COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

STATEMENT ON THE RISK ASSESSMENT OF THE EFFECTS OF COMBINED EXPOSURES TO CHEMICAL CARCINOGENS

Introduction

1. Testing and risk assessment are usually carried out on individual chemicals whereas humans are exposed to multiple chemicals both simultaneously and sequentially. At the horizon scanning exercise in 2007 we decided to review current developments in the testing and assessment of chemical mixtures with regard to carcinogenicity. For this review, “mixtures” was defined as combined exposure to more than one carcinogen, or to a carcinogen and other chemical(s) with potentially modifying effects, either simultaneously or at different times. The purpose of the review was to examine the data in the scientific literature on this topic, with a view to providing advice on the potential carcinogenic action of these combined exposures and on methods for testing and assessment of such effects.

2. Carcinogenicity is a multistage process. In simple terms, the main components of this process are initiation and promotion. Initiation is caused by changes in the cellular genetic material due to an induced or spontaneous mutation or gene rearrangement. The initiated cell has an altered response to external stimuli resulting in cell growth or programmed cell death (apoptosis) and is vulnerable to abnormal division or to escape from signals for apoptosis. Promotion is any process which gives the initiated cell a growth advantage over normal cells. Clonal proliferation of the initiated cell produces cancer. Chemicals can cause initiation and/or act to enhance promotion (promoters). The action of any particular chemical could potentially be influenced by other chemicals to which an individual is exposed, either simultaneously or at a different time.

3. When a chemical (or its metabolite) causes initiation by interacting directly with the genetic material, it is referred to as a “genotoxic carcinogen” and the process as “genotoxic carcinogenicity”. Chemicals which cannot be shown to interact directly with, or cause damage to, DNA in a number of short-term screening tests, but which are capable of inducing cancer, are referred to as non-genotoxic carcinogens.

4. Our sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM\(^a\)), has reviewed the literature pertaining to the evaluation of mixtures of potential mutagens (COM 2009). The COM focused on the possible occurrence of synergistic interactions, the possible mechanisms that may

\(^a\) A list of all abbreviations in this statement is given at the end of the document.
underpin these interactions, and whether these findings were likely to have any
implications for human health risk assessments. It concluded that there were some
examples where interaction with regard to mutagenicity occurred but that these required
further evaluation before the significance to public health could be determined. Our
attention was drawn to the COT report ‘Risk Assessment of Mixtures of Pesticides and
Similar Substances’ (COT 2002) and also to initiatives such as those organized by the
UK Interdepartmental Group on Health Risks from Chemicals (IGHRC 2009) and World
Health Organisation (WHO)/International Programme on Chemical Safety (IPCS, draft
document). Both of the latter organisations have developed framework procedures for
the risk assessment of combined exposures to multiple chemicals which will provide
solid guidance for anyone required to evaluate the toxicity of chemicals. However, we
note that, within these documents, there is no specific guidance on the assessment of
the impact of combined exposure to carcinogens or to carcinogens and other chemicals
with regards to cancer.

5. The papers presented to us on this topic discussed general principles and gave
some examples of where attempts had been made to evaluate combined actions of
different carcinogens. The different types of combined actions used to characterize the
possible outcomes between compounds in a mixture, as detailed in the COT report on
pesticides and similar substances, have been classified as follows:

1. Simple similar action (non-interaction, dose addition)
2. Simple dissimilar action (non-interaction, response addition)
3. Interaction (synergism/potentiation or antagonism/inhibition)

**Simple similar action** (also referred to as simple joint action) is the concept whereby
combinations of chemicals have the same target organ and act via the same
mechanism (or mode) of action. It is also occasionally referred to as ‘dose or
concentration addition’ although, strictly speaking, this is the effect, not the concept. In
simple similar action, the effect of the components of a mixture is determined by their
respective doses and potencies. The combined effect is estimated from the summation
of the potency-normalised doses and toxicity can be predicted from the dose response
curve of a ‘reference’ compound, to which the others are normalised.

