

## Annex 6

### FSA Project: T01051

#### Interpretation of margins of exposure for genotoxic carcinogens

##### Final Report for Objective 4

#### Use of existing epidemiological data to analyse the relationship between Margins of Exposure and human cancers and to estimate upper bounds for the incidences of unrecognised chemical-induced cancers

##### Aims

The aim of this part of the project was to compare risk estimates obtained from carcinogenicity data in experimental animals, using the MOE approach, with the measured risk in exposed subjects, or with upper bound estimates for the incidences of unrecognised chemical-induced cancers, obtained from epidemiological studies. In part 1, chemicals were sought for which there was sound evidence of carcinogenicity in humans, reasonably assumed to have arisen by a genotoxic mode of action, starting with IARC group 1 carcinogens. In part 2, carcinogens were sought from IARC groups 1, 2a and 2b for which there was good evidence that carcinogenicity in experimental animals was by a genotoxic mode of action, but for which there was no reported association with any increase over the background incidence of cancer in exposed subjects.

##### Methodology

For each of the case carcinogens data were obtained for:

1. Risk estimates associated with specific exposure levels in human studies; key literature, including reviews, meta-analyses and recent IARC monographs were reviewed.
2. Data from animal studies from which to obtain or derive BMD10 values and hence the POD; key issues to consider were which animal species was the most sensitive, the route of exposure, appropriate correction for exposure duration, the choice of target tissue

Dose-response modelling was performed with BMDS version 2.4, using default constraints for the models, as appropriate.

The following models were fitted to all data sets:

Gamma  
Logistic  
LogLogistic  
LogProbit  
Multistage (2 nested models)  
Multistage-Cancer (3 nested models)  
Probit  
Weibull  
Quantal-Linear

Models were rejected if  $P < 0.05$  (EFSA, 2009), unless otherwise indicated. Full details of model output are provided in Appendix 1 to this Report.

In the case of nested models (multistage and multistage-cancer), the model with the lowest AIC and/or chi-squared value was used where there was a large difference in the parameters. Where the difference was small, the model with the fewest parameters was used (EFSA, 2009).

The lowest acceptable BMDL10 value was used to estimate the exposure associated with a 1 in  $10^5$  risk (i.e. that associated with an MOE of 10,000), assuming a linear relationship between exposure and response. This was compared with the exposure from a human study for an excess cancer incidence of 1 in  $10^5$ .

## Case Carcinogens

### Objective 4.1

Chemicals were sought for which there is evidence of carcinogenicity in humans, reasonably assumed to have arisen by a genotoxic mode of action, starting with IARC group 1 carcinogens. Relevant data for humans, including estimates of the excess cancer incidence in exposed populations, were collated and reviewed. An estimate of the exposure that would be expected to be associated with an excess cancer incidence of 1 in  $10^5$  was determined. Key animal studies were located and BMDL10s were determined for all substances *de novo*, to ensure consistency in the modelling. Using an MOE of 10,000, the dose predicted to be associated with an excess risk of 1 in  $10^5$  (i.e. BMDL10/10,000) was calculated. The two estimates of exposure, animal and human, associated with an excess cancer incidence of 1 in  $10^5$  were compared. Where possible, quantitative information on uncertainty was obtained, for example 95% confidence intervals on risk estimates in humans. Other sources of uncertainty were identified and described.

A thorough evaluation of the IARC group 1 carcinogens revealed that in many cases, suitable data for this exercise were not available. Either there was inadequate information on exposure-response relationships in human subjects, or no suitable information was available for determination of a POD for carcinogenicity in experimental animals. The compounds for which adequate data could be retrieved were aflatoxin B1, benzidine, chromium VI and vinyl chloride monomer.

### Aflatoxin B1 (AFB1)

Aflatoxins are naturally occurring fungal products (i.e. mycotoxins) produced by *Aspergillus* species that can be present in some human foodstuffs such as grains, nuts, milk and dairy products. Contamination is widespread in hot and humid climates, including sub-Saharan Africa and Southeast Asia and contamination of foodstuffs in these regions is widespread; European populations become exposed through the importing of contaminated crops.

AFB1 is an IARC group I carcinogen (IARC, 2012a). Carcinogenicity, particularly liver cancer, has been evaluated in several epidemiological studies (ecological, cohort, case-control), mostly in Asia and Africa; occupational exposure through inhalation occurs in the food production industry, during loading and unloading of cargo and during rice and maize processing. Non-occupational exposure occurs through ingestion of contaminated foods. The risk of liver cancer from exposure to AFB1 has been shown to greatly increase in subjects who are hepatitis B virus (HBV) and certain other virus positive.

### Animal Data

The rat appears to be the most sensitive animal species to AFB1, with the most sensitive strain being the male Fischer rat. The primary tumour site in animals is the liver; tumours may also occur at other sites including lung, kidney, colon. Hence data from this strain were used for dose-response modelling. Benford et al (2010) have reviewed the animal data in order to derive a BMD and BMDL<sub>10</sub> through dose-response modelling of the available data. They considered that a study in male Fischer rats by Wogan et al (1974) was the most appropriate study for dose-response modelling. In the Wogan

study groups of male Fischer rats, weighing approximately 80 g, were fed diets containing 0, 1, 5, 15, 50, or 100 mg/kg diet of AFB1 (purity > 95%) until clinical deterioration of animals was observed, at which time all survivors in that treatment group were killed. The data were modelled by Benford et al using a range of models and model averaging. To ensure consistency with the modelling undertaken for other compounds, the data were modelled using each of the individual available models in BMDS 2.4 for the purpose of this project. This gave a range on BMDL<sub>10</sub> values for acceptable models from 220 to 427 ng/kg-bw/day. As recommended by EFSA (2009), the lowest value was used to calculate the MOE, i.e. 220 ng/kg-bw/day (multi-stage cancer model, third order; BMD<sub>10</sub> = 329 ng/kg-bw/day).

#### *Human Data*

The carcinogenicity of aflatoxins through ingestion has been examined in a number of population studies correlating levels of aflatoxin contamination of foodstuffs with liver cancer rates. These have mostly been carried out in Asia or Africa, where the presence of aflatoxins in foods may be relatively high, and HBV infection is very common. Major studies have been carried out in China and Taiwan. Eaton and Gallagher (1994) have reviewed data suggesting that the presence of hepatitis B infection may increase the risk of liver cancer from aflatoxin B1 by up to 60-fold.

There have also been several occupational studies of the adverse health effects of aflatoxin exposure through inhalation. Olsen *et al.*, (1988) assessed occupational cancers among male employees at 241 livestock feed processing companies in Denmark dating back to 1964 with past exposures to aflatoxins equivalent to 170 ng/day. The risks of liver cancer and cancers of the biliary tract within the cohort were increased significantly (2-3 times) after a 10 year lag period (Standardised Proportional Incidence Ratio (SPIR) of 2.46, 95% CI 1.08-4.86). The authors drew a parallel between their study and an assessment of the risk of liver cancer in the US associated with ingestion of aflatoxins from peanuts (Dichter 1984). They state 'on the basis of the dose-effect relationship seen for oral consumption of aflatoxins in the US study and of the expected live cancer incidence in the age groups represented by the workers (10 per 100,000), one could expect a 2.7-fold increase in risk for liver cancer following a daily exposure to 170 ng aflatoxin'. Assuming that aflatoxins are as carcinogenic to the liver after inhalation as by ingestion the authors point out that the estimate of 2.7 corresponds closely to their own estimated 2.46-fold increased risk for liver cancer found in their inhalation study after a latency of 10 years or more.

#### *Comparison of animal and human data*

##### Animal

POD = BMDL<sub>10</sub> of 220 ng/kg bw/day

Dose-response: assumed to be linear

Dose for MOE of 10,000, i.e. 1 in 10<sup>5</sup> excess incidence: 0.022 ng/kg bw/day

##### Human

RR = 2.5 (95% CI 1.08 – 4.86) @ approx 3 ng/kg bw/day (Olsen et al) assuming average body weight of 60 kg (170/60)

Background age-adjusted incidence of liver cancer in Europe = 6.7/100,000 men, 2.2/100,000 in women: 4.2/100,000 men+women (GLOBOCAN, 2008).

Excess risk associated with aflatoxin is 1.5\*4.2/100,000 @ approx 3 ng/kg bw/day, i.e. 6.3 in 100,000, i.e. 1 in 100,000 at 0.48 ng/kg bw/day. (Note: as aflatoxin exposure from ingestion is extremely rare in Europe we assume that the background incidence is all due to exposures other than aflatoxin e.g. alcohol)

Human data	Value	Animal data	Value
Study authors	Olsen et al 1988	Study authors	Wogan et al 1974 (Benford et al 2010)
Tumour type	Liver HCC	Species/strain/sex	Rat/Fischer/male
Relative Risk	2.5	Tumour site	Liver HCC
95% CI	1.08-4.86	Route	Oral (diet)
Exposure estimate	170 ng/day	BMD10 range*	0.329-0.497 µg/kg/d
Exposure range		Lowest BMD10	0.329 µg/kg/d
Exposure route	Inhalation scaled to oral	BMDL10 range	0.220-0.427 µg/kg/d
Converted dose (A)	3 ng/kg/d ( BW=60kg)	Lowest BMDL10	0.220 µg/kg/d
Background incidence M/F/both	6.7/2.2/4.2 per 10 <sup>5</sup>	Model for lowest BMDL10	Multistage (3 <sup>rd</sup> order)
Excess risk (RR-1)	1.5	Dose conversion	None
Excess cancer number (Background x [RR-1]) (B)	6.3 per 10 <sup>5</sup>	Dose for cancer excess of 1 in 10 <sup>5</sup> (BMDL10/10,000)	0.022 ng/kg/d
Dose for cancer excess of 1 in 10 <sup>5</sup> (A/B * 10 <sup>-5</sup> )	0.48 ng/kg/d		
<b>Dose in humans/dose in animals associated with excess risk of 1 in 10<sup>5</sup> = 22</b>			

\*Dose was adjusted for duration of exposure (animals were terminated when clinical deterioration was apparent, ranged from 54-104 weeks).

### Conclusion

The dose associated with 1 in 100,000 excess risk based on data from animal data is approximately 20 times less than that giving this excess risk in humans. This provides confidence that the MOE cutoff of 10 000 is adequate in predicting the excess risk from AFB1 in human.

