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**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD,  
CONSUMER PRODUCTS AND THE ENVIRONMENT**

**Horizon scanning exercise 2006**

Introduction

1. Members will wish to consider topics in the horizon scanning exercise conducted by the secretariat and DH Toxicology Unit and decide whether any of them merits a fuller review.
2. A limited literature search was undertaken using Medline and Toxline from 2005 to September 2006 based on the search terms: carcinogen\*, chemical\*; risk, and mechan\* . This produced 5832 references, the titles of which were scanned quickly, and papers on chemicals and generic areas of carcinogenicity which might contain relevant new information were identified. A short summary of papers of interest is given below. Members and assessors are also invited to suggest topics for further work. A short overview of the outcome of the 2004 horizon scanning exercise is also given below.

Review of the 2004 horizon scanning exercise

3. The topics identified for further work in the 2004 horizon scanning exercise and the outcome are listed in the table below. Members are asked whether they would still support further work on Hodgkin's lymphoma, mbreast cancer, hormesis and/or computational systems biology.

<b>Topic</b>	<b>Outcome</b>
Age-related differences in risk of carcinogenesis	Considered at the July 2006 meeting. Subject to be kept under review.
Trends in cancer incidence	Non-Hodgkin's lymphoma and testicular cancer identified for review. Testicular cancer considered in 2006 – no strong evidence of chemical causation. NHL review outstanding. Secretariat asked to come forward with a proposal for work on breast cancer: outstanding.
Comparative risk assessment	Supported work on this as a joint COT/COC/COM project. Work ongoing.
Hormesis	Further work desirable but not high priority.
<i>In utero</i> exposures to carcinogens	Considered as part of review of childhood cancer

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Computational systems biology	Suggested that committee should consider whether computational systems biology could be applied to its work. Outstanding.
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### Potential topics identified in the literature search

#### ***Information retrieved on some individual chemicals***

##### *Dibenzo(a,l)pyrene*

4. In 2003 the COC published a statement on dibenzo(a,l)pyrene (DB(a,l)P), which had been detected by air pollution monitoring. The committee concluded that DB(a,l)P should be considered a highly potent genotoxic carcinogen in experimental animals, and was likely to be 10-100 times more potent than benzo(a)pyrene. In a new study, Yu *et al* (2006) administered DB(a,l)P to pregnant mice (15 mg/kg bw by gavage) on day 17 of gestation resulted in significant mortality in the offspring at week 12 due to aggressive T-cell lymphoblastic lymphomas. All mice surviving 10 months after exposure had lung tumours, and some had liver tumours. To assess the role of the aryl hydrocarbon receptor (AHR) in these findings, the authors then crossed B6129SF1/J (AHR responsive) mice with 1291S1/SvIM (AHR non-responsive) mice. Offspring born to non-responsive mothers had greater susceptibility to lymphoma regardless of offspring phenotype. However, when the mother was responsive, an AHR phenotype in the offspring increased mortality 2-fold. The authors report this to be the first demonstration of a transplacental carcinogenic effect of DB(a,l)P.

##### *Formaldehyde*

5. IARC considers that there is “strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde”. This conclusion was tempered because it was not possible to identify a mechanism for leukaemia induction. Golden *et al* (2006) argue against their being a causal relationship for formaldehyde and leukaemia risk on the grounds that there is no evidence to suggest that formaldehyde reaches any target organ beyond the site of contact, no indication that formaldehyde is toxic to the bone marrow, and no credible evidence that formaldehyde induces leukaemia in experimental animals.

##### *Hexachlorobenzene (HCB)*

6. There is conflicting epidemiological evidence on whether environmental exposure to HCB is associated with increased risk of breast cancer. Randi *et al* (2006) report that co-administration of HCB to MNU-treated female rats significantly increased the incidence of mammary tumours. Treatment with HCB alone was associated with increased levels of insulin receptor, insulin-like growth factor-1 receptor and insulin receptor substrate-1 levels whereas treatment with NMU-HCB was associated with decreased insulin-like growth factor-1 receptor levels and insulin receptor substrate-1 phosphorylation. The

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authors claim that the study shows for the first time that HCB is a co-carcinogenic agent in NMU-induced mammary tumours in rats and that the insulin receptor or insulin-like growth factor-1 receptor signalling pathway may be involved in the mechanism of action.