**Simple dissimilar action** (also referred to as independent joint action, simple
independent action, effect/response addition) is assumed when individual chemicals
have different modes of action and, possibly, the nature and site of action also differ.
The effect of each chemical does not modulate or contribute towards the effects of the
other constituents of the mixture and, hence, the health effects of exposure to the
mixture are expected to be qualitatively and quantitatively similar to those produced by
the individual components when administered alone. Effect addition is the summation
of the individual responses of the different mixture components and toxicity is predicted
from the dose response curves of the individual chemicals.
Interaction is present when the observed effect of two or more exposures differs from the effect that would be expected if the exposures had additive effects. Synergism and potentiation are terms used to describe responses that are greater than additive, and antagonism and inhibition are used for responses which are less than additive.

6. The possible mechanisms underlying an interaction are often divided into three categories: direct chemical-chemical, toxico/pharmacokinetic, and toxico/pharmacodynamic mechanisms. It is emphasized that the nature of the interaction can change with altered exposure conditions (for example, dose, duration, sequence of exposure and the relative proportions of the components of the mixture). How these concepts and definitions can be applied to experimental and human epidemiological exposure scenarios are described in paragraphs 22 to 25. In both cases, the definition of non-additivity will depend on the nature of the outcome measured and the shape of the dose- (or exposure-) response model fitted.

7. The review was undertaken taking into account these theoretical classifications and principles. However, it is recognized that the nature of potential combination effects do not fall neatly into categories and some mixtures may have more than one type of effect. Initiation and promotion are discrete stages of carcinogenesis and therefore likely to be subject to the influence of different chemicals, as indicated by the development of initiation/promotion experimental carcinogenesis models. We also considered that it would facilitate the review if we examined examples of synergistic reactions which occur within the different stages of the carcinogenic process, as this may shed light on the mechanisms whereby carcinogens can interact. Finally, we sought to understand how the theoretical application of the general principles involved in evaluating the combined exposures to mixtures of chemicals can be applied to relevant environmental or occupational exposure scenarios.

8. With regard to evaluating synergistic responses, it was noted that the COM, in its review of mixtures, assessed papers according to the criteria laid out in Borgert (2001). The essential criteria were:

1. Dose-response relationships for the individual mixture components are adequately characterised.
2. An appropriate non-interaction or additivity hypothesis should be, a priori, explicitly stated and used as the basis for assessing combination effects.
3. Combination of mixture components should be assessed across a sufficient range of concentrations and mixture ratios to support the goals of the study.

However, we were unable to use these criteria for the papers we reviewed, as the requirement for detailed dose response data was rarely met. Mutagenicity/genotoxicity, which was the subject of the COM review, is, at most, only a contributory factor of the carcinogenic process. To evaluate accurately the effects of mixtures of chemicals on the entire carcinogenic process would necessitate life-time carcinogenicity studies of the mixtures. These studies would need to include groups of animals receiving different
doses of both the mixtures and the individual chemicals to determine the dose responses for both. This would entail large and complex studies which would be expensive and require ethical consideration in view of the high number of animals needed.

**Mode of Action concept and Simple similar action**

9. A widely applied principle when evaluating the effects of combined exposures to multiple chemicals is the Mode of Action (MOA) concept. MOA is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. Chemicals acting by dose addition can be said to act by the same MOA and the term common mechanism group (CMG) is frequently used in mixture risk assessment for a group of chemicals with the same MOA. Most simply, this applies to chemicals which act through the same molecular target to elicit the same effect(s), e.g. a receptor, such as the AhR receptor or the oestrogen receptor. More broadly, chemicals acting independently on the same rate-limiting key event would be anticipated to exhibit dose additivity in their carcinogenic response.

10. In the UK, the method used to assess the risk of carcinogens depends on their MOA. As noted above, genotoxic chemicals react with and mutate DNA, and non-genotoxic carcinogens act by other mechanisms. From what is known about the MOA of genotoxic carcinogens, it is currently assumed that, in the absence of mechanistic data to suggest a threshold for genotoxicity, no threshold for carcinogenicity exists. The predominant risk assessment advice is to keep exposures as low as reasonably practicable (ALARP) so as to minimise risk. Many non-genotoxic carcinogens induce tumours as a secondary adverse effect arising from an initial toxicological effect, which has a threshold. It follows that there is no carcinogenic risk at dose levels that do not produce the primary toxicological event i.e. at doses below the threshold. In these cases, the risk assessment approach relies on the elucidation of a No Observed Adverse Effect Level for carcinogenicity or a precursor event linked to tumour induction. This is divided by an appropriate uncertainty factor to generate a dose which is estimated to be without appreciable risk to human health over a lifetime i.e. a tolerable daily intake.