### Uncertainties

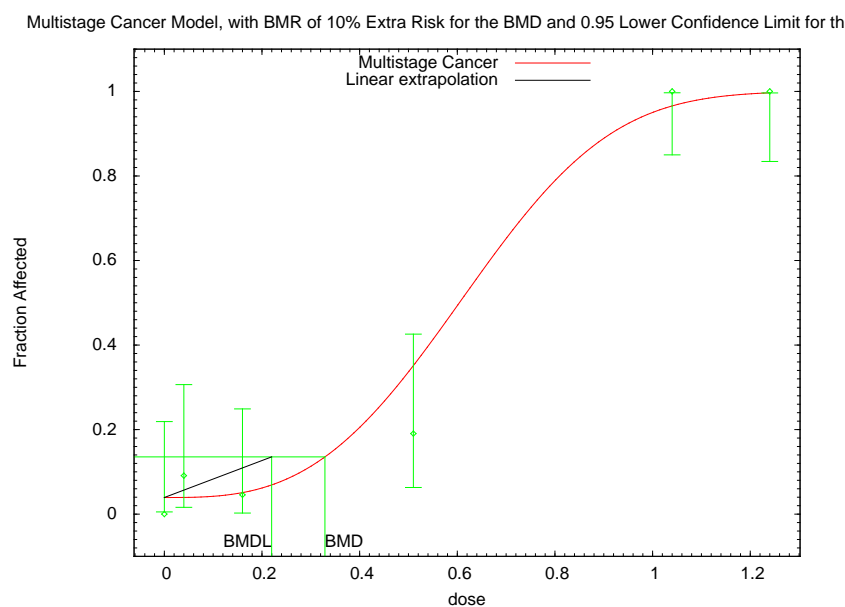
Most of the studies investigating the carcinogenicity of aflatoxins in humans have been population studies carried out in Africa or Asia where substantial quantities of aflatoxins occur in basic foodstuffs. HBV infection is very common in these countries and cancer of the liver is one of the most common cancers. Many of the studies are ecological/geographical and investigate the association between differences in aflatoxin contamination of foodstuffs and the occurrence of liver cancer. Both aflatoxin exposure assessment and ascertainment of incidences of liver cancer are uncertain in these studies. In addition it is difficult to disentangle the relationship between HBV and other possible confounders such as alcohol consumption in these studies and thus most are not relevant to the European situation where levels of HBV infection are low.

The study by Olsen et al (1988) which was chosen for comparison with the animal data was a study of Danish food processing workers where exposure to aflatoxin was through inhalation; no quantitative estimation was carried out in this study. However, the authors draw a parallel with a US study of oral consumption and assume that the level at which the same excess risk was found in the US study is applicable to their study. This assumes therefore that inhalation and oral exposures are equivalent regarding the carcinogenicity of aflatoxin.

A number of the uncertainties in the use of cancer data from studies in animals have been discussed in Benford et al (2010). Amongst these are the choice of the most sensitive species, strain and sex for dose-response modelling, the use of the lowest acceptable BMDL10 value for the calculations, the use

of the oral route for compound administration, the choice of a BMR of 10% and the assumption of linearity in the dose-response relationship between the POD and human exposure levels. In the animal studies, the dose-response relationship would have been influenced by the tumour promoting effects of hepatotoxicity at high doses.

The target tissue in both animals and humans was the liver, with hepatocellular carcinoma as the endpoint. Hence, there is no uncertainty in tissue concordance for this compound.



Multistage cancer (third-order) model for AFB1 (data of Wogan et al, 1974)

## Benzidine

Benzidine is an IARC class I carcinogen (IARC, 2102b). It causes bladder cancer in humans, as demonstrated by the extremely high incidence rate of bladder cancers in workers exposed in the dye manufacturing industry. It is a multi-organ carcinogen in animals: the primary tumour site varies with species. Benzidine and its salts are prohibited in many countries, including the EU (since 1998). Exposure to very low doses may occur in some jobs (mostly laboratories). Approximately 7000 workers are currently exposed in the EU (CAREX, 1999). Some benzidine-based dyes have been found to contain detectable levels of benzidine (quantitative data of specific levels that were found in some individual dye samples tested are given in IARC, 2010). Exposure may occur by contact with consumer goods (leather products, clothes, toys). Oral exposure may occur through food colourants such as tartrazine and sunset yellow (reported trace amounts < 5 to 270 ng/g – reference cited by IARC, 2010).

### *Animal Data*

Of species for which data are available, the mouse appears to be the most sensitive to benzidine. The primary tumour site in rodents is the liver whereas in non-rodents, such as the dog and in humans, the bladder is the primary site. The difference in target tissues is relatively well understood and is due to species differences in the metabolism and fate of benzidine. Liver and bladder tumours both arise from a P450-generated oxidation product produced in the liver. Data from male and female Balb/c x C57 B16/J F1 and so-called monohybrid cross (F1 x F1) mouse strains administered benzidine dihydrochloride in the drinking water for up to 33 months were modelled (Littlefield et al 1983, 1984). Dose-response modelling was on the basis of dose corrected for the molecular weight of the salt.

Female Balb/c x C57Bl6/J F1 mice were the most sensitive. The range on BMDL<sub>10</sub> values for acceptable models was from 376 to 749 µg/kg-bw/day. As recommended by EFSA (2009), the lowest value was used to calculate the MOE, i.e. 376 µg/kg-bw/day (log-logistic model; BMD10 = 626 µg/kg-bw/day).

#### *Human Data*

There are numerous epidemiological studies, both cohort and case-control studies, of bladder cancer occurring from inhalation exposure. Exposure levels are mostly not quantified; exposures are often mixed, or occur via more than one route (e.g. inhalation + dermal). One study (Zavon et al., 1973) gives quantification of exposure levels. EPA used data from this study to calculate risk estimates for benzidine based on human data. This study reported 11 cases of bladder cancer in 25 workers exposed to levels of benzidine from 0.005 to 17.6 mg/m<sup>3</sup> for a mean period of 11.46 years. A mean total accumulated dose of 130 mg/kg was estimated from urinary benzidine levels.

Another study carried out a 30-year follow-up of a cohort of workers at a benzidine manufacturing facility (Meigs et al. 1986). When compared with the population of Connecticut, a statistically significant excess incidence of bladder cancer was found in male workers exposed to the highest estimated level of benzidine (Standard Incidence Ratio = 3.43 95% CI (1.5-6.8), observed = 8, p<0.01). No quantification of exposure was reported. Of the eight cases of bladder cancer, three were long-term cigarette smokers. Risk increased with length of employment : <1year SIR=0 (0-3.2); 1-5 years SIR=3.4 (0.4-12.4); >5 years SIR=10.0 (0.6-21.7). Air concentrations measured in 1948 and 1949 found a mean of 0.018 mg/m<sup>3</sup> and a maximum of 0.087 mg/m<sup>3</sup>. Risk was greater for men employed before 1950 when major preventive measures were introduced in the plant. Most of the bladder cancers occurred in the high exposure group (SIR=13.0, 95% CI 4.79-28.4).

The ATSDR report (2001) cites a paper by Howard (1989) reporting occupational levels in the US ranging from 0.007-17.6 mg/m<sup>3</sup>

#### *Comparison of animal and human data*

##### **Animal**

POD = 376 µg/kg-bw/day

Dose-response: assumed to be linear

Dose for MOE of 10,000, i.e. 1 in 10<sup>5</sup> excess incidence: 0.0376 µg/kg-bw/day

##### **Human**

RR = 13.0 (95% CI 4.79-28.4) at approx 0.087 mg/m<sup>3</sup> (mean 0.018 mg/m<sup>3</sup>) (Meigs 1954; Meigs et al, 1986), representing the 'high' exposure level measured. This is equivalent to an oral dose (assuming 100% bioavailability) of 24.9 µg/kg-bw/day (mean 5.2 µg/kg-bw/day) (70 kg adult, 20 m<sup>3</sup> air per day).

Background incidence of bladder cancer in the US (GLOBOCAN, 2008): males = 21.1/100,000, females = 5.8/100,000, i.e. M+F = 12.7/100,000. A large proportion of these deaths will be due to smoking, some of which will be benzidine-related.

Excess risk associated with benzidine is 12 x 12.7 per 100,000, i.e. 152.4 per 100,000 at 24.9 µg/kg-bw/day (5.2 µg/kg-bw/day). Hence dose associated with excess risk of 1 in 100,000 is 0.16 µg/kg bw/day for the 'high' exposure estimate and 0.034 µg/kg bw/day for the mean exposure estimate.

Human data	Value	Animal data	Value
Study authors	Meigs 1954; Meigs et al, 1986	Study authors	Littlefield et al 1983, 1984
Tumour type	Bladder cancer	Species/strain/sex	Mouse/ Balb/c x C57 Bl6/J F1 & F1 x F1 monohybrid/M & F
Relative Risk	13.0	Tumour site	Liver
95% CI	4.79-28.4	Route/duration	Oral (drinking water)/33 months
Exposure estimate	'High': 0.087 mg/m <sup>3</sup>	BMD10 range (mg/kg) **	F1 – M: 1.64 – 3.21 F1 – F: 0.48 – 0.83 Mono – M: 3.17 – 5.12 Mono – F: 0.52
Exposure range	Mean: 0.018 mg/m <sup>3</sup>	Lowest BMD10 (mg/kg)	0.48
Exposure route	Inhalation	BMDL10 range (mg/kg)	F1 – M: 1.41 – 2.90 F1 – F: 0.38 – 0.75 Mono – M: 2.36 – 4.36 Mono – F: 0.43
Converted dose (A)*	'High': 24.9 µg/kg-bw/day Mean: 5.2 µg/kg-bw/day	Lowest BMDL10 (mg/kg)	0.38
Background incidence M/F/both	21.1/5.8/ <b>12.7</b> per 10 <sup>5</sup>	Model for lowest BMDL10	LogLogistic (F1 – F)
Excess risk (RR-1)	12.0	Dose conversion	Only as above
Excess cancer number (Background x [RR-1]) (B)	152.4 per 10 <sup>5</sup>	Dose for cancer excess of 1 in 10 <sup>5</sup> (BMDL10/10,000)	0.038 µg/kg bw per day
Dose for cancer excess of 1 in 10 <sup>5</sup> (A/B * 10 <sup>-5</sup> )	0.16 µg/kg bw per day (assume 'high' exposure) 0.034 µg/kg bw/day (assume mean exposure)		
Dose in humans/dose in animals associated with excess risk of 1 in 10 <sup>5</sup> = <b>4</b> when assume 'high' exposure <b>1</b> when assume mean exposure			

\*Assume 70 kg adult, breathing 20 m<sup>3</sup> air per day

Doses converted from benzidine dihydrochloride to benzidine equivalents

### Conclusion

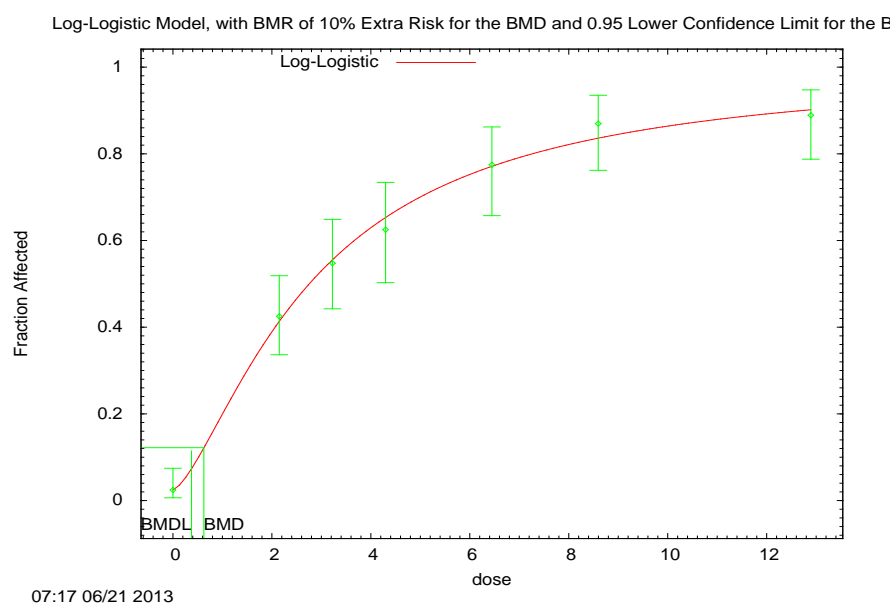
The dose associated with 1 in 100,000 increase in risk based on animal data is approximately 4 times less than that giving this excess risk in humans for the 'high' exposure estimate and is similar to that for the mean exposure estimate. This provides some confidence that the MOE cutoff of 10,000 would be protective for benzidine.

