Annex 5

FSA Project: T01051

Annex 5 (Objective 3, part 2.2)

Report of Second Expert workshop on differences in low-dose response relationship between various classes of genotoxic carcinogens (21st – 22nd March 2013)

Further detailed analysis of the findings of the Second Expert Workshop are currently underway (28 November, 2013) and will be submitted for publication in a peer reviewed journal.

The March 2013 workshop[1] built upon the conclusions of the May 2011 workshop, which employed expert elicitation to capture expert opinion on the general nature of low dose-response relationships for genotoxic carcinogens (see summary of first expert workshop in this report; Boobis et al, 2013 [Annex 4]). The decision on the need to adopt either a linear low-dose response extrapolation (US practice) or an MOE approach (EU practice) for a substance depends heavily on information available on its mode of action (MOA). In particular the interpretation of such information dictates whether the low-dose response of a substance would be expected to have a threshold or could be linear. However, one would need to consider that most MOAs are poorly understood. The question then becomes whether one can use MOA information, in parallel with information on inter-individual and intra-species factors, to reliably group carcinogens and further, to estimate the level of concern more accurately either for each class and/or for each carcinogen, at such low doses.

A two-day expert workshop was held in Fera, Sand Hutton (21st - 22nd March 2013[2]) with the aim of exploring differences in the form of the dose-response curve at human relevant exposures for genotoxic carcinogens where there may be dissimilar modes of action or different modifying factors. Four case studies were selected, to represent different classes of genotoxic carcinogens, in particular: aflatoxin B1, benzo[*a*]pyrene, ethylmethanesulfonate (EMS) and ethylnitrosourea (ENU). Experts were divided into two breakout groups and each group assessed two case studies[3]. Initially, experts were asked to build conceptual models (i.e. flowcharts of causal steps and factors influencing each step, including MOA) for the carcinogenic response in each case study (an example of the conceptual model for aflatoxin B1 and benzo[*a*]pyrene is shown in Figure 1). These models served as a coherent collective platform and facilitated the elicitation of quantitative estimates informing the shape of the dose response curve at human relevant exposures that followed (Figure 2).

Following the elicitation of quantitative estimates, experts assessed relevant uncertainties, whether each compound discussed could be a reliable representative of a particular class of genotoxic carcinogen, and whether more classes, and representative case studies, should be included in order to address coherently all genotoxic carcinogens.

[1] Results are being analysed and a peer-reviewed publication is being prepared

[2] Participants of March 2013 workshop: Prof. Alan Boobis; Dr Sue Barlow; Dr Philip Carthew; Dr Eduardo Cemeli; Dr Kenny Crump; Dr Shareen Doak; Dr Helmut Griem; Dr Gareth Jenkins; Prof Daniel Krewski; Dr John Larsen; Dr David Lovell; Barry Maycock; Dr Franz Oesch; Dr Frances Pollitt; Dr Lorenz Rhomberg; Dr Lesley Rushton; Dr Paul Schlosser; Dr Benjamin Smith;

[3] Breakout group 1 assessed: aflatoxin B1 and benzo[*a*]pyrene; breakout group 2 assessed: ethylmethanesulfonate (EMS) and ethylnitrosourea (ENU).