11. When there is evidence that the members of a group of chemicals elicit their effects by the same MOA, and do not themselves interact chemically, their combined effects can be determined by using Relative Potency Factors (RPF) or Toxic Equivalency Factors (TEF). These RPFs/TEFs are expressed relative to an ‘index compound’ and are used to normalize the toxicities of chemicals within such a common mechanism group to a single compound, which is generally the one for which toxicity and absorption/distribution/metabolism/excretion (ADME) profiles are best characterised. The RPF/TEF for each chemical is derived from information such as its point of departure for one or more end-points relative to that of the index chemical in *in vivo* and *in vitro* systems, QSAR and expert judgement. RPF/TEFs can be used either
to enable a risk assessment of a mixture of chemicals by using the tolerable daily intake of the best characterised member of the group (the 'index compound'), or to calculate a risk estimate for a mixture of genotoxic carcinogens. However, in the case of mixtures of genotoxic carcinogens, the predominant advice remains to keep exposures as low as reasonably practicable (ALARP), as stated above.

12. The TEF system was first developed to facilitate the risk assessment of polychlorinated dibenzo-p-dioxins and related chemicals. Detailed evaluations of the TEFs for dioxins and dioxin-like compounds have been undertaken and published by WHO/IPCS (van de Berg et al 2006). Carcinogenic potential is not an endpoint which has been used in the past when setting TEFs because of the lack of carcinogenicity data on individual congeners. A validation study has been carried out with 3 individual dioxins or dioxin-like compounds and this broadly supported the concept of dose addition and TEFs for carcinogenicity of mixtures of these chemicals (Walker et al 2005). However the database is very limited.

13. Oestrogens are also considered to form a CMG and there are some approaches using in-vitro screening which provide robust information on dose additivity (Charles et al 2002, Payne et al 2001). However, there is a paucity of studies investigating in vivo responses to mixtures of oestrogens. Moreover, there can be exceptions to the concept of dose additivity for groups of similar chemicals. For example, oestrogens may act through either ERα or ERβ to produce stimulation or inhibition of cell proliferation. In such cases, where the biological actions at each receptor are opposed, the effect will not necessarily be additive, and may be different in different organs depending on whether the oestrogen acts as an agonist, antagonist, or partial agonist in that organ or tissue. A further difficulty in assessing the carcinogenic potential of oestrogens is that, even if the biological effects can be benchmarked against a well characterised member of the oestrogen group such as 17β-oestradiol, the Toxic Equivalency approach cannot be used to calculate the potential increase in the risk of cancer because of the difficulty in identifying an appropriate point of departure for the tumour inducing effect in animals, or humans.

14. Other groups of similar chemicals may all demonstrate carcinogenic potential but may not necessarily act by the same MOA. In this case it would not be appropriate to use TEFs for evaluation of the potency of a mixture. For example, the available evidence indicates that it is inappropriate to use TEFs to assess the potential oral carcinogenicity of combined exposures to polycyclic aromatic hydrocarbons (PAHs), most of which have no oral carcinogenicity data. There are inconsistencies in the response to the different PAHs, dependent on the test system used to evaluate toxicities, evidence of interactions between different PAHs (see below) and no clearly appropriate index compound. An alternative approach has been derived for the carcinogenic risk assessment of mixtures of PAHs in food by the European Food Safety Authority (EFSA) (European Food Safety Agency, 2008). This entailed using a ‘surrogate marker’ approach, based on benchmark dose values derived from a 2-year carcinogenicity study in which mice were fed two mixtures of coal tar containing several
PAHs. A group of four PAHs (PAH4) was recommended as the appropriate surrogate marker for the presence of PAHs in food, based on their concentrations in food and in the tested mixtures. In this model, the possibility of interactions was taken into account. Whereas both methods involve uncertainties, we agree that, in this case, the EFSA surrogate marker approach is to be preferred to the Toxic Equivalency approach.

