

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT. (COM)

Initial discussion paper on mutagenicity of mixtures

Introduction

1. The COM has expressed an interest in the evaluation of the mutagenicity of chemical mixtures during the 2005 and 2006 horizon scanning exercises. One important recommendation was to consider the possibility occurrence of mutagenic synergy and the implications of such a finding for risk assessment. A number of strategies have been considered for the evaluation of chemical mixtures.¹ These include testing whole mixtures (integrative), fractionation of mixtures to determine mutagenic components (dissective, top-down approach), and investigations of interactions by testing simple combinations, recombined fractions, and spiking of mixtures/fractions (synthetic, bottom up approach). All of these approaches have been identified from literature searches with regard to mutagenicity testing, although relatively few studies of whole mixtures were identified. Approximately 50 research papers were identified for consideration in this initial discussion paper.

[Members will be aware of the COT Working Group report on risk assessment of mixtures of pesticides and similar substances which was published in . It was noted that although there were a large number of studies on mixtures relatively few had appropriate data on the nature of interactions between chemicals. The general principle reached from substantive consideration of data on pesticides across all toxicological end points was that in the absence of data to the contrary substances with similar modes of action could be assumed to act by dose-additivity and substances with dissimilar modes of action could be assumed to act by effect additivity. The term interaction could infer a range of effects such as synergism, potentiation, supra-additivity, sub additivity). In order to evaluate the nature of interactions it was necessary to understand the dose response for toxicological effects of the chemicals in any mixture to determine deviation from additivity. The section dealing with genotoxicity has been appended for information as MUT/07/05. The primary focus of the COT report was to develop practical approaches to risk assessment of mixtures of pesticides and other similar substances. There was no consideration of most appropriate approaches to mutagenicity testing of mixtures or development of mutagenicity testing approaches to identify particular interactions for mutagenicity which might require further consideration. The COT working group noted that it would be impossible to test all potential combinations of chemicals for all toxicological end points and also noted that some mechanisms of interaction such as effect of inhibition of DNA repair on DNA damage as a potential mechanism for potentiation.]

2. An example of a whole mixture mutagenicity approach is the evaluation of the mutagenicity using *Dlb-1^b* mutations in the small intestine of heterozygous mice fed diets containing fried beef, chicken, lamb, pork and

fish. No evidence of a mutagenic effect was identified in this study, although low levels of mutagens were present in the foods used.² These results and those from other studies of whole mixtures can be interpreted in terms of the overall mutagenic potency of the food and the sensitivity of the assay to detect an effect, but provides no information on the relative contribution, of mutagenic chemicals present in the food or the interactions between chemicals. Other investigators have noted that where there is evidence that components of a mixture do interact, and in particular where there is evidence of mutagenic synergy, then it might be prudent to evaluate whole mixtures as they exist to obtain appropriate information on mutagenic hazard.³ Anwar (1993) proposed the term 'total mutagenic burden' for whole mixtures.⁴ However the failure to detect mutagenicity when complex mixtures (fried foods) or fractions (e.g. catalytically cracked clarified oil) are tested either in-vitro or in-vivo doesn't prove the absence of potentially mutagenic compounds.^{5,6} Thus as the nature of interactions between mutagenicity of chemicals will vary with the approach used based on whole mixtures or fractions, it is likely that the results of such studies would have different uses.

3. It is proposed that mutagenicity testing of whole mixtures or fractions (dissective approach) can be primarily used for monitoring purposes to inform on risk reduction strategies. Synthetic studies (both in-vitro and in-vivo) of interaction between chemicals with regard to mutagenicity can be primarily used to identify hypotheses for interaction (such as synergy) and if confirmed in-vivo proposals for further investigation in biomonitoring studies or in epidemiological studies. A proposal is outlined in paras 21-26 of this discussion paper.

4. The focus of this initial review is to present information on four main areas of the evaluation of mutagenicity of chemical mixtures to help develop a rationale for evaluation of mixtures.

i) Approaches to dissection (fractionation/ concentration) of mixture, to provide information on a strategy for monitoring occupational and environmental sources for mutagenicity as an aid in risk reduction strategies.

ii) Approaches to evaluating interactions between exposure to chemicals which affect mutagenicity,

iii) An overview review of the published literature on mutagenic interactions which have been investigated. The data from this initial consideration might require additional literature searching and evaluation before conclusions on the identified interactions can be reached. One objective is that if a particular mutagenic interaction is considered to be of potential significance for public health risk assessment of mixture exposure, this might provide generic information on a mechanism of interaction for future studies. However since the default risk assessment approach to in-vivo mutagens is to assume no threshold and to apply 'As Low As Reasonably Practical' (ALARP) as a risk management approach, the only occasion where an interaction regarding mutagenicity might be of significance for risk management would be where there was clear evidence for mutagenic synergy

