used the machine smoked yield of the toxicant in tobacco smoke divided by the chronic reference exposure level (referenced from the USEPA or CalEPA databases) (Fowles and Dybing, 2003). This approach represented an important step forward in the arguments for the prioritisation of tobacco smoke toxicants within product regulation and risk reduction research.

However, there were certain areas where we considered that additional data would be valuable. For example, the indices calculated for some constituents lacked specific associations with any of the three main diseases associated with cigarette smoking, i.e., lung cancer, COPD and CVD. We have sought in this paper to use toxicity data sets linked to lung related diseases, thus capturing both lung cancer and COPD. Fowles and Dybing used USEPA/CalEPA chronic reference exposure levels which are based on the application of various uncertainty factors to NOAEL/Lowest Observed Adverse Effect Levels (LOAEL) data (Fowles and Dybing, 2003). We have sought, by using the MOE approach based on benchmark dosing, to eliminate the variability associated with the use of uncertainty factors and thus to allow more consistent estimates of relative toxicity. The BMD method is an alternative to the traditional NOAEL. A BMD utilises all available dose response data and the application of a $BMDL_{10}$ takes into account statistical uncertainty in the data (EFSA, 2005). Fowles and Dybing also estimated human exposure to tobacco smoke toxicants based on machine smoking yields from various sources (Fowles and Dybing, 2003). We have employed (in the majority of cases) yields for a reference cigarette from a single source in the peer-reviewed literature (Counts et al., 2005) using machine smoked yields at the Health Canada Intense regime. The aim of this was to ensure (where possible) that all toxicant yields were generated from a single cigarette type (with known design parameters), using a single smoking regime, ultimately ensuring consistency in our approach.

The MOE approach has a number of attractive features to allow for its application to tobacco smoke toxicants. MOEs have gained advocacy in the evaluation of genotoxic and carcinogenic compounds in food (EFSA, 2005), been reviewed by the scientific community (EFSA, 2006; O'Brien et al., 2006) and its use has started to spread to other industries. It is seen as a pragmatic approach for reviewing all available data and it can be used to compare and rank substances to set risk reduction strategies (EFSA, 2005). Importantly, it can also be used to evaluate a range of data sets, including genotoxic, carcinogenic, as well as non-cancer.

Although the critical value of 10,000 set by EFSA, specifically relates to uncertainty factors relevant to food, the use of 10,000 has also been used in this study. This can be considered to account for the following uncertainty factors which are applicable to the data used in the evaluation of tobacco smoke toxicants. It can be assumed that a factor of 10 covers any interspecies differences, another factor of 10 covers any intraspecies differences, a factor of 10 can be attributed to the fact that the POD used in our MOE calculations is not a NOAEL and the final factor of 10 can be used to account for extrapolations relating to route and/or length of exposure in the data sets used to generate the MOEs.

It is not our intention in this paper to derive MOE values for all known tobacco smoke toxicants, but rather to illustrate the utility of the approach with a handful of toxicants for which there already exists good dose response data. Our approach also explores the concept of using MOE values calculated from a range of studies for each toxicant to determine the consistency of the relationship within the available data sets. Whilst a precautionary approach to risk management will always utilise the lowest MOE calculated from a range of data sets, we believe that consistency is also important in giving credibility to the derived MOE. To support this, all the data sets that have been used in our calculations are included in the supplementary data associated with this paper.

The initial scope of this piece of research as described here only applies the MOE assessment to segregate individual tobacco smoke toxicants into either a high or low priority for risk reduction research. As a future development it may be possible to refine the approach for combined risk assessment of groups of constituents exhibiting similar structural and toxicological properties, to generate where possible a group MOE and begin to prioritise tobacco smoke toxicants as part of a complex mixture, rather than on an individual basis.

2. Materials and methods

2.1. Data sourcing

There are numerous diseases linked with smoking, however, for the purposes of this paper, lesion data used to generate MOEs will be restricted to those found in the lung. Two of the key diseases linked with tobacco smoke exposure are associated with the lung; lung cancer and COPD. COPD is a complicated disease with numerous associated pathologies. The definition of COPD has evolved over recent years and the more traditional terms of 'chronic bronchitis' and 'emphysema' are no longer used. The World Health Organization (WHO) uses the following definition: 'Chronic obstructive pulmonary disease (COPD) is a lung disease characterised by chronic obstruction of lung airflow that interferes with normal breathing and is not fully reversible' (WHO, 2010).

This paper has focused on the modelling of all available respiratory disease endpoints without segregating them into carcinogenic and non-carcinogenic categories, as for many of the toxicants investigated here their mode of action is not fully understood. This should ensure that any lesions possibly relevant to either lung cancer or COPD are included at this stage. Ultimately, each of these toxicants should also have a mode of action (MOA) review associated with them. This would then define the key disease events associated with each individual toxicant and therefore allow for the further refinement of the MOE assessment.

In this study, a detailed review of the published literature was performed in order to identify robust dose–response data, limiting the search to respiratory disease endpoints and the test compound. Initially, a TOXNET search was performed using the Chemical Abstracts Service (CAS) number of the test compound of interest. The results were assessed using the HSDB, TOXLINE, CCRIS, DART and GENE-TOX databases, relevance of the route and duration of exposure, effects of exposure, and links to the disease endpoint of interest. Where necessary, additional search terms were included (for example: inhalation, oral administration, chronic administration, human, cancer, COPD, epidemiology). Furthermore, a review of the secondary literature was performed for the constituent of interest, using the literature sources detailed in Table 1.

To ensure that the most appropriate available data sets were used, the quality of the experimental data was measured against the following series of criteria. To allow for the subsequent benchmark dose ($BMDL_{10}$) value to be calculated, the minimum requirement to be seen within any data set is a dose-related trend (USEPA, 2010). The maximum response from any given data set was specified to be above 10% and data were only selected where there were a minimum of three dose levels including zero. Data from either human epidemiological studies or from chronic animal inhalation studies with relevant respiratory lesions was considered most appropriate. However, although for numerous toxicants there are associated epidemiological studies, they did not meet the criteria described here. Therefore, for the purpose of generating

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Table 1
Source databases.

Data source	Websites
TOXNET (Toxicology Data Network)	http://toxnet.nlm.nih.gov/
HSDB (Hazardous Substances Data Bank)	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
TOXLINE (Toxicology Literature Online)	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE
CCRIS (Chemical Carcinogenesis Research Information System)	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS
DART (Developmental and Reproductive Toxicology Database)	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?DARTETIC
GENE-TOX (Genetic Toxicology Data Bank)	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX
11th Report on carcinogens (ROC)	http://ntp.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932
Environmental Health Criteria Monographs (EHCs)	http://www.inchem.org/pages/ehc.html
International Agency for Research on Cancer (IARC) Monographs	http://monographs.iarc.fr/ENG/Classification/index.php
Integrated Risk Information System (IRIS)	http://cfpub.epa.gov/ncea/iris/index.cfm
IARC summaries and evaluations	http://www.inchem.org/pages/iarc.html
NCBI (National Centre for Biotechnology Information) PubMed	http://www.ncbi.nlm.nih.gov/sites/entrez?db=PubMed
OEHHA (Office of Environmental Health Hazard Assessment) chronic reference exposure levels	http://www.oehha.ca.gov/air/chronic_rels/AllChrels.html
RAIS (Risk Assessment Information System)	http://rais.ornl.gov/
Chemicals Screening Information Dataset (SIDS) for high volume chemicals	http://www.chem.unep.ch/irptc/sids/oecdsids/sidspub.html
National Toxicology Program (NTP)	http://ntp.niehs.nih.gov/
International Programme on Chemical Safety (IPCS) Numerical List of CICADS (Concise International Chemical Assessment Documents)	http://www.who.int/ipcs/publications/cicad/cicad_numerical/en/index.html

a range of MOEs for each constituent, all available inhalation animal data has been utilised. In the cases where non-inhalation data was available, the studies were rated for potential relevance to tobacco smoke exposure (i.e. did they generate lung lesions), then used where little to no inhalation data was available and this was detailed in the accompanying MOE narrative. The route of exposure was not the only consideration for data selection; exposure duration is also important. Studies involving a daily exposure regime and chronic exposures should be prioritised where possible as they are most representative of smoking exposure. Where multiple papers for one toxicant were from the same laboratory, the information has been examined to ensure replicate data were not being assessed. Where multiple data sets were available for a given toxicant, any data sets where the only response seen was maximal (i.e. 100%) were excluded as these would give limited information regarding the shape of the dose response curve in the region of the BMR. In the same manner, if a data set only included a single response point above that of the control and this was above a 30% response, then these data sets were also excluded where superior data sets (according to the criteria given here) for that toxicant exist.

