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

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

6 **Introduction**

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

17 2 Carcinogenicity is a multistage process. In simple terms, the main components
18 of this process are initiation and promotion. *Initiation* is caused by changes in the
19 cellular genetic material due to an induced or spontaneous mutation or gene
20 rearrangement. The initiated cell has an altered response to external stimuli resulting in
21 cell growth or programmed cell death (apoptosis) and is vulnerable to abnormal division
22 or to escape from signals for apoptosis. *Promotion* is any process which gives the
23 initiated cell a growth advantage over normal cells. Clonal proliferation of the initiated
24 cell produces cancer. Chemicals can cause initiation and/or act to enhance promotion
25 (promoters). The action of any particular chemical could potentially be influenced by
26 other chemicals to which an individual is exposed, either simultaneously or at a different
27 time.

28 3 When a chemical (or its metabolite) causes initiation by interacting directly with
29 the genetic material, it is referred to as a “genotoxic carcinogen” and the process as
30 “genotoxic carcinogenicity”. Chemicals which cannot be shown to interact directly with,
31 or cause damage to, DNA in a number of short-term screening tests, but which are
32 capable of inducing cancer, are referred to as non-genotoxic carcinogens.

33 4. Our sister committee, the Committee on Mutagenicity of Chemicals in Food,
34 Consumer Products and the Environment (COM^a), has reviewed the literature pertaining
35 to the evaluation of mixtures of potential mutagens (COM 2009). The COM focused on
36 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.

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

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

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

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

31 **Simple dissimilar action** (also referred to as independent joint action, simple
32 independent action, effect/response addition) is assumed when individual chemicals
33 have different modes of action and, possibly, the nature and site of action also differ.
34 The effect of each chemical does not modulate or contribute towards the effects of the
35 other constituents of the mixture and, hence, the health effects of exposure to the
36 mixture are expected to be qualitatively and quantitatively similar to those produced by
37 the individual components when administered alone. Effect addition is the summation
38 of the individual responses of the different mixture components and toxicity is predicted
39 from the dose response curves of the individual chemicals.

1 **Interaction** is present when the observed effect of two or more exposures differs from
2 the effect that would be expected if the exposures had additive effects. Synergism and
3 potentiation are terms used to describe responses that are greater than additive, and
4 antagonism and inhibition are used for responses which are less than additive.

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

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

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

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

35 However, we were unable to use these criteria for the papers we reviewed, as the
36 requirement for detailed dose response data was rarely met. Mutagenicity/genotoxicity,
37 which was the subject of the COM review, is, at most, only a contributory factor of the
38 carcinogenic process. To evaluate accurately the effects of mixtures of chemicals on
39 the entire carcinogenic process would necessitate life-time carcinogenicity studies of the
40 mixtures. These studies would need to include groups of animals receiving different

1 doses of both the mixtures and the individual chemicals to determine the dose
2 responses for both. This would entail large and complex studies which would be
3 expensive and require ethical consideration in view of the high number of animals
4 needed.

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

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

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

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

1 to enable a risk assessment of a mixture of chemicals by using the tolerable daily intake
2 of the best characterised member of the group (the 'index compound'), or to calculate a
3 risk estimate for a mixture of genotoxic carcinogens. However, in the case of mixtures
4 of genotoxic carcinogens, the predominant advice remains to keep exposures as low as
5 reasonably practicable (ALARP), as stated above.

6 12. The TEF system was first developed to facilitate the risk assessment of
7 polychlorinated dibenzo-*p*-dioxins and related chemicals. Detailed evaluations of the
8 TEFs for dioxins and dioxin-like compounds have been undertaken and published by
9 WHO/IPCS (van de Berg et al 2006). Carcinogenic potential is not an endpoint which
10 has been used in the past when setting TEFs because of the lack of carcinogenicity
11 data on individual congeners. A validation study has been carried out with 3 individual
12 dioxins or dioxin-like compounds and this broadly supported the concept of dose
13 addition and TEFs for carcinogenicity of mixtures of these chemicals (Walker et al
14 2005). However the database is very limited.

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

30 14.. Other groups of similar chemicals may all demonstrate carcinogenic potential but
31 may not necessarily act by the same MOA. In this case it would not be appropriate to
32 use TEFs for evaluation of the potency of a mixture. For example, the available
33 evidence indicates that it is inappropriate to use TEFs to assess the potential oral
34 carcinogenicity of combined exposures to polycyclic aromatic hydrocarbons (PAHs),
35 most of which have no oral carcinogenicity data. There are inconsistencies in the
36 response to the different PAHs, dependent on the test system used to evaluate
37 toxicities, evidence of interactions between different PAHs (see below) and no clearly
38 appropriate index compound. An alternative approach has been derived for the
39 carcinogenic risk assessment of mixtures of PAHs in food by the European Food Safety
40 Authority (EFSA) (European Food Safety Agency, 2008). This entailed using a
41 'surrogate marker' approach, based on benchmark dose values derived from a 2-year
42 carcinogenicity study in which mice were fed two mixtures of coal tar containing several

1 PAHs. A group of four PAHs (PAH4) was recommended as the appropriate surrogate
2 marker for the presence of PAHs in food, based on their concentrations in food and in
3 the tested mixtures. In this model, the possibility of interactions was taken into account.
4 Whereas both methods involve uncertainties, we agree that, in this case, the EFSA
5 surrogate marker approach is to be preferred to the Toxic Equivalency approach.