### Uncertainties

The study chosen for the risk estimate was a small cohort of benzidine manufacturing workers; cancer incidence was ascertained from the local cancer registry. Both dermal and inhalation exposure occurred. Exposure assessment was based on work history and knowledge of exposure levels in different areas of the plant but was not sufficient to enable individual quantitative estimates of exposure to be made. Some effort had been made over the time period involved to reduce exposures at

the plant through engineering controls and use of personal protective equipment and also use of substituted benzidines; the authors state that it is not known if these chemicals could have caused bladder cancers.

There is some uncertainty associated with site concordance of cancer, liver in rodents and bladder in humans, though there are mechanistic data supporting this choice. The most sensitive species, strains and tumour site were chosen for dose-response modelling. The lowest acceptable BMDL10 value was used for the calculations; the oral route (drinking water) was used for compound administration in the animal studies, whilst exposure in humans was primarily by inhalation, necessitating route-to-route extrapolation. A BMR of 10% was chosen for determination of the POD and the dose-response relationship between the POD and human exposure levels was assumed to be linear.



Log-logistic model for benzidine (data of Littlefield et al 1981, 1984)

## Chromium VI

Chromium VI is an IARC class I carcinogen based on association with lung and sinonasal cancers (IARC, 2012c). IARC concluded that there is little evidence for association with cancers at other sites. Chromium (VI) rarely occurs naturally (the naturally occurring form is chromium (III)): it is generated mostly by industrial processes, particularly stainless steel manufacture, the production of alloys, in chrome-containing pigments, and in chrome-plating. Chromium (VI)-containing chemicals are used in products such as dyes, paints, wood and metal treatments and metalworking products. The formulation is important in the toxicity (e.g., mist, dust or spray, particle size, chemical formulation).

Exposure may occur in the general population via inhalation of ambient air (e.g., in the vicinity of anthropogenic source) or ingestion of contaminated drinking water. Chromium in food is reported to be essentially in the trivalent state (i.e. negligible level of chromium (VI)). Occupational exposure occurs through inhalation of dusts, mists and fumes and through dermal contact.

### Animal Data

The COC used chromium VI as a case study to explore the use of the MOE approach (COC, 2007 (CC/07/14 and annexes; available at <http://www.iacoc.org.uk/papers>)). It was concluded that the most suitable and sensitive tumour data for modelling were the combined incidences of adenomas and carcinomas of the small intestine (duodenum, jejunum or ileum) observed in a study by the NTP (2007; full report, 2008). Chromium VI as sodium dichromate dihydrate was administered in drinking



water for 2 years to female B6C3 F1 mice, at doses (correcting for the molecular weight of the salt) of 0, 0.38, 1.36, 3.1 and 8.7 mg/kg-bw/day. No data suitable for modelling by the inhalation route were available. All dichotomous models available in BMDS 2.4 were fitted to the data.

The range of BMDL<sub>10</sub> values for acceptable models was from 0.84 to 1.06 mg/kg-bw/day. As recommended by EFSA (2009), the lowest value was used to calculate the MOE, i.e. 0.84 mg/kg-bw/day (log-logistic model; BMD10 = 1.33 mg/kg-bw/day).

#### *Human Data*

Many studies have reported evaluations of occupational exposure levels associated with various industries (tabulated by IARC (1990), with descriptive updates of subsequent studies/data given in IARC (2012c)). Cole and Rodu (2005) reviewed 84 papers of 49 epidemiologic studies published since 1950, and undertook a range of meta-analyses relating CrVI exposure to mortality. A total of 47 studies examined lung cancer with a total of 2,454 deaths, whereas 1,741 were expected. This resulted in an overall SMR of 1.41 (95%CI=1.35-1.47). In 26 studies that controlled for smoking the SMR was reduced to 1.18 (95%CI=1.12-1.25) based on 1,325 cases whereas 1,118 were expected. Analysis of studies that did not control for smoking indicated that about 75% of the excess risk is probably due to smoking.

Data have also been summarised and modelled by several authors (see Haney et al., 2012; Thomson et al., 2011; Brown et al., 2012). Thomson (2011) focused on oral consumption and highlights the largely negative results for gastrointestinal cancers from the epidemiological studies. Haney et al (2012) focused on inhalation exposure and lung cancer. They review exposure models carried out in 3 cohorts of chromate production workers in order to obtain a POD: Painsville Ohio (Crump et al 2003, Luippold et al 2003); Germany (Birk et al 2006); Baltimore, Maryland (Park et al 2004, Park and Stayner 2006). These cohorts all found significantly raised risks for lung cancer but at varying levels of cumulative exposure. Haney et al use these studies to derive 3 'candidate' PODs for cumulative exposure (0.195, 0.26, 0.817 mg CrVI/m<sup>3</sup> yr) based on Birk et al (mean exposure duration of 9.8 years), Park and Stayner (mean exposure duration of 3.1 years) and Crump et al (mean exposure duration of 9.2 years). These correspond to estimated average air concentrations of 19.9, 83.9 and 88.8 µg Cr VI/m<sup>3</sup> respectively (e.g. 0.817 mg/m<sup>3</sup> yr/9.2 yr=0.0888 mg/m<sup>3</sup> or 88.8 µg/m<sup>3</sup>). The Birk paper measured chromium in urine and found an SMR=2.09 (95%CI 1.08-3.65) for the highest cumulative exposure category (> 200 µg Cr VI/l yr) which did not change when adjusted for smoking. Haney et al convert this to 0.760 mg/m<sup>3</sup> by dividing by 770. SMRs below this level were less than 1.

Haney et al select the POD estimate of 0.195 mg CrVI/m<sup>3</sup> yr based on Birk as being 'sufficiently conservative'. They assess uncertainty by constructing ranges for the cumulative exposure threshold of 0.260-0.760 mg CrVI/m<sup>3</sup> yr using bootstrapping methods. The upper bound of this range corresponds to the SMR of 2.09 in the Birk paper. Assuming an average duration of exposure of approximately 10, 2.09 corresponds an excess risk associated with 7.6 µg Cr VI/m<sup>3</sup> air concentration.

#### *Comparison of animal and human data*

POD = 0.84 mg/kg-bw/day

Dose-response: assumed to be linear

Dose for MOE of 10,000, i.e. 1 in 10<sup>5</sup> excess incidence: 0.084 µg/kg-bw per day

#### *Human*

RR = 2.09 (95% CI 1.08 – 3.65) at approx 7.6 µg Cr VI/m<sup>3</sup> air concentration from Haney et al and Birk et al. (smoking adjusted).

Age standardised incidence of lung cancer in Germany for males is 42.4/100,000, for females is 7.2/100,000 and for men and women together is 28.1/100,000 (GLOBOCAN 2008).

Excess risk associated with Cr VI is  $1.09 \times 28.1/100,000$  @ approx  $7.6 \mu\text{g}/\text{m}^3$ , i.e. 30.6 in 100,000, i.e. 1 in 100,000 at  $0.25 \mu\text{g}/\text{m}^3$ . This is equivalent to an oral dose of  $0.071 \mu\text{g}/\text{kg}$  bw per day, assuming average bw of 70 kg and  $20 \text{m}^3$  of air per day.

Human data	Value	Animal data	Value
Study authors	Birk et al, 2006; Haney et al, 2012	Study authors	NTP, 2008
Tumour type	Lung cancer	Species/strain/sex	Mouse/B6C3 F1/female
Relative Risk	2.09	Tumour site	Adenomas and carcinomas of duodenum, jejunum or ileum
95% CI	1.08 - 3.65	Route/duration	Oral (drinking water)/ 2 years
Exposure estimate	$7.6 \mu\text{g}$ per $\text{m}^3$	BMD10 range (mg/kg)**	1.33 – 1.37
Exposure range		Lowest BMD10 (mg/kg)	1.37
Exposure route	Inhalation	BMDL10 range (mg/kg)	0.84-1.06
Converted dose (A)*	$2.17 \mu\text{g}/\text{kg}$ bw per day	Lowest BMDL10 (mg/kg)	0.84
Background incidence M/F/both	$42.4/7.2/28.1$ per $10^5$	Model for lowest BMDL10	Log logistic
Excess risk (RR-1)	1.09	Dose conversion	None
Excess cancer number (Background x [RR-1]) (B)	$30.6$ per $10^5$	Dose for cancer excess of 1 in $10^5$ (BMDL10/10,000)	$0.084 \mu\text{g}/\text{kg}$ bw per day
Dose for cancer excess of 1 in $10^5$ (A/B * $10^{-5}$ )	$0.071 \mu\text{g}/\text{kg}$ bw per day		
Dose in humans/dose in animals associated with excess risk of 1 in $10^5 = 1$			

\* Assume 70 kg adult, breathing  $20 \text{m}^3$  air per day

\*\* Doses converted from sodium dichromate dihydrate to Cr VI equivalents

### Conclusion

The dose associated with 1 in 100,000 increase in risk based on animal data approx. the same as that giving this excess risk in humans. This provides some confidence that the MOE cutoff of 10,000 would be protective for chromium VI.

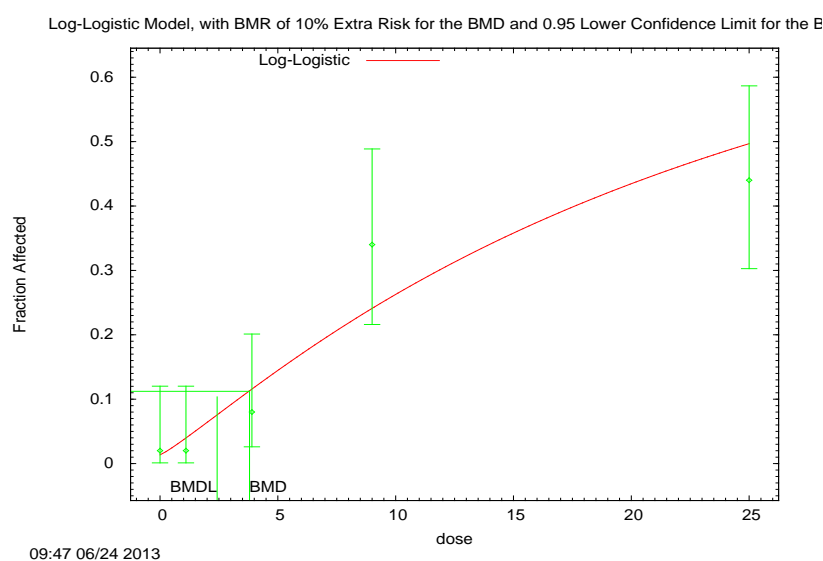
### Uncertainties

The risk estimate has been selected from a study of workers in the German Chromate industry and exposure measurements were from urine samples; the risk estimate did not change after adjustment for smoking. Because of the reduction of Cr(VI) in the airways and blood, urinary chromium is detected as Cr (III) so increased levels may reflect increased Cr(VI), Cr(III) or both. No co-exposure to other lung carcinogens was thought to be present in this study.