2.2. Cigarette yield/estimated human intake

The need for a comprehensive set of published smoke yield data on a combustible product (with the exceptions of ethylene oxide and vinyl chloride, for which details can be found in Sections 3.9 and 3.14 respectively) was fulfilled by using data from the University of Kentucky reference cigarette 1R4F (Counts et al., 2005). 1R4F cigarettes contain a blend of flue cured and burley tobacco with a non-ventilated cellulose acetate filter, with a machine-smoked Cambridge Filter Pad/International Organization for Standardization (ISO) tar yield of approximately 9 mg i.e. near the maximum permitted tar yield in the European Union (10 mg). The constituent yields from 20 cigarettes per day were used to calculate the value for daily exposure. This conservative cigarette consumption figure is in excess of the average consumption per smoker in the United Kingdom (UK) (13.5 cigarettes per day) (Office for National Statistics, 2006), in France (14.1 cigarettes per day) (Forey et al., 2002a), and in Germany (15.8 cigarettes per day) (Forey et al., 2002b). The constituent yield in smoke used for MOE computation was taken from 1R4F cigarettes under the Health Canada Intense machine-smoking regime. This regime uses the following parameters; 55 ml puff volume, 2 s duration, 30 s puff interval, 100% blocked filter ventilation (Health Canada, 1999). The 1R4F tar yield under this regime (26.3 mg tar per cigarette) is almost three times the yield values from ISO smoking conditions (Counts et al., 2005). Furthermore, we have assumed 100% retention of the constituent in the smoker's body. Consequently, these three assumptions used to estimate a human exposure, generate conservative MOE values. As used previously in calculations of inhalation derived risk assessment models, the daily yield of the constituent under examination is distributed into the average daily volume of air inhaled by a human subject of 20 m^3 (Fowles and Dybing, 2003; USEPA, 1997). Where required, an average human body weight of 70 kg was used (Dourson et al., 1985), to convert the units of our estimated human intake to mg/kg.

2.3. Benchmark dose calculation

A BMD lower limit (i.e. the lower limit of a one-sided 95% confidence interval on the BMD) for a disease incidence at 10% above control values ($BMDL_{10}$) was generated. This was calculated using the latest version of the USEPA Benchmark Dose Software (BMDS) v. 2.1.2, which can be downloaded from their website (USEPA, 2010).

Table 2
BMDS dichotomous (Quantal) model details.

Model Name	Constraints
Gamma	Restrict power $\geqslant 1$
Logistic	None
LogLogistic	Restrict power $\geqslant 1$ Restrict slope $\geqslant 1$
LogProbit	Restrict power $\geqslant 1$ Restrict slope $\geqslant 1$
Probit	None
Weibull	Restrict power $\geqslant 1$
Multistage ('Quad')	Restrict betas $\geqslant 1$
Multistage cancer ('Cubic')	None

The models listed in Table 2 were chosen to ensure that the minimum number of background response parameters (or assumptions) were contained within the model algorithms.

Within all models, the following default parameters were used:

Iteration = 250.
Relative function = 1 e^{-8}.
Parameter = 1 e^{-8}.

For each model, the BMD software generates a text output containing statistical data summaries, which can be imported into a spreadsheet, from which a graphical depiction can be produced. Analysis of χ^2 goodness of fit test p-values, Akaike's information criteria (AIC) values and scaled residuals, as well as visual inspection of graphical plots, allows assessment and comparison of the "goodness of fit" of models. The most appropriate model which adequately describes the data, especially in the region of the BMR from which the BMDL and $BMDL_{10}$ will be calculated, can then be identified. Guidelines from the USEPA BMDS website, the International Program on Chemical Safety (IPCS) and European Food Safety Authority (EFSA) documents were taken into consideration when selecting a model (USEPA, 2010; IPCS, 2009; EFSA, 2009).

2.4. Margin of Exposure (MOE)

MOEs were calculated as:

MOE = $BMDL_{10}$/estimated human intake (EFSA, 2006)

EFSA indicate that when assessing food safety, MOE values >10,000 "might be considered a low priority for risk management actions" (EFSA, 2006).

3. Results

3.1. Summary

In the following sections, summaries of MOE assessments of various toxicants are discussed.

Table 3
MOE's for individual smoke constituents.

Compound	References	Study length	Species/route[a]	Lesion[b]	MOE[c]
Acetaldehyde [75-07-0]	Woutersen et al. (1986)	28 months (then necropsied)	Rats	Nasal squamous cell carcinoma M	1268
				Nasal adenocarcinoma M	143
				Laryngeal squamous metaplasia/ hyperplasia M	1022
				Nasal adenocarcinoma F	437
				Nasal squamous metaplasia F	1131
				Laryngeal squamous metaplasia/ hyperplasia F	693
	Appelman et al. (1982)	4 weeks (then necropsied)	Rats	Nasal degeneration with hyper/ metaplasia M	1187
				Tracheal degeneration with hyper/ metaplasia M	1338
				Laryngeal degeneration with hyper/ metaplasia M	1197
				Nasal degeneration with hyper/ metaplasia F	1264
				Tracheal degeneration with hyper/ metaplasia F	1469
				Laryngeal degeneration with hyper/ metaplasia F	1181
Acrolein [107-02-8]	Cassee et al. (1996)	3 days (then necropsied)	Rats	Disarrangement, necrosis, thickening and desquamation of epithelium – moderate M	2
	Dorman et al. (2008)	65 days (then necropsied)	Rats	Nasal respiratory epithelial hyperplasia lateral wall (II) M	4
				Nasal respiratory epithelial squamous metaplasia septum (I) M	4
				Nasal olfactory epithelial squamous metaplasia dorsal meatus (II) M	11
				Laryngeal epithelial squamous metaplasia M	1
		65 days + 60 days recovery (then necropsied)	Rats	Nasal respiratory epithelial hyperplasia dorsal meatus (tip) M	9
				Nasal respiratory epithelial squamous metaplasia septum (I)	4
				Laryngeal epithelial squamous metaplasia M	3
Acrylonitrile [107-31-1]	Ghanayem et al. (2002)	2 years (then necropsied)	Mice oral gavage	Lung adenoma or carcinoma – terminal rates F	790
	Quast et al. (1980)	2 years (then necropsied)	Rats	Hyperplasia nasal turbinates M	230
				Hyperplasia nasal mucous secreting cells M	58
				Focal inflammation nasal turbinates F	244
				Flattening of respiratory epithelium nasal turbinates F	42
Benzo(a)pyrene [50-32-8]	Thyssen et al. (1981)	Lifetime exposure period (allowed to die before autopsy ~95 weeks)	Hamsters	Tracheal tumours M	1.3×10^6
				Laryngeal tumours M	2.8×10^5
				Pharyngeal tumours M	3.0×10^5
	Feron et al. (1973)	52 weeks (necropsied at 78 weeks)	Hamsters intratracheal instillation	Bronchioloalveolar tumour M	1.6×10^5
				Tracheal tumour M	1.4×10^5
				Bronchial tumour M	7.0×10^5
	Culp et al. (1998)	2 years (then necropsied)	Mice feeding	Laryngeal papillomas and/or carcinomas F	1.5×10^6
1,3-Butadiene [106-99-0]	NTP (1984)	61 weeks (then necropsied)	Mice	Alveolar/bronchiolar carcinoma M	3743
				Alveolar/bronchiolar adenoma or carcinoma M	114
				Alveolar/bronchiolar adenoma F	2773
				Alveolar/bronchiolar adenoma or carcinoma F	2296
	Melnick et al. (1990)	65 weeks (then necropsied)		Alveolar/bronchiolar neoplasm M	905
				Alveolar/bronchiolar epithelial hyperplasia M	752
				Alveolar/bronchiolar neoplasm F	640
Cadmium chloride [10108-64-2]	Takenaka et al. (1983)	18 months + 13 months recovery (then necropsied)	Rats	Lung epidermoid carcinoma M	92
				Lung adenocarcinoma M	38
				Total lung carcinoma M	40
Cadmium sulphide [1306-23-6] Cadmium oxide [1306-19-0]	Glaser et al. (1990)	18 months (maximum time before necropsied 31 months)		Bronchio–alveolar adenomas M	135
				Bronchio–alveolar adenomas F	3283
				Bronchio–alveolar adenomas F (Dust)	122
				Adenocarcinoma F (Dust)	27
				Any lung tumours F (Dust)	6
	NTP (1995)	13 weeks (then necropsied)	Rats	Mesenteric lymph node inflammation M	328
				Mesenteric lymph node inflammation F	104

(*continued on next page*)

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Table 3 (*continued*)