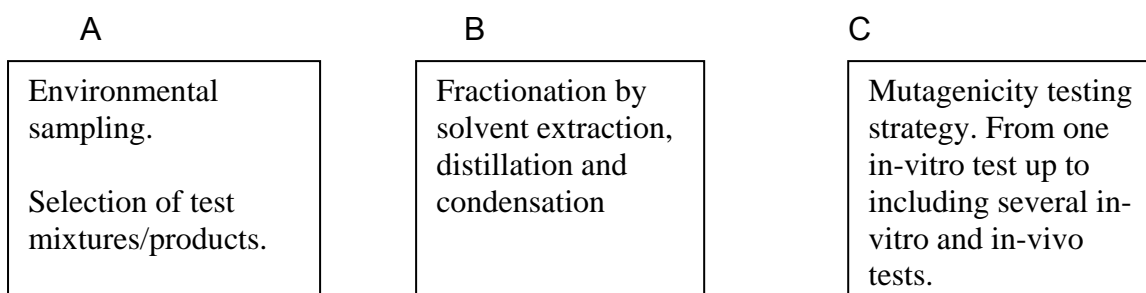
or potentiation occurring at exposure levels appropriate to human exposure. This might possibly raise the priority for the application of ALARP for one or more compounds or exposure scenarios.

iv) Seek comments from COM members for completion of the review, including possible identification of other interactions for consideration.

5. Stewart-Houk and Waters (1996) reviewed strategies for genetic toxicology risk assessment of mixtures and considered the most important potential area for future work was the use of mutational fingerprints of individual chemicals and biomonitoring data to assess potential interactions regarding mutagenicity of mixture exposures in exposed human populations.⁷ Some information on these areas has been incorporated into this review, but overall there is comparatively little information on the nature of mutational interactions in exposed populations and this remains a long term objective of research.

Approaches to dissection (fractionation/ concentration) of mixture.

6. An overview of the published literature is provided. The key elements to approaches are;



Sampling

7. A detailed review of environmental sampling for mutagenicity evaluation of mixtures is beyond the scope of the COM review. A number of papers are cited to illustrate the potential impact of sampling procedures in the results of mutagenicity tests. Crisp and Fisher reviewed the approaches to mutagenicity evaluation of airborne particles and noted the impact of sample mass and volume collected, the size distribution of particles sampled, the potential for chemical reaction of adsorbed particles on filters (e.g. oxidation and nitration of PAHs), the temperature of emissions where samples are taken and combustion conditions pertaining to the sampled material (e.g. coal fly ash). Additional meteorological factors affect environmental sampling strategies, such as wind conditions, precipitation. Geographical factors need to be considered (e.g. high mutagenicity of samples collected at Norwegian coasts reflects particulate matter from continental Europe and Britain).⁸ The conditions of storage of samples prior to preparation for and testing in mutagenicity assays can also influence the results of such assays. Thus the direct acting mutagenicity of diesel exhaust particles reduced with length of refrigeration which was speculated to be due to an increase in bacterial toxic

components during storage.⁸ The factors outlined in this paragraph would also pertain to approaches used to sample cigarette smoke. The condensation of cigarette smoke is considered in the next section.

8. Overall, sampling strategies can significantly influence the estimation of mutagenicity of chemical mixtures and there is thus a need for consistency between sampling in order to generate data that are comparable.

Fractionation by solvent extraction, distillation and condensation

9. A brief tabulation of examples of the approaches used in monitoring studies of mutagenicity of mixtures is given below. There are a considerable number of studies predating the 1980s.⁸ Most investigators use a single step extraction procedure for monitoring studies. The use of more complex approaches involving several solvents (possibility of varying polarity or pH) in investigative studies of particular components is generally not used in monitoring studies. One of the clearest difficulties in developing a monitoring strategy is optimising mutagenic response whilst avoid excessive toxicity to the indicator organisms. Multi-step procedures can result in loss or modification of mutagenic components. Some investigators attempt to sum up the mutagenic responses of fractions and compare o the whole mixture which may be problematic in that the concentrations and interactions between compounds will be altered during the fractionation process.⁸ In an IPCS led collaborative study of the mutagenicity of mixtures (urban air samples, diesel particles and coal tar solution) significant interlaboratory and intralaboratory variance in the results of *Salmonella typhimurium* TA98 and TA100 with or without exogenous metabolic activation was noted, which was partly due to the method of extraction (either soxhlet or ultrasonication) using dichloromethane as a solvent as well as the mutagenicity test procedures used.^{9,10} The final step in the procedures usually involves evaporation of extracts and resuspension in a solvent (usually DMSO) which is more compatible with cell culture and in-vivo mutagenicity test systems. This step may also introduce variance into the procedure.

