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

**DRAFT STATEMENTS ON THE ASSESSMENT OF THE CARCINOGENICITY OF CHEMICAL MIXTURES**

The Committee discussed a first draft statement on the carcinogenicity of chemical mixtures at the last meeting and suggested that it would be appropriate for it to be split into two – one addressing concomitant exposures to more than one chemical in a mixture, and the other addressing interaction between exposures to more than one chemical but not necessarily at the same time. The DH Toxicology Unit has therefore prepared two draft statements, which incorporate comments made by the Committee at the last meeting. These are attached at Annexes 1 and 2 and Members' comments are invited.

Secretariat

July 2009

## DRAFT STATEMENTS ON THE ASSESSMENT OF THE CARCINOGENICITY OF CHEMICAL MIXTURES

### [1] APPRAISAL OF GENERAL PRINCIPLES

#### Introduction

1. At the horizon scanning exercise in 2007 it was suggested that we review current developments in the assessment of chemical mixtures with regard to carcinogens, potential interactions and their modes of action. Similarly, our sister committee, the Committee on Mutagenicity (COM), has reviewed the literature pertaining to the evaluation of mixtures of potential mutagens. The COM had focused on the possible occurrence of synergistic interactions, the possible mechanism that may underpin these interactions, and whether these findings were likely to have any implications for human health risk assessments. It was concluded that there were some examples where interaction with regards to mutagenicity occurred but that these mechanisms required further evaluation before the significance to public health could be determined. Our attention was drawn to the COT WiGRAMP<sup>a</sup> report on the risk assessment of mixtures of pesticides and similar substances (COT 2002) which provides comprehensive information specifically on the mixtures arising from dietary exposure to residues of pesticides or veterinary medicines, and also to current initiatives such as those organized by the UK Interdepartmental Group on Health Risks from Chemicals (IGHRC) and World Health Organisation (WHO)/ International Programme on Chemical Safety (IPCS). Both have developed framework procedures for the risk assessment of combined exposures to multiple chemicals which will provide 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 chemicals on carcinogenic potential.

2. The papers presented to us on this topic discussed general principles and provided some examples of where attempts had been made to evaluate the effects of interactions between carcinogens. The different types of combined actions used to characterize the possible outcomes between compounds in a mixture, as detailed in the COT report 'Risk Assessment of Mixtures of Pesticides and Similar Substances' (COT 2002), 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, dose or concentration addition) is the principle applied to combinations of chemicals which are assumed to have the same target organ acting via the same mechanism (or mode) of action, and bearing similar pharmacokinetic profiles.

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<sup>a</sup> Working Group for the Risk Assessment of Mixtures of Pesticides and Similar Substances

1 **Simple dissimilar action** (also referred to as independent joint action, simple independent  
2 action, effect/response addition) is assumed when individual chemicals have different modes of  
3 action and, possibly that the nature and site of actions also differ and thus do not modulate or  
4 contribute towards the effects of the other constituents of the mixture. Effect addition is the  
5 summation of the individual responses of the different mixture components and toxicity is  
6 predicted from the dose response curves of the individual chemicals.

7  
8 **Interaction** (also referred to when positive as synergism, potentiation supra-additivity, or when  
9 negative as antagonism, sub-additivity inhibition) implies a deviation from expected effects, or  
10 from dose or response additions resulting in toxicities either greater or less than the anticipated  
11 additive responses of the individual components of the chemical mixture. Synergism and  
12 potentiation are terms used to describe heightened responses, antagonism and inhibition for  
13 diminished responses.