Compound	References	Study length	Species/route[a]	Lesion[b]	MOE[c]
				Laryngeal epithelial degeneration M	51
				Nasal respiratory epithelium Inflammation M	328
				Nasal respiratory epithelium inflammation F	328
				Alveolar histiocytic infiltrate M	131
				Alveolar histiocytic infiltrate F	131
				Alveolar epithelial hyperplasia M	131
				Alveolar epithelial hyperplasia F	131
				Lung inflammation M	525
				Lung inflammation F	525
				Lung fibrosis M	268
				Lung Fibrosis F	268
				Tracheobronchial lymph node inflammation M	191
				Nasal olfactory epithelium degeneration M	929
				Nasal olfactory epithelium degeneration F	765
				Nasal olfactory epithelium respiratory metaplasia F	1476
			Mice	Lung inflammation M	257
				Lung inflammation F	175
				Tracheobronchial lymph node hyperplasia F	39
				Alveolar epithelial hyperplasia M	98
				Alveolar epithelial hyperplasia F	268
				Lung fibrosis M	197
				Lung fibrosis F	224
				Nasal olfactory epithelium degeneration M	301
				Nasal olfactory epithelium degeneration F	426
				Nasal olfactory epithelium respiratory metaplasia M	601
				Respiratory epithelium hyaline droplets M	765
				Respiratory epithelium hyaline droplets F	765
m/p Cresols [108-39-4]/[106-44-5]	NTP (2008)	104 weeks (then necropsied)	Rats feeding	Nasal goblet cell hyperplasia M	1500
				Nasal epithelial hyperplasia M	7281
				Nasal squamous metaplasia M	41771
				Nasal inflammation M	52160
			Mice feeding	Lung bronchial hyperplasia F	648
	NTP (1992)	13 weeks (then necropsied)	Rats feeding	Nasal respiratory epithelium glandular hyperplasia M	3677
				Nasal respiratory epithelium hyperplasia F	34106
			Mice feeding	Nasal respiratory epithelium hyperplasia M	90983
				Nasal respiratory epithelium hyperplasia F	97473
Ethylene oxide [75-21-8]	NTP (1987)	102 weeks (then necropsied)	Mice	Alveolar/bronchiolar adenoma M	5310
				Alveolar/bronchiolar carcinoma M	4375
				Alveolar/bronchiolar carcinoma or adenoma M	2239
				Alveolar epithelial hyperplasia M	10941
				Alveolar/bronchiolar adenoma F	8470
				Alveolar/bronchiolar carcinoma F	9381
				Alveolar/bronchiolar carcinoma or adenoma F	6890
	Adkins et al. (1986)	6 months (then necropsied)		Pulmonary tumour M + F	4293
Formaldehyde [500-00-0]	Kerns et al. (1983)	24 months + 6 months recovery (then necropsied)	Rats	Nasal squamous cell carcinoma M	157
				Nasal squamous cell carcinoma F	154
	Woutersen et al. (1989)	Physical nasal damage + 28 months (then necropsied)		Nasal squamous cell carcinoma M	119
	Monticello et al. (1996)	Up to 24 months (then necropsied)		Nasal squamous cell carcinoma M	154
	Wilmer et al. (1989)	13 weeks interrupted exposure (then necropsied)		Respiratory epithelium squamous metaplasia M	11
	Rusch et al. (1983)	26 weeks (then necropsied)		Nasal squamous metaplasia/hyperplasia M + F	19

Table 3 (*continued*)

Compound	References	Study length	Species/route[a]	Lesion[b]	MOE[c]
	Kamata et al. (1997)	28 months (then necropsied)		Nasal cavity epithelial cell hyperplasia with squamous cell metaplasia M	10
	Maronpot et al. (1986)	13 weeks (then necropsied)	Mice	Nasal squamous metaplasia M	63
				Nasal squamous metaplasia F	78
				Laryngeal squamous metaplasia M	186
				Laryngeal squamous metaplasia F	198
				Tracheal squamous metaplasia M	259
				Tracheal squamous metaplasia F	158
				Bronchial squamous metaplasia F	402
	Swenberg et al. (1980)	18 months + 6 months recovery (then necropsied)	Rats	Nasal squamous metaplasia M + F	8
	Woutersen et al. (1987)	13 weeks (then necropsied)		Rhinitis M	38
				Nasal focal respiratory epithelial hyperplasia – slight M	34
				Nasal focal respiratory epithelial keratinisation – slight M	58
				Nasal focal respiratory epithelial hyperplasia – Slight F	10
				Nasal focal respiratory epithelial keratinisation – very slight F	40
				Nasal focal respiratory epithelial keratinisation – slight F	86
Isoprene [78-79-5]	Placke et al. (1996)	80 weeks + 24 week recovery (then necropsied)	Mice	Alveolar/bronchiolar carcinoma M	2010
	NTP (1994)	6 months + 6 months recovery (then necropsied)		Alveolar/bronchiolar adenoma M	2273
				Alveolar/bronchiolar carcinoma M	6565
				Alveolar epithelial hyperplasia M	3266
				Nasal turbinate olfactory epithelial degeneration M	325
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [64091-91-4]	Hoffmann et al. (1984)	20 weeks (autopsied on death)	Rats subcutaneous injection	Nasal tumours M	1685
				Nasal tumours F	13364
				Lung tumours M	973
				Lung tumours F	18319
	Rivenson et al. (1988)	Lifetime exposure (autopsied on death ~100 weeks)	Rats drinking water	Nasal tumours M	1881
				Lung tumours M	338
	Hoffmann et al. (1993)	7 weeks + 30 weeks recovery (then necropsied)	Mice ip injection	Lung tumours F	4180
	Hecht et al. (1990)	1 single dose + 16 weeks recovery (then necropsied)		Lung adenomas F	1.1×10^5
	Hecht et al. (1989)	1 single dose + 3.5 months recovery (then necropsied)		Lung adenomas F	23937
	Hecht et al. (1983)	1 single dose + 72 weeks sham smoking (then necropsied)	Hamsters subcutaneous injection	Lung Tumours M	1.2×10^5
				Nasal mucosa tumours M	3.7×10^5
				Respiratory tract tumours M	85,128
				Nasal mucosa tumours F	2.9×10^5
N-Nitrosonornicotine (NNN) [80508-23-2]	Hoffmann et al. (1984)	20 weeks (autopsied on death)	Rats subcutaneous	Nasal tumours M	3295
				Nasal tumours F	9078
	Hoffmann et al. (1993)	7 weeks (necropsied at 30 weeks)	Mice ip injection	Lung tumours F	71168
	McCoy et al. (1981)	25 weeks (autopsied on death ~ 15–18 months from beginning of exposure)	Hamsters ip injection	Nasal cavity tumours M	1.9×10^5
				Tracheal tumours M	87793
	Griciute et al. (1986)	78 weeks (autopsied on death or at 120 weeks of age)	Rats intragastric instillation	Malignant nasal tumours M	20824
				Malignant nasal tumours F	15674
Vinyl chloride [75-01-4]	Hehir et al. (1981)	Single 1 h exposure (autopsied on death)	Mice	Bronchio–alveolar adenoma M	1.5×10^9
	Feron et al. (1981)	Maximum exposure of 2.7 years (autopsied on death)	Rats oral	Lung angiosarcoma M	1.9×10^6
	Lee et al. (1978)	12 months (then necropsied)	Mice	Bronchio–alveolar adenoma M	8.0×10^6
				Bronchio–alveolar adenoma F	1.3×10^7
	Hong et al. (1981)	6 months + 12 months recovery (then necropsied)	Mice	Lung bronchioalveolar tumours M	4.8×10^6
				Lung hemangiosarcoma M	9.9×10^6
		10 months + 12 months recovery (then necropsied)	Rats	Lung hemangiosarcoma F	3.3×10^7
	Suzuki (1981)	4 weeks + up to 40 weeks recovery (then necropsied)	Mice	Pulmonary tumours M	1.0×10^6

[a] Unless stated route of administration is inhalation.
[b] M = Male, F = Female.
[c] Blue = "High priority for risk management actions".

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3.2. Acetaldehyde

Acetaldehyde is a reactive compound which is miscible in water and most organic solvents, is known to react with cellular macromolecules (IPCS, 1995) and is also known to be a direct acting contact site toxicant (Morris et al., 1996). It is classified by the International Agency for Research on Cancer (IARC) under Group 2B "possibly carcinogenic to humans" based upon sufficient evidence in experimental animals (IARC, 1999a). The IARC assessment was based on inhalation exposure and intratracheal instillation dosing which induced tumours of the respiratory tract, particularly adenocarcinomas and squamous-cell carcinomas.

The literature search identified a multi-component independent inhalation study, which contained rat tumour dose response data (Woutersen et al., 1984, 1986; Woutersen and Feron, 1987). From this inhalation study nasal adenocarcinoma, nasal squamous cell carcinoma, nasal squamous metaplasia and larynx squamous metaplasia/hyperplasia were identified. In this study, the highest inhalation dose had to be modified during the in-life phase, gradually reducing from 3000 to 1000 ppm, because of severe growth retardation and early mortality in this group. Due to this mid-study alteration, the results generated from the group treated with the top dose were not included in our calculations. A second subchronic inhalation study contained data on degeneration with hyper/metaplasia of olfactory epithelium in the nose, trachea and larynx of both male and female rats (Appelman et al., 1982).

A total of 12 MOE values were computed using data from the above references. Using an acetaldehyde yield from cigarette smoke of 1448 μg per cigarette (Counts et al., 2005), these MOEs covered the range from 143 to 1469 (Table 3) indicating a high priority for exposure reduction research for acetaldehyde in tobacco smoke.

3.3. Acrolein

Acrolein is classified by IARC under Group 3 as "not classifiable as to its carcinogenicity to humans" (IARC, 1995). However, acrolein is widely accepted as having high reactivity with mammalian tissues, with a high retention following inhalation and classified as being highly cytotoxic (IPCS, 1991). Two papers were identified providing a broad range of respiratory lesions. Dorman et al. (2008), considered five different exposure regimes, of which an exposure of 65 days with or without a recovery period was considered to be the most relevant to model tobacco smoke toxicants. Using these criteria, the paper contained a total of 49 potentially relevant lesions across a range of tissue types. Using the criteria set out in Sections 2.1 and 2.4; this number was reduced to seven MOEs, ranging from 1 to 11. Analysis of the data from Cassee et al. (1996), gave an additional MOE value of two. These two data sets exhibit a consistent response for acrolein at a cigarette smoke yield of 122.4 μg per cigarette (Counts et al., 2005), indicating a high priority for exposure reduction research for acrolein in tobacco smoke. Furthermore, these data using three exposure periods ranging from 3 days to 18 months demonstrate a high consistency of the derived MOEs.