Type of sample	Procedures used	Comments
Particles (e.g. diesel) ^{12,13,14} collected occupationally or environmentally, or directly from exhausts ^{19,21} , or fumes (coke oven, roofing tar) ¹⁶	Extraction predominantly in dichloromethane ^{12,13,16} (methanol ¹⁴) using soxhlet or ultrasonication processes ^{19,21} evaporate, and resuspend in DMSO prior to testing	Approaches can include additional fractionation and recombination steps ^{12,24} or spiking with known compounds ¹³
Organic based liquids, such as sample products, e.g. oils. ^{15,22}	Solubilise (e.g. cyclohexane) and then evaporate and resuspend in DMSO ²² or add directly to tests system. Use Tween 80 to aid dispersion in in-vitro test systems. ¹⁵	Additional steps can be taken similar to those outlined above for particles/fumes for investigative studies of interactions.
Condensates or particles from pyrotechnic mixtures (e.g. cigarette smoke ^{8,11,18} or mixtures of known compounds ¹⁸). Pyrotechnic	Undertake condensation on filters at room temperature or using dry ice to collect chemicals. ^{8,18} Extraction phases (e.g. diethyl ether ¹⁸ or	Additional steps as outlined for particles above. May include pyrolysis of spiked compounds. ¹¹

procedures may involve electric ignition in closed systems, a pyrolyser machine using defined conditions or calibrated smoking machines	DMSO ¹¹) followed by evaporation to dryness and resuspend in DMOS for testing. Filters can be extracted directly into DMSO. Condensate may be applied directly to test system.	
Hazardous wastes including industrial process effluents (e.g. coke ovens, herbicide plants, paint manufacturers) and municipal sludges. ¹⁷	The physical presentation of samples may vary from solids to sludges and liquids. Extraction procedures include ethanol and/or DMSO extracts. A thin layer chromatography process has been investigated. ¹⁷ Extracts or spots (identified visually with/without UV light) can be incorporated into agar for mutagenicity testing. ¹⁷	Studies generally aimed at monitoring. TLC/Salmonella assay was validated with known mutagens.
Water samples taken from drinking water at various points in the distribution system, rivers at intakes, outflows to rivers, which can be timed to include or exclude agricultural run off. ²⁰	Samples require concentration (evaporation/use of XAD-resin), followed by testing or fractionation and testing. Filter sterilisation may be required prior to testing. ²⁰	Studies can be directed towards monitoring and/or investigative studies of interaction. ²⁰

Mutagenicity testing strategy

10. The primary objective of the mutagenicity testing strategy for chemical mixtures should be to identify hazard in the tested material or mixture. A comparison of the mutagenicity test data for test mixtures derived from the same sources and subject to the same extraction and fractionation procedures may provide information for monitoring hazard of environmental samples, commercial products, pyrolysis products and hazardous wastes. Considerable scope for variance in sampling of mixtures for testing has been reported above and also between and within laboratories regarding the results of monitoring studies has already been noted above with regard to extraction and fractionation procedures using the same source mixtures.^{9,10} The IPCS collaborative study also reported considerable variance with regard to strain of Salmonella used, the activation conditions and between replicate mutagenicity tests within the same laboratory.^{9,10} It is therefore likely that any approach to monitoring mutagenic hazard in chemical mixtures over a period of time would use defined and fixed sampling, extraction and fractionation procedures and mutagenicity testing procedures using a high degree of quality control and possibly undertaking mutagenicity testing at one laboratory for all tests. It is noted that apart from the IPCS trial, there were no published accounts of interlaboratory studies of the monitoring of mutagenicity in chemical mixtures retrieved.

11. A brief overview of the mutagenicity testing strategies that investigators have used is provided below followed by consideration and adaptation of the proposal by Waters MD et al (1990)²³ to use genetic activity profiles in the testing and evaluation of chemical mixtures.

12. The majority of studies identified for this review use *Salmonella typhimurium* test strains as the only mutagenicity test.^{2,8,12,13,17,19,22,24} These studies may include metabolic activation selected to increase the number of revertant colonies formed for a particular tested mixture or to test for the mutagenicity of particular groups of compounds in a mixture (e.g. use of hamster S-9)²² or selection of particular *Salmonella* strains (e.g. use of nitroreductase (NR) deficient strains¹⁹, and NR and O-acetyltransferase deficient strains²⁴) or treatments (use of ROS scavengers such as α -tocopherol and/or ascorbate¹⁴) to monitor the mutagenicity of particular groups of mutagenic chemicals within the mixture. This approach is likely to be applicable to all sources of mixtures identified in this review. More recently investigators have used a combination of *Salmonella* bacterial assays for mutagenicity and inhibition of gap junction communication in rat liver epithelia cells to provide information genotoxicity and tumour promotion for fractions from Diesel particles and combinations of fractions.²⁴