15. When assessing the risks from exposure to combinations of chemicals, it is considered important to understand dose-response relationships. Extrapolation of the effects seen at high doses to possibly more relevant low doses is likely to be especially complex if there are a number of chemicals to be taken into account, particularly if the MOAs are not well characterised. In-vitro studies are frequently used to investigate hypotheses that relate to combined exposures to chemicals and some examples of these studies were evaluated and are described below (para 18). Some of these studies are valuable in that they provide information about MOAs or specific molecular targets, confirm whether a chemical within a group acts as an agonist of antagonist, and/or provide insight into the mechanism of an interaction. However, as it is not possible to derive points of departure (POD) or benchmark indices for the critical effect, we consider that information from in vitro studies should be used as a qualitative measure only, and over-interpretation of dose-response relationships is to be avoided.

**Simple dissimilar action**

16. Application of this principle to the evaluation of cancer as an endpoint is complicated and there are insufficient experimental data on how chemicals with diverse MOAs would act in combination with regard to the induction of tumours. Consequently, an examination of the potential complexities of combined exposures to such chemicals was considered to be outside of the scope of the current review. However, in general terms, it would be appropriate to use response addition to assess the combined effects of two carcinogens which act by different modes of action and which do not interact.

**Interactions**

**Toxicological data**

17. An interaction at a key event in the carcinogenic process may be reflected in non-additive effects on carcinogenic response and we aimed to examine the potential for chemicals to interact at different stages. The following stages in the carcinogenic process were identified as examples of potential points for interaction: ADME processes, DNA adduction, mutagenicity, early preneoplastic changes, proliferation, apoptosis and neoplastic transformation. Initially, the toxicological literature was reviewed for examples of interactions and we examined in the first instance polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs). It is noteworthy that most studies of interactions, including studies conducted in vitro, did not conform to the criteria laid out by Borgert, as described previously.
18. PAHs are a group of chemicals which have been evaluated with the consideration that human populations are exposed to mixtures, including complex mixtures such as those found in coal tar and urban dust particulate matter. *In vitro* and *in vivo* approaches were used in the papers retrieved to assess potential synergistic responses including: the production of PAH-DNA adducts, tumour formation using initiation promotion models, and effects on the cytochrome P450 (CYP) family of enzymes, particularly CYP1A1 and CYP1B1. There was some evidence that some PAHs, including those within a complex mixture, may have the potential to decrease the potency of others by altering metabolism. For example, a significant reduction in PAH-DNA adducts was observed when coal tar extract (Standard Reference Material, SRM$_{1597}$) was co-administered with benzo[a]pyrene (B[a]P) and dibenzo[a,l]pyrene (DB[a,l]P). In human breast epithelial cells (MCF-10A), reduced DNA binding was associated with induction of CYP1A1 and 1B1 (Mahadevan et al 2005). In V79 cells expressing CYP1A1 or 1B1, reduction in DNA adducts was more apparent in the CYP1B1 expressing cells (Mahadevan et al 2007). EROD activity indicated that SRM competitively inhibited the activity of both isoforms, more strongly on CYP1B1. *In vivo*, SRM$_{1597}$ reduced the number of tumours induced by DB[a,l]P in a SENCAR mouse skin model, but did not have the same effect on B[a]P induced lesions (Marston et al 2001).

19. The studies provided some examples of how chemicals, including complex environmental mixtures, can impact on the carcinogenic potential of other PAHs. In testing the hypothesis of competitive inhibition of enzymes responsible for the metabolic activation of PAHs, it was broadly demonstrated that tumour promotion and DNA adduction were affected by the mixtures and that this could be explained, in part, by altered CYP activity. For example, it is proposed that B[a]P is more readily activated by CYP1A1 than by CYP1B1, such that the competitive inhibition of the former isoform would result in reduced activity. Furthermore, it was generally shown that the effects of environmental mixtures on the metabolism of DB[a,l]P were different from their effects on the metabolism of B[a]P. This probably indicates the complexity of the interactions, both metabolic and genotoxic, involved in the processes and the dose dependency of these interactions. Moreover, the majority of interactions described involved toxicokinetic alterations and it is difficult to put these into context with interactions downstream in the carcinogenic process.

