

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

THIRD DRAFT STATEMENT ON THE ASSESSMENT OF THE CARCINOGENICITY OF CHEMICAL MIXTURES

The secretariat has redrafted this statement following the comments received at, and subsequent to, the last meeting. As explained previously, it was decided that the summary of the Committee's consideration of examples of multiple exposures and potential interactions in humans, previously drafted as a second statement, was insufficient to form a standalone statement and so has been incorporated into the main statement as an annex. The Committee is asked for final comments on the statement, which is attached at Appendix 1. In particular, members are asked whether they agree with the additional recommendations which have been included.

Paragraph 9 now refers to the approach derived by the European Food Safety Authority for the carcinogenic risk assessment of mixtures of PAHs in food, as an example of an approach derived for mixtures of chemicals which do not act by the same mode of action. For Members unfamiliar with the assessment, it is attached at Annex 2.

Secretariat
October 2009

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DRAFT STATEMENT ON THE ASSESSMENT OF THE CARCINOGENICITY OF CHEMICAL MIXTURES

Introduction

1. Testing and risk assessment are usually carried out on single chemicals whereas humans are usually exposed to mixtures of chemicals either simultaneously or sequentially. At the horizon scanning exercise in 2007 we decided to review current developments in the assessment of chemical mixtures with regard to carcinogens, potential interactions and their modes of action. 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 combined carcinogenic action of mixtures of carcinogens, or of mixtures of a carcinogen and other chemical(s), and on methods for testing and assessment of such effects.

2. Our sister committee, the Committee on Mutagenicity (COM^a), has reviewed the literature pertaining to the evaluation of mixtures of potential mutagens. The COM focused on the possible occurrence of synergistic interactions, the possible mechanisms 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 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) and World Health Organisation (WHO)/ International Programme on Chemical Safety (IPCS). Both of the latter 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 chemicals on carcinogenic potential.

3. 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:

^a A list of all abbreviations in this statement is given at the end of the document.

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

4
5 Simple similar action (also referred to as simple joint action) is the concept whereby
6 combinations of chemicals have the same target organ acting via the same mechanism
7 (or mode) of action. (It is also occasionally referred to as 'dose or concentration
8 addition' although, strictly speaking, this is the effect, not the concept). In simple similar
9 action, the effect of the components of a mixture is determined by their respective doses
10 and potencies. The combined effect is estimated from the summation of the potency-
11 normalised doses and toxicity is predicted from the dose response curve of a 'reference'
12 compound, to which the others are normalised.

13
14 Simple dissimilar action (also referred to as independent joint action, simple
15 independent action, effect/response addition) is assumed when individual chemicals
16 have different modes of action and, possibly, the nature and site of action also differ.
17 The effect of each chemical does not modulate or contribute towards the effects of the
18 other constituents of the mixture and, hence, the health effects of exposure to the
19 mixture are expected to be qualitatively and quantitatively similar to those produced by
20 individual components when administered alone. Effect addition is the summation of
21 the individual responses of the different mixture components and toxicity is predicted
22 from the dose response curves of the individual chemicals.

23
24 Interaction is present when the observed effect of two or more exposures differs from
25 the effect that would be expected if the exposure had additive effects. Synergism and
26 potentiation are terms used to describe responses that are greater than expected, and
27 antagonism and inhibition are used for responses which are less than expected.

28
29 4. The possible mechanisms underlying an interaction are often divided into three
30 categories: direct chemical-chemical, toxico/pharmacokinetic, and
31 toxico/pharmacodynamic mechanisms. It is emphasized that the nature of the
32 interaction can change with altered exposure conditions (for example, dose, duration,
33 sequence of exposure and the relative proportions of the components of the mixture).

34
35 5. The review was undertaken taking into account these theoretical classifications
36 and principles. However, it is recognized that the nature of potential combination effects
37 do not fall neatly into categories and some mixtures may have more than one type of
38 effect. Furthermore, we considered that it would facilitate the review if we identified
39 examples of potential synergistic reactions which occurred at different stages of the
40 carcinogenic process, as this may shed light on the mechanisms whereby carcinogens
41 can interact. With regard to evaluating synergistic responses, it was noted that the
42 COM, in its review of mixtures, assessed papers according to the criteria laid out in
43 Borgert (2001). The essential criteria were:

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

7 However, we were unable to use these criteria for the papers we reviewed, as the
8 requirement for detailed dose response data was rarely met. To evaluate accurately the
9 effects of mixtures of chemicals on the entire carcinogenic process would necessitate
10 life-time carcinogenicity studies of mixtures of carcinogens. These studies would need
11 to include groups of animals receiving different doses of both the mixtures and the
12 individual chemicals to determine the dose responses for both. This would entail large
13 and complex studies which it would be hard to justify in terms of either expense or
14 animals.