13. Relatively fewer studies use additional in-vitro and in-vivo tests. The approaches are summarised briefly in the following table as illustrative examples. Overall additional in-vitro tests can extend the potential for monitoring mutagenic hazard over a wider range of chemicals present in the mixture. In-vivo mutagenicity tests are usually incorporated into testing strategies to confirm the potential for a compound of unknown mutagenic potential to induce effects in-vivo. With regard to monitoring mixtures, the focus of monitoring is to bioassay change in the levels of chemicals some of which are already known to be in-vivo mutagens and thus there is less value in conducting in-vivo tests in a monitoring scheme for mixtures particularly where the levels of known in-vivo mutagens may be below the level of detection in in-vivo tests. One potentially useful approach inclusion of in-vivo tests in a strategy for monitoring complex mixtures was provided by Williams and Lewtas 1985¹⁶ who correlated the mutagenic response (slope of dose-response) to organic extracts from diesel, coke oven, roofing tar and cigarette smoke emissions from in-vitro tests (*Salmonella typhimurium* TA98 +S-9 (rat or hamster), and mouse lymphoma mutagenicity) with response in mouse skin tumour initiation assays. Having correlated mutagenic potency in-vitro and in-vivo (in this case between different mixtures) it would therefore be possible to continue monitoring of the comparative ranking of these mixtures with an in-vitro mutagenicity test strategy. It is possible to reach this conclusion as there was relatively good knowledge of the chemical composition of the mixtures included in the study, and a key hypothesis under test would have been the investigation of mixtures of PAHs which helped to define the in-vitro and in-vivo parts of the testing strategy.

Test strategy used	Test mixtures used	Comments
1. <i>Salmonella typhimurium</i> strains +/- S-9 plus in-vitro MN assay ¹¹	TPM from cigarette smoke extracted into DMSO	Positive in ST but negative results obtained in in-vitro MN assay. Studies specifically designed to investigate addition of

		additives to combustion of cigarettes. Some monitoring information available for constituents of pyrolysis of cigarettes available.
2. Salmonella typhimurium strains +/- S-9 (plus CA in CHO cells 3h exposure +/- S-9 for motorcycle exhaust particles (MEPs)) and additional in-vivo BM MN assay.	Tested materials, MEPs(a) and pyrolysis products of chemical mixtures, including titanium dioxide/hexachloroethane, and zinc/hexachloroethane(b)	MEPs positive in ST, CAs (both +/- S-9), and positive dose-response in in-vivo i.p BMMN tests. Focus of study was to investigate effect of antioxidants. Chemical constituents of MEP were unknown. For pyrolysis of mixtures (b) positive dose-response in TA 98/100 in absence of S-9. BMMN i.p assays negative. Some information available on pyrolysis products.
3. Salmonella typhimurium/SCEs in PBLs, in-vivo sperm abnormality test (i.p), BMMN (i.p) and BM SCEs (i.p) In-vitro tests +/- S-9 ¹⁵	Comparison of Eastern and Western US shale oils, crude petroleum and coal-derived oil.	Investigators found samples were not soluble in DMSO and used suspension in Tween 80. Investigators report ranking of oils using ST data. Slight SCE induction by all samples except petroleum crude oil in-vitro for SCEs. Negative results in in-vivo assays reported. Rationale for study was to extend previous studies of oil, use a different solubilising system and to include in-vivo tests in comparative study.
4. Salmonella typhimurium TA98/100 (+S-9 rat or hamster), mammalian cell mutagenicity (MLA) and in-vivo skin dermal bioassay ¹⁶	Comparison of organic extracts from diesel, coke oven, roofing tar and cigarette smoke emissions (Condensate).	Relative ranking in all tests was similar; coke oven>roofing tar>cigarette condensate>diesel.

Initial comments on a possible strategy for monitoring mutagenicity of complex mixtures

14. Waters MD and colleagues²³ (Annex 1 to this discussion paper) suggested that information from published databases of Genetic Activity Profiles could be used in combination with data on chemical composition of mixtures (e.g. on air pollutants) along with information on the lowest effective dose in mutagenicity tests to define an appropriate testing strategy for mutagenicity testing of chemical mixtures. The authors accepted that the approach would be limited by inadequate chemical and biological characterisations of chemical constituents in mixtures, and the assumptions

made in using the data (regarding interactions between chemicals), but the approach would be useful in optimising use of resources.

15. Combining the information presented in this discussion paper with the proposals made by Waters MD and colleagues, it is possible to tentatively suggest a possible staged approach to monitoring strategies for chemical mixtures. The approach has similarities to the tiered approach to toxicological testing of mixtures suggested by Schaeffer DJ (1987).²⁵ Members are asked to comment and/or refine this proposal.