20. There are many reservations when interpreting these data. Although it is known that PAHs are inducers of xenobiotic metabolism, induction would be thresholded and the extent of induction would be dependent on dose, dose route and tissue examined. Differences were observed between results obtained *in vitro* and *in vivo* and between different models. The relevance of the SENCAR mouse skin model for the evaluation of carcinogenicity is also questionable. As such, it is difficult to extrapolate the altered risk of chemicals observed in the models used and the implications for human risk assessment are uncertain. It was concluded that analysis of *in vivo* studies with regard to potential interactions is complicated since pathways of activation and detoxification are inextricably linked and it is difficult to determine how these toxicokinetic interactions
may contribute to the overall carcinogenic process, particularly at the low levels of PAHs likely to occur following dietary or environmental exposure.

21. Heterocyclic amines (HCAs) are another class of chemicals which have the potential to interact with one another. A number of studies were retrieved which had assessed potential interactions of food heterocyclic amines using liver foci initiation promotion models in rats. The HCAs examined were Trp-P-1, Glu-P-2, IQ, MelIQ and MelIQx, Trp-P-2, Glu-P-1, MeAaC, AeC and PhIP (see list of abbreviations). As an example, these were administered as 1/1, 1/5, 1/10, 1/25 or 1/100 of the known carcinogenic dose\(^b\) and as combinations of the first four HCAs at 1/5 and 1/25 of the dose or all 10 at 1/10 and 1/100 of the dose. GST-P-positive foci >0.1mm were the selected endpoint (Ito et al 1991, Hasegawa et al 1994 a,b). It was claimed that some HCAs may act synergistically in promoting tumours through a hypothesised CYP induction mechanism and this was apparent at low doses claimed by the authors to be relevant as a human consumption scenario. However, we find it difficult to draw useful conclusions from these studies for a number of reasons. Firstly, the initiation-promotion study protocols which have been used to examine interactions between the HCAs were overly complex. The partial hepatectomy protocol fixes mutations occurring during the period of regrowth and, since there was no consistent synergistic response in this very sensitive model, the relevance to human health is questionable. The way in which the authors have analysed the results (subtracting a high background incidence from the induced incidence) is likely to be subject to significant error. In addition to the high variability and high background tumour incidence, only limited dose response data were provided. No null hypothesis was given and, therefore, no statistical comparison of the tested hypotheses was possible. We do not agree with the conclusion from these studies that there was clear evidence of synergy close to the observed NOEL for CYP induction. This may be artefactual. It is unlikely that subtle effects seen at high doses will occur at low, environmentally relevant exposures. Furthermore, the studies which evaluated HCAs were unconvincing and we suggest that less complex protocols might lead to more informative studies.

**Epidemiological data**

22. In the absence of clear evidence of interactions in carcinogenicity from the toxicological literature studied, we also examined the epidemiological literature for examples of evaluations of the effect of combinations of exposures on cancer incidence and the potential impact on public health. The two examples which we considered were combined exposure to alcohol and tobacco smoking on the incidence of a number of cancer endpoints, and combined exposure to asbestos and tobacco smoking on the incidence of lung cancer. From these data it was hoped to determine whether an understanding of the mechanisms which lead to interactions with regard to carcinogenicity could be useful in improving the assessment of the risk of mixtures of

\(^b\) Described in Ito et al (1991) as ‘the dose used in the carcinogenicity studies’.
chemicals to man. Our comments on the data reviewed are given in the Annex to this statement.

23. In epidemiology, as in toxicology, interaction is present when the observed effect of two or more exposures differs from the effect expected if the exposure had additive, joint effects (Siemiatycki et al 1981). The term “additive effects” has to be interpreted in terms of the model fitted to the data. It is possible to work on the scale of absolute measures, such as cumulative risks, or on relative scales, such as relative risks. The epidemiologic literature refers to both types of scale, with the null hypothesis of no interaction modelled as additive on the absolute scale (de Klerk et al 1989), and as multiplicative on the relative scale (as in logistic regression).