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

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

28 7. When there is evidence that the members of a group of chemicals elicit their
29 effects by the same MOA, and do not themselves interact chemically, their combined
30 effects can be determined by using Relative Potency Factors (RPF) or Toxic
31 Equivalency Factors (TEF). These RPFs/TEFs are expressed relative to an 'index
32 compound' and are used to normalize the toxicities of chemicals within such a common
33 mechanism group to a single compound, which is generally the one for which toxicity
34 and absorption/distribution/metabolism/excretion (ADME) profiles are best
35 characterised. The RPF/TEF for each chemical is derived from information such as its
36 point of departure for one or more end-points relative to that of the index chemical in *in*
37 *vivo* and *in vitro* systems, QSAR and expert judgement.

38 8. The TEF system was first developed to facilitate risk assessment for
39 polychlorinated dibenzo-*p*-dioxins and related chemical classes. Detailed evaluations of
40 the TEFs for dioxins and dioxin-like compounds have been undertaken and published

1 by WHO/IPCS (van de Berg et al 2006). Carcinogenic potential is not an endpoint
2 which has been used in the past when setting TEFs because of the lack of
3 carcinogenicity data on individual congeners. A validation study has been carried out
4 with 3 individual dioxins or dioxin-like compounds and this broadly supported the
5 concept of dose addition and TEFs for carcinogenicity of mixtures of these chemicals
6 (Walker et al 2005). However the database is very limited. Oestrogens are also
7 considered to form a CMG and there are some approaches using *in-vitro* screening
8 which provide robust information on dose additivity (Charles et al 2002, Payne et al
9 2001). However, there is a paucity of studies investigating *in vivo* responses to mixtures
10 of oestrogens. Moreover, there can be exceptions to the concept of dose additivity for
11 groups of similar chemicals. For example, oestrogens may act through either ER α or
12 ER β to produce either inhibitory or stimulatory effects.

13 9. Other groups of similar chemicals may all demonstrate carcinogenic potential but
14 may not necessarily act by the same MOA. In this case it would not be appropriate to
15 use TEFs for evaluation of the potency of a mixture. For example, the available
16 evidence supports the view that it is inappropriate to use TEFs to assess the potential
17 carcinogenicity of combined exposures to polycyclic aromatic hydrocarbons (PAHs),
18 where there are no carcinogenicity data on most of the individual PAHs. There are
19 inconsistencies in the response to the different PAHs, dependent on the test system
20 used to evaluate toxicities, evidence of interactions between different PAHs (see below)
21 and limitations in the selection of an appropriate index compound. An alternative
22 approach has been derived for the carcinogenic risk assessment of mixtures of PAHs in
23 food by the European Food Safety Authority (EFSA) (European Food Safety Agency,
24 2008), although this does not take account of possible interactions between PAHs. This
25 approach entailed using a 'surrogate marker' approach, based on benchmark dose
26 values derived from the 2 year carcinogenicity study in which mice were fed two
27 mixtures of coal tar containing several PAHs. A group of four PAHs was recommended
28 as the appropriate surrogate marker for the presence of PAHs in food (PAH4), based on
29 a collation of food survey data. Whereas both methods involve uncertainties, we agree
30 that, in this case, the EFSA surrogate marker approach is to be preferred to the TEF
31 approach.

32

33 **Simple dissimilar action**

34

35 10. Application of this principle to the evaluation of cancer as an endpoint is
36 complicated and there are insufficient experimental data on how chemicals with diverse
37 modes of action would act in combination with regard to the induction of tumours.
38 Consequently, an examination of the potential complexities of combined exposures to
39 such chemicals was considered to be outside of the scope of the current review.

40

41 **Interactions**

42

43 11. We aimed to examine the potential for chemicals to interact at different stages in
44 the carcinogenic process. The following points in the carcinogenic process were
45 identified as examples of potential sites for interaction: ADME processes, DNA

1 adduction, mutagenicity, early preneoplastic changes, proliferation, apoptosis and
2 neoplastic transformation. Initially, the toxicological literature was reviewed for
3 examples of interactions and we examined in the first instance polycyclic aromatic
4 hydrocarbons (PAHs) and heterocyclic amines (HCAs). It is noteworthy that most
5 studies of interactions, including studies conducted *in vitro*, did not conform to the
6 criteria laid out by Borgert, as described previously.