There is largely negative evidence in human studies of stomach and GI cancers but consistent evidence of an increased risk of lung cancer. The target organ in the animal studies was the small

intestine in mice; thus there was lack of concordance in target sites in the comparison of the animal and human data. In addition, exposure routes differed, being inhalation in humans and ingestion via drinking water in mice. Hence, in humans and mice, respectively, the target site for carcinogenesis was related to the portal of entry of Cr VI, introducing appreciable uncertainty into the comparison. Route-to-route extrapolation of dose metrics was also necessary. A number of other assumptions were necessary in these calculations e.g. the average duration of exposure in the Birk study, conversion of urinary concentrations to inhaled concentrations etc.

The most sensitivity species, sex and tumour site were chosen for dose-response modelling. The lowest acceptable BMDL10 value was used for the calculations. A BMR of 10% was chosen for determination of the POD and the dose-response relationship between the POD and human exposure levels was assumed to be linear. Data for adenomas and carcinomas from several sites in the small intestine were combined for the modelling.



Log-logistic model for chromium VI (data of NTP, 2007, 2008) (NB Dose not corrected for mol wt)

### Vinyl Chloride Monomer (VCM)

VCM is an IARC class I carcinogen (IARC, 2012d) and has been commercially available since the 1920s; it has been used since the 1930s to manufacture polyvinyl chloride (PVC) resin. It is not known to occur naturally and exposure is predominantly occupational. The highest exposure is known to occur during the cleaning of the reactors in which VC is polymerized to make PVC, a process that traditionally was done manually by workers who would have sustained exposures to VC as high as 1000 ppm (2600 mg/m<sup>3</sup>). General environmental exposure to VCM is very low/ negligible, except in the vicinity of specific emission sources where concentrations ranging up to 2600 µg/m<sup>3</sup> have been detected. Historically, occupational exposure levels have decreased from very high levels (several thousands of mg/m<sup>3</sup> in the 1940s/1950s to several hundreds of mg/m<sup>3</sup> in the 1960s/early 1970s). Current occupational exposure standards in most countries are around 13-26 µg/m<sup>3</sup> [5-10 ppm] (set in the mid-1970s when vinyl chloride was recognised as a human carcinogen) (IARC, 2008b).

Vinyl chloride causes liver cancer, particularly angiosarcoma of the liver (ASL), which is very rare in the general population, and hepatocellular carcinoma (HCC). Cohort studies have shown a high incidence of cancers in workers in industries where vinyl chloride exposures were historically very high (pre-1970s).

### *Animal Data*

Of species for which data are available, the rat appears to be the most sensitive to vinyl chloride monomer. The primary tumour site in rodents is the liver (angiosarcoma) which is the same primary site as in humans. Maltoni et al. (1981, 1984) have undertaken a number of studies in which Sprague-Dawley rats were exposed to various concentrations of vinyl chloride by inhalation, for 4 h/day, 5 days per week for 52 weeks. The data for several studies were pooled and summarised/tabulated by EPA/IRIS (IRIS, 2000) and by IARC (2012d) for the purposes of dose-response modelling. IARC pooled sixteen different dose groups from 5 different studies, and presented information for the incidences of liver angiosarcomas in males and females combined. IRIS pooled 13 different dose groups for the combined incidences of liver angiosarcomas, angiomas, hepatomas, and neoplastic nodules in female rats. No model provided an acceptable fit to either data set ( $P < 0.01$ ), despite the fact that there was a biological gradient in tumour response with dose. Following the recommendation of Bolger et al (2010) and EFSA (2009), BMDL10 values from those fits that were satisfactory on the basis of visual inspection were used for selection of a suitable value for the MOE calculation.

The dataset compiled by IRIS resulted in the lower BMDL10 values. The range of BMDL10 values was from 860 to 3795  $\text{mg}/\text{m}^3$ . As recommended by EFSA (2009), the lowest value was used to calculate the MOE, i.e. 860  $\text{mg}/\text{m}^3$  (multistage cancer (third order) model; BMD10 = 1160  $\text{mg}/\text{m}^3$ ).

[Using the data set compiled by IARC, the range of BMDL10 values was from 8900 to 34700  $\text{mg}/\text{m}^3$ . The lowest value was 8900  $\text{mg}/\text{m}^3$  (log-logistic model; BMD10 = 12350  $\text{mg}/\text{m}^3$ .)]

### *Human Data*

There have been several case studies and epidemiological studies of liver cancer occurring in workers exposed to VCM. A review by Kielhorn *et al.* (2000), in which epidemiologic studies of mortality amongst VC/PVC workers from several countries were combined in a meta-analysis, gave a meta-SMR of 5.33 (95% CI 4.23- 6.62), primarily due to an excess risk of ASL, with a 45-fold increase in ASL being seen in workers exposed to >10,000 ppm-years compared with workers exposed to <2000 ppm years. In the European study by Simonato *et al.* (1991), a significantly raised excess risk of liver cancer was observed (SMR = 2.86, 95% CI 1.83 – 4.25) with a significant exposure-response relationship ( $p < 0.001$ ) being demonstrated by exposure (ppm) categories: <50 RR=1.19 (95%CI 0.25-3.47); 50-499 RR=1.61 (95%CI 0.33-4.71); >500ppm RR=5.67 (2.93-9.91). In addition, histological analysis was performed and 16 of the 24 cases of liver cancer in the study cohort were verified as ASL. The follow-up to this study (Ward et al, 2001) only reported results by cumulative exposure. There are also several papers reporting results of follow-up from a large US cohort study of VCM-exposed workers. However, these papers do not report results by exposure levels.

### *Comparison of animal and human data*

#### Animal

POD = 860  $\text{mg}/\text{m}^3$  (4 h/day, 5 days per week for 52 weeks) = 102  $\text{mg}/\text{m}^3$  average exposure per day

Dose-response: assumed to be linear

Dose for MOE of 10,000, i.e. 1 in  $10^5$  excess incidence: 10.2  $\mu\text{g}/\text{m}^3$

#### Human

RR = SMR=1.19 at 25ppm (63.9  $\text{mg}/\text{m}^3$ ) taking the midpoint of the lowest exposure category from Simonato et al. 16 of the 24 cases in this paper were ASL.

Background incidence of ASL in Europe = 0. There are very few ASL cases and we can assume that these are probably all due to VCM exposure. Hence, comparison will need to be with background incidence of liver cancer. Background age-adjusted incidence of liver cancer in Europe = 6.7/100,000 men, 2.2/100,000 in women: 4.2/100,000 men+women (GLOBOCAN, 2008). Assume that all of cancers in Simonato *et al.*, (1991) were angiosarcoma, i.e. 0.80 in 100,000.

Hence, 1 in 100,000 risk associated with exposure to 79.9 mg/m<sup>3</sup>

Human data	Value	Animal data	Value
Study authors	Simonato et al, 1991	Study authors	Maltoni et al, 1981, 1984 Summarised by IRIS, Summarised by IARC, 2012
Tumour type	Liver cancer (particularly angiosarcoma)	Species/strain/sex	Rats/Sprague-Dawley/M & F
Relative Risk	1.19	Tumour site	IRIS: hepatic angiosarcomas, angiomas, hepatomas, and neoplastic nodules in F IARC: angiosarcomas in M & F combined
95% CI	0.25 – 3.47	Route/duration	Inhalation; 4 h/d, 5 d/week, 52 weeks**
Exposure estimate	63.9 mg/m <sup>3</sup>	BMD10 range (mg/m <sup>3</sup> ) <sup>+</sup>	IRIS: 1161-4650 IARC: 12348-40396
Exposure range		Lowest BMD10 (mg/m <sup>3</sup> )	IRIS: 1161 IARC: 12,348
Exposure route	Inhalation	BMDL10 range (mg/m <sup>3</sup> )	IRIS: 860-3795 IARC: 8883-34707
Converted dose (A) [none]	63.9 mg/m <sup>3</sup>	Lowest BMDL10 (mg/m <sup>3</sup> )	IRIS: 860 IARC: 8,883
Background incidence* M/F/both	6.7/2.2/4.2 per 10 <sup>5</sup>	Model for lowest BMDL10	IRIS: Multi-stage cancer (3 <sup>rd</sup> order) IARC: Log logisitc
Excess risk (RR-1)	0.19	Dose conversion	/24*4 (h/d) /7*5 (d/wk)
Excess cancer number (Background x [RR-1]) (B)	0.80 per 10 <sup>5</sup>	Dose for cancer excess of 1 in 10 <sup>5</sup> (BMDL10/10,000)	IRIS: 86 µg/m <sup>3</sup> = 10.2 µg/m <sup>3</sup> (daily average) IARC: 0.888 mg/m <sup>3</sup> = 106 µg/m <sup>3</sup> (daily average)
Dose for cancer excess of 1 in 10 <sup>5</sup> (A/B * 10 <sup>5</sup> )	79.9 mg/m <sup>3</sup>	Convert to oral (0.223 m <sup>3</sup> air/d, 350 g)	IRIS: 6.5 µg/kg bw/d IARC: 67.5 µg/kg bw/d
Convert to oral (20 m <sup>3</sup> air /d, 70 kg)	22.8 mg/kg bw/d		
Dose in humans/dose in animals associated with excess risk of 1 in 10 <sup>5</sup> = IRIS: <b>7800</b> (inh); <b>3500</b> (oral) IARC: <b>750</b> (inh); <b>338</b> (oral)			

\*For hepatocellular carcinoma

\*\*Study termination at 147 weeks

+ No model was acceptable at  $P < 0.01$ . Range is for all models except log logistic, which was an outlier (BMD10 487  $\text{mg}/\text{m}^3$ )

### Conclusion

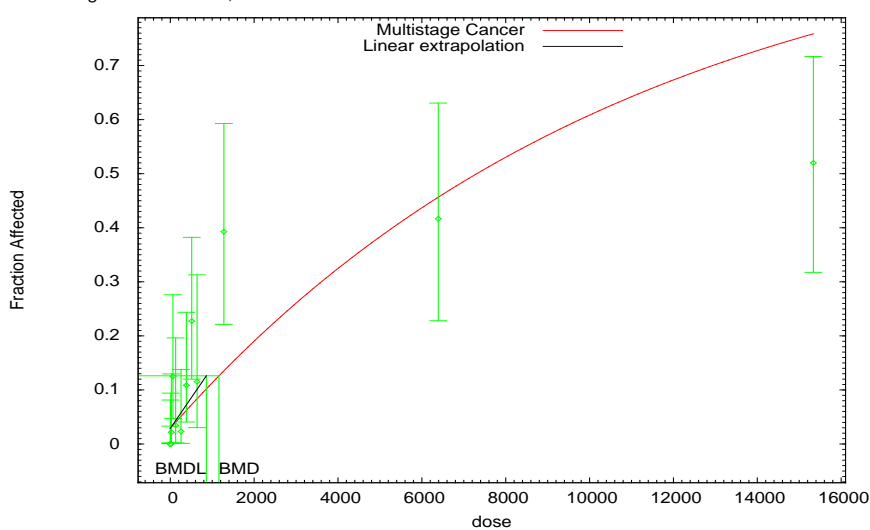
The dose associated with 1 in 100,000 excess risk based on animal data is almost 8000 times less than that giving this excess risk in humans. This supports confidence that the MOE cutoff of 10,000 would be adequately protective for vinyl chloride monomer and shows that the predicted excess risk in humans is much greater than the actual risk. Note that as the comparison is for exposure by the inhalation route, an MOE of 3,000 may be more appropriate as the cutoff for a level of concern, based on the normal inter-species extrapolation factor of 3 for this route, rather than 10 as for the oral route.