3.4. Acrylonitrile

Acrylonitrile is classified under IARC Group 2B "possibly carcinogenic to humans", based on "sufficient evidence in experimental animals" (IARC, 1999b). An epidemiological study of acrylonitrile where exposure is via the inhalation route is available (O'Berg, 1980) and was used by the CalEPA as part of their Office of Environment Health Hazard Assessment (OEHHA) Air Toxics Hot Spots Program as the critical paper, giving an inhalation unit risk value of 2.9×10^{-4} per μg/m^3 (OEHHA, 2009). However, the O'Berg paper does not include any quantitative exposure data and therefore cannot be used for MOE computation. Following a literature search, a total of two suitable animal studies were identified. Ghanayem et al. (2002), reported the incidence of respiratory adenomas and carcinomas in female mice after gavage administration. Upon application of the data criteria from Sections 2.1 ***and*** 2.4, this yielded a single MOE value. Using an acrylonitrile cigarette smoke yield of 29.3 μg per cigarette (Counts et al., 2005), the computed MOE was 790 (Table 3). From the single inhalation study available (Quast et al., 1980), four lesions with data which met the criteria set out in Sections 2.1 ***and*** 2.4 were identified. The lesions included: respiratory epithelium hyperplasia, hyperplasia of the mucous secreting cells, focal inflammation of the nasal turbinates and flattening of the respiratory epithelium in the nasal turbinates. The MOEs generated were 230, 58, 244 and 42 respectively (Table 3). All five available MOEs indicate a high priority for exposure reduction research for acrylonitrile in tobacco smoke.

3.5. Benzo(a)pyrene (B(a)P)

Probably one of the most well known tobacco smoke toxicants, benzo(a)pyrene (B(a)P) has a Group 1 IARC classification: "carcinogenic to humans" (IARC, 1983). Four papers covering inhalation and instillation exposure, plus one feeding study, with subsequent lesions of the respiratory tract have been analysed. Using a B(a)P cigarette smoke yield of 13.62 ng per cigarette (Counts et al., 2005), produces three MOEs based on data for hamsters following inhalation; 2.8×10^5, 3.0×10^5 and 1.3×10^6, three MOEs based on data for hamsters following a single instillation; 1.4×10^5, 1.6×10^5 and 7.0×10^5 and a single MOE based on data for mice following exposure via the diet of 1.5×10^6 (Table 3). All of these MOEs indicate B(a)P would be considered a low priority for exposure reduction research in tobacco smoke.

3.6. 1,3-Butadiene

1,3-Butadiene as a constituent of tobacco smoke has recently been re-reviewed by IARC. It has a Group 1 "carcinogenic to humans" status and is considered to be a leukaemogen in man via inhalation, although it has not been reported to induce lung tumours (IARC, 1999c, 2008a). 1,3-Butadiene carcinogenesis has been examined in three species; rat, mouse and humans. The metabolic pathway and proximal genotoxic metabolite are known and bio-monitoring data are available to track these metabolic transformations in all three species. In summary, mice are reported to have the most heterogeneous yield of tumours including lung, whilst rats mainly have an increase in levels of spontaneous tumours (which do not include lung) and the epidemiological analysis in man indicates an increase in leukaemia (IARC, 1999c, 2008a). As a potential explanation for these data, the components of metabolic pathways can be traced in these species. A cascade of decreasing levels of genotoxic metabolites by multiple orders of magnitude can be seen from mouse to rat to man (IARC, 1999c, 2008a).

The lung cancer data for MOE assessment as a result of inhalation exposure to 1,3-butadiene is consequently only available from the mouse model. From two publications (NTP, 1984; Melnick et al., 1990) and using a 1,3-butadiene cigarette smoke yield of 105 μg per cigarette from a 1R4F product (Counts et al., 2005), a total of seven MOE values can be produced. All seven are below 10,000 (Table 3), indicating a high priority for exposure reduction research for 1,3-butadiene in tobacco smoke. As demonstrated in the IARC reviews (IARC, 1999c, 2008a), 1,3-butadiene exposure in man is not explicitly linked to respiratory tumours. It may be that using data sets from respiratory lesions in mice may not adequately reflect disease in man. It is important to note from the EFSA guidelines that MOEs should never be quoted in isolation

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and that a commentary is required (EFSA, 2005). In this instance, the murine respiratory lesion data sets indicate a high priority for exposure reduction research in tobacco smoke. However, the underlying toxicology suggests that these respiratory lesions may not be relevant for man and therefore any conclusion based on this data may be questionable.

3.7. Cadmium

Cadmium and cadmium compounds are currently assessed by IARC as Group 1 "carcinogenic to humans" (IARC, 1993). Whilst epidemiological data identifies the human lung as a target site following cadmium inhalation these data sets are not compatible with the MOE paradigm as the reported data does not include sufficient detail on exposure to allow direct comparison with estimated human intake from smoking. However, three inhalation studies using cadmium chloride, sulphide or oxide with suitable data have been identified, giving 37 different MOE values (Table 3), 25 of which are based on data from rat studies and 12 on data for mice. At a cigarette smoke yield of 160.1 ng per cigarette (Counts et al., 2005), all 37 computed MOEs are less than 3283, which would indicate that cadmium compounds in tobacco smoke would be classified as a high priority for exposure reduction research in tobacco smoke. As a matter of note, the form of cadmium in tobacco smoke has not been specifically identified (Rodgman and Perfetti, 2009). However, considering that the IARC assessment is for cadmium compounds as a class and that the +2 oxidation state of cadmium compounds is the most stable form, further identification is unlikely to change the status of the MOE assessment.

3.8. m- and p-Cresols (3- and 4-methylphenol)

Cresol data are usually considered as a compound group or as mixtures of meta and para isomers. Chronic inhalation studies of cresols with analysable data to compute MOEs were not available. However, two National Toxicology Program (NTP) feeding studies using rats and mice based on 13 weeks (NTP, 1992) and 2 years exposure are available (NTP, 2008). These studies report respiratory lesions including; hyperplasia, inflammation and metaplasia. Using a yield of 10.1 μg per cigarette (Counts et al., 2005), nine MOEs were calculated, spanning a range from 648 to 97473. From these two studies, the one which could be considered most relevant with regard to smoking exposure would be the chronic 2 year study. This study generates three MOEs which segregate below 10,000, whereas three out of the four MOEs generated by the 13 week study segregate above 10,000. Using the consensus from the chronic study suggests that m- and p-cresols can be considered as high priorities for exposure reduction research. However, the lack of inhalation data and the inconsistency in the segregation of the MOEs indicate that further investigation into tissue dose and route extrapolation (potentially using physiologically based pharmacokinetic modelling), as well as an understanding of the mode of action would aid in interpretation of this MOE assessment.

3.9. Ethylene oxide

Ethylene oxide has been assessed as Group 1 "carcinogenic to humans" by IARC, where the epidemiological evidence from 14 cohort studies was examined, focusing on lymphatic and haematopoietic cancers and cancers of the breast, stomach, pancreas and brain (IARC, 2008c). Two experimental inhalation studies in mice were available, generating eight MOEs. Ethylene oxide does not feature within the 44 identified tobacco smoke components routinely measured, as reported by Counts et al. (2005). Instead, an average ethylene oxide yield taken from four references has been used to estimate a smoke yield to be included in the MOE calculation. Using a yield of 7 μg per cigarette (IARC, 2004, 2008c; Hoffmann and Hoffmann, 1998, 2001), the eight MOEs generated range from 2239 to 10941. Once again, applying a precautionary approach would suggest that given the weight of supporting evidence (i.e. seven MOE values below 10,000), then ethylene oxide would be considered as a high priority for exposure reduction research in tobacco smoke.

3.10. Formaldehyde

As a result of widespread use of formaldehyde within industrial settings, its toxicology has been extensively studied. It has been assessed by IARC and allocated a Group 1 status "carcinogenic to humans" resulting in induction of nasopharyngeal cancer (IARC, 2006). Nine papers following a range of rat inhalation exposure scenarios and one mouse inhalation paper have been analysed for respiratory tract lesions (Table 3). These papers subsequently generated MOE values from lesions including squamous cell carcinoma, squamous metaplasia, respiratory epithelial hyperplasia, respiratory epithelial keratinisation and rhinitis. Using a cigarette smoke yield of 60.5 μg per cigarette (Counts et al., 2005), 21 data-points are consistent in generating MOEs less than 10,000, indicating that formaldehyde may be considered a high priority for exposure reduction research in tobacco smoke. In addition, when nasal tissue with pre-existing physical damage was exposed to formaldehyde to investigate the potential role of cytotoxicity in formaldehyde induced tumours, data indicate MOEs are of the same order of magnitude as in the absence of physical damage (Woutersen et al., 1989) (Table 3).