7. What comments do Members have on the above studies and would they wish to carry out an updated review on any of these chemicals?

### ***Studies with transgenic animals***

8. Hovik et al (2005) report that a single oral dose of 90 mg/kg MNU increases lymphoma of the haematopoietic system in the thymus, spleen, liver, lungs and kidney in p53+/- transgenic mice maintained for 13 weeks. The authors suggest that this treatment could be used as a positive control. A series of studies were reported in the same edition of the International Journal of Toxicology which investigate the carcinogenic potential of clofibrate in alternative animal models under investigation by the US International Life Sciences Institute (ILSI) (Nesfield et al, 2005a, 2005b; Torrey et al, 2005a, b and c). The most interesting finding was the lack of carcinogenicity in the Tg.AC mouse when clofibrate was administered via the oral route but a positive finding (cutaneous tumours) when it was administered via the dermal route (at dose levels 3 times the human systemic exposure). Negative findings were reported in the neonatal mouse and in the p53+/- mouse, and positive findings in the RasH2 mouse. What inferences can be drawn from these data?

### ***Toxicogenomics***

9. There have been a number of publications in the past year in the field of toxicogenomics. A brief description of a small number of these studies is provided here, divided into broad sections.

#### ***Studies on Individual chemicals or tumour types***

10. Diodovich et al (2005) investigated gene and protein expressions in cord blood cells following exposure to acrylonitrile, a high volume industrial chemical used primarily in the manufacture of plastics and rubber. Using techniques such as macroarray hybridization analysis, CFU-GM assay and western blotting, their data showed that acrylonitrile modulates some genes implicated in cell differentiation, cell-cycle progression and clonogenic potential of human cord blood cells. Seidel et al (2006) attempted to elucidate dose-response changes in gene expression in the liver of rats following exposure to a known genotoxic carcinogen, 2-acetylaminofluorene (AAF), and a known non-genotoxic carcinogen, phenobarbital (PB). Using a highly focused Clontech Rat Toxicology II microarray (465 genes), hybridised with a 32P-labeled cDNA target, 14 genes were altered by AAF and 18 by PB. Several of the genes that showed modulation of transcriptional activity following AAF and PB treatment displayed an atypical dose-response

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relationship such that the expression at the higher doses tended to be similar to that of control.

11. Kim et al (2006) attempted to characterize the gene expression profile and to identify the major carcinogenic pathways involved in rat peritoneal mesothelioma (RPM) formation following treatment of Fischer 344 rats with *o*-nitrotoluene (*o*-NT) or bromochloroacetic acid (BCA). The expression profile using oligo-arrays of over 20,000 genes was studied in 8 peritoneal mesotheliomas from F344 rats treated with *o*-NT or BCA. A non-transformed mesothelial cell line (Fred-PE) was used as the reference for the expression profile. The 1173 genes that consistently displayed altered expression patterns in both chemical groups and throughout the biological replicates were analyzed using Ingenuity Pathway Analysis (IPA) software to identify signaling pathways involved in the RPM tumourigenesis. The categories that were revealed with large numbers of signature genes included cellular movement, cellular growth and proliferation, cell to cell signalling and interaction, tissue development, cancer and cell death. Other categories included in the global analysis were immune response, cell morphology, cell cycle and lipid and amino acid metabolism. The p38 MAPK, insulin-like growth factor 1 (IGF-1), *Wnt/β-catenin*, and integrin signalling pathways were identified by the IPA analysis. Because the study demonstrated that mesotheliomas in rats were similar to mesotheliomas in humans, at least at the cellular and molecular level, the authors concluded that chemical-induced RPM animal models may be useful for studying of the progression of mesothelioma and for identifying diagnostic markers and/or targets of therapy for the human mesotheliomas.