24. There are several limitations in epidemiological studies that attempt to investigate interactions: (a) investigation of interactions requires the data to span a range of combinations of the variables concerned, and an observational study may not necessarily exhibit this range; (b) statistical power is usually limited, because one needs a sample size approximately four times larger than for a single exposure to investigate the joint effect of two exposures; (c) in epidemiological studies where the exposure assessment is weak and/or prone to misclassification, estimates of risks and of interactions may be distorted. Low statistical power may lead to both false positive and false negative results, while exposure misclassification mainly leads to false negatives. Also, technical issues arise when managing large sets of data with high-degree order interactions (typically in the context of gene-environment interaction or genome-wide association studies). Although mathematical and computational tools have become available to tackle such complex analyses, it remains very difficult to go beyond a two-way interaction with confidence.

25. A potential important improvement of the study of interactions in humans might come from the development of intermediate biomarkers, but this field is currently underdeveloped. Using biomarkers it is possible to follow the fates of the individual active components of a mixture in the body, to investigate their links/reactions with relevant target molecules, and eventually to devise risk assessment models.

26. In general, it was considered that the assessment of potential interactions between carcinogenic chemicals was fraught with difficulties. Firstly, it is recognised that extrapolating data from the majority of methodologies used to substitute for carcinogenicity bioassays to possible carcinogenic responses in humans is extremely difficult. In vitro studies can give qualitative information on the relative carcinogenic hazard at best. The complexities involved in the carcinogenic process, including the possibility that two chemicals could be present in the body at very different times, yet provoke a synergistic response, make the evaluation of risks posed by potentially carcinogenic chemicals entirely different from the evaluation of the vast majority of chemical toxicities.
27. It could be postulated that the combination of any chemical which causes a mutation with one that induces proliferation will act synergistically with regards to the induction of tumours. This is analogous to the well-established phenomenon of initiation-promotion. It is also of note that dose responses to chemicals can be more complex than simple high or low dose effects; it is possible that MOAs will also change with increasing dose, thus further complicating the interpretation of data. Metabolic interactions may occur although it is considered more likely that they will impact on a genotoxic event in the carcinogenesis process as this will only require a short period of alteration; a non-genotoxic mode of action will be affected only by a metabolic change over a prolonged period. In addition, the extended time taken for tumours to occur following chemical exposure make it difficult at present to evaluate responses in test systems other than life-time bioassays in rodents. Epidemiological studies are expensive and investigation of interactions necessitates the existence of populations that have been exposed to the individual components of the mixture and other populations that have been exposed to the mixture. This is not a common situation for chemicals, for example, occupational and environmental exposure to carcinogenic PAHs is always to a mixture of PAHs. Thus, epidemiological studies are not a practical alternative to animal studies in this case.

Conclusions

28. Humans are exposed to mixtures of chemicals, including carcinogens, and it is not possible for the risk assessment process to account for the combined action of every possible mixture of carcinogens at all possible levels of exposures over all possible time frames. Nevertheless, some general principles can be stated:

- Mixtures of chemicals which act via the same MOA and which do not react chemically with one another, such as polychlorinated dibenzo-p-dioxins, can be assessed using the concept of dose additivity and relative potency factors/toxic equivalency factors.
- Although there may be a substantial margin between exposure to a carcinogen and either its no observed adverse effect level (in the case of a non-genotoxic carcinogen) or another point of departure (in the case of a genotoxic carcinogen), it is possible that simultaneous exposure to two carcinogens which have the same MOA may result in a lower margin of exposure. Risk assessors should be alert to this possibility when assessing a chemical which commonly occurs together with one or more other chemicals which have the potential to cause cancer.
- There are several stages in the carcinogenic process at which carcinogens might interact, for example: ADME processes, DNA adduction, mutagenicity, early preneoplastic changes, proliferation, apoptosis and neoplastic transformation. MOA analysis may be of value here, in determining critical steps at which interaction might be anticipated. Potential interactions in genotoxic MOAs have been addressed in the statement by the COM.
It is postulated that otherwise non-carcinogenic chemicals, such as anti-apoptotic chemicals or chemicals which interfere with cell cycle regulation, which alter ADME processes or which increase permeability of the skin or oral mucosa, might have the potential to interact synergistically with known carcinogens.

The assessment of potential interactions in the context of carcinogenicity is complex due to the multistage nature of the process. However, we do not advocate standard carcinogenicity studies on mixtures of chemicals except in exceptional circumstances. Such studies would be costly and would require ethical consideration in view of the high number of animals required.