14 3. The possible mechanisms underlying an interaction are often divided into three  
15 categories: direct chemical-chemical, toxico/pharmacokinetic, and toxico/pharmacodynamic  
16 mechanisms. It is emphasized that the nature of the interaction can change with altered  
17 exposure conditions (for example, dose, duration, sequence, relative proportions of the  
18 components)

19 4. **Simple similar action:** When there is evidence that the members of a group of  
20 chemicals elicit their effects by the same mode of action, and do not themselves interact, their  
21 combined effects can be determined by using Relative Potency Factors (RPF) or Toxic  
22 Equivalency Factors (TEF). RPFs/TEFs are potency factors, expressed relative to an 'index  
23 compound', and are used to normalize the toxicities of chemicals within such a common  
24 mechanism group to the 'index compound'. The TEF system was first developed to facilitate  
25 risk assessment for polychlorinated dibenzo-*p*-dioxins ("dioxins") and related chemical classes.  
26 The TEF for each chemical is derived from its point of departure for one or more end-points  
27 relative to that of the index chemical, which is generally the one for which toxicity and ADME  
28 profiles are best characterised. The term common mechanism group (CMG) is frequently used  
29 in mixture risk assessment and applies to a class of chemical with the same mode of action.  
30 Most simply, this applies to chemicals which act through the same molecular target, e.g. a  
31 receptor, such as the Ah receptor or the oestrogen receptor.

32 5. Detailed evaluations of the TEFs for dioxins and dioxin-like compounds have been  
33 undertaken and published by WHO/IPCS (van de Berg et al 2005). It is noted that carcinogenic  
34 potential is not a considered endpoint when setting TEFs. We have reviewed a few studies  
35 which broadly demonstrate the concept of dose additivity for dioxins as a CMG when using  
36 tumours as the endpoint (Walker et al 2005). However the database is very limited.  
37 Oestrogens are also considered to form a CMG and there are some approaches using *in-vitro*  
38 screening which provide robust information on dose additivity (Charles et al 2002, Payne et al  
39 2001). However, there is a paucity of studies investigating *in vivo* responses to mixtures of  
40 oestrogens. Moreover, there can be exceptions to the concept of dose additivity for similar  
41 groups of chemicals. For example, oestrogens may act through either ER $\alpha$  or ER $\beta$  to produce  
42 either inhibitory or stimulatory effects.

43 6. We consider that the use of TEFs for the evaluation of combined exposures to  
44 polyaromatic hydrocarbons (PAHs) with regards to carcinogenic potential is limited. There are  
45 inconsistencies in the response of the different PAHs and it is difficult to select an appropriate

1 marker compound. However, we note that the principle is applied by some authorities during the  
2 risk assessment of PAH contaminated air or land.

3  
4 7. **Simple dissimilar action:** The application of this principle to the evaluation of cancer as  
5 an endpoint is complicated. It is possible that different carcinogens producing tumours at the  
6 same sites, but as a consequence of different modes of action (e.g genotoxic, and non-  
7 genotoxic), could be considered to fulfill these criteria.

8  
9 8. **Interactions:** We examined the potential for chemicals to interact synergistically at  
10 different stages in the carcinogenic process. The following points in the carcinogenic process  
11 were identified as examples of potential sites for interaction: ADME processes, DNA adduction,  
12 mutagenicity, early preneoplastic changes, and neoplastic transformation. The literature was  
13 reviewed for examples of interactions and, in the first instance, we examined polycyclic aromatic  
14 hydrocarbons (PAHs) and heterocyclic amines (HCAs).

15  
16 9. The COM, in its review of mixtures, assessed papers according to the criteria laid out in  
17 Borgert (2001). The essential criteria were:

- 18 1. Dose-response relationships for the individual mixture components are adequately  
19 characterised.
- 20 2. An appropriate non-interaction or additivity hypothesis should be, a priori, explicitly  
21 stated and used as the basis for assessing combination effects.
- 22 3. Combination of mixture components should be assessed across a sufficient range of  
23 concentrations and mixture ratios to support the goals of the study  
24

25  
26 However, this was not considered appropriate for the papers we reviewed as the requirement  
27 for detailed dose response data was usually not met. To evaluate accurately the potential  
28 interactions of chemicals during the entire carcinogenic process would necessitate life-time  
29 carcinogenicity studies including groups to determine dose responses for the individual  
30 chemicals as well as the combinations. This would entail large and complex studies which  
31 would be expensive.