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

19 **Simple dissimilar action**

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

27 **Interactions**

28 *Toxicological data*

29 17. An interaction at a key event in the carcinogenic process may be reflected in
30 non-additive effects on carcinogenic response and we aimed to examine the potential
31 for chemicals to interact at different stages. The following stages in the carcinogenic
32 process were identified as examples of potential points for interaction: ADME
33 processes, DNA adduction, mutagenicity, early preneoplastic changes, proliferation,
34 apoptosis and neoplastic transformation. Initially, the toxicological literature was
35 reviewed for examples of interactions and we examined in the first instance polycyclic
36 aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs). It is noteworthy that
37 most studies of interactions, including studies conducted *in vitro*, did not conform to the
38 criteria laid out by Borgert, as described previously.

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

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

33 20. There are many reservations when interpreting these data. Although it is known
34 that PAHs are inducers of xenobiotic metabolism, induction would be thresholded and
35 the extent of induction would be dependent on dose, dose route and tissue examined.
36 Differences were observed between results obtained *in vitro* and *in vivo* and between
37 different models. The relevance of the SENCAR mouse skin model for the evaluation of
38 carcinogenicity is also questionable. As such, it is difficult to extrapolate the altered risk
39 of chemicals observed in the models used and the implications for human risk
40 assessment are uncertain. It was concluded that analysis of *in vivo* studies with regard
41 to potential interactions is complicated since pathways of activation and detoxification
42 are inextricably linked and it is difficult to determine how these toxicokinetic interactions

1 may contribute to the overall carcinogenic process, particularly at the low levels of PAHs
2 likely to occur following dietary or environmental exposure.

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

30 *Epidemiological data*

31 22. In the absence of clear evidence of interactions in carcinogenicity from the
32 toxicological literature studied, we also examined the epidemiological literature for
33 examples of evaluations of the effect of combinations of exposures on cancer incidence
34 and the potential impact on public health. The two examples which we considered were
35 combined exposure to alcohol and tobacco smoking on the incidence of a number of
36 cancer endpoints, and combined exposure to asbestos and tobacco smoking on the
37 incidence of lung cancer. From these data it was hoped to determine whether an
38 understanding of the mechanisms which lead to interactions with regard to
39 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'.

1 chemicals to man. Our comments on the data reviewed are given in the Annex to this
2 statement.

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

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

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

30 26. In general, it was considered that the assessment of potential interactions
31 between carcinogenic chemicals was fraught with difficulties. Firstly, it is recognised
32 that extrapolating data from the majority of methodologies used to substitute for
33 carcinogenicity bioassays to possible carcinogenic responses in humans is extremely
34 difficult. *In vitro* studies can give qualitative information on the relative carcinogenic
35 hazard at best. The complexities involved in the carcinogenic process, including the
36 possibility that two chemicals could be present in the body at very different times, yet
37 provoke a synergistic response, make the evaluation of risks posed by potentially
38 carcinogenic chemicals entirely different from the evaluation of the vast majority of
39 chemical toxicities.

1 27. It could be postulated that the combination of any chemical which causes a
2 mutation with one that induces proliferation will act synergistically with regards to the
3 induction of tumours. This is analogous to the well-established phenomenon of
4 initiation-promotion. It is also of note that dose responses to chemicals can be more
5 complex than simple high or low dose effects; it is possible that MOAs will also change
6 with increasing dose, thus further complicating the interpretation of data. Metabolic
7 interactions may occur although it is considered more likely that they will impact on a
8 genotoxic event in the carcinogenesis process as this will only require a short period of
9 alteration; a non-genotoxic mode of action will be affected only by a metabolic change
10 over a prolonged period. In addition, the extended time taken for tumours to occur
11 following chemical exposure make it difficult at present to evaluate responses in test
12 systems other than life-time bioassays in rodents. Epidemiological studies are
13 expensive and investigation of interactions necessitates the existence of populations
14 that have been exposed to the individual components of the mixture and other
15 populations that have been exposed to the mixture. This is not a common situation for
16 chemicals, for example, occupational and environmental exposure to carcinogenic
17 PAHs is always to a mixture of PAHs. Thus, epidemiological studies are not a practical
18 alternative to animal studies in this case.