In vitro studies of interactions should be hypothesis driven, attempt to characterize the dose-response and use models relevant to in vivo carcinogenicity. These studies should adhere to the criteria laid out in Borgert et al (2001). Models used to evaluate the synergistic interactions between PAHs and between HCAs were, in general, complex and may not truly reflect the situation for carcinogenesis. Thus extrapolation of results for risk assessment in humans is difficult.

Overall, in vitro studies can be used to confirm molecular targets or provide insight into MOA identification but are not of value for the evaluation of relative potencies of chemicals or interactions at environmentally relevant exposure levels.

In terms of the risk assessment of potential interactive effects of carcinogens, exposure to a non-genotoxic carcinogen at or below the no-effect level for the critical effect contributing to the interaction is unlikely to result in an interaction with a chemical which has a different MOA. In the case of genotoxic carcinogens, in principle, effects could occur at any level of exposure which could lead to interaction. This supports the view that exposure to genotoxic carcinogens should be as low as reasonably practicable.
Examples of multiple exposures and potential interactions in humans

Alcohol and tobacco smoking:

1. Alcohol and tobacco smoking are each known to be major risk factors for a number of cancers i.e. cancers of the mouth, neck and squamous cell carcinoma of the oesophagus. The studies reviewed show that these two factors act in a greater than additive manner to produce these cancers with effects apparent at moderate as well as high intakes (Lagergren et al 2000, Lee et al 2008). In some instances, the multiplicative increases are very large (odds ratios of up to 177). However, this synergism is not apparent for oesophageal adenocarcinoma and cancers of the gastric cardia (Sjodahl et al 2006).

2. The mechanism for the synergistic effect is not well understood and we considered a number of plausible hypotheses. Firstly, the induction of cytochrome P450 (CYP) enzymes by ethanol is suggested as a potential mechanism. There is evidence that ethanol induces CYP isoforms which are capable of metabolically activating some carcinogenic nitrosamines found in tobacco smoke. Induction of the CYP 2E1 isoform at extra-hepatic sites such as the oesophagus, combined with decreased first-pass metabolism of tobacco associated nitrosamines in the liver due to competitive inhibition by ethanol, is predicted to lead to increased concentrations of DNA-reactive nitrosamine metabolites leading to elevated cancer risk (Lecheveral et al 1999, Godoy et al 2002, Anderson et al 1995). A second plausible hypothesis, based on in vitro data which are convincing but not extensive, suggests that alcohol increases the permeability of the oral mucosa to carcinogenic nitrosamines. This may also contribute to the synergistic effect observed (Du et al 2000, Azzi et al 2005).

3. We agree that the metabolic interaction hypothesis is plausible. However, we concluded that, although the permeability mechanism looks reasonable, it was not clear whether the in vitro results could be extrapolated to the in vivo situation. We suggest that consideration should also be given to the interaction of alcohol and growth factors and the effect of local irritation of tissues. In addition, although the metabolic argument is convincing, this scenario could also be true of exposures to other chemicals which induce CYP2E1 and it was noted that there are no clear indications that there are similarly other synergistic carcinogenic interactions with alcohol.

Cigarette smoking and asbestos

4. Exposure independently to cigarette smoke or to asbestos causes lung cancer and it has been claimed that combined exposure results in a synergistic effect on lung cancer induction (Selikoff et al 1968, Lee 2001). The exact nature of the interaction between asbestos and tobacco smoking in the induction of lung cancer has been debated among researchers. From the published literature, most systematic reviews
have found a marked heterogeneity in the magnitude of the joint effect, with the interaction ranging from less than additive in some studies to multiplicative in other studies. Despite extensive investigations exploring the interaction between cigarette smoke and asbestos, the precise mechanisms involved at the cellular and molecular level are unclear. Asbestos and tobacco are both complex carcinogens and it is believed that they can both act at more than one stage of carcinogenesis and, hence, have interdependent effects on the multistage process of lung cancer (Vainio and Boffetta, 1994).