Preliminary considerations

- A. Collect information on chemical composition, and mutagenicity of chemicals in mixture. Define purpose of monitoring approach (is this to monitor overall mutagenic hazard, or to monitor selected levels of chemicals or groups of chemicals).
- B. Review literature for appropriate sampling, extraction and testing of similar mixtures. Review mutagenicity test data on specific chemicals in mixture or chemicals selected for monitoring.

With regard to mutagenicity testing

- C. Define in-vitro testing strategy with focus on optimising and standardising the approach.
- D. Undertaken in-vitro monitoring to validate approach and identify sources of variance and their impact.
- E. Consider if necessary to develop an in-vivo segment to strategy. (For known in-vivo mutagens in a mixture, the default is to assume no threshold for mutagenicity in-vivo.)

Review of strategy

- F. Implement strategy and use data to inform on risk reduction strategies. Review strategy or add additional fractionation and identification of chemicals in monitoring scheme, only if a defined need is identified (e.g. sudden change in results of monitoring data).

Approaches to evaluating interactions using mutagenicity tests

16. A number of approaches may be used. In this initial discussion paper, a number of papers from the Ostby L and Eide I and colleagues from a number of research institutes in Norway have been appended as Annex 2^{11,12,24} to illustrate the complexity of such studies and the need for consideration of expected patterns of mutagenic response in tester strains, the design of a testing strategy to limit the number of combinations tested to a minimum required to evaluate the nature of any interactions in mutagenicity

tests (by selecting concentrations of test materials taking into account the dose-response of individual compounds or fractions in the tester strains, the consideration of the need for replicate experiments), and the consideration of the most appropriate approach to statistical analysis of data.

17. Ostby and colleagues evaluated the mutagenicity of crude extracts from diesel particles and combinations of fractions prepared from the crude extract to identify the mutagenic responses in *Salmonella typhimurium* TA 98 (and 98 NR and 98 O-acetyltransferase deficient strains, TA 100, (and 100NR)). A factorial design depending on the number of mixtures or fractions to be tested (e.g. 3 or 5 mixture fractions) was used to determine the number of combinations to test. The data were analysed by projections to latent structures (PLS) approach. The data for the experiments with diesel particles have been interpreted as showing additivity between the various tested fractions.¹²

18. In a further study from the same group, the mutagenicity of crude extract from diesel particles was investigated in *Salmonella typhimurium* TA 98 (98NR) TA 100 (100NR) in the presence of exogenous metabolic activation using a spiking approach where benzo(a)pyrene, benzo(a)anthracene, pyrene and fluoranthrene were added. A factorial experimental design was used. The investigators report additivity.¹³

19. Members are asked for generic comments on the approaches used for combination and spiking experiments. What alternative approaches could be used for experimental design and for statistical analysis? Presumably the experimental design advocated by Ostby and colleagues is applicable to other mutagenicity assays including in-vivo approaches but is most practical when applied to relatively cheap and easy to undertake in-vitro assay such as using *Salmonella typhimurium*. One potential discussion point to consider is when evidence for mutagenic synergy identified in in-vitro experiments need further evaluation in in-vivo studies and what approach should taken.

20. A number of other potential approaches for studying interactions between chemicals with regard to mutagenicity were identified in the following section which may help to take these discussions points forward.

Overview of studies on interaction in mutagenicity studies (Synthetic approach).

21. The objective of this section is to highlight some potential interactions which have been studied and those which might be the subject of further consideration by COM where there is evidence for a mechanistic rationale which might result in interaction (i.e. departure from mutagenic additivity involving potentiation or synergy) with regard to mutagenicity and for potential for relevance to public health risk assessment can be demonstrated. The need for information on dose-response of components in interaction studies was emphasised in the introduction to this discussion paper. Demonstration of potential relevance for public health would possibly include mechanistic rationale, in-vitro and in-vivo evidence for interaction with regard to

mutagenicity. This might lead to the formation of a reasonable hypothesis for further study such as in biomonitoring studies.

22. A number of studies were identified where the rationale for the investigation of potential interaction regarding mutagenicity related to a specific situation (e.g. interaction between p-phenylenediamine in oxidative hair dye mixtures and hydrogen peroxide to form Bandrowski's base)²⁶ or where chemicals had been selected as being commonly present in mixtures (e.g. chemicals commonly found at hazardous waste sites in the USA).³ These particular types of investigation have not been the main topics covered in this discussion paper.

23. There are also a number of other studies where a clear antagonistic interaction regarding mutagenicity has been identified (e.g. benzo(a)pyrene has an antagonistic effect on 1-nitropyrene mediated mutagenicity in *Salmonella typhimurium* TA98 mediated by inhibition of nitroreduction of 1-NP).^{27,28} Another example is the inhibition of bacterial mutagenicity of benzo(a)pyrene by the presence of 2,4,6-trinitrotoluene.²⁹ Investigations identifying antagonism of mutagenicity have not been the main focus of this section since such effects in-vivo would not require any change in risk assessment approach and would not suggest any additional public health risks worthy of further investigation in biomonitoring studies.