### *Studies investigating mechanisms*

12. Marin-Kuan et al, (2006) adopted a toxicogenomic approach to study the potential involvement of epigenetic mechanisms in the carcinogenicity of the mycotoxin Ochratoxin A (OTA) under exposure conditions producing a significant incidence of renal tumours without overt toxicity. Gene expression profiles were analyzed in kidney and liver of male Fischer (F-344) rats given dietary OTA over various periods ranging from 7 days to 12 months. Renal tumours were discovered during the last 6 months of the study. OTA-modulated gene expression profiles were obtained in both kidney and liver. The effect on abundance of certain mRNAs correlated with the change in expression of respective proteins. In the kidney, genes involved in xenobiotic metabolism and oxidative stress response were generally down-regulated by OTA, whereas this group of genes was much less modulated in the liver. Other gene classes, such as several enzymes involved in fatty acid metabolism and cytochrome P450, were also selectively down-regulated in the kidney of OTA-treated animals whereas only small changes occurred in expression of genes known to be involved in DNA synthesis and repair or in genes induced as a result of DNA damage and little or no effects were found on expression of apoptosis-related genes. Alterations of gene expression indicating effects on calcium homeostasis and a disruption of pathways regulated by the transcription factors hepatocyte nuclear factor 4 alpha (HNF4 $\alpha$ ) and nuclear factor-erythroid 2-related factor 2 (Nrf2) were observed

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in the kidney but not in the liver. Expression of many transporter genes was down-regulated in the kidney and several genes known as markers of kidney injury, cell regeneration, and oncogenesis were significantly modulated by OTA. The authors conclude that depletion of Nrf2-regulated enzymes is likely to impair the cellular defence potential, resulting in chronic elevation of oxidative stress in the kidney, which could contribute to OTA-mediated carcinogenicity.

13. The rasH2 mouse is a hemizygous transgenic mouse carrying three copies of the prototype human c-Ha-ras gene with its own promoter integrated into the genome in a tandem array. These mice have been used in several 6-month short-term carcinogenicity tests and have shown high and rapid susceptibility to various carcinogens. In order to clarify the mechanisms underlying the enhancement of carcinogenesis in rasH2 mice, Okamura et al (2006) analysed the gene expression profiles and mRNA expression of the transgene and some molecules involved in the Ras pathway in urethane-induced lung tumours in these mice. Comparing data from previous microarray analysis of ethylnitrosourea (ENU)-induced squamous cell carcinomas of the forestomach in rasH2 mice, genes were selected from among those that showed a similar expression pattern in ENU- and urethane-induced tumours to identify the candidate genes responsible for the enhanced carcinogenesis in these mice. Analysis of the mRNA expression of the transgene and some molecules involved in the Ras pathway in urethane-induced lung tumours confirmed the over-expression of the transgene in comparison with its expression in normal lung tissue, similar to that observed in ENU-induced forestomach squamous cell carcinomas. The authors suggest that the overexpression of the transgene plays an important role in the enhanced carcinogenesis in rasH2 mice. The genes related to carcinogenesis or ras-mediated malignant transformation were selected as the candidate genes responsible for the enhanced carcinogenesis in rasH2 mice.

14. Liu et al (2006) explored the molecular mechanism of transplacental arsenic hepatocarcinogenesis following exposure to inorganic arsenic *in utero*. The authors had previously shown that exposure to inorganic arsenic *in utero* produces hepatocellular carcinoma (HCC) in adult male mice. A genome-wide analysis was carried out through the National Center for Toxicogenomics using the Agilent 22K chip arrays. Changes in expression of specific genes of interest were confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis. Total RNA was isolated from samples of control mouse liver, arsenic-exposed nontumorous normal liver, and arsenic-induced HCC taken at autopsy, and subjected to microarray analysis. The expression of 2,010 genes were significantly altered in arsenic-exposed normal liver samples compared with control and 2,540 genes were altered in arsenic-induced HCC. The 22K mouse chip revealed several novel pathways and gene expression alterations associated with arsenic-induced HCC and in arsenic-exposed, non tumorous normal liver samples. A central network role of *c-myc* was seen in this study and is consistent with *c-myc* activation in arsenic-transformed rat liver in other