7
8 12. PAHs are a group of chemicals which have been evaluated with the
9 consideration that human populations are commonly exposed to mixtures of these,
10 including complex mixtures such as those found in coal tar and urban dust particulate
11 matter. *In vitro* and *in vivo* approaches were used in the papers retrieved to assess
12 potential synergistic responses including: the production of DNA adducts, tumour
13 formation using initiation promotion models, and effects on the P450 (CYP) family of
14 enzymes, particularly CYP1A1 and CYP1B1. There was some evidence that some
15 PAHs, including those within a complex mixture, may have the potential to decrease the
16 potency of others by altering metabolism. For example, a significant reduction of DNA
17 adducts was observed when coal tar extract (Standard Reference Material, SRM₁₅₉₇)
18 was co-administered with benzo[a]pyrene (B[a]P) and dibenzo[a,l]pyrene (DB[a,l]P). In
19 human breast epithelial cells (MCF-10A), reduced DNA binding was associated with
20 induction of CYP1A1 and 1B1 (Mahadevan et al 2005). In V79 cells expressing
21 CYP1A1 or 1B1, reduction in DNA adducts was more apparent in the CYP1B1
22 expressing cells (Mahadevan et al 2007). EROD activity indicated that SRM
23 competitively inhibited the activity of both isoforms, more strongly on CYP1B1. *In vivo*,
24 SRM₁₅₉₇ reduced the number of tumours induced by DB[a,l]P in a SENCAR mouse skin
25 model, but did not have the same effect on B[a]P induced lesions (Marston et al 2001).

26
27 13. The studies provided some examples of how chemicals, including complex
28 environmental mixtures, can impact on the carcinogenic potential of other PAHs. In
29 testing the hypothesis of competitive inhibition of enzymes responsible for the metabolic
30 activation of PAHs it was broadly demonstrated that tumour promotion and DNA
31 adduction were affected by the mixtures and that this could be explained, in part, by
32 altered CYP activity. For example, it is proposed that B[a]P is more readily activated by
33 CYP1A1 than by CYP1B1, such that the competitive inhibition of the former isoform
34 would result in reduced activity. Furthermore, it was generally shown that the effects of
35 environmental mixtures on the metabolism of DB[a,l]P differs from those of B[a]P. This
36 probably indicates the complexity of the interactions, both metabolic and genotoxic,
37 involved in the processes and the dose dependency of these interactions. Moreover,
38 the majority of interactions described involved toxicokinetic alterations and it is difficult
39 to put these into context with interactions downstream in the carcinogenic process.

40
41 14. There are many reservations when interpreting these data. Although it is known
42 that PAHs are inducers of xenobiotic metabolism, induction would be thresholded and
43 the extent would be dependent on dose, dose route and tissue examined. Differences
44 were observed between results obtained *in vitro* and *in vivo*. The relevance of the
45 SENCAR mouse skin model for the evaluation of carcinogenicity is also questionable.

1 As such, it is difficult to extrapolate the altered risk of chemicals observed in the models
2 used and the implications for human risk assessment are uncertain. It was concluded
3 that analysis of *in vivo* studies with regard to potential interactions is complicated since
4 pathways of activation and detoxification are inextricably linked and it is difficult to
5 determine how these toxicokinetic interactions may contribute to the overall
6 carcinogenic process, particularly at low levels of PAHs likely to occur following dietary
7 or environmental exposure.