24. Even when mutagenic potentiation has been demonstrated in-vitro, it is still possible that the data might not suggest a clear rationale for public health significance. Thus Catterall and colleagues reported that black tea thefulvins, a fraction of thearubigins, which are a major component of polyphenols of black tea possessed antimutagenic activity against indirect acting food carcinogens such as heterocyclic amines, PAHs³⁰ and anticarcinogenic activity towards tobacco –specific carcinogens in the mouse lung has also been reported.³¹ Catterall and colleagues recently demonstrated that black tea theafulvins potentiated the mutagenicity of aflatoxin B₁ in *Salmonella typhimurium* most likely through decreased deactivation by reducing conjugation with glutathione.³⁰ Thus to suggest a potential interaction which might be of potential importance for public health, there needs to be not only a mechanistic rationale, in-vitro evidence of interaction and in-vivo evidence of interaction but the information must consistently point towards a synergistic/potentiation interaction with no evidence of potential beneficial antagonistic effects.

25. The following table summarising a number of types of interaction which might be considered further. No definite conclusions have been reached on the basis of the literature reviewed to date. No papers have been appended at this stage for member's consideration.

Type or Groups of chemical with interaction	Experimental evidence identified in initial discussion paper.	Comments.
<u>Metals</u> 1. Arsenite and B(a)P	1. <i>Arsenite and B(a)P</i> ; co-exposure increases adducts 18 fold in Hepa-1 cells and	1. Mechanism may be complex and not necessarily related to effects on DNA

<p>2. Cadmium and B(a)P</p> <p>3. Inhibition of DNA repair by nickel, cobalt, arsenic, cadmium cited as general mechanism of possible interaction.³⁶</p> <p>4. Chromium and B(a)P³⁷</p>	<p>increases <i>hprt</i> mutations 8 fold.³² In-vivo studies showed arsenite (in drinking water) and topical application of B(a)P show increase B(a)P adducts related to arsenite tissue concentrations.³³</p> <p>2. <i>Cadmium and B(a)P</i> Cadmium inhibited BPDE alkylation in the major groove of DNA but not minor groove.³⁴ In a small biomonitoring study, <i>hprt</i> mutation frequency related to the number of Lipophilic DNA adducts (measured by ³²P-postlabelling) correlated with Cadmium concentrations on cord blood.³⁵</p> <p>3. Authors cite interaction between Ni and B(a)P with regard to formation and stability of B(a)P induced DNA adducts.³⁶</p> <p>4. <i>Chromium and B(a)P</i>; Cr enhanced BPDE-DNA adducts in human lung fibroblasts.³⁷</p>	<p>repair. Some epidemiological evidence to support an interaction with regard to smoking and arsenic co-exposure.</p> <p>2. Authors hypothesised that co-exposure possibly through tobacco smoke might result in significant mutagenic effects. Authors cite the possible DNA repair inhibition of Cd another metals as a possible mechanism.³⁵</p> <p>3. Authors acknowledge different molecular targets for metals, but also focus on zinc finger structure in DNA repair proteins as possible targets.³⁶</p> <p>4. Authors note possible co-exposure in tobacco smoke.</p>
<p><u>Pesticides</u></p> <p>1. Paraoxon and 2AAAF³⁸</p> <p>2. Paraoxon and a range of aromatic amines.³⁹</p> <p>3. Pentachlorophenol and 2AAAF⁴⁰</p> <p>4. Paraoxon and heterocyclic amines including PhIP.⁴¹</p>	<p>1. Paraoxon enhanced the mutagenicity of 2AAAF in a range of Salmonella tester strains. However authors noted that previous studies indicated that paraoxon suppressed the mutagenicity of 2AAAF in CHO cells.³⁸</p> <p>2. Evidence of mutagenic synergy reported for Salmonella tester strains.³⁹</p> <p>3. PCP reduced degradation of 2AAAF and enhanced mutagenicity in Salmonella tester strains.⁴⁰</p> <p>4. Evidence for mutagenic synergy reported in Salmonella tester strain (YG1024) in presence of S-9.⁴¹</p>	<p>1. No mechanism could be advanced for these findings</p> <p>2. No mechanism could be advanced. Effect not related to generation of new mutagenic products or to modification of stability of activated amine products.</p> <p>3. Unknown if the mechanism reported would be relevant to in-vivo situation.</p> <p>4. No clear mechanism for effect established.</p>