32  
33 10. PAHs are a group of chemicals which have been evaluated with the consideration that  
34 human populations are exposed to mixtures of these, including complex mixtures such as those  
35 found in coal tar and urban dust particulate matter. *In vitro* and *in vivo* approaches were used in  
36 these papers to assess potential synergistic responses, including the production of DNA  
37 adducts, tumour formation using initiation promotion models and effects on the cytochrome  
38 P450 (CYP) family of enzymes, particularly CYP1A1 and CYP1B1. There was some evidence  
39 that some PAHs, including those within a complex mixture, may have the potential to decrease  
40 the potency of others by altering metabolism. For example, a significant reduction of DNA  
41 binding was observed when coal tar extract (Standard Reference Material, SRM<sub>1597</sub>) was co-  
42 administered with benzo[a]pyrene (B[a]P) and dibenzo[a,l] pyrene (DB[a,l]P). In human breast  
43 epithelial cells (MCF-10A), reduced DNA binding was associated with induction of CYP1A1 and  
44 1B1 (Mahadevan et al 2005). In V79 cells expressing CYP 1A1 or 1B1, the reduction in DNA  
45 binding was more apparent in the CYP1B1 expressing cells (Mahadevan et al 2007). EROD  
46 activity indicated that SRM competitively inhibited the activity of both isoforms, more strongly on  
47 1B1. *In vivo*, SRM<sub>1597</sub> reduced the number of tumours induced by DB[a,l]P in a SENCAR  
48 mouse skin model, but did not have the same effect on B[a]P induced lesions (Marston et al  
49 2001).

1  
2 11. The studies provided some examples of how chemicals, including complex  
3 environmental mixtures, can impact on the carcinogenic potential of other PAHs. In testing the  
4 hypothesis of competitive inhibition of enzymes responsible for the metabolic activation of PAHs  
5 it was broadly demonstrated that tumour promotion and DNA adduction were affected by the  
6 mixtures and that this could be in part be explained by altered CYP expression. For example, it  
7 is proposed that B[a]P is more readily activated by CYP1A1 than by CYP1B1, such that the  
8 competitive inhibition of this isoform would result in reduced activity. Furthermore, it was  
9 suggested that the effects of environmental mixtures on the metabolism of DB[a,l]P differs from  
10 B[a]P although this is not supported by all the available data. This probably indicates the  
11 complexity of the interactions, both metabolic and genotoxic, involved in the processes and the  
12 dose dependency of these interactions. Moreover, the majority of interactions described  
13 involved toxicokinetic alterations and it is difficult to put these into context with interactions  
14 downstream in the carcinogenic process.  
15

16 12. There are many reservations when interpreting these data. Although it is known that  
17 PAHs are inducers of xenobiotic metabolism, the extent of the induction would be largely  
18 dependent on dose, dose route and tissue examined and differences are often observed  
19 between results obtained *in vitro* and *in vivo*. The relevance of the SENCAR mouse skin model  
20 for the evaluation of carcinogenicity is also questionable, as it is essentially a genotoxicity  
21 assay. As such, it is difficult to extrapolate the altered risk of chemicals observed in the models  
22 used and the implications for human risk assessment are uncertain. Additionally, it is  
23 questionable whether CYP activity actually drives carcinogenic risk *in vivo*, as it is known that  
24 effects are still seen in aryl hydrocarbon receptor knockout mice. It was concluded that analysis  
25 of *in vivo* studies with regards to potential interactions is complicated since pathways of  
26 metabolism, activation and detoxification are inextricably linked and it is difficult to comprehend  
27 how these toxicokinetic interactions may contribute to the overall carcinogenic process.  
28