8
9 15. Heterocyclic amines (HCAs) are another class of chemicals which have the
10 potential to interact with one another. A number of studies were retrieved which had
11 assessed potential interactions of food heterocyclic amines using liver foci initiation
12 promotion models in rats. The HCAs examined were Trp-P-1, Glu-P-2, IQ, MeIQ and
13 MeIQx, Trp-P-2, Glu-P-1, MeAaC, AaC and PhIP (see abbreviations). As an example,
14 these were administered as 1/1, 1/5, 1/10, 1/25 or 1/100 of a known carcinogenic dose^b
15 and as combinations all of the first five at 1/5 and 1/25 of the dose or all 10 at 1/10 and
16 1/100. GST-P-positive foci >0.1mm were the selected endpoint (Ito et al 1991,
17 Hasegawa et al 1994 a,b). It was claimed that some HCAs may act synergistically in
18 promoting tumours through a hypothesised CYP induction mechanism and this was
19 apparent at low doses claimed by the authors to be relevant as a human consumption
20 scenario. However, we find it difficult to draw useful conclusions from these studies for
21 a number of reasons. Firstly, the initiation-promotion study protocols which have been
22 used to examine interactions between the HCAs were overly complex. The partial
23 hepatectomy protocol fixes mutations occurring during the period of regrowth and, since
24 there was no consistent synergistic response in this very sensitive model, the relevance
25 to human health is questionable. The way in which the authors have analysed the
26 results (subtracting a high background incidence from the induced incidence) is likely to
27 be subject to significant error. In addition to the high variability and high background
28 tumour incidence, only limited dose response data was provided. No null hypothesis
29 was given and, therefore, no statistical comparison of the tested hypotheses was
30 possible. We do not agree with the conclusion from these studies that there was clear
31 evidence of synergy. Furthermore, even if synergy was shown to occur in the model
32 system used, its applicability to human exposure is highly questionable.

33
34 16. From these studies we conclude that there can be apparent interaction at doses
35 close to the observed threshold for CYP induction but this may be artefactual and it is
36 not clear whether interaction would be seen at higher doses, when the response could be
37 determined more reliably. Furthermore, the studies which evaluated HCAs were
38 unconvincing and we suggest that less complex protocols might lead to more
39 informative studies.

40
41 17. In the absence of clear evidence of interactions in carcinogenicity from the
42 toxicological literature studied, we also examined the epidemiological literature for
43 examples of evaluations of the effect of combinations of exposures on cancer incidence

^b Described in Ito et al (1991) as 'the dose used in the carcinogenicity studies'.

1 and the potential impact on public health. The two examples which we considered were
2 combined exposure to alcohol and tobacco smoking on the incidence of a number of
3 cancer endpoints, and combined exposure to asbestos and tobacco smoking on the
4 incidence of lung cancer. From these data it was hoped to determine whether an
5 understanding of the mechanisms which lead to interactions with regard to
6 carcinogenicity could be useful in improving the assessment of the risk of combination
7 of chemicals following exposure to man. Our comments on the data reviewed are given
8 in the Annex to this statement.

9
10 18. In epidemiology, as in toxicology, interaction is present when the observed effect
11 of two or more exposures differs from the effect expected if the exposure had additive
12 effects. However, the term “additive effects” has to be interpreted in terms of the model
13 fitted to the data, which may or may not itself be additive. For example, if the
14 cumulative risk of cancer is 1% for exposure A and 2% for exposure B, the expected
15 joint effect under an additive model will be 3%. This works on a scale of absolute
16 measures (such as cumulative risks). If a relative scale is used instead (e.g. relative
17 risks), complications arise and, according to the additive model, the expected joint effect
18 is $RR_{AB} = (RR_A + RR_B - 1)$. This is what is proposed by some, but not all, epidemiologists
19 as the standard. However, the additive model for relative measures conflicts with the
20 fact that, from a statistical point of view, lack of interaction (i.e. statistical independence)
21 is given by the product of probabilities. This is consistent with the observation that in
22 multivariate (logistic regression) the expected value for the interaction term is the
23 product of the relative risks (null hypothesis of no interaction).

24
25 19. Since there is disagreement between a model based on additivity used for public
26 health purposes, and one based on multiplicativity derived from statistical premises, it is
27 necessary to state upfront which model one uses whenever “interaction” or “synergy”
28 are referred to. In the case of the literature on asbestos and tobacco, many estimates
29 are compatible with a multiplication of the relative risks, others with their sum.
30 According to a public health point of view, there is interaction in the first instance, but
31 not in the second instance. According to a statistical approach, instead, there is no
32 interaction in the first case and there is “antagonism” in the second.