3.11. Isoprene

Isoprene has been assessed by IARC as Group 2B "possibly carcinogenic to humans" (IARC, 1999d). This is based on evidence in humans that there is a correlation between isoprene exposure in rubber production workers and upper respiratory effects, such as degeneration of the olfactory tract (IARC, 1999d). Two mouse inhalation studies are available, which generate five MOE values (Table 3). At a cigarette smoke yield of 952 μg per cigarette (Counts et al., 2005), the MOEs range from 325 to 6565, indicating that isoprene would be classified as a high priority for exposure reduction research in tobacco smoke.

3.12. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

There are no available data on the human carcinogenicity of NNK, however, on the basis of animal experimentation by a number of routes of administration, IARC has allocated a Group 1 status to NNK, "carcinogenic to humans" (IARC, 2007a). Considering the available information, 28 experimental studies covering NNK have been identified. However, there is no report of NNK administered via inhalation, rather intraperitoneal or subcutaneous routes predominate. More than half of the studies (16) use a single dose and all studies report induction of respiratory tract tumours. The analysable data meeting the criteria to be able to run the MOE analytical model using a cigarette smoke yield of 199.9 ng per cigarette (Counts et al., 2005) are given in Table 3. Six papers, yielding 13 sets of respiratory tract lesions, give a range of MOEs both below and above the critical value of 10,000. Two of these MOEs are based on data from a chronic exposure study, both segregating below 10,000. All other studies look at exposures of 20 weeks or less. Using the most relevant chronic exposure studies would suggest that NNK is a high priority for exposure reduction research. However, it should be noted that these MOEs rely on a single paper which, although is a lifetime study, the exposure occurs via drinking water and may be considered as an inappropriate

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exposure scenario for tobacco smoke. In order to support these conclusions there is the potential need for further work. For example, a chronic NNK inhalation study (which in this instance would be the most relevant exposure route) could be conducted, or the use of physiologically based pharmacokinetic (PBPK) modelling, which has the capability to use alternative exposure scenario data sets to predict lung potency of inhaled NNK.

3.13. N-Nitrosonornicotine (NNN)

The evaluation of NNN and the spectrum of data available follows a similar pattern to that of NNK. There are no available data on the human carcinogenicity of NNN, however, on the basis of animal experimentation and following a number of different routes of administration, IARC has allocated a Group 1 status to NNN, "carcinogenic to humans" (IARC, 2007b). A total of 29 reviewed papers covered NNN, with 12 from single dose experiments and there are no reports of administration via the inhalation route. Overall, 21 papers reported induction of respiratory tumours. Four papers generating seven analysable data sets, covering three respiratory lesions, are available. Using a cigarette smoke yield of 231.1 ng per cigarette (Counts et al., 2005) to generate the MOE values, the range was both above and below the critical 10,000 value (Table 3), as with NNK. These data sets have been generated using exposure scenarios which are not of particular relevance for cigarette smoke (one via subcutaneous injection, one via intragastric instillation and two by i.p. injection). Consequently, as with NNK, there is a potential need for the generation of relevant chronic data via the inhalation route on NNN as a lung carcinogen before a firm conclusion can be drawn from the MOE analysis.

3.14. Vinyl chloride

Vinyl chloride has been assessed as Group 1 "carcinogenic to humans" by IARC, causing angiosarcomas of the liver and hepatocellular carcinoma (IARC, 2008b). Specifically, IARC states in a summary of epidemiological data that "among vinyl chloride workers overall, there was no evidence of an increased risk for lung cancer" (IARC, 2008b). In total, six experimental papers surveyed gave details relating to respiratory lesions. However, an application of the criteria given in Sections 2.1 and 2.4 left five papers (a rat dietary study, three mouse inhalation studies and a inhalation study covering both rats and mice) with eight MOE's (Table 3). As in the case of ethylene oxide, vinyl chloride is not routinely measured in smoke and therefore cigarette smoke yield data was not reported in the Counts et al. (2005). Instead an average yield of 10.5 ng per cigarette (Hoffmann et al., 1976) was used to calculate the MOEs. The eight MOEs generated range from 1.0×10^6 to 1.5×10^9. As a consequence, vinyl chloride may be considered a low priority for exposure reduction research in tobacco smoke.

4. Discussion

Within this paper, we have described how fundamental risk assessment principles can be applied to a number of toxicants known to be present in mainstream tobacco smoke using an MOE model similar to that adopted by EFSA and other regulatory bodies. A key feature of our approach is that in deriving $BMDL_{10}$ values, we have tried to identify from the literature those studies that are most relevant to the inhalation of tobacco smoke toxicants. This includes consideration of the route of dosing and the development of lesions that are pertinent to the toxicology of the respiratory tract.

Whereas a purely regulatory approach to this problem would involve the identification of a single critical study for computation of a $BMDL_{10}$, we have, where relevant, taken all available toxicological studies and computed $BMDL_{10}$ and MOEs for each. From a risk assessment perspective, the lowest computed MOE would always take precedence but we believe that by computing all possible MOEs from qualifying data sets, we can in most cases show a degree of consistency between MOEs for any given smoke toxicant and thus lend some credibility to the approach.

In the data presented here, representative examples for a range of tobacco smoke constituents, acetaldehyde, acrolein, acrylonitrile, cadmium, ethylene oxide, formaldehyde and isoprene all segregate with MOEs lower than 10,000 and must therefore be considered as high priority for exposure reduction research. Vinyl chloride and benzo(a)pyrene segregate with MOEs considerably higher than 10,000 and could be considered as a low priority for exposure reduction research. For 1,3-butadiene, all computed MOEs are below 10,000 indicating it to be a high priority toxicant, although it could be questioned whether the mouse respiratory lesion is relevant for human tobacco smoke-related disease since 1,3-butadiene is an acknowledged human leukaemogen. For NNK and NNN, there is insufficient data to calculate MOEs with any confidence, highlighting a need for additional research. From the single chronic study for NNK, there is a suggestion that this could be considered as a high priority. In the case of NNN, due to the lack of chronic studies the prioritisation using MOE is ambiguous. However, given NNN is a recognised human carcinogen then it is also likely to be of high priority. For m- and p-cresols, the values for computed MOEs are both below and above 10,000 and there is a clear need for chronic inhalation data to allow a firm conclusion to be drawn from the MOE analysis. At this time there are no known additional factors (i.e. IARC classifications or other regulatory positions) to cause the prioritisation of these compounds for exposure reduction research.

Overall, we propose that the MOE approach, when applied to tobacco smoke toxicants, represents a useful first segregation into high or low priority for exposure reduction research. However, as in the cases of NNN, NNK and m- and p-cresols, MOE can also be valuable in identifying the toxicants and areas where additional data needs to be generated from inhalation studies.

As previously discussed, the use of 10,000 as a critical value for MOEs has been well documented for use in the evaluation of genotoxic and carcinogenic compounds present in food where a single toxicant is being evaluated. We have adopted the same principle here initially as a starting point for the prioritisation of tobacco smoke toxicants where the MOEs tend to segregate below or above 10,000. However, when considering a complex mixture such as smoke, this simple segregation may not always be sufficient in order to directly compare and prioritise toxicants. The ranking of toxicants according to MOEs may provide a more useful insight into the respective contribution of each toxicant. However, considering that MOEs are calculated from a variety of studies with various routes of administration and exposure lengths, it may be argued that this direct comparison of values may not be valid. An alternative may be to increase the number of groupings for MOEs i.e. 1–10 (top priority), 10–100 (very high priority), 100–1000 (high priority), 1000–10,000 (medium priority), 10,000–1,000,000 (low priority), >1,000,000 (very low priority). This may prove to be a more useful system than just using 10,000 to categorise a toxicant as a high or low priority. Application of this proposed system to the toxicants described in this paper would result in the following ranking.

- Top priority (1–10): acrolein.
- Very high priority (10–100): formaldehyde.
- High priority (100–1000): acrylonitrile, 1,3-butadiene, cadmium.
- Medium priority (1000–10,000): acetaldehyde, ethylene oxide, isoprene.

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- Low priority (10,000–1,000,000): benzo(a)pyrene.
- Very low priority (>1,000,000): vinyl chloride.
- Unranked: m-/p-cresols, NNK, NNN.

In each case, ranking is based on the majority view for each toxicant. For example, in the case of acrolein the MOEs range from one to eleven, with only one of seven MOEs being larger than 10 and therefore its overall classification would be as a top priority.

For the majority of toxicants investigated here, this separation is fairly straightforward, with toxicants showing a high level of consistency and segregating easily into the bands. For the higher priority toxicants in particular, this second level of banding can be useful to further prioritise toxicants and focus future research into manageable stages. However, for the compounds that do not initially segregate above or below 10,000, including NNN, NNK and m- and p-cresols, this ranking system becomes more ambiguous and can be misleading. For example, applying the majority view (as described above) for NNK would place it into a low priority band, however, considering only the MOEs based on a chronic study would give rise to a high priority banding. This could be argued as being more appropriate. The alternative would be to take a precautionary risk assessment approach and use the lowest MOE available but again this may not represent all data available and may be confounded by outliers. These issues highlight the importance of demonstrating consistency in an MOE assessment. We suggest that each toxicant should be considered on a case by case basis, with relevant dialogue to explain the decision for prioritisation.