19 Conclusions

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

- 24 • Mixtures of chemicals which act via the same MOA and which do not react
25 chemically with one another, such as polychlorinated dibenzo-*p*-dioxins, can be
26 assessed using the concept of dose additivity and relative potency factors/toxic
27 equivalency factors.
- 28 • Although there may be a substantial margin between exposure to a
29 carcinogen and either its no observed adverse effect level (in the case of a non-
30 genotoxic carcinogen) or another point of departure (in the case of a genotoxic
31 carcinogen), it is possible that simultaneous exposure to two carcinogens which
32 have the same MOA may result in a lower margin of exposure. Risk assessors
33 should be alert to this possibility when assessing a chemical which commonly
34 occurs together with one or more other chemicals which have the potential to
35 cause cancer.
- 36 • There are several stages in the carcinogenic process at which
37 carcinogens might interact, for example: ADME processes, DNA adduction,
38 mutagenicity, early preneoplastic changes, proliferation, apoptosis and neoplastic
39 transformation. MOA analysis may be of value here, in determining critical steps
40 at which interaction might be anticipated. Potential interactions in genotoxic
41 MOAs have been addressed in the statement by the COM.

- 1 • It is postulated that otherwise non-carcinogenic chemicals, such as anti-
2 apoptotic chemicals or chemicals which interfere with cell cycle regulation, which
3 alter ADME processes or which increase permeability of the skin or oral mucosa,
4 might have the potential to interact synergistically with known carcinogens
- 5 • The assessment of potential interactions in the context of carcinogenicity
6 is complex due to the multistage nature of the process. However, we do not
7 advocate standard carcinogenicity studies on mixtures of chemicals except in
8 exceptional circumstances. Such studies would be costly and would require
9 ethical consideration in view of the high number of animals required.
- 10 • *In vitro* studies of interactions should be hypothesis driven, attempt to
11 characterize the dose-response and use models relevant to *in vivo*
12 carcinogenicity. These studies should adhere to the criteria laid out in Borgert et
13 al (2001). Models used to evaluate the synergistic interactions between PAHs
14 and between HCAs were, in general, complex and may not truly reflect the
15 situation for carcinogenesis. Thus extrapolation of results for risk assessment in
16 humans is difficult.
- 17 • Overall, *in vitro* studies can be used to confirm molecular targets or
18 provide insight into MOA identification but are not of value for the evaluation of
19 relative potencies of chemicals or interactions at environmentally relevant
20 exposure levels.
- 21 • In terms of the risk assessment of potential interactive effects of
22 carcinogens, exposure to a non-genotoxic carcinogen at or below the no-effect
23 level for the critical effect contributing to the interaction is unlikely to result in an
24 interaction with a chemical which has a different MOA. In the case of genotoxic
25 carcinogens, in principle, effects could occur at any level of exposure which could
26 lead to interaction. This supports the view that exposure to genotoxic
27 carcinogens should be as low as reasonably practicable.

28

1 Annex

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

3 **Alcohol and tobacco smoking:**

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

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

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

33 **Cigarette smoking and asbestos**

34 4. Exposure independently to cigarette smoke or to asbestos causes lung cancer
35 and it has been claimed that combined exposure results in a synergistic effect on lung
36 cancer induction (Selikoff et al 1968, Lee 2001). The exact nature of the interaction
37 between asbestos and tobacco smoking in the induction of lung cancer has been
38 debated among researchers. From the published literature, most systematic reviews

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

9 5. 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 2002,
15 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 KRAS, 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 6. Overall, it was difficult to draw conclusions from the studies evaluating the
32 proposed synergy between asbestos and tobacco as the interaction models need to be
33 studied in depth to understand whether the interaction is additive or multiplicative and to
34 evaluate in detail the hypothesised mechanisms for the interactions and whether they
35 are relevant to understanding risk in man. The definition of additivity in an experiment
36 appears to depend upon which model fits the individual chemicals evaluated.
37 Furthermore, the importance of different types of asbestos needs to be addressed;
38 different types of asbestos may fit different dose response models. Exposure
39 misclassification might also lead to substantial uncertainty in epidemiological studies;
40 this distortion in risk estimates means it is impossible to differentiate between interaction
41 models. We consider that there is some evidence that there might be a synergistic
42 interaction, but it is not strong. It should be noted that, whilst mesothelioma risk stays

1 constant over time following cessation of inhalation of asbestos, lung cancer risk
2 reduces in reformed smokers. This probably reflects the fact that asbestos fibre
3 remained in the lung whereas the amount of smoke residue is considered to be
4 significantly reduced once smoking stopped.

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

14

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- 33

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 = ethoxyresorufin-O-deethylase

13 GST-P = glutathione-S-transferase-placental

14 HCA = heterocyclic amine

15 MOA = mode of action

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

17 PAH: polyaromatic hydrocarbon

18 POD: Point of Departure

19 SRM₁₅₉₇ = coal tar extract Standard Reference Material,

20 TEF = toxic equivalency factor;

21 V79 = a Chinese hamster cell line

22

23 HCA Abbreviations:

24

25 Trp-P-1 = 3-amino-1,4-dimethyl-5*H*-pyrodo[4,3-*b*]indole,

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

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

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

29 IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline

30 MeIQ = 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoline,

31 MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline,

32 MeAaC = 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole,

33 AaC = 2-amino-9*H*-pyrido[2,3-*b*]indole,

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