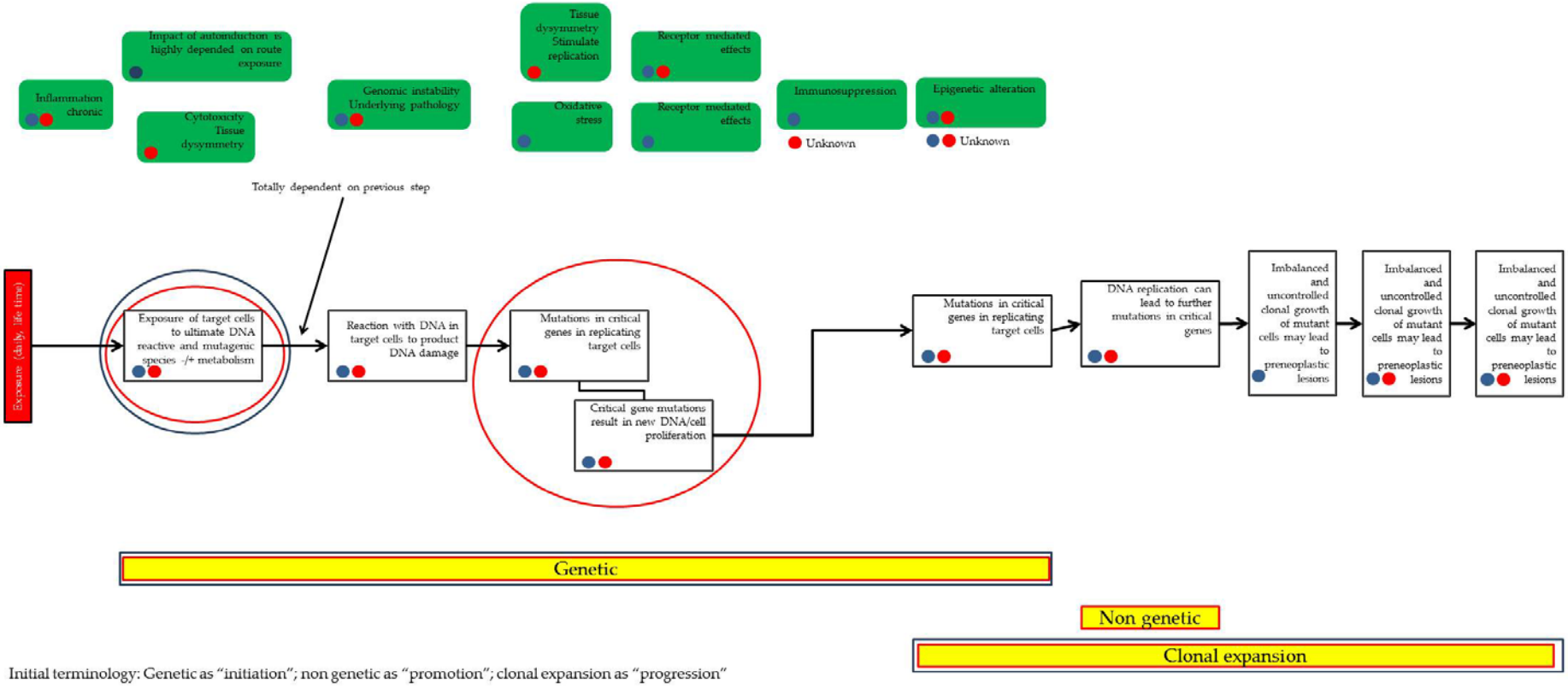


Figure 1: Conceptual model detailing experts' views on the process of carcinogenesis for aflatoxin B1 (red circle) and benzo[*a*]pyrene (blue circle). Main causal steps described in Preston and Williams (2005), apart from the first one, i.e. uptake and distribution within the body, were taken into account. Causal steps (white background rectangles) and major modifying factors (rectangles highlighted in green) are mapped in sequence for the two compounds (relevance with for aflatoxin B1 and benzo[*a*]pyrene indicated by red and blue dots, respectively). Most important key causal steps for either compound are circled in red and blue, respectively. Experts built the conceptual model at the start of the workshop. This process allowed experts to disseminate, document and share their knowledge and provided a coherent view of carcinogenesis, and facilitated the elicitation of quantitative estimates of linear low dose extrapolation for either compound. Three key mechanistic biological steps were recognised to underlie the carcinogenesis process: (a) genetic changes, initially thought of as "initiation short term"; (b) non-genetic (epigenetic) changes (e.g. methylation of DNA; could be reversible unlike the genetic changes), initially thought of as "promotion longer term"; (c) clonal expansion (includes proliferation of similar types of cells and selective proliferation), initially thought of as "progression", although this view was not necessarily held by all experts.