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studies. The activation of various oncogenes was also evident. Conversely, the tumour suppressor genes BRCA1 and BRCA2 were decreased. The authors suggest that the downregulation of BRCA1 and BRCA2 could play a role in the overexpression of ER- $\alpha$  and ER- $\alpha$ -linked gene expression observed in this model for arsenic carcinogenesis. Western blot analysis confirmed the overexpression of ER- $\alpha$  in adult male liver after *in utero* arsenic exposure. These findings lead to the hypothesis that arsenic could somehow produce estrogenic like effects, possibly directly or indirectly through stimulation of ER- $\alpha$ , thus resulting in tumour formation. Overexpression of ER- $\alpha$  is associated with the feminisation pattern of male liver bearing arsenic induced HCC. Altered expression of genes that encode gender-related metabolic enzymes such as the cytochrome P450 were evident. The authors also demonstrated that genes involved in cell-cycle dysfunction and cell-cell communication may be involved in arsenic carcinogenesis in the liver.

### *Use of toxicogenomics as a predictive tool*

15. Tsujimura et al (2006) conducted gene expression profiling of cultured rat hepatoma (MN1C1) cells exposed to 39 chemicals with the aim of providing a basis for rapid and reliable prediction of carcinogenicity using microarray technology. Their microarray analysis identified a set of genes that differentiated hepatocarcinogens from non-carcinogens and all carcinogens from non-carcinogens, by statistical methods.

16. A large gene expression database built using cDNA microarrays and liver samples treated with over 100 compounds was mined by Nie et al (2006) to determine gene expression signatures for non-genotoxic carcinogens. A semiexhaustive, nonredundant gene selection algorithm yielded six genes which identified non-genotoxic carcinogens with 88.5% prediction accuracy estimated by cross-validation. The authors also found that this six genes signature set predicted non-genotoxic carcinogens with 84% accuracy when samples were hybridized to commercially available CodeLink oligo-based microarrays.

17. In view of the above papers, is there any further work which Members would like to pursue on toxicogenomics, for example: a review of recent developments in methodology, or an examination of its recent use in investigating mechanisms or in predicting carcinogenicity? Would Members would like to consider any of the above papers in more detail?

### ***Thresholds for genotoxic carcinogens***

18. The findings of several recent papers indicate that there may be an effect threshold for at least some genotoxic carcinogens (e.g. NDEA, MeIQx,). These findings are based either on newly acquired experimental data or on the re-analysis of existing dose-response data.

19. Wanibuchi *et al* (2006) exposed groups of F344 rats to increasing doses of the genotoxic liver carcinogen MeIQx in drinking water, either alone

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or with 10% ethanol in drinking water for 16 weeks and assessed GST-P positive foci formation in the liver. In rats treated with MeIQx alone, the number of foci was significantly increased in rats treated with 100 ppm MeIQx whereas in rats co-exposed to ethanol, significant increases in foci formation was observed at both 100 and 10 ppm MeIQx. At all MeIQx doses, foci formation was enhanced in the co-treated rats as compared to the rats exposed to MeIQx alone. Based on the foci results (and supportive findings for cell proliferation and adduct formation), the authors conclude that the study suggests a no effect level for liver foci formation (and, by implication, tumour formation) for MeIQx. Do Members consider that this conclusion is justified? If so, what are the implications of the finding?

20. Waddell (2006) re-analysed the data for 2-acetylaminofluorene (AAF), *N*-nitrosodiethylamine (NDEA), aflatoxins and 30 other chemicals for which National Toxicology Program Technical Reports have been published. As part of the analysis, the author re-expressed the dose data on a logarithmic (Rozman) scale and in units of molecules (rather than mg) per kg per day and concludes that the findings support application of the threshold concept to genotoxic carcinogenesis. In another paper, Waddell and Fukushima (2006) used the same method to re-analyse the data from three previous studies looking at the liver carcinogenicity of NDEA. In addition, the authors evaluated whether expressing the dose data on a “per day” basis or on a “cumulative dose” basis provided better agreement between the dose-response curves from the underlying studies. They concluded that concordance among the NDEA dose-response curves was highest when the data are expressed in terms of cumulative (molecules per kg) dose. Further, the authors report that the threshold doses for DNA adduct and foci formation were below the threshold dose for tumour formation ( $10^{20.3}$  molecules per kg). Other authors have criticised Waddell for using the x-intercept of the log scale plots as the threshold dose. Andersen and Renwick (2005) commented that the choice of the Rozman scale (log-dose from  $10^0$  to  $10^{23}$ ) increases the appearance of a threshold and potentially masks any non-linearity. They also criticise use of administered dose rather than a more biological relevant dose metric (e.g. tissue dose). Would Members wish to consider Waddell’s work in more detail or to seek an independent statistical opinion?