33
34 20. In general, it was considered that assessing the potential interactions that may
35 occur during the biological responses to carcinogenic chemicals (both increased and
36 decreased effects) was fraught with difficulties. Firstly, it is recognised that
37 extrapolating data from the majority of methodologies used to substitute for
38 carcinogenicity bioassays to possible carcinogenic responses in man is extremely
39 difficult. *In vitro* studies can give qualitative information on the relative carcinogenic
40 hazard at best and extrapolating from the results to propose modes of action is
41 questionable. The complexities involved in the carcinogenic process, including the
42 possibility that two chemicals could be present in the body at very different times, yet
43 provoke a synergistic response, make evaluating risks posed by potentially carcinogenic
44 chemicals entirely different from evaluating the vast majority of chemical toxicities.
45 Furthermore, the extended time taken before tumours occur following chemical

1 exposure make it difficult at present to evaluate responses in test systems other than
2 life time bioassays in rodents. Epidemiological studies are expensive and investigation
3 of interactions necessitates the existence of populations which have been exposed to
4 the individual components of the mixture and other populations which have been
5 exposed to the mixture. This is not a common situation for chemicals, for example,
6 occupational and environmental exposure to the carcinogenic PAHs is nearly always to
7 a mixture of PAHs. Thus, epidemiological studies are not a practical alternative to
8 animal studies in this case.

10 **Conclusions**

11
12 21. It should be recognised that humans are commonly exposed to mixtures of
13 chemicals, including carcinogens and it is not possible for risk assessment to account
14 for every possible combined action of mixtures of carcinogens, nor of mixtures of a
15 carcinogen and other chemical(s) which might have an additive or interactive effect.
16 Nevertheless, some recommendations can be made:

- 17 • Mixtures of chemicals which act via the same MOA, which do not interact with
18 one another, such as polychlorinated dibenzo-*p*-dioxins, can be assessed using
19 the concept of dose additivity and relative potency factors/toxic equivalency
20 factors.
- 21 • Although there may be an adequate margin between exposure to a carcinogen
22 and either its no observed adverse effect level (in the case of a non-genotoxic
23 carcinogen) or another parameter such as a BMDL₁₀ (in the case of a genotoxic
24 carcinogen), it is possible that simultaneous exposure to two carcinogens which
25 have the same MOA may result in an unacceptably low margin of exposure.
26 Risk assessors should be alert to this possibility when assessing a chemical
27 which commonly occurs together with one or more other chemicals which have
28 the potential to cause cancer.
- 29 • There are several potential sites in the carcinogenic process at which
30 carcinogens might interact, for example, ADME processes, DNA adduction,
31 mutagenicity, early preneoplastic changes, proliferation, apoptosis and neoplastic
32 transformation. MOA analysis may be of value here, in determining critical steps
33 at which interaction might be anticipated.
- 34 • It could be postulated that the combination of a chemical which causes a
35 mutation with one that induces proliferation will act synergistically with regards to
36 the induction of tumours. This is analogous to the well-established phenomenon
37 of initiation-promotion.
- 38 • It is postulated that otherwise non-carcinogenic chemicals, such as anti-apoptotic
39 chemicals or chemicals which interfere with cell cycle regulation, which alter
40 ADME processes or which increase permeability of the skin or oral mucosa,
41 might have the potential to interact synergistically with classical carcinogens.
- 42 • It was considered that there were a number of possible ways to proceed in this
43 area. Unfortunately, the assessment of potential interactions in the context of
44 carcinogenicity is complex due to the multi-stage nature of the process and the
45 high cost of carcinogenicity studies.

- 1 • *In vitro* studies of interactions must be hypothesis drive, attempt to characterise
2 the dose-response and use models relevant to *in vivo* carcinogenicity. These
3 studies should adhere to the criteria laid out in Borgert et al (2001). Models used
4 to evaluate the synergistic interactions between PAHs and between HCVs were,
5 in general, overly complex and may not truly reflect the situation for
6 carcinogenesis. Thus extrapolation of results for risk assessment in man is
7 difficult.
- 8 • We do not advocate standard carcinogenicity studies on mixtures of chemicals
9 except in exceptional circumstances. Such studies would be costly and would
10 involve the use of a considerable number of animals.
- 11 • In terms of the risk assessment of carcinogens for potential interactive effects,
12 exposure to a non-genotoxic carcinogen at or below the no-effect level for the
13 critical effect contributing to the interaction will not result in an interaction with a
14 chemical which has a different MOA. In the case of genotoxic carcinogens, in
15 principle, effects could occur at any level of exposure which could lead to
16 interaction. This supports the view that exposure to genotoxic carcinogens
17 should be as low as reasonably practicable.