Clearly the MOE model lacks complexity and additional refinements may be necessary in order to customise it for the special situation of human exposure to tobacco smoke. As previously described in Section 2.2, three conservative assumptions regarding cigarette consumption, smoke exposure and retention have been made when estimating this exposure. Future developments to this model should focus on refining these. For example, there is an assumption within this model that there is a consistent level of exposure to any given tobacco smoke toxicant (based on the yield from 20 cigarettes per day distributed evenly through a 20 m^3 daily human breathing volume). This is a very simplistic representation; in reality smokers are exposed to sequential acute exposures via inhalation. One of the challenges for the development of improved risk assessment paradigms is to utilise contemporary tools such as PBPK models and computational fluid dynamic models to estimate more accurately human exposure to individual tobacco smoke toxicants during smoking.

Tobacco smoke is a complex mixture of over 5300 chemicals and it would be naïve to assume that there are no interactions when these chemicals impact on biological systems. Certain chemical classes of compound, e.g. aldehydes, that are found in tobacco smoke may well have common MOAs and therefore the MOE approach described here could be further refined to incorporate groups of such structurally (and toxicologically) related chemicals leading to the concept of a group MOE. To proceed with such a model requires careful consideration of the MOA for groups of structurally related chemicals together with a more rigorous evaluation of the tissue dose for individual chemicals. The approach described here, estimating the MOE for individual smoke toxicants, represents a first step towards prioritising tobacco smoke toxicants for research, and towards development of more complex, but physiologically more relevant risk assessment paradigms.

Conflict of Interest

The authors (Fiona Cunningham, Stacy Fiebelkorn, Marie-Louise Johnson and Clive Meredith) are either employees of or contractors to British American Tobacco, which has funded this research as part of its harm reduction programme. The authors declare that no financial or personal conflicts of interest exist with regard to the submission of the manuscript entitled "A novel application of the Margin of Exposure approach: Segregation of tobacco smoke toxicants".

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fct.2011.07.019.

References

Adkins Jr., B., Van Stee, E.W., Simmons, I.E., Eustis, S.L., 1986. Oncogenic response of strain A/J mice to inhaled chemicals. Journal of Toxicology and Environmental Health 17, 311–322.

Appelman, L.M., Woutersen, R.A., Feron, V.J., 1982. Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. Toxicology 23, 293–307.

Aylward, L.L., Goodman, J.E., Charnley, G., Rhomberg, L.R., 2008. A margin-of-exposure approach to assessment of non-cancer risks of dioxins based on human exposure and response data. Environmental Health Perspectives 116, 1344–1351.

Barlow, S., Renwick, A.G., Kleiner, J., Bridges, J.W., Busk, L., Dybing, E., Edler, L., Eisenbrand, G., Fink-Gremmels, J., Knaap, A., Kroes, R., Liem, D., Müller, D.J.G., Page, S., Rolland, V., Schlatter, J., Tritscher, A., Tueting, W., Würtzen, G., 2006. Risk assessment of substances that are both genotoxic and carcinogenic. Report of an International Conference organized by EFSA and WHO with support of ILSI Europe. Food and Chemical Toxicology 44, 1636–1650.

Benford, D., Bolger, P.M., Carthew, P., Coulet, M., DiNovi, M., Leblanc, J.-C., Renwick, A.G., Setzer, W., Schlatter, J., Smith, B., Slob, W., Williams, G., Wildemann, T., 2010. Application of the margin of exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. Food and Chemical Toxicology 48, S2–S24.

Boobis, A.R., Ossendorp, B.C., Banasiak, U., Hamey, P.Y., Sebestyen, I., Moretto, A., 2008. Cumulative risk assessment of pesticide residues in food. Toxicology Letters 180, 137–150.

Burns, D.M., Dybing, E., Gray, N., Hecht, S., Anderson, C., Sanner, T., O'Connor, R., Djordevic, M., Dresler, C., Hainaut, P., Jarvis, M., Opperhuizen, A., Straif, K., 2008. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. Tobacco Control 17, 132–141.

Cassee, F.R., Groten, J.P., Feron, V.J., 1996. Changes in nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde and acrolein. Fundamental and Applied Toxicology 29, 208–218.

Counts, M.E., Morton, M.J., Laffoon, S.W., Cox, R.H., Lipowicz, P.J., 2005. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine smoking conditions. Regulatory Toxicology and Pharmacology 41, 185–227.

Crump, K.S., 1984. A new method for determining allowable daily intakes. Fundamental and Applied Toxicology 4 (5), 854–871.

Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S., Beland, F.A., 1998. A Comparison of the tumours induced by coal tar and benzo(a)pyrene in a 2-year bioassay. Carcinogenesis 19 (1), 117–124.

Department of Justice Canada, 2000. Tobacco reporting regulations. SOR/2000-273. Available from: <http://laws-lois.justice.gc.ca/PDF/SOR-2000-273.pdf>.

Dorman, D.C., Struve, M.F., Wong, B.A., Marshall, M.W., Gross, E.A., Willson, G.A., 2008. Respiratory tract responses in male rats following subchronic acrolein inhalation. Inhalation Toxicology 20, 205–216.

Dourson, M.L., Hertzberg, R.C., Hartung, R., Blackburn, K., 1985. Novel methods for the estimation of acceptable daily intake. Toxicology and Industrial Health 1 (4), 23–33.

EC, 2009. European Commission (EC), Scientific Committee on Health and Environmental Risks/Scientific Committee on Consumer Products/Scientific Committee on Emerging and Newly Identified Health Risks (SCHER/SCCP/SCENIHR) 2009, Scientific opinion on 'Risk assessment methodologies and approaches for mutagenic and carcinogenic substances'. Available from: <http://ec.europa.eu/health/ph_risk/committees/04_scher/docs/scher_o_113.pdf>.

EFSA, 2005. European Food Safety Authority (EFSA) Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic (Request No. EFSA-Q-2004-020). Adopted on 18 October 2005. Available at: <http://www.efsa.europa.eu/en/efsajournal/doc/282.pdf>.

EFSA, 2006. European Food Safety Authority (EFSA) meeting summary report, 2006. EFSA/WHO international conference with support of ILSI Europe on risk assessment of compounds that are both genotoxic and carcinogenic. 16–18 November 2005 Brussels. ISBN: 92-9199-028-0.

EFSA, 2009. European Food Safety Authority (EFSA), 2009. Scientific Opinion: use of the benchmark dose approach in risk assessment. Guidance of the Scientific Committee. The EFSA Journal 1150, 1–72.

Feron, V.J., De Jong, D., Emmelot, P., 1973. Dose-response correlation for the induction of respiratory tract tumours in Syrian golden hamsters by intratracheal instillations of benzo[a]pyrene. European Journal of Cancer 9, 387–390.

Feron, V.J., Hendriksen, C.F.M., Speek, A.J., Til, H.P., Spit, B.J., 1981. Lifespan oral toxicity study of vinyl chloride in rats. Food and Chemical Toxicology 19, 317–333.

Forey, B., Hamling, J., Lee, P. and Wald, N., 2002a. International Smoking Statistics. A collection of historical data from 30 economically developed countries. 2nd Edition. Wolfson Institute of Preventive Medicine, London and Oxford University Press, Oxford. pp. 210-211. ISBN: 019-8508565.

Forey, B., Hamling, J., Lee, P. and Wald, N., 2002b. International Smoking Statistics. A collection of historical data from 30 economically developed countries. 2nd Edition. Wolfson Institute of Preventive Medicine, London and Oxford University Press, Oxford. pp. 250-253. ISBN: 019-8508565.

Fowles, J. and Bates, M., 2000. The chemical constituents of cigarettes and cigarette smoke: priorities for harm reduction. A report by the Institute of Environmental Science and Research for the New Zealand Ministry of Health. Porirua: Institute of Environmental Science and Research. Available from: <http://www.ndp.govt.nz/moh.nsf/pagescm/1003/$File/chemicalconstituentscigarettespriorities.pdf>.

Fowles, J., Dybing, E., 2003. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. Tobacco Control 12, 424–430.

Ghanayem, B.I., Nyska, A., Haseman, J.K., Bucher, J.R., 2002. Acrylonitrile is a multisite carcinogen in male and female B6C3F1 mice. Toxicological Sciences 68, 59–68.

Glaser, U., Hochrainer, D., Otto, F.J., Holdiges, H., 1990. Carcinogenicity and toxicity of four cadmium compounds inhaled by rats. Toxicological and Environmental Chemistry 27, 153–162.

Green, C.R., 2009. Book review – The chemical components of tobacco and tobacco smoke. Beiträge zur Tabakforschung International 23 (4), 227–230.

Gregg, E., Williamson, J., Proctor, C. and O'Reilly, D., 2008. A proposed framework for PREP assessment. Poster presented at the 14th Annual Meeting of the Society for Research on Nicotine and Tobacco, Portland, Oregon, USA, February 27–March 1, 2008. Available from: <http://www.bat-science.com/groupms/sites/BAT_7AWFH3.nsf/vwPagesWebLive/DO7AXLK3?opendocument&SKN=4>.