<u>Effect of UV/sunlight on chemical mediated mutation</u> 1. UV/sunlight irradiation of N-nitrosodimethylamine (NDMA). ⁴² 2. UVB/UVC and B(a)P ⁴³	1. Formation of DNA adducts identified in absence of S-9 in bacterial and calf thymus DNA. Mutagenesis in Salmonella or E.coli tester strains in absence of S-9. ⁴² 2. Increase in frequency of BPDE mutation signatures in co-exposure in SupF mutation assay using kidney cell line. ⁴³	1. Photoactivation of NDMA identified, but relevance to in-vivo situation unclear. 2. Mechanism complex but may involve enhancement of mutagenicity of BPDE-DNA adducts. ⁴⁴ In-vivo relevance unclear. Noted order of exposure affected results.
<u>Mutagenicity of N-acetoxy-acetylaminofluorene with AflatoxinB₁ 8-9-epoxide⁴⁵</u>	Pretreatment of TA 98/1538 with AFB ₁ -8,9-epoxide enhanced mutagenicity of N-AcO-AAF. ⁴⁵	Binding of AFB ₁ reactive metabolite to DNA affected access and binding of second mutagen. ⁴⁵ In-vivo relevance unclear.

B(a)P can signify that studies used BPDE.

26. Using the criteria suggested above in paragraph 25, it would appear that further evaluation of the preliminary data reported in this discussion paper on metals and aromatic hydrocarbon mutagenicity is warranted but there is insufficient evidence currently available regarding the other scenarios reviewed in the table above. Do members agree?

COM questions for consideration.

27. Members are asked to consider the following generic discussion questions.

- i) Can any conclusions be reached regarding the derivation of general guidance on an approach for monitoring occupational and environmental sources for mutagenicity as an aid in risk reduction strategies? What further information might be required?
- ii) Can any conclusions be reached regarding approaches to evaluating interactions between exposure to chemicals which affect mutagenicity? What other approaches might be useful?
- iii) Can any conclusions be reached regarding the specific interactions identified in this discussion paper with regard to priority for review? Are the criteria for consideration appropriate?
- iv) Do members have any suggestions for further topics for consideration within this review?

Secretariat January 2007.

References

1. Eide I (1996). Strategies for Toxicological Evaluation of Mixtures. *Food Chem Toxicol*, 34, 1147-1149.
2. Heddle JA et al (2001). A test of the mutagenicity of cooked meats in-vivo. *Mutagenesis*, 16, 103-107.
3. Ma TH et al (1992). Synergistic and antagonistic effects on genotoxicity of chemicals commonly found in hazardous waste sites. *Mutation Research*, 270, 71-77.
4. Anwar WA (1993). Chemical Interaction: Enhancement and inhibition of clastogenicity. *Environmental Health perspectives*, 101 (suppl3), 203-206.
5. Lee H et al (1994). Bacterial mutagenicity, metabolism, and DNA adduct formation by binary mixtures of benzo(a)pyrene and 1-nitropyrene. *Environ Mol Mutagen*, 24, 229-234.
6. Przygoda RT et al (1999). Assessment of the utility of the micronucleus tests for petroleum-derived materials. *Mutation Research*, 438, 145-153.
7. Stewart Houk V, and Waters MD (1996). Genetic toxicology and risk assessment of complex environmental mixtures. *Drug and Chemical Toxicology*, 19, 187-219.
8. Crisp CE and Fisher GL (1980). Mutagenicity of airborne particles. *Mutation Research*, 76, 143-164.
9. Krewski D et al (1992). Sources of variation in the mutagenic potency of complex chemical mixtures based on the Salmonella/microsome assay. *Mutation Research*, 276, 33-59.
10. Claxton LD et al (1992). Results of the IPCS collaborative study on complex mixtures. *Mutation Research*, 276, 23-32.
11. Baker RB et al (2004). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food Chemical Toxicology*, 42S, S53-S83.
12. Østby L et al (1997). Mutagenicity of organic extracts of diesel exhaust particles after fractionation and recombination. *Archives of Toxicology*, 71, 314-319.
13. Bostrom E et al (1998). Mutagenicity testing of organic extracts of diesel exhaust particles after spiking with polycyclic aromatic hydrocarbons (PAH). *Archives of Toxicology*, 72, 645-649.