### ***Mechanisms of action***

21. Sielken et al (2005) use dose-response modelling to provide statistical insight into the relative likelihood of different mechanisms of action in cancer dose-response studies. They provide two illustrative examples based on time-to-tumor data on mammary fibroadenoma and adenocarcinoma in female Sprague-Dawley rats using 34 different dose metrics. They found that the usual dose metric based on administered dose had some explanatory ability but not nearly as much as the dose metrics more directly related to hormonal mechanisms of action. Would Members like to review this paper in more detail?

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### ***Potential biomarker: Nitritive DNA damage as a biomarker for carcinogenesis with reference to inflammation***

22. Kawanishi et al (2006) investigated the formation of 8-nitroguanine (8-NG), a nitritive DNA lesion, in a number of models of carcinogenesis (including colonic epithelial cells of mice) and in patients with ulcerative colitis. The authors considered that 8-NG was formed at the sites of carcinogenesis regardless of aetiology. Could 8-NG have a role in the evaluation of inflammation related risk of carcinogenesis, and could it be relevant to the evaluation of environmental chemical exposure?

### ***A possible framework to derive quantitative risk estimates based on cancer epidemiological data***

23. Goldbohm et al (2006) describe a systematic approach for the quantitative risk assessment of carcinogens based on epidemiological rather than animal data. The paper explains the three steps required to derive a risk estimate: Step 1 – selection of suitable data; Step 2 – fitting an appropriate mathematical model to the exposure data to generate a relative risk ratio (RR); Step 3 – methods used to calculate the Excess Lifetime Risk (ELR) in the target population from the estimated RR (the life table approach is thought to produce a more accurate estimate of risk). The paper also notes the importance of estimating the amount of uncertainty, and the use of validation studies to estimate the level of bias, exposure measurement error and confounding.

24. The authors illustrate the above approach by deriving an ELR estimate of lung cancer for workers exposed to hexavalent chromium. It was noted that use of the log-linear model produces RR that were unrealistically high at high exposure levels. The paper concludes that the aforementioned approach is transparent, reproducible and sufficiently flexible such that other forms of epidemiological data, including incomplete data, can be used to derive quantitative risk estimates. The authors hope that this will help increase confidence in using epidemiological data for the risk assessment of carcinogens. What do Members think of this proposal? Could this framework also be applied to existing epidemiological data on environmental carcinogens evaluated by the COC?

### ***Methodological flaws in epidemiological studies linking carcinogen-DNA adduct levels with cancer risk***

25. Members will recall a paper by Okona-Mensah and colleagues (2005) which proposed a novel biomonitoring approach to evaluate the role of high potency PAHs in air pollution-related lung cancer. It was proposed that an occupational study examining specific DNA adducts for dibenzo[a,h]anthracene and dibenzo[a,l]pyrene in nasal cells would help to evaluate the extent to which these high potency PAHs might contribute to the increased risk of lung cancer from air pollution. However, a recent paper published by Rundle (2006) suggests that epidemiological studies which seek to establish

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an association between carcinogen-DNA adduct levels and cancer risk often fail to incorporate fundamental epidemiological principles into their study methods. The paper suggests that several methodological issues must be addressed in any future work if the potential of carcinogen-DNA adducts as a tool in epidemiological research is to be realised.