5. A number of authors have proposed a synergistic interaction between cigarette smoke and asbestos and various mechanisms have been proposed as the potential explanation. These include:

- cytotoxic, genotoxic and clastogenic nature of asbestos and tobacco smoke – supra-additive effects have been noted for mutation frequency, sister chromatid exchange, and DNA strand breaks in a variety of test systems (Lohani et al 2002, Kelsey et al 1986, Jung et al 2000)
- the generation of oxidative damage - both cigarette smoke and asbestos fibres generate reactive oxygen species and synergistic responses in models evaluating this have been observed. However mechanistic insights into or hypotheses about this interaction are not well developed.
- enhancement of the penetration and accumulation of asbestos in the lung by tobacco smoke – demonstrated in a number of models including following the assessment of asbestos fibres in the airways of smokers and non-smokers (McFadden et al 1986 a,b).
- the potential for asbestos to act as a delivery system for tobacco carcinogens into the lung, for example by enhancing the diffusion of lipophilic carcinogens, was shown to be unlikely (Gerde et al 1994).
- the enhancement of somatic mutations in KRAS, FHIT and p53 genes. – some associations of smoking and/or asbestos exposure and lung cancer with these genes have been postulated although specific mechanisms have not been not described.

6. Overall, it was difficult to draw conclusions from the studies evaluating the proposed synergy between asbestos and tobacco as the interaction models need to be studied in depth to understand whether the interaction is additive or multiplicative and to evaluate in detail the hypothesised mechanisms for the interactions and whether they are relevant to understanding risk in man. The definition of additivity in an experiment appears to depend upon which model fits the individual chemicals evaluated. Furthermore, the importance of different types of asbestos needs to be addressed; different types of asbestos may fit different dose response models. Exposure misclassification might also lead to substantial uncertainty in epidemiological studies; this distortion in risk estimates means it is impossible to differentiate between interaction models. We consider that there is some evidence that there might be a synergistic interaction, but it is not strong. It should be noted that, whilst mesothelioma risk stays
constant over time following cessation of inhalation of asbestos, lung cancer risk reduces in reformed smokers. This probably reflects the fact that asbestos fibre remained in the lung whereas the amount of smoke residue is considered to be significantly reduced once smoking stopped.

7. Overall, without an understanding of the specific mechanisms, it is concluded that it is hard to interpret the short term studies retrieved, although it is possible to suggest plausible hypotheses. Epigenetic mechanisms may also play a part, or asbestos exposure might increase uptake of carcinogens from tobacco smoke. We consider that examination of the p53 mutational spectra might offer some insights, as this is well defined for mutations arising as a result of exposure to tobacco smoke. It might also be interesting to examine the anatomical location of lung tumours, for example at bifurcations of the airway, which might help elucidate a mechanical mechanism.
References


IPCS - Draft WHO/IPCS Framework for Risk Assessment of Combined Exposures to Multiple Chemicals


General Abbreviations:

ADME = absorption, distribution, metabolism, excretion
B[a]P = benzo[a]pyrene
CMG = common mechanism group
COM = committee on mutagenicity
COT = committee on toxicity
CYP = cytochrome P450
DB[a,l]P = dibenzo[a,l]pyrene
DNA = deoxyribonucleic acid
ER = oestrogen receptor
EROD = ethoxyresorufin-O-deethylase
GST-P = glutathione-S-transferase-placental
HCA = heterocyclic amine
MOA = mode of action
MCF-10A = a human breast epithelial cell line;
PAH: polyaromatic hydrocarbon
POD: Point of Departure
SRM1597 = coal tar extract Standard Reference Material,
TEF = toxic equivalency factor;
V79 = a Chinese hamster cell line

HCA Abbreviations:

Trp-P-1 = 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole,
Trp-P-2 = 3-amino-1-methyl-5H-pyrido[4,3-b]indole,
Glu-P-1 = 2-amino-6 methyl(dipyrido[1,2-a:3',2'-d]imidazole,
Glu-P-2 = 2-amino-dipyrido[1,2-a:3',2'-d]imidazole,
IQ = 2-amino-3-methylimidazo[4,5-f] quinoline
MeIQ = 2-amino-3,8-dimethylimidazo [4,5-f] quinoline,
MeIQx = 2-amino-3,8-dimethylimidazo[4,5-f] quioxaline,
MeAaC = 2-amino-3-methyl-9H-pyrido[2,3-b]indole,
AaC = 2-amino-9H-pyrido[2,3-b]indole,
PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine