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

## Preface 2012

The Committee on Mutagenicity (COM) provides advice on potential mutagenic activity of specific chemicals at the request of UK Government Departments and Agencies. Such requests generally relate to chemicals for which there are incomplete, non-standard or controversial data sets for which independent authoritative advice on potential mutagenic hazards and risks is required. Frequently recommendations for further studies are made.

During 2012, the Committee completed interim guidance on a strategy for genotoxicity testing and mutagenic hazard assessment of impurities in chemical substances and a guidance document on the human health significance of mutagenicity. The finalised strategy and guidance documents were published on the COM website. The COM considered the *in vitro* genotoxicity assessment of nanomaterials, the genotoxicity of chlorophenols and completed a review of the current scientific progress regarding cell transformation assays for the prediction of carcinogens. Statements on these topics were also published on the COM website.

The Committee also considered the evidence for hormesis in mutagenicity dose-response relationships and a Food Standards Agency funded research project on the genotoxic consequences of exposure to mixtures of food-derived chemical carcinogens. Horizon scanning was discussed at the October meeting.

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## Interim Guidance on a Strategy for Genotoxicity Testing and Assessment of Impurities

2.1 The COM has a remit to provide UK Government Departments and Agencies with advice on the most suitable approaches to testing chemical substances for genotoxicity. The COM has been asked to advice on the need for a generic strategy to test and evaluate the genotoxicity of impurities present in chemical substances. The COM has not previously published guidance on impurities. The published strategy represents the most appropriate at the time of publication.

2.2 The COM recommended a staged approach to genotoxicity data assessment which is presented in the guidance statement.

2.3 The COM concluded that the genotoxicity assessment of impurities present in chemical substances is guided by knowledge of the structure, estimated exposure and the application of the Threshold of Toxicological Concern (TTC) concept to select impurities which require evaluation (i.e. 0.15 µg/person (0.0025 µg/kg body weight/day for a 60 kg adult) proposed by Kroes et al 2004).[1] In situations where it is not possible to undertake an estimation of exposure, or the structure of the impurity has not been or cannot be determined, then a pragmatic cut off concentration of 0.1% can be used as a guide for priority setting for genotoxicity assessment. The genotoxicity testing strategy needs to be derived on a case-by-case basis but should, where the structure of the impurity is known, include (quantitative) structure-activity relationship ((Q)SAR) evaluation of impurities selected for genotoxicity assessment, coupled with expert judgement and reference to genotoxicity data on similar substances. Genotoxicity testing of isolated or synthesised impurities should be undertaken where a (Q)SAR evaluation indicates potential for mutagenicity, and where exposure cannot be confirmed to be

[1] Kroes et al (2004), list of exceptions to the use of the TTC

- High potency carcinogens (e.g. aflatoxin-like, azoxy- or N-nitroso substances)
- Steroids
- Inorganic substances
- Metals, including essential metals
- Polymers, oligomers
- Proteins
- Substances known/predicted to bioaccumulate
- Insoluble nonmaterials
- Radioactive substances

Substances likely to exert local effects (on GI tract, respiratory tract or skin)

below the TTC, and should include an Ames test and an *in vitro* micronucleus (MNvit) test. In situations where the structure of the impurity has not been or cannot be determined and is unknown, and where exposure cannot be confirmed to be below the TTC, then the first step in the evaluation for impurities selected for genotoxicity assessment should be to conduct an Ames test and an MNvit test. If the available evidence suggests that an impurity should be considered to be mutagenic then levels should be controlled to as low as reasonably practical.

2.4 The full guidance statement can be found at http://www.iacom.org.uk/guidstate/documents/COM2012S2impuritiesfinal2012finalforinternetr.pdf

**Human Health Significance of Chemical Induced Mutagenicity**

2.5 The purpose of this introductory guidance was to provide information on chemically induced mutagenesis relevant to human health to the informed lay reader. It includes information on the role of mutagenesis in cancer, inherited genetic disease (and teratogenesis) and a glossary.

2.6 The full guidance statement can be found at http://www.iacom.org.uk/guidstate/documents/humanhealthsignificancefinal.pdf

**Genotoxicity Assessment of Nanomaterials**

2.7 The COM has previously advised on nanomaterials genotoxicity in 2005 as part of a joint review undertaken with the Committees on Toxicity and Carcinogenicity. The COM considered recent literature on the *in vitro* genotoxicity testing of nanomaterials outlining some discrepancies in *in vitro* data and the potential confounding factors when existing standard assays are used for the testing of nanomaterials.