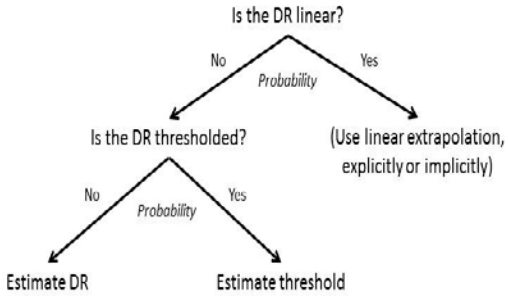


Figure 2: Individual probabilities were elicited of whether (a) the dose-response is linear at human relevant exposures, and (b) there is a threshold for each compound assessed. Thereafter, experts' individual quantitative estimates were elicited of (a) exposures that would cause additional cancers in the human population (i.e. 1 in 10,000; 1 in 100,000; 1 in 1,000,000), and (b) a threshold. Experts disseminated the rationale underlying their estimates.

Research gaps and uncertainties were flagged during group discussions held in the breakout groups[4], and experts emphasised that quantitative estimates elicited reflect mainly one or a small number of key events and considerations. The experts also emphasised that further consideration of all available evidence may lead to revised estimates, and therefore the elicited quantitative estimates should be perceived rather as "starting points" than final points. Linearity of dose response at human relevant exposures was questioned in all case studies (Figure 3). For aflatoxin B1 there was agreement that the dose-response would likely deviate sub-linearly from the POD to human relevant exposures, and for benzo[*a*]pyrene experts had a mixed view: experts agreed that there would be differences depending on the particular route of exposure (i.e. oral or inhalation) and that there would be some deviation from linearity; however, there was uncertainty as to how much (Figure 3). Experts in this group thought that most probably there is no threshold either for aflatoxin B1 or for benzo[*a*]pyrene. Experts in the group that assessed EMS and ENU had more diverse views regarding the linearity of dose-response at human relevant exposures (Figure 3), and they thought that it is more probable that EMS is thresholded than EMU. Regardless of the compound concerned, there was significant variation amongst experts in their quantitative estimates (Figure 4).

Experts agreed that there is something more than just DNA reactivity that drives carcinogenicity, and rephrased the question "Representativeness of selected case studies?" to "Should the same MOE (possibly different than 10,000) be used for all genotoxic carcinogens or are they too diverse for this?" It was suggested to take a range of genotoxic carcinogens for which there was evidence for deviation from linearity below the POD and determine the minimum deviation from linearity, if the difference between compounds was not too great, to determine a suitable MOE for such compounds. Otherwise one would have to create a number of different groups of genotoxic carcinogens or treat each compound individually on a case-by-case basis. It appeared that each case study was unique, and that there is a lot of uncertainty on "how far off" from linearity and chemical specific information is needed for each to decide on what level of MOE would raise concern – an open question from the

[4] E.g. Aflatoxin B1: Not all of relevant data were available to the group for systematic review; lack of a good concordance study that would address (a) cytotoxicity, (b) genotoxicity, (c) carcinogenicity (i.e. endpoints should be assessed in animal studies; for example, what is POD for cytotoxicity for aflatoxin B1?); Kinetics by different routes of exposure (target tissue exposure); Quantitative information on species differences and critical metabolic routes. Benzo[*a*]pyrene: Data : What enzymes are induced, in which tissue, at what time and at what dose? Clear definition of (a) time, (b) dose, (c) route dependency of effects? Lack of high quality dose-response data at low doses for both oral and inhalation routes? Physiology: Kinetics in target tissue unknown; what is the contribution of other mechanisms beyond genotoxicity? High dose problem – but what is the impact on POD –what is implication for low dose extrapolation? What effects are apparent below POD? What effects are not apparent below POD?

workshop was whether the carcinogenic process is too complex to group genotoxic carcinogens or whether it would be possible to create small groups of genotoxic carcinogens.