### Uncertainties

The study used for the risk estimate was a large cohort study combining data from 12 plants in 4 countries. The majority of the liver cancer cases were confirmed as ASL. Job exposure matrices were used for estimation of exposure thus incurring some imprecision in the estimates; a clear dose-response was seen however. There was no adjustment for alcohol consumption or hepatitis infection in this study. A hospital-based case-control study by Mastrangelo et al (2004) found that VCM exposure was an independent risk factor for both liver cirrhosis and hepatocellular liver cancer. Case reports have indicated that cirrhosis may be present in angiosarcoma cases but that it is of a non-alcoholic form. It was necessary to use the background incidence of liver cancer to obtain an estimate of the excess risk for angiosarcomas.

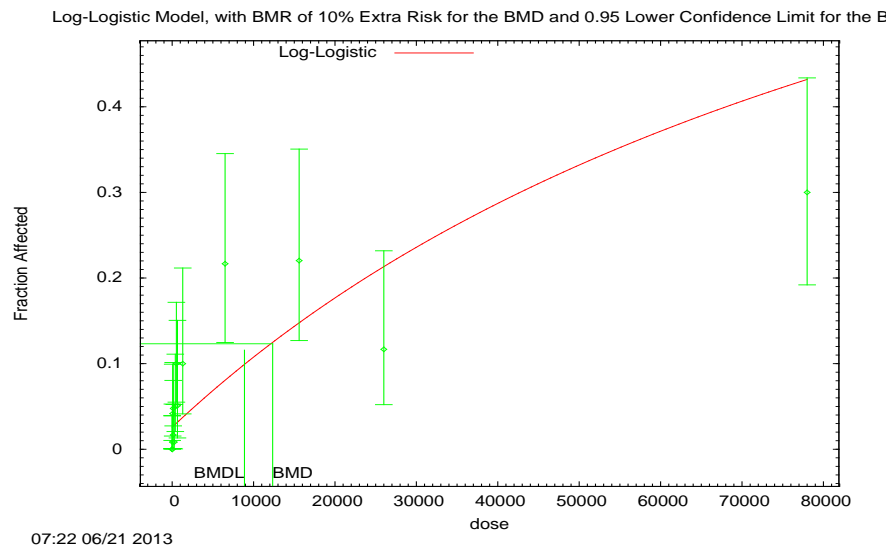
Additional uncertainties included the choice of the most sensitive species, strain and tumour site for dose-response modelling. The liver was the target site in both rats and humans, with concordance in tumour type. However, the lowest BMDL10 values were obtained using a data set in which all liver tumour types were combined. A BMR of 10% was chosen for determination of the POD. Data from several studies were pooled and it was not possible to test for heterogeneity between studies. Despite the existence of a relationship between dose and response, apparent visually, none of the models provided acceptable fits to the data. The lowest BMDL10 value from amongst all of the models was used for the calculations. The dose-response relationship between the POD and human exposure levels was assumed to be linear. Some conversion of dose metrics was necessary, necessitating the use of default assumptions for physiological parameters.

Multistage Cancer Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for th



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Multistage cancer (third order) model for vinyl chloride monomer (data for all liver tumours of Maltoni et al, 1981; 1984: compiled by IRIS)



Log-logistic model for vinyl chloride monomer (data for liver angiosarcomas of Maltoni et al, 1981; 1984: compiled by IARC)

## Objective 4.2

Chemicals were sought for which there is good evidence that they cause carcinogenicity in experimental animals by a genotoxic mode of action, but for which there is no association with any increase in the background incidence of cancer in humans. Existing epidemiological data was retrieved and used to estimate upper bounds for the incidences of any unrecognised chemical-induced cancers. IARC group 1, 2a and 2b carcinogens were evaluated to identify substances for which there were suitable experimental data to enable estimation of the experimental BMDL10 and epidemiological information on exposed populations in whom the relative risk was not significantly affected by the compound. The upper 95 % confidence interval was used to estimate the lower bound of an exposure that could be associated with an increase in the background incidence of cancer of 1 in  $10^5$ . Choice of tumour type was not obvious, and is discussed for each of the case chemicals below. A key consideration was that as site concordance could not be assumed for a genotoxic carcinogen, but no cancer in humans was significantly elevated on exposure to the compound, the greatest possible risk (in terms of population attributable risk) would be for that cancer type with the highest background incidence. Key animal studies were located and BMDL10s were determined for all substances *de novo*, to ensure consistency in the modelling. Using an MOE of 10,000, the dose predicted to be associated with an excess risk of in in  $10^5$  (i.e. BMDL10/10,000) was calculated. The two estimates of exposure, animal and human, associated with an excess cancer incidence of 1 in  $10^5$  were compared. Where possible, quantitative information on uncertainty as obtained, for example 95% confidence intervals on risk estimates in humans. Other sources of uncertainty were identified and described.

A thorough evaluation of the IARC group 1, 2a and 2b carcinogens revealed that in many cases, suitable data for this exercise were not available. Either there was no or inadequate information on exposure-response relationships in human subjects, or no suitable information was available for determination of a POD for carcinogenicity in experimental animals. The compounds for which adequate data could be retrieved were acrylamide, ethylene oxide and tamoxifen (liver cancer only).

## Acrylamide

### *Introduction*

Acrylamide has been shown to be a genotoxic carcinogen in experimental animals. It is metabolised by CYP2E1 to glycidamide (highly reactive with DNA). Acrylamide and its metabolites are rapidly eliminated in the urine. Tumours in multiple tissue types occur in animal bioassays. It is also possible that acrylamide may cause cancer by non-genotoxic mode(s) of action, but the evidence is currently considered to be insufficient. There is also discussion of PBTK modelling to compare internal doses of acrylamide and the metabolite, glycidamide, in humans and rats. IARC has classified acrylamide as probably carcinogenic to humans (Group 2A). It is present in many foods and has been shown to be associated with an increase in pancreatic cancer in workers exposed to acrylamide monomer.

### *Animal Data*

The rat appears to be the most sensitive animal species to acrylamide, with the most sensitive strain and sex being the female Fischer F344 rat. The primary tumour site is the mammary gland; tumours also occur at other sites including peritesticular mesothelioma, thyroid follicular adenoma and CNS tumours of glial origin. Hence data from this strain were used for dose-response modelling. Bolger et al (2010) have reviewed the animal data in order to derive a BMD10 and BMDL10 through dose-response modelling of the available data. They considered that a study in female Fischer F344 rats by Johnson et al (1986) resulted in the lowest BMD(L) values. In the Johnson et al study, groups of female Fischer rats, received via their drinking water, doses of 0, 0.01, 0.1, 0.5 and 2 mg/kg-bw per day of acrylamide for 2 years. The data on mammary gland tumours (fibroadenomas and adenocarcinomas) (Johnson et al 1986; compiled by Rice et al, 2005) were modelled by Bolger et al using a range of models and model averaging. To ensure consistency with the modelling undertaken for other compounds, the data were modelled using each of the individual available models in BMDS 2.4 for the purpose of this project.



This gave a range of BMDL10 values for acceptable models from 307 to 692 µg/kg-bw/day. As recommended by EFSA (2009), the lowest value was used to calculate the MOE, i.e. 307 µg/kg-bw/day (log-logistic model; BMD10 = 544 µg/kg-bw/day).

#### *Human data*

There have been several follow-ups of a cohort of 8,854 workers employed at four plants where inhalation exposure to acrylamide monomer occurred, three in the United States and one in the Netherlands. The latest update by Marsh *et al* (2007) in the period 1925 – 2002 reported that the SMR for pancreatic cancer for workers in the US plants in the highest cumulative exposure category (>30 mg/m<sup>3</sup> –years) had decreased from a statistically significant value of 2.26 (P<0.05) to a non-significant SMR of 1.71. However, further analysis of mortality risk for this cancer, using relative risk regression models that related internal cohort rates to potential confounders (such as smoking history), found an increasing risk with mean intensity of exposure in the early period of follow-up (unexposed SMR= 0.80 (0.54-1.14); 0.001-0.019 mg/m<sup>3</sup> SMR=1.69 (0.46-4.32); 0.02-0.29 mg/m<sup>3</sup> SMR=1.50 (0.49-3.49); ≥ 0.30 mg/m<sup>3</sup> SMR=2.31 (0.75-5.40)) but the pattern was less clear when the whole period was used (unexposed SMR= 0.78 (0.55-1.08); 0.001-0.019 mg/m<sup>3</sup> SMR=1.34 (0.44-3.14); 0.02-0.29 mg/m<sup>3</sup> SMR=1.11 (0.36-2.60); ≥ 0.30 mg/m<sup>3</sup> SMR=1.85 (0.68-4.03)).

A number of epidemiological studies have investigated possible associations between dietary acrylamide intake (estimated in various ways) and risk of any, or specific types of, cancer. Two recent reviews (Lipworth *et al* 2012, Pelucchi *et al* 2011) have noted that the studies (which all have various limitations particularly with regard to exposure estimation) have not identified any consistent associations between acrylamide exposure and cancer risk or shown any evidence of a dose-response relationship, although individual studies occasionally reported elevated risks for particular cancers. Pelucchi *et al* carried out a meta-analysis of dietary exposure and found meta-RRs close to 1 for 10 µg/day of acrylamide intake (0.98 for oesophageal cancer to 1.01 for colon, endometrial, ovarian and kidney cancer) and similar ranges for high quartile of exposure versus low with the highest estimate being for kidney cancer. It should be noted that several of these results are based on only 2 studies. Using 2 studies of occupational inhalation exposure to acrylamide (one being the Marsh 2007 paper described above) Pelucchi *et al* estimate a combined SMR for high occupational exposure of 1.69 (95%CI 0.83-2.99) for pancreatic cancer and using 4 studies of dietary exposure, 1 cohort and 3 case-control, of 2.22 (95% 0.81-4.84) for kidney cancer.

Whilst some individual studies have reported a slight increase in the risk of some forms of cancer, such as of the kidney, there is no clear and consistent association between exposure to acrylamide and an increase in incidence of cancer at any site. Hence, to obtain a worst case upper bound estimate of risk, the cancer type with the highest background incidence was used, together with the 95<sup>th</sup> percentile upper confidence interval, this yielding the highest value for the population attributable risk.

#### *Comparison of animal and human data*

##### *Animal*

POD = 307 µg/kg-bw/day

Dose-response: assumed to be linear

Dose for MOE of 10,000, i.e. 1 in 10<sup>5</sup> excess incidence: 0.031 µg/kg-bw per day = 31 ng/kg bw/day.

##### *Human*

The relative risk for breast cancer in non-smoking females exposed to acrylamide in the diet with the highest upper bound CI was 1.5 (95% CI 0.6-3.6) from the study of Olesen *et al*, 2008 (Lipworth *et al*, 2012).

Background incidence of breast cancer in females in Europe is: 62.8 per 100,000 (GLOBOCAN, 2008).

Maximum excess risk associated with acrylamide is  $2.6 \times 62.8$  per 100,000, i.e. 163.4 per 100,000 at  $10 \mu\text{g}/\text{day} = 0.17 \mu\text{g}/\text{kg bw}/\text{day}$  (assuming average female body weight of 60 kg).

Hence dose associated with excess risk of 1 in 100,000 is  $1.02 \text{ ng}/\text{kg bw}/\text{day}$ .

Human data	Value	Animal data	Value
Study authors	Olesen et al, 2008 (in Lipworth et al, 2012)	Study authors	Johnson et al, 1986 (in Rice et al, 2005)
Tumour type*	Breast cancer	Species/strain/sex	Rat/Fischer/female
Relative risk ( $\pm$ 95%CI)**	1.5 (0.6-3.6)	Tumour site	Fibroadenomas and adenocarcinomas of mammary gland
Upper bound relative Risk (95% CI)	3.6	Route/duration	Oral (drinking water)/2 years
Exposure estimate	RR per $10 \mu\text{g}/\text{day}$	BMD10 range (mg/kg)	0.544-1.044
Exposure range		Lowest BMD10 (mg/kg)	0.544
Exposure route	Oral	BMDL10 range (mg/kg)	0.307-0.623
Converted dose (A)	$0.167 \mu\text{g}/\text{kg bw}$ per day (F BW = 60 kg)	Lowest BMDL10 (mg/kg)	0.307
Background incidence M/F/both	F: $62.8 \text{ per } 10^5$	Model for lowest BMDL10	Log logistic
Excess risk (RR-1)	2.6	Dose conversion	None
Excess cancer number (Background $\times$ [RR-1]) (B)	$163.3 \text{ per } 10^5$	Dose for cancer excess of 1 in $10^5$ (BMDL10/10,000)	$30.7 \text{ ng}/\text{kg bw}$ per day
Dose for cancer excess of 1 in $10^5$ (A/B $\times 10^{-5}$ )	$1.02 \text{ ng}/\text{kg bw}$ per day		
Dose in humans/dose in animals associated with excess risk of 1 in $10^5 = \mathbf{0.033}$			

\*Chosen as background incidence is highest

\*\*For study with highest upper 95% CI

### Conclusion

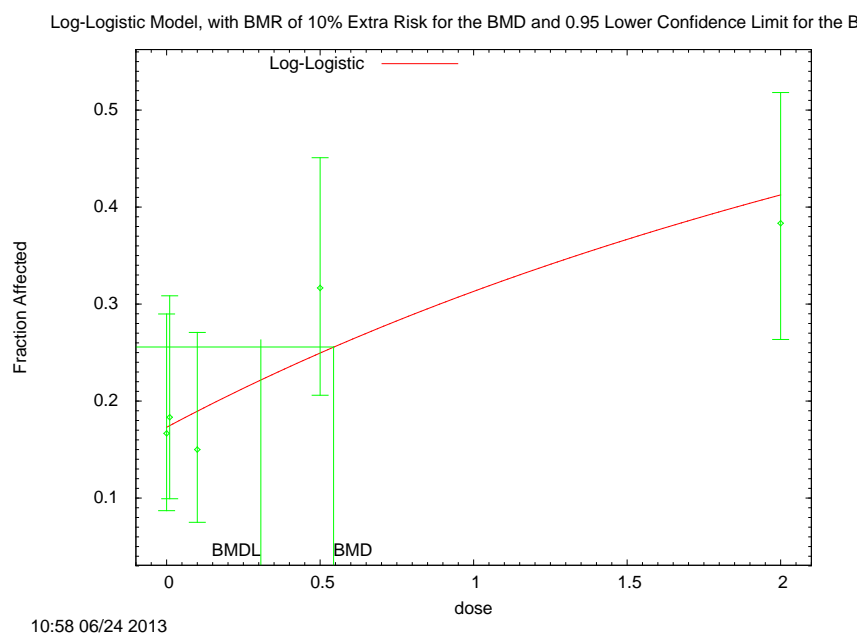
The dose associated with 1 in 100,000 excess risk based on animal data is approximately 30 times that giving the worst case upper bound for excess risk in humans. This, together with the very low margins of exposure reported by Bolger et al (2010) for exposure to acrylamide in high and average consumers (40 and 160, respectively), emphasises the uncertainty in the possible risk to humans from exposure to acrylamide.

### Uncertainties

Studies of dietary intake of acrylamide and cancer risk in humans may suffer from several potential sources of bias including uncontrolled confounding, lack of adjustment for other relevant dietary components and/or lifestyle factors, and misclassification of acrylamide exposure due the limitations of the food intake data collection methods. The tumour type with the highest background incidence (breast cancer in females) and the highest 95 % CI were used in the calculations.

Additional uncertainties included the choice of the most sensitive species, strain, sex and tumour site for dose-response modelling. The mammary gland was the target site in rats but the target site, if any,

in humans is not known. A BMR of 10% was chosen for determination of the POD. The lowest BMDL10 value from amongst the acceptable models was used for the calculations. The dose-response relationship between the POD and human exposure levels was assumed to be linear.



Log-logistic model for acrylamide (data for mammary gland tumours (fibroadenomas and adenocarcinomas) (Johnson et al 1986; compiled by Rice et al, 2005)

## Ethylene oxide

### Introduction

Ethylene oxide (EO) is used widely as a sterilising agent, disinfectant, and pesticide. It is also an intermediary in the chemical synthesis of ethylene glycol (antifreeze), non-ionic surfactants, resins and films, and other derivatives in smaller quantities. Human exposure occurs in hospitals, in the production of certain chemicals and in the manufacture of plastics and drugs. Although the epidemiological evidence is limited, it has been classified by IARC as carcinogenic to humans (Group 1) based primarily upon sufficient evidence in animals and genotoxic considerations (IARC, 2012e).

### Animal data

The carcinogenicity of ethylene oxide by inhalation has been investigated in a number of studies in rats and mice. The most sensitive sites were the lung, brain, testes and bone marrow (MCL). Data from studies of Adkins et al (1986) in A/J mice, NTP (1987) in B6C3F1 mice, Lynch et al (1984) in Fischer F344 rats, and Snellings et al (1984) and Garman et al (1985, 1986) in Fischer F344 rats were modelled.

The most sensitive endpoint was lung tumours in female A/J mice (Adkins et al, 1986). In this study animals were exposed to concentrations of ethylene oxide of 0, 126 and 360 mg/m<sup>3</sup>, 6 h/day, 5 days/week for 6 months. The range on BMDL10 values for acceptable models was from 16.0 to 32.1 mg/m<sup>3</sup>. As recommended by EFSA (2009), the lowest value was used to calculate the MOE, i.e. 16.0 mg/m<sup>3</sup> (multi-stage cancer (first order) model; BMD10 = 23.3 mg/m<sup>3</sup>).

### Human data

The initial concern about the health risk of EO was raised by studies in Sweden when a cluster of cases was observed among a cohort of EO-exposed workers employed by a company producing EO since the 1940s (Hogstedt *et al*, 1979). The cohort consisted of 241 men and was followed up between 1961 and 1977. A total of nine cancers were observed (SMR=2.65, 95%CI=1.12-5.02), of which two were from leukaemia (SMR=14.3, 95%CI=1.71-51.6), seen among full-time exposed workers. Among maintenance workers there was one leukaemia death (SMR=7.69, 95%CI=0.20-42.9). No cases were seen among unexposed workers. Updates of the cohort increased its size to 733 exposed workers (Hogstedt *et al*, 1986, Hogstedt, 1988). In this analysis eight cases of leukaemia were observed, with 0.8 expected. This resulted in a SMR of 10.0 (95%CI=4.32-19.7).

There have been several other studies in different countries in Europe including in the UK and a large cohort study in the USA (tabulated in IARC 2008a). Coggon *et al*. (2004) extended to 2000 the follow-up of cancer risk among 2,876 men and women with definite or potential exposure to EO in the chemical industry or in hospital sterilising units. There were only five leukaemia deaths (SMR=1.08, 95%CI=0.35-2.51), four among chemical manufacturers (SMR=1.41, 95%CI=0.39-3.62) and one in hospitals (SMR=0.55, 95%CI=0.01-3.06). Among the chemical workers all of the deaths were workers with definite exposure to EO (SMR=2.29, 95%CI=0.62-5.85), and those in hospital were among workers with continual exposure (SMR=1.08, 95%CI=0.03-5.99).

Shore and colleagues published a meta-analysis of a number of cohorts (Shore *et al*, 1993), and obtained a meta-SMR of 1.06 (95%CI=0.73-1.48), based on 31 leukaemia deaths among 29,800 workers. In 1999 Teta and her colleagues (1999) published an update of this meta-analysis, including 17 studies of ten unique cohorts of nearly 33,000 exposed workers with more than 800 cancers (mostly deaths, but some incident cases) (Teta *et al*, 1999). The cancer meta-SMR was 0.94 (95%CI=0.85-1.05), and that for leukaemia (based on 35 observed cases) was 1.08 (95%CI=0.61-1.93). However, for leukaemia, if the Swedish studies of Hogstedt were removed, the meta-SMR was reduced to 0.95 (95%CI=0.64-1.35). The Hogstedt study was excluded in the second analysis because it accounted for the largest amount of heterogeneity in leukaemia risk.

In an update of the largest cohort, 18,235 US workers were followed-up through 1998 (Steenland *et al*, 2004). In total there were 2,852 deaths, 860 from cancer (SMR=0.92, 95%CI=0.86-0.98). There were a total of 29 leukaemia deaths resulting in an SMR of 0.99 (95%CI=0.71-1.36). A negative exposure-response relationship with cumulative exposure (ppm-days) was observed: 0-1199 RR= 1.15 (0.55-2.11); 1200-3679 RR= 1.06 (0.39-2.31); 3680-13499 RR= 0.93 (0.34-2.02); 13500+ RR= 0.43 (0.09-1.26). A significant SMR was found for non-Hodgkin's lymphoma (NHL) for a cumulative exposure of 13500+ ppm-days (SMR=2.37 (95%CI 1.02-4.67)). Internal analyses using Cox's regression found positive trends for all lymphohaematopoietic cancers with cumulative exposure, which were limited to males (15-year lag): 0 RR= 1; >0-646 RR= 1.23 (0.32-4.73); 647-2779 RR= 2.52 (0.69-9.22); 2780-12321 RR= 3.13 (0.95-10.37); 1232+ RR=3.42 (1.09-10.73). The trend was driven by lymphoid tumours (non-Hodgkin's lymphoma, myeloma, lymphocytic leukaemia): 0 RR= 1; >0-646 RR= 0.90 (0.16-5.24); 647-2779 RR= 2.89 (0.65-12.86); 2780-12321 RR= 2.74 (0.65-11.55); 1232+ RR=3.76 (1.03-13.64). An earlier publication from this cohort gives the average duration of exposure as approximately 5 years (Stayner *et al* 1993). So the highest cumulative exposure group is approximately 13500/(5X365) ppm i.e. 7.4ppm or 13.3mg/m<sup>3</sup> (1ppm=1.8mg/m<sup>3</sup>).

The only positive associations reported were in males and only after a 15 year lag, following subgroup analyses. Meta-analyses of an appreciable number of studies failed to find a significant association between exposure to ethylene oxide and an increased risk of cancer. Hence, the maximum upper bound estimate of excess risk of cancer on exposure to ethylene oxide was calculated using the results of the meta-analysis of Teta *et al* (1999). The 95% CIs with and without exclusion of the Hogstedt study, which was very heterogeneous relative to the other studies, were used. These were respectively, 1.35 and 1.93.

### Comparison of animal and human data

#### Animal

POD (lung tumours, female A/J mice) = 16.0 mg/m<sup>3</sup> 6 h/day, 5 days/week for 6 months, equivalent to an average daily exposure of 2.86 mg/m<sup>3</sup> for 6 months.

POD (MCL, female Fischer F344 rats) = 22.5 mg/m<sup>3</sup> 6 h/day, 5 days/week for 2 years, equivalent to an average daily exposure of 4.02 mg/m<sup>3</sup> for 2 years.

Dose-response: assumed to be linear

Dose for MOE of 10,000, i.e. 1 in 10<sup>5</sup> excess incidence: 0.29 µg/m<sup>3</sup> (lung) and 0.40 µg/m<sup>3</sup> (MCL).

#### Human

Using Teta et al 1999, upper 95% CI = 1.93 (all studies) and 1.35 (Hogstedt study omitted) for leukaemia. Exposure was estimated as 13.3 mg/m<sup>3</sup> from Stayer et al (1993).

Background incidence of leukaemia: in US: 12.1/100,000 men, 7.9/100,000 women, 9.9/100,000 men+ women (GLOBOCAN, 2008)

Maximum excess risk associated with ethylene oxide is 0.93 x 9.9 per 100,000, i.e. 9.2 per 100,000 (all studies) and 0.35 x 9.9 per 100,000, i.e. 3.5 per 100,000 (Hogstedt study omitted) at 13.3 mg/m<sup>3</sup>

Hence, 1 in 100,000 risk associated with exposure to 1.45 mg/m<sup>3</sup> (all studies) and 3.8 mg/m<sup>3</sup> (Hogstedt study omitted).

Human data	Value	Animal data	Value
Study authors	Teta et al, 1999	Study authors	Adkins et al, 1986 (mouse) Snellings et al, 1984 (rat)
Tumour type	Leukaemia	Species/strain/sex	Mouse - A/J – female Rat – Fischer - female
Relative risk (± 95%CI)	All studies: 1.08 (0.61-1.93) Hogstedt study omitted*: 0.95 (0.64-1.35)	Tumour site	Mouse: lung Rat: mononuclear cell leukaemia (MCL)
Upper bound relative Risk (95% CI)	All studies: 1.93 Hogstedt study omitted: 1.35	Route/duration	Inhalation Mouse: 6 h/day, 5 days/week; 6 months Rat: 6 h/day, 5 days/week; 2 years
Exposure estimate	13.3 mg/m <sup>3</sup> (Stayer et al, 1993)	BMD10 range (mg/m <sup>3</sup> )	Mouse: 23.26- 41.44 Rat: 35.47-84.90
Exposure range		Lowest BMD10 (mg/m <sup>3</sup> )	Mouse: 23.26 Rat: 35.47
Exposure route	Inhalation	BMDL10 range (mg/m <sup>3</sup> )	Mouse: 16.05-32.07 Rat: 22.54-66.70
Converted dose (A)	13.3 mg/m <sup>3</sup>	Lowest BMDL10 (mg/m <sup>3</sup> )	Mouse: 16.05 Rat: 22.54
Background incidence M/F/both	12.1/7.9/9.9 per 10 <sup>5</sup>	Model for lowest BMDL10	Mouse: Multi-stage cancer (1 <sup>st</sup> order)

			Rat: Log logistic
Excess risk (RR-1)	All: 0.93 - Hogstedt: 0.35	Dose conversion	Mouse: /24*6 (h/d) /7*5 (d/wk) Rat: /24*6 (h/d) /7*5 (d/wk)
Excess cancer number (Background x [RR-1]) (B)	All: 9.21 per 10 <sup>5</sup> - Hogstedt: 3.47 per 10 <sup>5</sup>	Dose for cancer excess of 1 in 10 <sup>5</sup> (BMDL10/10,000)	Mouse: 0.29 µg/m <sup>3</sup> Rat: 0.40 µg/m <sup>3</sup>
Dose for cancer excess of 1 in 10 <sup>5</sup> (A/B * 10 <sup>-5</sup> )	All: 1.44 mg/m <sup>3</sup> - Hogstedt: 3.83 mg/m <sup>3</sup>	Convert to oral (Mouse: 0.035 m <sup>3</sup> air/d, 30 g); Rat: 0.223 m <sup>3</sup> air/d, 350 g)	Mouse: 0.338 µg/kg bw/d Rat: 0.255 µg/kg bw/d
Convert to oral (20 m <sup>3</sup> air/d, 70 kg)	All: 0.41 mg/kg bw/d - Hogstedt: 1.09 mg/kg bw/d		
Dose in humans/dose in animals associated with excess risk of 1 in 10 <sup>5</sup> = All - Mouse: <b>5000</b> (inh), <b>1200</b> (oral); All – Rat: <b>3600</b> (inh), <b>1600</b> (oral) -Hogstedt - Mouse: <b>13,200</b> (inh), <b>3200</b> (oral); -Hogstedt – Rat: <b>9,600</b> (inh), <b>4300</b> (oral)			

\*This showed considerable heterogeneity relative to the other studies

### Conclusion

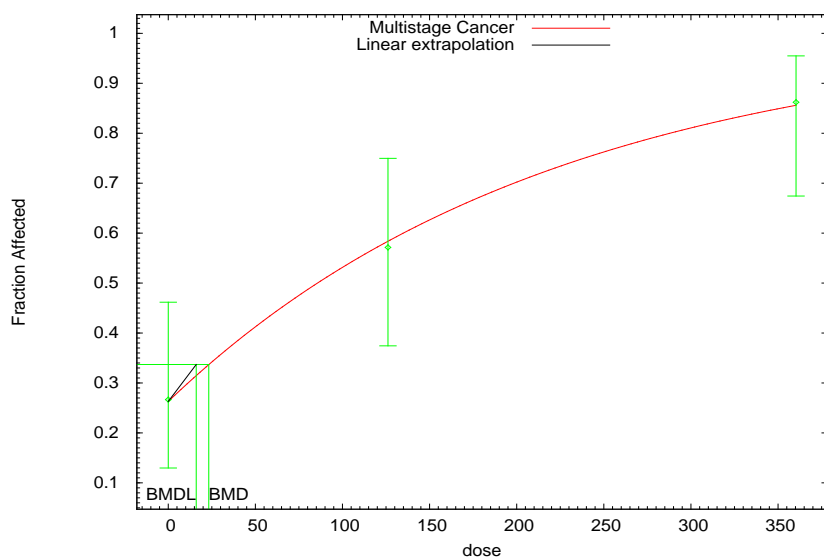
The dose associated with 1 in 100,000 excess risk based on animal data is approx. 5000 (lung) and 3600 (MCL) times less than that giving the upper bound excess risk for leukaemia in humans (all studies) and approx. 13,000 (lung) and 9,500 (MCL) times (Hogstedt study omitted). This supports confidence that the MOE cutoff of 10,000 would be adequately protective for ethylene oxide and shows that the predicted excess risk in humans is much greater than the actual risk. Note that as the comparison is for exposure by the inhalation route, an MOE of 3,000 may be more appropriate as the cutoff for a level of concern, based on the normal inter-species extrapolation factor of 3 for this route, rather than 10 as for the oral route.

### Uncertainties

Data for leukaemia were used for the calculations but the target site, if any, for the carcinogenicity of ethylene oxide is not known. The background incidence for the US was used in the calculations, but the studies used in the meta-analysis were from a number of countries, adding some uncertainty to the estimates. The background incidence will vary with tumour type and hence influence the population attributable upper bound excess risk. There is some uncertainty in the relative risk estimates, with one study showing considerable heterogeneity compared to the others. The upper 95% CI was used in the calculations to provide an upper bound estimate of any excess risk. Exposure was based on a large number of observed industrial hygiene measurements and a validated model to estimate past exposures.

Additional uncertainties included the choice of the most sensitive species, strain, sex and tumour site for dose-response modelling. The lung and haematopoietic system were the target sites chosen in rats and the epidemiological data were for tumours of the haematopoietic system. A BMR of 10% was chosen for determination of the POD. The lowest BMDL10 value from amongst the acceptable models was used for the calculations. The dose-response relationship between the POD and human exposure levels was assumed to be linear.

Multistage Cancer Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for th



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Multistage cancer (first order) model for ethylene oxide (data for lung tumours in mice) (Adkins et al (1986))

## Tamoxifen

### Introduction

Tamoxifen has been available since the 1970s for the treatment of metastatic breast cancer in postmenopausal women. It is used to reduce the risk of invasive breast cancer following surgery and radiation therapy. It is also currently being considered as a chemopreventive agent to reduce the risk of breast cancer in high risk women. Side effects include hot flushes, sweats, nausea and weight gain and potentially an increased risk of thrombosis.

### Animal data

The rat appears to be the most sensitive animal species to the carcinogenic effects of tamoxifen (IARC, 2012f). The primary site in animals for tumours arising from a genotoxic mode of action is the liver; tumours may also occur at other sites including the mammary gland and endometrium, but this is through a hormonal mode of action. The most appropriate and suitable data sets for dose-response modelling were those from the studies of Hard et al (1993) and Greaves et al (1993). Male Alderley, Wistar-derived rats (Greaves et al, 1993) were most sensitive from amongst those studied in these investigations. In this study, groups of male Alderley rats were administered daily doses of 0, 5, 20 and 35 mg/kg tamoxifen citrate (doses expressed as tamoxifen base) by gastric intubation as a suspension in 0.5% hydroxypropyl methylcellulose in 0.1% aqueous polysorbate 80 for 2 years. Tamoxifen was associated with increases in the incidences of hepatocellular adenomas and hepatocellular carcinomas, as well as a small increase in the incidence of hepato/cholangiocellular carcinoma. As data on the combined incidences of adenomas and hepatocellular carcinomas was not provided, modelling of only the latter was undertaken. The dose-response for adenomas in males was not suitable for modelling separately.

These data gave a range on BMDL10 values for acceptable models from 1480 to 3780  $\mu\text{g}/\text{kg}\text{-bw}/\text{day}$ . As recommended by EFSA (2009), the lowest value was used to calculate the MOE, i.e. 1480  $\mu\text{g}/\text{kg}\text{-bw}/\text{day}$  (log-logistic model; BMD10 = 3010  $\mu\text{g}/\text{kg}\text{-bw}/\text{day}$ ).

### *Human data*

There have been a large number of cohort studies, case-control studies and randomised controlled trials investigating the potential carcinogenic effects in humans of tamoxifen (summarised by IARC). The most consistent effect, found in many studies is an increased risk of endometrial cancer among women with breast cancer, with many risk estimates being more than doubled. The results from studies investigating gastrointestinal cancers have varied with most studies not reporting statistically significant excesses of oesophageal, stomach or colorectal cancer in breast cancer patients treated with tamoxifen compared with those not treated. However, one study found a borderline excess for colorectal cancer after 5 years use (SIR=1.47, 95%CI 1.00-2.15) (Newcomb et al 1999) and 2 other studies showing significant excesses from stomach cancer compared with the general population (the IARC working group thought the latter might be biased and that comparison with a non-treated group was preferable). In a meta-analysis (Braithwaite et al 2003), tamoxifen was associated with significantly increased risks of gastrointestinal cancers (16 trials) with a RR-1.31 (95%CI 1.01-1.69).