Griciute, L., Castegnaro, M., Herezia, J.C., Cabral, J.R.P., 1986. Influence of ethyl alcohol on the carcinogenicity activity on *N*-Nitrosonornicotine. Cancer Letters 31, 267–275.

Health Canada, 1999. Determination of "Tar", nicotine and carbon monoxide in mainstream tobacco smoke. Official Method T-115. Available from: <http://www.hc-sc.gc.ca/hc-ps/alt_formats/hecs-sesc/pdf/tobac-tabac/legislation/reg/indust/method/_main-principal/nicotine-eng.pdf>.

Hecht, S.S., 2006. Cigarette smoking: cancer risks, carcinogens and mechanisms. Langenbeck's Archives of Surgery 391, 603–613.

Hecht, S.S., Adams, J.D., Numoto, S., Hoffmann, D., 1983. Induction of respiratory tract tumours in Syrian golden hamsters by a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and the effect of smoke inhalation. Carcinogenesis 4 (10), 1287–1290.

Hecht, S.S., Morse, M.A., Amin, S., Stoner, G.D., Jordan, J.D., Choi, C.-I., Chung, F.-L., 1989. Rapid single-dose model for lung tumour induction in A/J mice by 4-(methylnitrosamino)-1-(3-piridyl)-1-butanone and the effect of diet. Carcinogenesis 10, 1901–1904.

Hecht, S.S., Jordan, K.G., Choi, C.-I., Trushin, N., 1990. Effects of deuterium substitution on the tumorigenicity of 4-(methylnitrosamino)-1-(3-piridyl)-1-butanone and 4-(methylnitrosamino)-1-(3-piridyl)-1-butanonol in A/J mice. Carcinogenesis 11, 1017–1020.

Hehir, R.M., McNamara, B.P., McLaughlin, J., Willigan, D.A., Bierbower, G., Hardisty, J.F., 1981. Cancer induction following single and multiple exposures to a constant amount of vinyl chloride monomer. Environmental Health Perspectives 41, 63–72.

Herzfeld, L., 1982. A Prophylactic Plan for Reducing Risks to Smokers. Notabene Medici 12, 808–816.

Hoffmann, D., Hecht, S.S., 1990. Advances in tobacco carcinogenesis. In: Cooper, C.S., Grover, P.L. (Eds.), Chemical Carcinogenesis and mutagenesis I. Handbook of Experimental Pharmacology, 94. Springer-Verlag, Heidelberg, pp. 63–102 (ISBN: 03-8751-182-2).

Hoffmann, D., Hoffmann, I., 1997. The changing cigarette, 1950–1995. Journal of Toxicology and Environmental Health 50, 307–364.

Hoffmann, D., Hoffmann, I., 1998. Tobacco smoke components. Beiträge zur Tabakforschung International 18 (1), 49–52.

Hoffmann, D., Hoffmann, I., 2001. The Changing Cigarette: Chemical Studies and Bioassays. In: Burns, D.M., Benowitz, N.L. (Eds.), Risks Associated with Cigarettes with Low Machine-Measured Yields of Tar and nicotine. NCI Smoking and Tobacco Control. Monograph 13. United States Department of Health and Human Services, Bethesda, MD, pp. 159–161 (Chapter 5).

Hoffmann, D., Patrianakos, C., Brunnemann, K.D., Gori, G.B., 1976. Chromatographic determination of vinyl chloride in tobacco smoke. Analytical Chemistry 48, 47–50.

Hoffmann, D., Rivenson, A., Amin, S., Hecht, S.S., 1984. Dose–response study of the carcinogenicity of tobacco-specific *N*-Nitrosamines in F344 rats. Journal of Cancer Research and Clinical Oncology 108, 81–86.

Hoffmann, D., Djordjevic, M.V., Rivenson, A., Zang, E., Desai, D., Amin, S., 1993. A study of tobacco carcinogenesis. LI. Relative potencies of tobacco-specific *N*-Nitrosamines as inducers of lung tumours in A/J mice.. Cancer Letters 71, 25–30.

Hong, C.B., Winston, J.M., Thornburg, L.P., Lee, C.C., Woods, J.S., 1981. Follow up study on the carcinogenicity of vinyl chloride and vinylidene chloride in rats and mice: tumour incidence and mortality subsequent to exposure. Journal of Toxicology and Environmental Health 7, 909–924.

IARC, 1983. International Agency for Research on Cancer (IARC), 1983. IARC monographs on the evaluation of carcinogenic risk to humans. Benzo[a]pyrene 32, 211–225. ISBN: 92-832-1532.

IARC, 1993. International Agency for Research on Cancer (IARC), 1993. IARC monographs on the evaluation of carcinogenic risk to humans. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry 58, 119–237. ISBN: 92-832-1258-4.

IARC 1995. International Agency for Research on Cancer (IARC), 1995. IARC monographs on the evaluation of carcinogenic risk to humans. Acrolein 63, 337–371. ISBN: 92-832-1263-0.

IARC 1999a. International Agency for Research on Cancer (IARC), 1999a. IARC monographs on the evaluation of carcinogenic risk to humans. Acetaldehyde 71, 319–382. ISBN: 92-832-1271-1.

IARC, 1999b. International Agency for Research on Cancer (IARC), 1999b. IARC monographs on the evaluation of carcinogenic risk to humans. Acrylonitrile 71, 43–108. ISBN: 92-832-1271-1.

IARC, 1999c. International Agency for Research on Cancer (IARC), 1999c. IARC monographs on the evaluation of carcinogenic risk to humans. 1,3-Butadiene. Volume 71. pp. 109-225. ISBN: 92-832-1271-1.

IARC, 1999d. International Agency for Research on Cancer (IARC), 1999d. IARC monographs on the evaluation of carcinogenic risk to humans. Isoprene 71, 1015–1025. ISBN: 92-832-1271-1.

IARC, 2004. International Agency for Research on Cancer (IARC), 2004. IARC monographs on the evaluation of carcinogenic risk to humans. Tobacco Smoke and Involuntary Smoking 83, 82. ISBN: 92-832-1283-5.

IARC, 2006. International Agency for Research on Cancer (IARC), 2006. IARC monographs on the evaluation of carcinogenic risk to humans. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol 88, 39–325. ISBN: 92-832-1288-6.

IARC, 2007a. International Agency for Research on Cancer (IARC), 2007a. IARC monographs on the evaluation of carcinogenic risk to humans. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) 89, 457–468. ISBN: 978-92-832-1289-8.

IARC, 2007b. International Agency for Research on Cancer (IARC), 2007b. IARC monographs on the evaluation of carcinogenic risk to humans. *N*-Nitrosonornicotine (NNN) 89, 471–478. ISBN: 978-92-832-1289-8.

IARC, 2008a. International Agency for Research on Cancer (IARC), 2008a. IARC monographs on the evaluation of carcinogenic risk to humans. 1,3-Butadiene, ethylene oxide and vinyl halides 97, 45–184. ISBN: 978-92-832 1297-3.

IARC, 2008b. International Agency for Research on Cancer (IARC), 2008b. IARC monographs on the evaluation of carcinogenic risk to humans. 1,3-Butadiene, ethylene oxide and vinyl halides 97, 311–443. ISBN: 978-92-832-1297-3.

IARC, 2008c. International Agency for Research on Cancer (IARC), 2008c. IARC monographs on the evaluation of carcinogenic risk to humans. 1,3-Butadiene, Ethylene Oxide and Vinyl Halides 97, 185–309. ISBN: 978-92-832-1297-3.

ILSI, 2009. International Life Sciences Institute (ILSI), 2009. ILSI Europe Report Series: Application of the Margin of Exposure Approach to compounds in food which are both genotoxic and carcinogenic. ISBN: 9789078637165.

IPCS, 1991. International Program on Chemical Safety (IPCS), 1991. Acrolein. Environmental Health Criteria 127. ISBN: 92-4-157127-6.

IPCS, 1995. International Program on Chemical Safety (IPCS), 1995. Acetaldehyde. Environmental Health Criteria 167, ISBN 92 4 157167 5.

IPCS, 2009. International Program on Chemical Safety (IPCS), 2009. Principles for modelling dose-response for the risk assessment of chemicals. Environmental Health Criteria 239. ISBN 978 92 4 157239 2.

Kamata, E., Nakadate, M., Uchida, O., Ogawa, Y., Suzuki, S., Kaneko, T., Saito, M., Kurokawa, Y., 1997. Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fischer-344 rats. The Journal of Toxicological Sciences 22, 239–254.

Kerns, W.D., Pavkov, K.L., Donofrio, D.J., Gralla, E.J., Swenberg, J.A., 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Research 43, 4382–4392.

Lee, C.C., Bhandari, J.C., Winston, J.M., House, W.B., Dixon, R.L., Woods, J.S., 1978. Carcinogenicity of vinyl chloride and vinylidene chloride. Journal of Toxicology and Environmental Health 4 (1), 15–30.

Leuchtenberger, C., Leuchtenberger, L., Zbinden, I., 1974. Gas vapour phase constituents and SH reactivity of cigarette smoke influence lung cultures. Nature 247, 565–567.

Lowe, F.J., Gregg, E.O., McEwan, M., 2009. Evaluation of biomarkers of exposure and potential harm in smokers, former smokers and never smokers. Clinical Chemistry and Laboratory Medicine 47, 311–320.