14. Cheng YW et al (2004). Genotoxicity of Motorcycle exhaust particles in-vivo and in-vitro. *Toxicological Sciences*, 81, 103-111.
15. Lockard JM et al (1982). Comparative study of the genotoxic properties of Eastern and Western U.S shale oils, crude petroleum, and coal-derived oil. *Mutation Research*, 102, 221-235.
16. Williams K and Lewtas J (1985). Metabolic activation of organic extracts from diesel, coke oven, roofing tar, and cigarette smoke emissions in the Ames assay. *Environmental Mutagenesis*, 7, 489-500.
17. Stewart-houk V and Claxton LD (1986). Screening complex hazardous wastes for mutagenic activity using a modified version of the TLC/Salmonella assay. *Mutation Research*. 169, 81-92.
18. Karlsson N et al (1991). Mutagenicity testing of condensates of smoke from titanium dioxide/hexachloroethane and zinc/hexachloroethane pyrotechnic mixtures. *Mutation research*, 260, 39-46.
19. Clark CR et al (1981). Mutagenicity of diesel exhaust particle extracts: Influence of cart type. *Fundamental and Applied Toxicology*, 1, 260-265.
20. DeMarini DM et al (1982). Use of four short –term tests to evaluate the mutagenicity of municipal water. *Journal of toxicology and Environmental Health*, 9, 127-140.
21. Brooks AL et al (1984). A comparison of genotoxicity of automotive exhaust particles from laboratory and environmental sources. *Environmental Mutagenesis*, 6, 651-668.
22. Blackburn GR et al (1986). Predicting carcinogenicity of petroleum distillation fractions using a modified Salmonella mutagenicity assay. *Cell Biology and toxicology*, 2, 63-84.
23. Waters MD et al (1990). Genetic activity profiles in the testing and evaluation of chemical mixtures, *Teratogenesis, Carcinogenesis, and Mutagenesis*, 10, 147-164.
24. Reveal E et al (2003). Supplemental role of the Ames mutation assay and gap junction intercellular communication studies of possible carcinogenic compounds from diesel particles. *Archives of Toxicology*, 77, 533-542.
25. Schaeffer DJ (1987). A new approach for using short-term tests to screen complex mixtures. *Regulatory Toxicology and pharmacology*, 7, 417-421.
26. Bracher M et al (1990). Studies on the potential mutagenicity of p-phenylenediamine in oxidative hair dyes. *Mutation research*, 241, 313-323.

27. Lee H et al (1994). Bacterial mutagenicity, metabolism, and DNA adduct formation by binary mixtures of benzo(a)pyrene and 1-nitropyrene. *Environmental and Molecular Mutagenesis*, 24, 229-234.
28. Cherng SH et al (1996). Modulatory effects of polycyclic aromatic hydrocarbons on the mutagenicity of 1-nitropyrene: a structure-activity relationship study. *Mutation Research*, 367, 177-185.
29. Donnelly KC, et al (1998). Mutagenic interactions of model chemical mixtures. *Chemosphere*, 37, 1253-1261.
30. Catterall F et al (2003). Effects of black tea theafulvins on aflatoxin B₁ mutagenesis in the Ames test. *Mutagenesis*, 18, 145-150.
31. Yang GY (1997) *Carcinogenesis*, 18, 2361-2365.
32. Maier A et al (2002). Arsenic co-exposure potentiates benzo(a)pyrene genotoxicity. *Mutation Research*, 517, 101-111.
33. Evans CD et al (2004). Effect of arsenic on benzo(a)pyrene DNA adduct levels in mouse skin and lung. *Carcinogenesis*, 25, 493-497.
34. Prakash AS et al (1998). Cadmium inhibits BPDE alkylation of DNA in the major groove but not the minor groove. *Biochemical and Biophysical Research Communications*, 244, 198-203.
35. Godshalk R et al (2005). Interaction between cadmium and aromatic DNA adducts in hprt mutagenesis during foetal development. *Mutagenesis*, 20, 181-185.
36. Hartwig A and Schwerdtle T (2002). Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicology Letters*, 127, 47-54.
37. Feng Z et al (2003). Chromium (VI) exposure enhances polycyclic aromatic hydrocarbon-DNA binding at the p53 gene in human lung cells. *Carcinogenesis*, 24, 771-778.
38. Gichner T et al (1996). Mutagenic synergy between paraoxon and plant-activated m-phenylenediamine or 2-acetoxyacetylaminofluorene. *Environmental and Molecular Mutagenesis*, 27, 59-66.
39. Wagner ED et al (1997). Mutagenic synergy between mammalian or plant activated aromatic amines. *Environmental and molecular Mutagenesis*, 30, 312-320.
40. Gichner T et al (1998). Pentachlorophenol-mediated mutagenic synergy with aromatic amines in *Salmonella typhimurium*, 420, 115-124.

41. Wagner ED et al (2003). Modulation of the mutagenicity of heterocyclic amines by organophosphate insecticides and their metabolites. *Mutation research*, 536, 103-115.
42. Arimoto-Kobayashi S et al (1999). Mutation and DNA modification in *Salmonella* exposed to N-nitrosodimethylamine under UVA-and sunlight irradiation. *Mutation Research*, 444, 413-419.
43. Routledge MN et al (2001). Presence of benzo(a)pyrene diol epoxide adducts in target DNA leads to an increase in UV-induced DNA single strand breaks and *supF* mutations. *Carcinogenesis*, 22, 1231-1238.
44. McLuckie KEI et al (2004). Effects of the order of exposure to a binary mixture of mutagens on the induced mutation spectra of the *supF* gene. *Mutagenesis*, 19, 137-141.
- 45.