29 13. We also identified heterocyclic amines (HCAs) as a class of chemicals which have the  
30 potential to interact with one another. A number of studies were retrieved which had assessed  
31 potential interactions of food heterocyclic amines using liver foci initiation promotion models in  
32 rats. The HCAs examined were Trp-P-1, Glu-P-2, IQ, MeIQ and MeIQx, Trp-P-2, Glu-P-1,  
33 MeAαC, AαC and PhIP (see Abbreviations). As an example, these were administered as 1/1,  
34 1/5, 1/10, 1/25 or 1/100 of the given dose (the known carcinogenic dose) and as combinations  
35 all of the first at 1/5 and 1/25 of the dose or all 10 at 1/10 and 1/100. GST-P-positive foci  
36 >0.1mm were the selected endpoint (Ito et al 1991, Hasegawa et al 1994 a,b). It was claimed  
37 that some HCAs may act synergistically in promoting tumours through a hypothesised CYP  
38 induction mechanism and this was apparent at low doses claimed by the authors to be relevant  
39 as a human consumption scenario. However, we find it difficult to draw useful conclusions from  
40 these studies for a number of reasons. Firstly, the initiation-promotion study protocols which  
41 have been used to examine interactions between the HCAs were overly complex. The partial  
42 hepatectomy protocol fixes mutations occurring during the period of regrowth and, since there  
43 was no consistent synergistic response in this very sensitive model, the relevance to human  
44 health is questionable. The way in which the authors have analysed the results (subtracting a  
45 high background incidence from the induced incidence) is likely to be subject to significant error.  
46 In addition to the high variability and high background tumour incidence, only limited dose  
47 response data was provided. No null hypothesis was given and, therefore, no statistical  
48 comparison of the tested hypotheses was possible. We do not agree with the conclusion from  
49 these studies that there was clear evidence of synergy. Furthermore, even if synergy was

1 shown to occur in the model system used, its applicability to human exposure is highly  
2 questionable.

3  
4 14. From these studies we conclude that there can be apparent synergy at doses close to the  
5 threshold for effect but it is not clear whether that this would be apparent at higher doses.  
6 Furthermore, the studies which evaluated HCAs were unconvincing and we suggest that less  
7 complex protocols might lead to more informative studies.

## 8 9 **Conclusions**

10  
11 15. Overall, we reached the following conclusions in the discussion of mechanisms by which  
12 chemicals may interact during the carcinogenic process:

- 13  
14 • Mixtures of chemicals acting via the same mechanism, which do not interact with one  
15 another, such as polychlorinated dibenzo-*p*-dioxins, can be assessed using the concept  
16 of dose additivity.
- 17  
18 • There are several potential sites for interaction between carcinogens in the carcinogenic  
19 process, for example, ADME processes, DNA adduction, mutagenicity, early  
20 preneoplastic changes, and neoplastic transformation.
- 21  
22 • It could be postulated that t a chemical which causes a mutation and one that induces  
23 proliferation will interact synergistically with regards to the induction of tumours.
- 24  
25 • It is also likely that otherwise non-carcinogenic chemicals, such as anti-apoptotic  
26 chemicals or chemicals which interfere with cell cycle regulation, alter ADME processes  
27 or increase permeability of skin/oral mucosa, might reasonably be expected to interact  
28 synergistically with classical carcinogens.
- 29  
30 • There were a number of possible ways to proceed in this area. Unfortunately, the  
31 assessment of potential interactions in the context of carcinogenicity is complex due to  
32 the multi-stage nature of the process and the high cost of carcinogenicity studies.
- 33  
34 • *In vitro* studies of interactions must be hypothesis driven, attempt to characterise the  
35 dose-response and use models relevant to *in vivo* carcinogenicity. These studies should  
36 adhere to the criteria laid out in Borgert et al (2001). Models used to evaluate the  
37 synergistic interactions between PAHs and HCAs were, in general, overtly complex and  
38 may not truly reflect the situation for carcinogenesis. Thus, extrapolation for risk  
39 assessment in man is difficult.
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41 compounds. *Environ.Health. Perspect.* **113** 43-48  
42  
43

1 **General Abbreviations**

- 2  
3 ADME = absorption, distribution, metabolism, excretion  
4 B[a]P = benzo[a]pyrene;  
5 CMG = common mechanism group  
6 COM = committee on mutagenicity  
7 COT = committee on toxicity  
8 CYP = cytochrome P450;  
9 DB[a,l]P= dibenzo[a,l] pyrene;  
10 DNA = deoxyribonucleic acid;  
11 ER = oestrogen receptor  
12 EROD = ethoxy resorufin-o-deethylase  
13 GST-P = glutathione-S-transferase-placental  
14 HCA = heterocyclic amine  
15 MCF-10A = a human breast epithelial cell line;  
16 SRM<sub>1597</sub> = coal tar extract Standard Reference Material,  
17 TEF = toxic equivalency factor;  
18 V79 = a Chinese hamster cell line