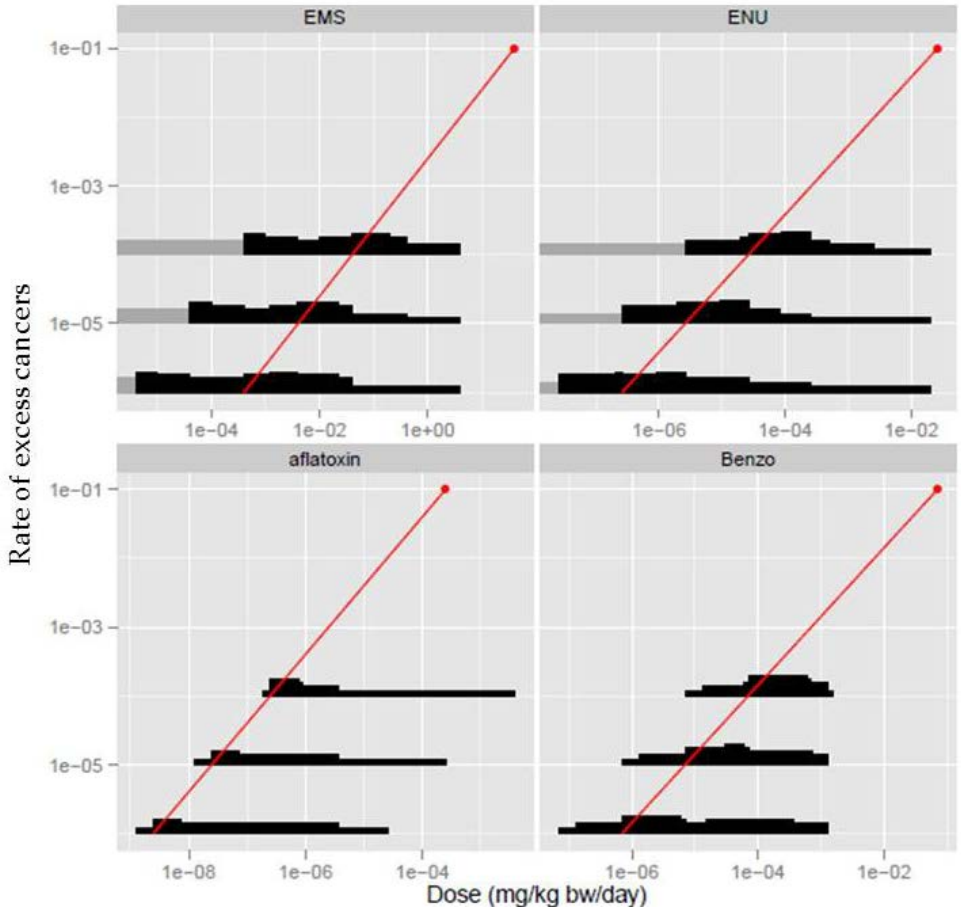
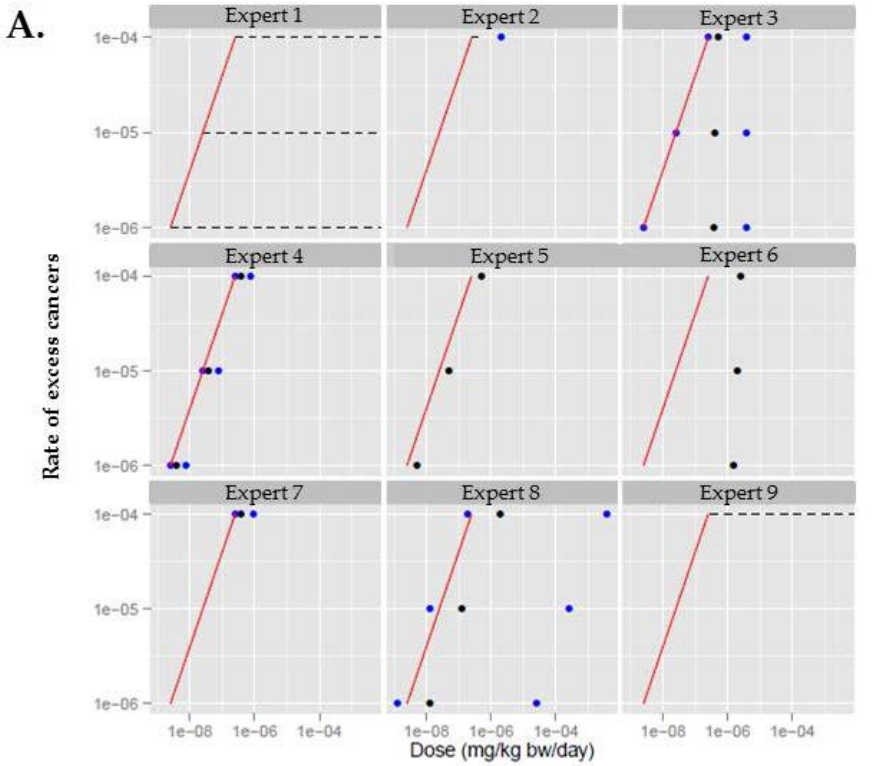
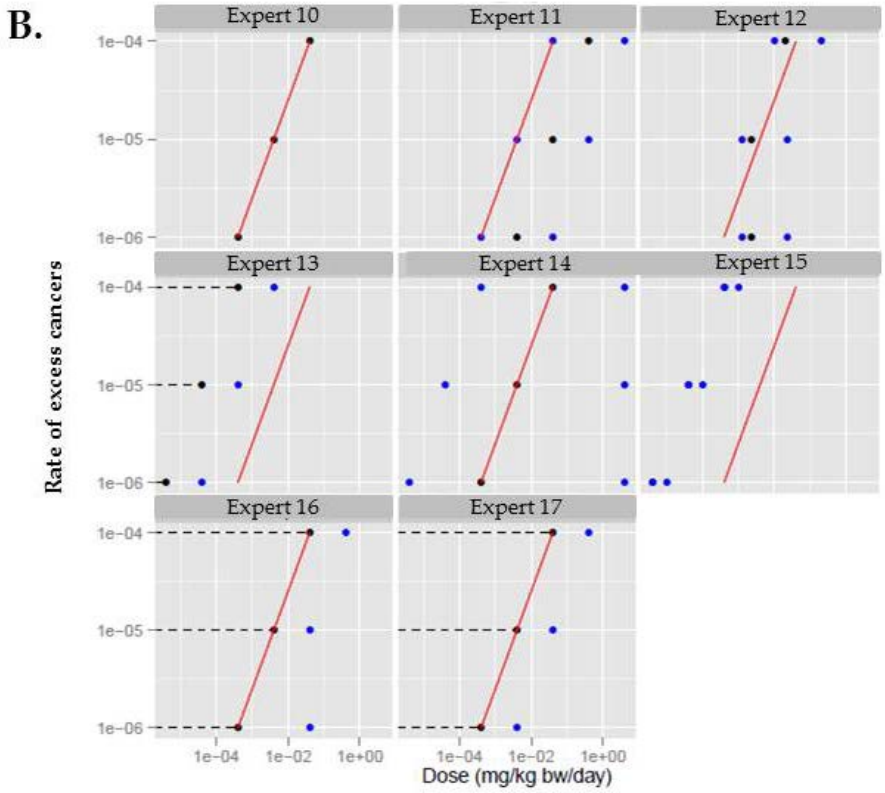