1 Annex

2
3 **Examples of multiple exposures and potential interactions in humans**

4
5 **Alcohol and tobacco smoking:**

6
7 1. Alcohol and tobacco smoking are each known to be predominant risk factors for
8 a number of cancers i.e. cancers of the mouth, neck and squamous cell carcinoma of
9 the oesophagus. The studies reviewed show that these two factors act in a greater than
10 additive manner to produce these cancers with effects apparent at moderate as well as
11 high intakes (Lagergren et al 2000, Lee et al 2007). In some instances, the
12 multiplicative increases are very large (odds ratios of up to 177). However, this
13 synergism is not apparent for oesophageal adenocarcinoma and cancers of the gastric
14 cardia (Sjodahl et al 2006).

15
16 2. The mechanism for the synergistic effect is not well understood and we
17 considered a number of plausible hypotheses. Firstly, the induction of cytochrome
18 P450 (CYP) enzymes by ethanol is suggested as a potential mechanism. There is
19 evidence that ethanol induces CYP isoforms which are capable of metabolically
20 activating some carcinogenic nitrosamines found in tobacco smoke. Induction of the
21 CYP 2E1 isoform at extra-hepatic sites such as the oesophagus, combined with
22 decreased first pass metabolism of tobacco associated nitrosamines in the liver due to
23 competitive inhibition by ethanol, is predicted to lead to increased concentrations of
24 DNA-reactive nitrosamine metabolites leading to elevated cancer risk (Lecheveral et al
25 1999, Godoy et al 2002, Anderson et al 1995). A second plausible hypothesis, based
26 on *in vitro* data which is convincing but not extensive, suggests that alcohol increases
27 the permeability of the oral mucosa to carcinogenic nitrosamines. This may also
28 contribute to the synergistic effect observed (Du et al 2000, Azzi et al 2005).

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

37
38 **Cigarette smoking and asbestos**

39
40 6. Exposure independently to cigarette smoke or to asbestos causes lung cancer
41 and it has been claimed that combined exposure results in a synergistic effect on lung
42 cancer induction (Selikoff et al 1968, Lee 2001). The exact nature of the interaction
43 between asbestos and tobacco smoking in the induction of lung cancer has been
44 debated among researchers. From the published literature, most systematic reviews
45 have found a marked heterogeneity in the magnitude of the joint effect, with the

1 interaction ranging from less than additive in some studies to multiplicative in other
2 studies. Despite extensive investigations exploring the interaction between cigarette
3 smoke and asbestos, the precise mechanisms involved at the cellular and molecular
4 level are unclear. Asbestos and tobacco are both complex carcinogens and it is
5 believed that they can both act at more than one stage of carcinogenesis and, hence,
6 have interdependent effects on the multistage process of lung cancer (Vainio and
7 Boffetta, 1994).

8
9 7. A number of authors have proposed a synergistic interaction between cigarette
10 smoke and asbestos and various mechanisms have been proposed as the potential
11 explanation. These include:

- 12 • cytotoxic, genotoxic and clastogenic nature of asbestos and tobacco smoke –
13 supra-additive effects have been noted for mutation frequency, sister chromatid
14 exchange, and DNA strand breaks in a variety of test systems (Lohani et al
15 2002, Kelsey et al 1986, Jung et al 2000)
- 16 • the generation of oxidative damage - both cigarette smoke and asbestos fibres
17 generate reactive oxygen species and synergistic responses in models
18 evaluating this have been observed. However mechanistic insights into or
19 hypotheses about this interaction are not well developed.
- 20 • enhancement of the penetration and accumulation of asbestos in the lung by
21 tobacco smoke – demonstrated in a number of models including following the
22 assessment of asbestos fibres in the airways of smokers and non-smokers
23 (McFadden et al 1986 a,b).
- 24 • the potential for asbestos to act as a delivery system for tobacco carcinogens
25 into the lung, for example by enhancing the diffusion of lipophilic carcinogens,
26 was shown to be unlikely (Gerde et al 1994).
- 27 • the enhancement of somatic mutations in k-ras, FHIT and p53 genes. – some
28 associations of smoking and/or asbestos exposure and lung cancer with these
29 genes have been postulated although specific mechanisms have not been not
30 described.