19  
20 **HCA Abbreviations**

- 21  
22 Trp-P-1 = 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole,  
23 Trp-P-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole,  
24 Glu-P-1 = 2-amino-6-methyl-dipyrido[1,2- $\alpha$ :3',2'-*d*]imidazole ,  
25 Glu-P-2 = 2-amino-dipyrido[1,2- $\alpha$ :3',2'-*d*]imidazole,  
26 IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline  
27 MeIQ = 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoline,  
28 MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline,  
29 MeAaC = 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole,  
30 AaC = 2-amino-9*H*-pyrido[2,3-*b*]indole,  
31 PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

## DRAFT STATEMENTS ON THE ASSESSMENT OF THE CARCINOGENICITY OF CHEMICAL MIXTURES

### [2] EXAMPLES OF MULTIPLE EXPOSURES TO CHEMICALS AND POTENTIAL INTERACTIONS IN HUMANS

#### Introduction

1. As part of a review of current developments in the assessment of chemical mixtures with regard to carcinogenicity, we evaluated the published literature with the aim of identifying potential interactions between chemicals which may have an impact on human health risk assessments. We conclude that there is support for the concept that mixtures of chemicals acting via the same mechanism, such as dioxin-like compounds, can be assessed using the model of dose additivity. In addition, there is some evidence for interactions between chemical carcinogens resulting in greater or lesser effects than would be seen if the same compounds were given independently (see statement [1]).

2. However, there is a paucity of published epidemiological studies which have evaluated the effect of combinations of chemicals on cancer incidence and the potential impact on public health. In statement [1], we reviewed the available data on polyaromatic hydrocarbons and heterocyclic amines, groups of chemicals to which the public are exposed. These have been assessed in animal models and *in vitro* but no study reports were retrieved which had attempted to assess the impact of mixtures of combinations of these chemical groups in man or any further investigations to determine the mechanistic plausibility of these interactions in man, as discussed in statement [1]. We identified the following additional examples of combinations for which interactions may be of relevance in one or more cancer type: alcohol and tobacco (several cancer sites), bacterial and nitrosamine production (oesophageal cancer),  $\beta$ -carotene and tobacco (lung cancer), and asbestos and tobacco (lung cancer). Two of these were selected for further review: alcohol and tobacco, and asbestos and tobacco, and we reviewed the extent of evidence for synergism and potential mechanisms of interaction. From these data we hoped to determine whether an understanding of the mechanisms which lead to interactions would be useful in improving the assessment of the risk of human exposure to combinations of chemicals.

#### Alcohol and tobacco

3. Alcohol and tobacco are both known to be predominant 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 2007). In some instances the multiplicative increases are very large (odd ratios of up to 32). However, this synergism is not apparent for oesophageal adenocarcinoma and cancers of the gastric cardia (Sjodahl et al 2006).

4. 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

1 ethanol is suggested as a potential mechanism. There is evidence to indicate that ethanol  
2 induces CYP isoforms which are capable of metabolically activating some carcinogenic  
3 nitrosamines found in tobacco smoke. Induction of the 2E1 isoform at extra-hepatic sites such  
4 as the oesophagus, combined with decreased first pass metabolism of tobacco associated  
5 nitrosamines in the liver due to competitive inhibition by ethanol, is predicted to lead to  
6 increased concentrations of DNA-reactive nitrosamine metabolites leading to elevated cancer  
7 risk (Lecheveral et al 1999, Godoy et al 2002, Anderson et al 1995). A second hypothesis, for  
8 which there are limited but convincing data *in vitro*, suggests that alcohol increases the  
9 permeability of the oral mucosa to carcinogenic nitrosamines, which would account for the  
10 synergistic effect observed (Du et al 2000, Azzi et al 2005).