Figure 3: Summary of 90% individual expert ranges (width of horizontal black bars) for the case studies assessed at the 2nd workshop. Diagonal red line indicates the linear extrapolation from POD (red dot). Histograms' height is indicative of the number of experts who believed that a particular exposure could lead to a specified number of additional cancer cases (shown on the Y-axis). The lowest height shown in the graph indicates the contribution from one expert. The horizontal grey shading shown for EMS and ENU denote instances where an expert (or multiple experts) gave 0 as the lower end of their 90% range for the dose leading to the specified excess risk. The extent of agreement amongst the experts varied depending on the compound concerned, and their individual estimates differed.

A number of key issues were identified to take into account for a future strategy: (a) several processes are ongoing in carcinogenicity – is the POD a poor index to represent them all? (b) compare POD in the *in vivo* studies to that in *in vitro* studies to find a scaling factor that would enable account to be taken of key factors in order to modify the MOE; (c) how will it be possible to avoid the possibility of being very under (or over) predictive?; (d) nature and determinants of dose-response at low doses is largely unknown; there are many factors that might cause an increase or decrease in response; (e) candidate key processes to group genotoxic carcinogens, e.g. repair, saturation of metabolic steps, autoinduction?; (f) impact of these key processes on deviation from linearity; (g) biomarkers of key events; (h) metabolism – PBPK principles.


A.
Expert 1
Expert 2
Expert 3
Expert 4
Expert 5
Expert 6
Expert 7
Expert 8
Expert 9
Rate of excess cancers
1e−04
1e−05
1e−06
1e−08
1e−06
1e−04
Dose (mg/kg bw/day)


B.
Expert 10
Expert 11
Expert 12
Expert 13
Expert 14
Expert 15
Expert 16
Expert 17
Rate of excess cancers
1e−04
1e−05
1e−06
1e−02
1e+00
Dose (mg/kg bw/day)

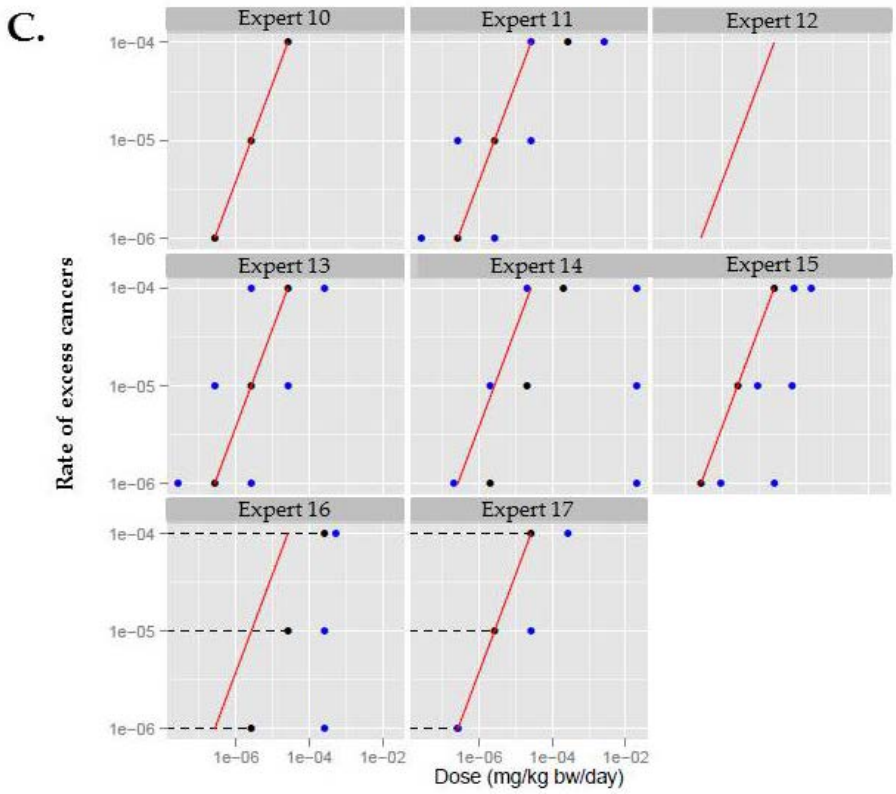


Figure 4: Individual quantitative estimates of exposure (dose in mg/kg BW/day) that could lead to additional cancers in the general human population for aflatoxin B1 (A), ethylmethanesulfonate (EMS) (B), and ethylnitrosourea (ENU) (C). Red line indicates the linear extrapolation from PoD of each compound. We elicited the median (50% quantile; shown in black dot) and 5% and 95% quantiles (shown in blue dots). Data for benzo[*a*]pyrene are currently being re-analysed (28/11/13).

Reference

Preston RJ and Williams GM (2005). DNA-reactive carcinogens: mode of action and human cancer hazard. *Crit Rev Toxicol*, 35, 673-683