Tamoxifen has been associated with rare instances of idiosyncratic, clinically apparent liver injury, typically arising within the first six months of treatment and having variable presentations with cholestatic, mixed or hepatocellular pattern of enzyme elevations. Immunoallergic features (fever, rash, eosinophilia) are uncommon, as are autoantibodies. Some instances have been severe with signs of hepatic failure, but most cases are self-limited. Long term tamoxifen therapy has also been linked to isolated cases of peliosis hepatis, hepatic cysts. Very few studies give results for liver cancer and these are largely negative and based on very small numbers of cases: Davies et al (2013) RR=0.99 (95%CI 0.20-4.90); Vogel et al (2006) 20mg/d RR=0.96 (95%CI 0.56-1.64); Rutqvist et al (1995) 40mg/d RR=3.3 (95%CI 0.92-12.1).

### *Comparison of animal and human data*

#### Animal

POD = 1480 µg/kg-bw/day

Dose-response: assumed to be linear

Dose for MOE of 10,000, 1 in 10<sup>5</sup> excess incidence: 0.15 µg/kg-bw/day

#### Human

Maximum excess risk associated with tamoxifen (upper 95% CI for liver cancer in females):

4.90 at 20 mg/day = 0.33 mg/kg bw/day (assuming average body weight of 60 kg) (Davies et al, 2002)

1.64 at 20 mg/day = 0.33 mg/kg bw/day (Vogel et al, 2006)

12.1 at 40mg/day = 0.67 mg/kg bw/day (Rutqvist et al, 1995)

Background age-adjusted incidence of liver cancer in Europe = 2.2/100,000 in women (GLOBOCAN, 2008).

Hence upper bound excess risk associated with tamoxifen is:

3.9 x 2.2 per 100,000, i.e. 8.6 per 100,000 at 333 µg/kg bw/day (Davies et al, 2002) = 1 in 100,000 at 38.7 µg/kg bw/day

0.64 x 2.2 per 100,000, i.e. 1.4 per 100,000 at 333 µg/kg bw/day (Vogel et al, 2006) = 1 in 100,000 at 238 µg/kg bw/day



11.1 x 2.2 per 100,000, i.e. 24.4 per 100,000 at 667 µg/kg bw/day (Rutqvist et al, 1995) = 1 in 100,000 at 27.3 µg/kg bw/day

Human data	Value	Animal data	Value
Study authors	i. Davies et al, 2002 ii. Vogel et al, 2006 iii. Rutqvist et al, 1995	Study authors	Greaves et al, 193
Tumour type	Liver (HCC)	Species/strain/sex	Rat/Alderley Wistar/male
Relative risk (± 95%CI)	i. 0.99 (0.20-4.90) ii. 0.96 (0.56-1.64) iii. 3.3 (0.92-12.1)	Tumour site	Liver (HCC)
Upper bound relative Risk (95% CI)	i. 4.90 ii. 1.64 iii. 12.1	Route/duration	Oral (gavage)/2 years
Exposure estimate	i. 20 mg/day ii. 20 mg/day iii. 40 mg/day	BMD10 range (mg/kg)*	2.71-4.57
Exposure range		Lowest BMD10 (mg/kg)	2.71
Exposure route	Oral	BMDL10 range (mg/kg)	1.48-3.78
Converted dose (A)**	i. 0.33 mg/kg bw per day ii. 0.33 mg/kg bw per day iii. 0.67 mg/kg bw per day	Lowest BMDL10 (mg/kg)	1.48
Background incidence M/F/both	F: 2.2 per 10 <sup>5</sup>	Model for lowest BMDL10	Log logistical
Excess risk (RR-1)	i. 3.90 ii. 0.64 iii. 11.1	Dose conversion	None
Excess cancer number (Background x [RR-1]) (B)	i. 8.58 per 10 <sup>5</sup> ii. 1.41 per 10 <sup>5</sup> iii. 24.42 per 10 <sup>5</sup>	Dose for cancer excess of 1 in 10 <sup>5</sup> (BMDL10/10,000)	0.15 µg/kg bw per day
Dose for cancer excess of 1 in 10 <sup>5</sup> (A/B * 10 <sup>-5</sup> )	i. 38.8 µg/kg bw per day ii. 236 µg/kg bw per day iii. 27.3 µg/kg bw per day		
Dose in humans/dose in animals associated with excess risk of 1 in 10 <sup>5</sup> = Davies et al, 2002(i): <b>260</b> Vogel et al, 2006 (ii): <b>1570</b> Rutqvist et al, 1995 (iii): <b>180</b>			

\* Doses converted from tamoxifen citrate to tamoxifen base equivalents

\*\*Assume average female body weight of 60 kg

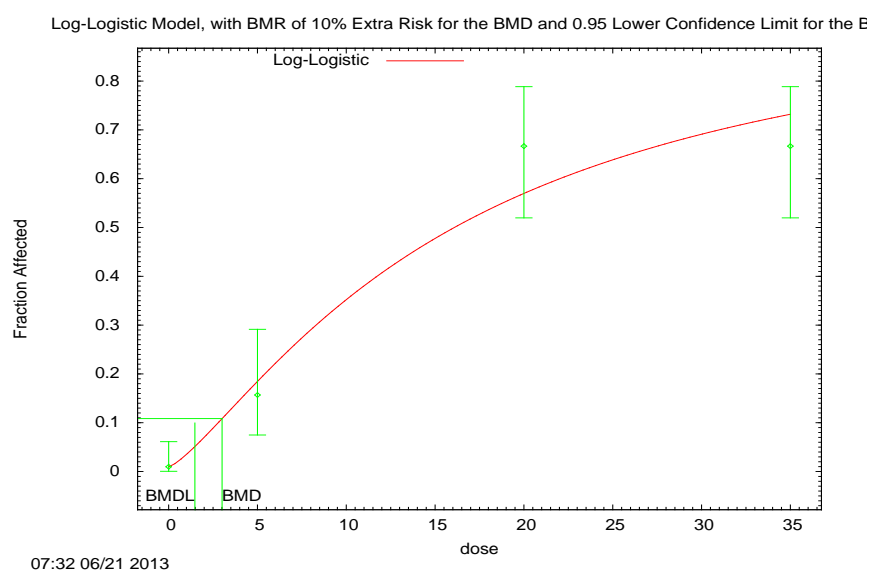
### Conclusion

The dose associated with 1 in 100,000 excess risk based on animal data ranges from 180 - 1600 times less than that giving this excess risk in humans, as an upper bound. This supports confidence that the MOE cutoff of 10,000 would be adequately protective for tamoxifen and shows that the predicted excess risk in humans is much greater than the actual risk.

### Uncertainties

The cohorts studied were relative small, resulting in wide confidence intervals. The upper 95% CI was used in the calculations, and this varied appreciably amongst the available studies, leading to additional uncertainty. It is assumed that liver is the potential target tissue in humans, but site concordance for genotoxic carcinogens is uncertain. As the background cancer rate varies with site and tumour type, this introduces a degree of uncertainty into the upper bound risk estimate.

Additional uncertainties included the choice of the most sensitive species, strain and sex for dose-response modelling. The liver was the target site chosen in both rats and humans, as this is the tissue for which there is the strongest evidence for carcinogenicity in rodents by a genotoxic mode of action. A BMR of 10% was chosen for determination of the POD. The lowest BMDL10 value from amongst the acceptable models was used for the calculations. The dose-response relationship between the POD and human exposure levels was assumed to be linear.



Log-logistic model for tamoxifen (data for hepatocellular carcinoma in males (Greaves et al, 1993))

### Summary and Overall Conclusions

In this part of the project we aimed to use existing epidemiological data to analyse the relationship between margins of exposure and human cancers, and to estimate upper bounds for the incidences of unrecognised chemical-induced cancers.

In 4.1 we selected four case carcinogens and obtained data on risk estimates associated with specific exposure levels in human studies and used data from animal studies to derive BMD10 values and hence the POD. The lowest acceptable BMDL10 value was used to estimate the exposure associated with a 1 in  $10^5$  risk (i.e. that associated with an MOE of 10,000), assuming a linear relationship between exposure and response. This was compared with the exposure from a human study for an excess cancer incidence of 1 in  $10^5$ .

The dose associated with  $1 \times 10^5$  increase in risk based on animal data was much less for aflatoxin (20 times) and VCM (8000 times), slightly less for benzidine at high human exposure (4 times) but similar to that giving this excess risk in humans for benzidine at low human exposure and for chromium.

In 4.2 we evaluated IARC group 1, 2a and 2b carcinogens to identify chemicals for which there is good evidence that they cause carcinogenicity in experimental animals by a genotoxic mode of action and for which there were suitable experimental data to enable estimation of the BMDL10 and epidemiological information on exposed populations but for which there is little or no association with any increase in the background incidence of cancer in humans. Epidemiological data was retrieved and used to estimate upper bounds for the incidences of any unrecognised chemical-induced cancers. The upper 95 % confidence interval was used to estimate the lower bound of an exposure that could be associated with an increase in the background incidence of cancer (the cancer type with the highest background incidence was used) in humans of 1 in  $10^5$ . In many cases, suitable data for this exercise were not available. The compounds for which adequate data could be retrieved were acrylamide, ethylene oxide and tamoxifen (liver cancer only).

For ethylene oxide and tamoxifen the dose associated with 1 in 100,000 excess risk based on animal data is much less than that giving this excess risk in humans, approximately 180-1600 times for tamoxifen and for ethylene oxide 5000 (lung) and 3600 (MCL) for leukaemia. This supports confidence that the MOE cutoff of 10,000 would be adequately protective for both tamoxifen and ethylene oxide and shows that the predicted excess risk in humans is much greater than the actual risk. However for acrylamide the dose associated with 1 in 100,000 excess risk based on animal data is 30 times that giving the worst case upper bound for excess risk in humans. This, together with the very low margins of exposure reported by Bolger et al (2010) for exposure to acrylamide in high and average consumers (40 and 160, respectively), emphasises the uncertainty in the possible risk to humans from exposure to acrylamide.

These results generally provide some confidence that the MOE cutoff of 10,000 would be adequately protective for several of the case carcinogens. Uncertainties in the data, e.g. from poor exposure assessment in human studies, heterogeneity in both animal and human studies, differences in routes of exposure and target organs between animal and humans etc. are unlikely to greatly affect this conclusion. However, for benzidine at low human exposures and chromium the doses associated with  $1 \times 10^5$  increase risk were similar for animal and humans. For acrylamide, there is appreciable uncertainty about the adequacy of the MOE cutoff of 10,000. The available information was uninformative in this respect. Overall, the uncertainties in the data and assumptions made in our estimates may, however, affect these conclusions. For example, inaccuracy of the exposure estimates in the human studies might lead to an under- or over-estimate of the dose associated with the relative risk. We have highlighted the inadequacies of the data generally available and this generally hampers quantitative assessment of the impact of the sources of uncertainty. For these three chemicals our results indicate that more detailed evaluation may be warranted.

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