Maronpot, R.R., Miller, R.A., Clarke, W.J., Westerberg, R.B., Decker, J.R., Moss, O.R., 1986. Toxicity of formaldehyde vapour in B6C3F1 mice exposed for 13 weeks. Toxicology 41, 253–266.

McCoy, G.D., Hecht, S.S., Katayama, S., Wynder, E.L., 1981. Differential effect of chronic ethanol consumption on the carcinogenicity of *N*-Nitrosopyrrolidine and *N*'-Nitrosonornicotine in male Syrian golden hamsters. Cancer Research 41, 2849–2854.

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Melnick, R.L., Huff, J.E., Roycroft, J.H., Chou, B.J., Miller, R.A., 1990. Inhalation toxicology and carcinogenicity of 1,3-butadiene in B6C3F1 mice following 65 weeks of exposure. Environmental Health Perspectives 86, 27–36.

Monticello, T.M., Swenberg, J.A., Gross, E.A., Leininger, J.R., Kimbell, J.S., Seilkop, S., Starr, T.B., Gibson, J.E., Morgan, K.T., 1996. Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Research 56, 1012–1022.

Morris, J.B., Robinson, D.E., Vollmuth, T.A., Brown, R.P., Domeyer, B.E., 1996. A parallelogram approach for safety evaluation of ingested acetaldehyde. Regulatory Toxicology and Pharmacology 114, 140–146.

NTP, 1984. National Toxicology Program (NTP), 1984. NTP Technical report on toxicology and carcinogenicity studies of 1,3-butadiene (CAS No. 106-99-0) in $B6C3F_1$ mice (inhalation studies). NTP TR 288, NIH publication 84-2544. Available from: <http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr288.pdf>.

NTP, 1987. National Toxicology Program (NTP), 1987. Toxicology and carcinogenesis studies of ethylene oxide (CAS No. 75-21-8) in $B6C3F_1$ mice (inhalation studies). NTP TR 326, NIH publication 88-2582. Available from: <http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr326.pdf>.

NTP, 1992. National Toxicology Program (NTP), 1992. Toxicity studies of cresols (CAS Nos. 95-48-7, 108-39-4, 106-44-5) in F344/N rats and B6C3F1 mice (feed studies). NTP TOX 9, NIH publication 92-3128. Available from: <http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox009.pdf>.

NTP, 1994. National Toxicology Program (NTP), 1994. Toxicity studies of isoprene (CAS No. 78-79-5) administered by inhalation to F344/N rats and B6C3F1 mice. NTP TOX 31, NIH publication 94-3354. Available from: <http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox031.pdf>.

NTP, 1995. National Toxicology Program (NTP), 1995. Toxicity studies of Cadmium oxide (CAS No. 1306-19-0) administered by inhalation to F344/N rats and $B6C3F_1$ mice. NTP Tox 39, NIH Publication No. 95-3388. Available from: <http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox039.pdf>.

NTP, 2008. National Toxicology Program (NTP), 2008. Toxicology and carcinogenesis studies of cresols (CAS No. 1319-77-3) in male F344/N rats and female B6C3F1 mice (feeding studies). NTP TR 550, NIH Publication No. 08-5891. Available from: <http://ntp.niehs.nih.gov/files/550_FINAL_WEB.pdf>.

O'Berg, M.T., 1980. Epidemiologic study of workers exposed to acrylonitrile. Journal of Occupational Medicine 22, 245–252.

O'Brien, J.O., Renwick, A.G., Constable, A., Dybing, E., Müller, D.J.G., Schlatter, J., Slob, W., Tueting, W., van Benthem, J., Williams, G.M., Wolfreys, A., 2006. Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. Food and Chemical Toxicology 44, 1613–1635.

OEHHA, 2009. Office of Environmental Health Hazard Assessment (OEHHA), 2009. Technical support document for cancer potency factors: methodologies for derivation, listing of available values and adjustments to allow for early life stage exposures. Appendix B, B-17–B-28. Available from: <http://www.oehha.ca.gov/air/hot_spots/2009/AppendixB.pdf>.

Office of National Statistics, 2006. General Household Survey. Available from: <http://www.ic.nhs.uk/webfiles/publications/Smoking%20bulletin/Smoking%202008/Final%20Smoking%20Excel%20Tables%20v3.xls>.

Placke, M.E., Griffis, L., Bird, M., Bus, J., Persing, R.L., Cox Jr., A.L., 1996. Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. Toxicology 110, 253–262.

Quast, J. F., Schuetz, D. J., Balmer, M. F., Gushow, T. S., Park, C. N. and McKenna, M. J., 1980. A two-year toxicity and oncogenicity study with acrylonitrile following inhalation exposure of rats. Dow Chemical Co., Toxicology Research Laboratory, Midland MI. Cited in: Office of Environmental Health Hazard Assessment (OEHHA). Acrylonitrile Chronic Toxicity Summary 2001. Available from: <http://www.oehha.ca.gov/air/chronic_rels/pdf/2ApnA_Acrylonitrile_chloropicrin.pdf>.

Rickert, W. S., Kaiserman, M. J., 1998. Development of an index for mainstream tobacco smoke based on chemical composition. Relative exposure index. Presented at the 52nd Tobacco Scientists' Research Conference.

Rivenson, A., Hoffmann, D., Prokopczyk, B., Amin, S., Hecht, S.S., 1988. Induction of lung and exocrine pancreas tumours in F344 rats by tobacco-specific and Arecia-derived *N*-Nitrosamines. Cancer Research 48, 6912–6917.

Rodgman, A., Green, C., 2003. Toxic chemicals in cigarette mainstream smoke–hazard and hoopla. Beiträge Zur Tabakforschung International 20, 481–538.

Rodgman, A., Perfetti, T.A., 2009. The Chemical Components of Tobacco and Tobacco Smoke. CRC press, Florida, USA, ISBN 978-1-4200-7883-1 (pp. 1259).

Rusch, G.M., Clary, J.J., Rinehart, W.E., Bolte, H.F., 1983. A 26 week inhalation toxicity study with formaldehyde in the monkey, rat and hamster. Toxicology and Applied Pharmacology 68, 329–343.

Smith, C.J., Hansch, C., 2000. The relative toxicity of compounds in mainstream cigarette smoke condensate. Food and Chemical Toxicology 38, 637–646.

Stratton, K., Shetty, P., Wallace, R. and Bondurant, S., 2001. Executive summary. In: Clearing the smoke. Assessing the science base for tobacco harm reduction. Nat. Acad. Press, Washington, pp. 1. ISBN: 0-309-07282-4.

Suzuki, Y., 1981. Neoplastic and nonneoplastic effects of vinyl chloride in mouse lung. Environmental Health Perspectives 41, 31–52.

Swenberg, J.A., Kerns, W.D., Mitchell, R.I., Gralla, E.J., Pavkov, K.L., 1980. Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapour. Cancer Research 40, 3398–3402.

Takenaka, S., Oldiges, H., König, H., Hochrainer, D., Oberdörster, G., 1983. Carcinogenicity of cadmium chloride aerosols in W rats. Journal of the National Cancer Institute 70, 367–373.

Thyssen, J., Althoff, J., Kimmerie, G., Mohr, U., 1981. Inhalation studies with benzo[a]pyrene in Syrian golden hamster. Journal of the National Cancer Institute 66, 575–577.

USEPA, 1997. US Environment Protection Agency (USEPA) Exposure Factor Handbook, 1997. Exposure Factor Handbook, pp. 38, (Chapter 5). Available from: <http://www.epa.gov/ncea/efh/pdfs/efh-chapter05.pdf>.

USEPA, 2010. US Environment Protection Agency (USEPA), 2010. Benchmark Dose Software (BMDS), supporting documents. Available from: <http://www.epa.gov/ncea/bmds/index.html>.

WHO, 2010. World Health Organization (WHO), 2010. COPD: Definition. Available from: <http://www.who.int/respiratory/copd/definition/en/index.html>.

Wilmer, J.W.G.M., Woutersen, R.A., Appelman, L.M., Leeman, W.R., Feron, V.J., 1989. Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. Toxicology Letters 47, 287–293.

Woutersen, R.A., Feron, V.J., 1987. Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure*. Toxicology 47, 295–305.

Woutersen, R.A., Appelman, L.M., Feron, V.J., van der Heijden, C.A., 1984. Inhalation toxicity of acetaldehyde in rats II. Carcinogenicity study: interim results after 15 months. Toxicology 31, 123–133.

Woutersen, R.A., Appelman, L.M., van Garderen-Hoetmer, A., Feron, V.J., 1986. Inhalation toxicity of acetaldehyde in rats III Carcinogenicity study. Toxicology 41, 213–231.

Woutersen, R.A., Appelman, L.M., Wilmer, J.W.G.M., Falke, H.E., Feron, V.J., 1987. Subchronic (13-week) inhalation toxicity study of formaldehyde in rats. Journal of Applied Toxicology 7, 43–49.

Woutersen, R.A., van Garderen-Hoetmer, A., Bruijntjes, P., Zwart, A., Feron, V.J., 1989. Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. Journal of Applied Toxicology 9, 39–46.

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