11 5. We agree that the metabolic interaction hypothesis is plausible. However, we conclude  
12 that although the permeability mechanism looks reasonable, it is not clear whether the *in vitro*  
13 results can be extrapolated to the *in vivo* situation. We suggest that consideration should also  
14 be given to the interaction of alcohol and growth factors and the effect of local irritation of  
15 tissues. Also, although the metabolic argument is convincing, this scenario could also be true of  
16 exposures to other chemicals which induce CYP2E1 and there are no indications that there are  
17 similarly other synergistic interactions with alcohol

18

### 19 **Cigarette smoking and asbestos**

20

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

32

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

- 36 • cytotoxic, genotoxic and clastogenic nature of asbestos and tobacco smoke – supra-  
37 additive effects have been noted for mutation frequency, sister chromatid exchange, and  
38 DNA strand breaks in a variety of test systems (Lohani et al 2002, Kelsey et al 1986,  
39 Jung et al 2000)
- 40 • the generation of oxidative damage - both cigarette smoke and asbestos fibres generate  
41 reactive oxygen species and synergistic responses in models evaluating this have been  
42 observed. However mechanistic insights into or hypotheses about this interaction are  
43 not well developed.
- 44 • enhancement of the penetration and accumulation of asbestos in the lung by tobacco  
45 smoke – demonstrated in a number of models including following the assessment of  
46 asbestos fibres in the airways of smokers and non-smokers (McFadden et al 1986 a,b).
- 47 • the potential for asbestos to act as a delivery system for tobacco carcinogens into the  
48 lung, for example by enhancing the diffusion of lipophilic carcinogens was shown to be  
49 unlikely (Gerde et al 1994).

- 1       • the enhancement of somatic mutations in k-ras, FHIT and p53 genes. – some  
2       associations of smoking and/or asbestos exposure and lung cancer with these genes  
3       have been postulated although specific mechanisms have not been not described.  
4

5       8. Overall, it is difficult to draw conclusions from the studies evaluating the proposed  
6       synergy between asbestos and tobacco as the interaction models need to be studied in depth to  
7       understand whether the interaction is additive or multiplicative and to evaluate in detail the  
8       hypothesised mechanisms for the interactions. The definition of additivity in an experiment  
9       appears to depend upon which model fits the individual chemicals evaluated. Furthermore, the  
10      importance of different types of asbestos needs to be addressed; different types of asbestos  
11      may fit different dose response models. Exposure misclassification might also lead to  
12      substantial uncertainty in epidemiological studies; this distortion in risk estimates means it is  
13      impossible to differentiate between interaction models. We consider that the evidence is  
14      insufficient to preclude the null hypothesis, although there's some evidence that there might be  
15      a synergistic interaction. It should be noted that, whilst mesothelioma risk stays constant over  
16      time following cessation of exposure to asbestos, lung cancer risk reduces in reformed smokers.  
17      This probably reflects the fact that tobacco smoke is both an initiator and promoter of cancer.  
18

19      9. Overall, without an understanding of the specific mechanisms, it is hard to interpret the  
20      short term studies retrieved; although it is possible to suggest plausible hypotheses. Epigenetic  
21      mechanisms may also play a part, or asbestos exposure might increase uptake of carcinogens  
22      from tobacco smoke. We consider that examination of the p53 mutational spectra might offer  
23      some insights, as this is well defined for mutations arising as a result of exposure to tobacco  
24      smoke. It might also be interesting to examine the anatomical location of lung tumours, for  
25      example at bifurcations of the airway, which might help elucidate a mechanical mechanism.  
26

## 27      Conclusions

28  
29      10. It is clear that multiple exposures to specific carcinogens can result in a greater than  
30      additive risk of some cancers in humans. Investigations have demonstrated that it is possible  
31      to suggest plausible hypotheses for why these interactions occur. However, at present it is  
32      uncertain how an understanding of these interactions can impact on human health risk  
33      assessment strategies.

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