26. Rundle(2006) identifies a number of methodological flaws in the studies reviewed and concludes that, with the exception of one study, they all fail to provide evidence to show that the use of carcinogen-DNA adduct measurements can implicate environmental exposures in carcinogenesis. It is suggested that studies should allow for measurements of DNA adduct levels in tissues collected years prior to cancer diagnosis (e.g. a retrospective cohort study in individuals treated for benign conditions of the target organ of interest and then followed up in a nested case control study). This approach would allow the use of valid controls from whom target tissues can be sampled. It also enables investigators to see the prospective temporal relationship between adducts and cancer diagnosis. What are Members views? Should this paper be further considered at a meeting? Should this approach be used to evaluate the strength of association for future biomarker evaluations conducted by the Committee?

### ***Mode of Action (MOA)***

27. The COC has previously discussed the the Human Relevance Framework (HRF), a tool which uses the MOA for a chemical established as a carcinogen in animals to evaluate the human relevance of the tumours. Holsapple et al (2006) reports the outcome of a workshop at the 2005 SOT meeting to determine if the HRF could be used to analyse 5 MOAs for rodent liver tumours. The MOAs considered included phenobarbital (PB)-like P450 induction, metal overload, porphyrinogenicity, hormone perturbation (ie oestrogen) and cytotoxicity.

28. It was concluded that, for those compounds where there are robust data for a PB-like MOA, the carcinogenic response is not relevant to humans. Using the HRF, examination of the MOA for metal overload (copper and iron) and porphyrinogenic chemical-induced rodent hepatocellular cancer induction led the working group to conclude that, without additional scientific proof, neither metal produces rodent or human liver cancer. However, using porphyrinogenic agents such as hexachlorobenzene, the working group concluded that porphyrinogenic compounds have a definable cytotoxic MOA for rodent hepatic cancer. The key events in rodent liver carcinogenesis following exposure to estrogenic agents are perturbation of hormone level or function, altered cell proliferation to apoptosis balance and development of altered foci of cellular alteration. Because this MOA is receptor mediated, the use of the HRF allows initial development of quantitative risk assessment that can be applied across species. Cytotoxicity is a generally accepted MOA and has defined for a number of nongenotoxic rodent carcinogens, including chloroform induced liver tumours. Liver tumours formed as a result of sustained cytotoxicity and regenerative proliferation are considered relevant

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for evaluating human cancer risk, if appropriate metabolism occurs in the animal models and in humans. However, because of the dose-response relationship for cytotoxicity and proliferation that is present for this MOA, a non-linear model should be used for human health risk assessment. Do Members have any comments on these conclusions? Is further work on the HRF indicated?

### ***Items identified by the COM***

29. The COM considered horizon scanning topics at its recent meeting and considered that further work should be carried out with the COC on mixtures (potential synergistic effects) and on the use of mutational fingerprints in assessing cancer aetiology (see Annex 1 for the relevant extracts from the COM paper). Do members have any comments or views on how these topics should be taken forward?

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**Extract from COM Horizon Scanning Paper 2006; MUT/06/19**

### **Mutagenicity evaluation of mixtures**

1. The COM considered an approach to evaluate mixtures of benzimidazoles and other tubulin binding compounds at its May 2006 meeting. Members questioned the premise that dose-additivity would be the default approach in cases where there were no data to support this approach. An extract from the relevant section on mixtures from the 2005 horizon scanning paper is given below.

It is widely recognised that we are exposed to complex mixtures of chemicals environmentally, occupationally, therapeutically or via a combination of these. However toxicology testing strategies (including genotoxicity/mutagenicity) are largely based upon the evaluation of single chemicals. The risk assessment of chemical mixtures is characterised by models in which chemicals within a mixture are considered to fit either dose additive, response additive or interactive profiles (Jonker et al 2004). In general it is believed that genotoxic chemicals fit the dose additive model whereby they are assumed to behave similarly in terms of mode of action and combined responses can be calculated from dose responses of the constituents of the mixture. 'Bottom-up' investigative approaches, where knowledge of the toxicities of individual chemicals are modelled to give an overall assessment of risk, can be performed when the mixture is simple (few components, well defined) and/or when sufficient data is available (examples; a pesticide product). 'Top-down' approaches are used for complex mixtures (many components, ill defined) and involve testing the whole mixture and comparing results to those achieved with individual components. Examples of complex mixtures include petroleum hydrocarbons, contaminated land/waste sites and drinking water.