31
32 8. Overall, it was difficult to draw conclusions from the studies evaluating the
33 proposed synergy between asbestos and tobacco as the interaction models need to be
34 studied in depth to understand whether the interaction is additive or multiplicative and to
35 evaluate in detail the hypothesised mechanisms for the interactions and whether they
36 are relevant to understanding risk in man. The definition of additivity in an experiment
37 appears to depend upon which model fits the individual chemicals evaluated.
38 Furthermore, the importance of different types of asbestos needs to be addressed;
39 different types of asbestos may fit different dose response models. Exposure
40 misclassification might also lead to substantial uncertainty in epidemiological studies;
41 this distortion in risk estimates means it is impossible to differentiate between interaction
42 models. We consider that there is some evidence that there might be a synergistic
43 interaction, but it is not strong. It should be noted that, whilst mesothelioma risk stays
44 constant over time following cessation of inhalation of asbestos, lung cancer risk
45 reduces in reformed smokers. This probably reflects the fact that asbestos fibre

1 remained in the lung whereas smoke residue would be cleared out once smoking
2 stopped.

3

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

References

Borgert CJ, Price B, Wells CS et al (2001) Evaluating chemical interaction studies for mixture risk assessment. *Human Ecol Risk Assessment* **7** 259-306

Charles, G.D., Gennings, C., Zacharewski, T.R. et al (2002) An approach for assessing estrogen receptor-mediated interactions in mixtures of three chemicals: a pilot study. *Toxicol. Sci.* **68** 349-360

COT (2002) WiGRAMP report - 'Risk Assessment of Mixtures of Pesticides and Similar Substances' . <http://www.food.gov.uk/cotwg/wigramp/wigrampfinalreport>

European Food Safety Authority (2008), Polycyclic Aromatic Hydrocarbons in Food - Scientific Opinion of the Panel on Contaminants in the Food Chain.

Hasegawa R, Miyata E, Futakuchi M et al (1994a) Synergistic enhancement of hepatic foci development by combined treatment of rats with 10 heterocyclic amines at low doses. *Carcinogenesis* **15** 1037-1041

Hasegawa, Tanaka H, Tamono S et al (1994b) Synergistic enhancement of small and large intestinal carcinogenesis by combined treatment of rats with five heterocyclic amines in a medium-term multi organ bioassay. *Carcinogenesis* **15** 2567-2573

Ito N, Hasegawa R, Shirai et al (1991) Enhancement of GST-P positive liver cell foci development by combined treatment of rats with five heterocyclic amines at low doses. *Carcinogenesis* **12** 767-772

Mahadevan B, Marston CP, Dashwood WM et al (2005) Effect of a standardized complex mixture derived from coal tar on the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons in human cells in culture. *Chem.Res.Toxicol.* **18** 224-231

Mahadevan B, Marston CP, Luch A, et al (2007) Competitive inhibition of carcinogen-activating CYP1A1 and CYP1B1 enzymes by a standardised complex mixture of PAH extracted from coal tar. *Int.J.Cancer* **120** 1161-1168

Marston CP, Pereira C, Ferguson J et al (2001) Effect of a complex environmental mixture from coal tar containing polycyclic aromatic hydrocarbons (PAH) on the tumour initiation, PAH-DNA binding and metabolic activation of carcinogenic PAH in mouse epidermis. *Carcinogenesis* **22** 1077-1086

Payne, J., Scholze, M., Kortenkamp, A. (2001) Mixtures of four organochlorines enhance human breast cancer cell proliferation. *Environ. Health Perspec.* **109** 391-397

Van den Berg, M., Birnbaum, LS, et al (2006) The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci* **93**(2) 223-241

Walker, N.J., Crockett, P.W et al (2005) Dose-additive carcinogenicity of a defined mixture of dioxin-like compounds. *Environ.Health. Perspect.* **113** 43-48

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 = ethoxy resorufin-o-deethylase

GST-P = glutathione-S-transferase-placental

HCA = heterocyclic amine

MOA = mode of action

MCF-10A = a human breast epithelial cell line;

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-5*H*-pyrido[4,3-*b*]indole,

Trp-P-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole,

Glu-P-1 = 2-amino-6-methyldipyrido[1,2- α :3',2'-*d*]imidazole,

Glu-P-2 = 2-amino-dipyrido[1,2- α :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*]quinoxaline,

MeA α C = 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole,

A α C = 2-amino-9*H*-pyrido[2,3-*b*]indole,

PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

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THIRD DRAFT STATEMENT ON THE ASSESSMENT OF THE CARCINOGENICITY OF CHEMICAL MIXTURES

European Food Safety Authority. Polycyclic Aromatic Hydrocarbons in Food [1] - Scientific Opinion of the Panel on Contaminants in the Food Chain. 9 June 2008. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902034842.htm