For genotoxic carcinogens in the environment, the requirement is to maintain levels as low as is reasonably practicable (ALARP). With this in mind, there are two potential issues which may arise when considering the mutagenicity/genotoxicity of a mixture:

- 1) Is the assumption that all genotoxins will adhere to the dose additive model correct? (Expressed mathematically as :  $E_{AB}(d_A, d_B) = E_A(d_A) + E_B(d_B)$  : where A and B are two chemicals in the mixture and E and d represent the effects and dose of individual compounds respectively), or are there scenarios that interactions (either synergistic or antagonistic) will occur, when the presence of a first mutagen affects the potency of a second?

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Said et al (1999) demonstrated the enhancement of the bacterial mutagenicity induced by N-acetoxy-acetylaminofluorene by pre-treatment with AFB1-8,9-epoxide and suggested that pre-existing adducts may distort DNA thus leading to potentiation of intercalating agent effects. It is also suggested that the ability of arsenic to modulate DNA repair *in vitro* enhances genotoxicity in CHO cells (Lee-Chen 1993, Wiencke and Yager 1992) and similar conclusions have been reached regarding the potential mechanism of cobalt induced genotoxicity (De Boeck et al 1998).

2) If a complex mixture is tested and found to be positive *in-vitro* or *in-vivo*, should the mixture simply be regarded as a mutagen or should efforts be made to tease out the active components. For example, in a study assessing the mutagenicity of settled house dust, it was demonstrated that only 25% of the mutagenicity could be accounted for by known PAHs (Maertens et al 2004). Furthermore, how should the variability of complex mixtures such as drinking water be accounted for when assessing the risk?

This concern is borne out by the analyses of available data presented in the WiGRAMP report on risk assessment of chemical mixtures. Weaknesses were revealed in the design of many studies which had attempted to assess the genotoxicity of mixtures, as it was not possible to draw conclusions on potential interactions (e.g. individual compounds not tested separately, inadequate dose responses).

Do the Committee think further assessment of the potential interactions between genotoxic chemicals is warranted?

Should specific genotoxicity testing strategies be developed to aid those involved in the risk assessment of chemical mixtures?

2. The COM evaluation of benzimidazoles was undertaken in the context of providing advice on approaches to risk assessment of combined exposures for a group of compounds that are regulated by a threshold approach. A key piece of COM advice was the need to establish whether compounds in the potential common mechanisms group acted by dose additivity (i.e. same mode of action) before considering which chemicals to include the common mechanism and attempting to rank potency.
3. One recent study on potential interactions between mutagenic chemicals has been published. Lutz W et al (Toxicological Sciences, 86, 318-323, 2005) undertook mutagenicity studies for the production of micronuclei in LY5178Y cells using three binary mixtures of the methylating agents methylmethanesulphonate (MMS) and N-methyl-N-nitrosourea (MNU) and the topoisomerase

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inhibitor genestin. ( $2 \times 10^5$  cells using  $\leq 1\%$  DMSO as a vehicle, with 4h incubation, 20 h expression time during which 5ug/ml cytochalasin B was added). The induction of MN was assessed for both response and dose additivity. The authors report the use of the concept of “envelope of additivity” to quantitatively define responses between response and dose additivity. Evidence of synergism or antagonism would occur for responses reported to be outside the “envelope of additivity.” For MMS and MNU the effect was compatible with dose addition. For MMS and GEN the response was above response addition but below dose addition. For MNU and GEN the response was below response addition indicative of antagonism. The observed difference between MNU and MMS when tested with GEN would not have been predicted on the basis of a simplistic interpretation of methylation.

4. The approach used by these authors does highlight the potential difficulties in interpreting studies where there has been no evaluation of dose and response additivity.
5. What further work should COM be undertaking with regard to mixtures. Initial literature searches suggest there is a relatively small number of studies using both top down (e.g. diesel exhaust Ostby L Arch Tox, 71, 314-319, 1997, Oh SM Tox Letts, 161, 226-235, 2006) and bottom up approaches (e.g chlorination byproducts MakiPaakkanen J in Environ Mol Mutagen, 43, 217-225, 2004) which could be reviewed. (Data have not been screened for adequacy at the present time.) Any generic guidance which could be developed might be of value in a number of areas such as;
  - i) developing advice on possible strategies for assessing the mutagenicity of chemical mixtures in order to help define potential marker compounds or mixture specific approaches to regulation.
  - ii) developing advice on possible strategies to assess the adequacy of regulatory standards for mixtures which are based on single marker compound. (It is noted that COC/COM have agreed that mutagenic potency and DNA binding at the site of contact can act as a surrogate for site of contact carcinogenic potency for PAHs.)
6. The COM raised the possibility of interactions between mutagenic chemicals not being additive during discussions on benzimidazoles. One possible approach might be to review the literature for evidence of clear synergistic interactions and to consider whether the available data suggests the need for generic guidance on possible approaches to testing or advice on mechanisms of interaction with respect to mutagenicity which might need to be considered more widely.

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## Mutation “fingerprints” in assessing cancer aetiology.

7. The COM previously considered whether mutational fingerprints resulting from exposure to genotoxic carcinogens can be established from mutational hotspot analysis of tumours a number of years ago (e.g in 1999 when some preliminary data relating to exposure to ozone was considered and 2001 when some data on p53 mutational spectra in lung tumours of tobacco smokers was considered). The general advice provided has been that with the exception of a number of exposure scenarios such as tobacco smoking, mutational fingerprints for exposure to genotoxic carcinogens cannot be elucidated since mutations arising during carcinogenesis cannot be differentiated from those originating from exposure to genotoxic carcinogens.
8. Besaratinia A and Pfeifer G have published a review on this subject. In brief it is proposed that in-vitro studies of specific genes (e.g hprt) and use of rodent transgenic *in-vivo* mutation assays (e.g *lacI* and *cII* in Big Blue <sup>TM</sup> mice) are cited as potential approaches to the identification of mutational fingerprints which can be used to derive hypotheses for investigation in molecular epidemiology studies (e.g p53 and *ras*). Examples of specific PAHs present in tobacco smoke and aflatoxin B1 are cited with regard to in-vitro studies of mutational fingerprints using normal bronchial tissue and Hep2G cells respectively. The data from these studies are compatible with the data from molecular epidemiology studies of cancers from individuals exposed to these chemicals. In assessing data from such studies the cascade hypothesis suggested by Loeb LA (Proc Natl Acad Sci USA, 100, 776-781, 2003) is cited. Essentially Loeb argued that specific exposure-associated mutation spectrum recovered from a cancer related gene in a tumour is of relevance to cancer etiology only if i) the mutations occur early on in the carcinogenic process and ii) the gene in which mutations occur is essential and required for tumourigenesis and retained throughout the tumour selection process.
9. Do members agree with the approach suggested by Besaratinia and Pfeifer regarding approaches to the use of mutational fingerprints for carcinogen identification. Can the approach be used for any tumours given that appropriate hypotheses can be identified from available *in-vitro* and *in-vivo* approaches in cell lines and rodent studies respectively?
10. In a recently published investigation, different p53 mutation patterns were reported for colorectal tumours from smokers compared to non smokers. In brief, 153 tumour tissues samples (63 smokers, 90 non smokers) were available and were examined for p53 mutation and p53 protein expression by direct sequencing and

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immunohistochemistry. P53 mutations were identified in 77 of the 153 colorectal tumours. (22 smokers, and 31 non smokers). There were no statistically significant differences for transition or transversion mutations (it was noted that G:C→A:T transition was relatively more common in non smokers than smokers. A statistically significant increase in the number of smokers with deletion mutation was reported (7 compared to 1). Immunohistochemistry showed immunoreactivity correlated with p53 mutated tumours. The authors considered the higher occurrence of deletion mutation as evidence for a mutational pattern associated with smoking. Would members agree with this conclusion?