2.8 The COM reached the following conclusions:

i) The standard protocols as used in genotoxicity testing of chemicals could not be directly applied to nanomaterials and that test conditions should be modified/optimised using a case-by-case approach. However each test would require physico-chemical characterisation of the nanomaterials in the test medium and direct evidence for uptake into cells. The most appropriate *in vitro* package was gene mutation in mammalian cells (*hprt* gene mutation assay) and the *in vitro* micronucleus test. Because nanomaterials may not penetrate bacterial cell walls and because of the inability of bacterial cells to phagocytose particles, bacterial gene mutation tests should not be used for nanomaterials.

ii) There was currently no convincing evidence that nanomaterials induce DNA-mediated genotoxic effects. There is evidence for oxidative DNA damage with many of the nanomaterials that have been tested for genotoxicity, and evidence that carbon nanotubes may induce aneuploidy by interaction with the mitotic spindle apparatus. There was a need for more investigations into possible modes of genotoxic action by nanomaterials.

iii) More information on nanomaterials' uptake and persistence in cells should be the priority for further research.

iv) At the present time, the limited available genotoxicity data on nanomaterials did not allow definitive conclusions to be reached. There remained considerable uncertainty regarding the nature of genotoxic hazards identified in the available studies. These uncertainties do not permit a generic approach to hazard characterisation to be identified.

2.9 The full guidance statement can be found at http://www.iacom.org.uk/statements/documents/nanomaterialsfinal2012.pdf

**Chlorophenols**

2.10 The Food Standards Agency has asked for advice on the genotoxicity of chlorophenol compounds to assist in developing advice to consumers on the implications of the occurrence of chlorophenol contaminants in wine.

2.11 The COM reached the following conclusions:

i) The amount of data available for assessing the mutagenicity of chlorophenols is generally limited, with a particular paucity of *in vivo* genotoxicity data. The majority of tests, particularly for cytogenetic effects, were conducted to old protocols and the data have limitations. Chlorophenols can contain numerous impurities, including dioxins, which are carcinogenic but not genotoxic, though the occurrence of genotoxic impurities cannot be ruled out.

ii) The chlorophenols that have been tested did not cause mutations in bacterial cells.

iii) Clastogenic effects have been reported *in vitro* for a number of chlorophenols, particularly, the trichlorophenols, may have been due to the generation of free radicals from redox cycling of quinones and semi-quinones or auto-oxidation and may not be relevant *in vivo*, particularly at low levels of exposure.

iv) There was insufficient evidence to conclude on whether or not the clastogenicity was via a direct or indirect mechanism, and whether there was a threshold for any mode of action.

v) Although there is some limited evidence to suggest that trichlorophenols could be considered as a common mechanism group, overall the data on genotoxicity and mode of genotoxic action for chlorophenols did not allow conclusions on a common mechanism group to be drawn.

vi) There was insufficient information to establish whether the chloroanisoles would have the same genotoxic properties as the chlorophenols.

vii) Conclusions on individual compounds are presented in the statement.

2.12 The full statement can be found at http://www.iacom.org.uk/statements/documents/Chlorophenolsstatement2.pdf

**Cell Transformation Assays for the Prediction of Carcinogens**

2.13 The Committee on Carcinogenicity (COC) has asked the COM for a review of the current scientific progress regarding cell transformation assays for the prediction of carcinogens. The COM considered cell transformation assays based on: Syrian hamster embryo (SHE) cells (at pH 6.7 or pH ≥ 7.0), and the mouse cell lines BALB/c 3T3, C3H10T1/2 and Bhas 42. The assessment of the SHE *in vitro* test system was previously considered by the COM in 1994 and 1996.

2.14 The COM reached the following conclusions:

i) The SHE CTAs as currently used do not discriminate between genotoxic and non-genotoxic carcinogens.

ii) The SHE cell transformation assay would be inappropriate for regulatory screening of chemicals such as pharmaceuticals and agrochemicals for potential carcinogenicity, and further that it should not be used in place of an *in vivo* rodent carcinogenicity study.

iv) The available evidence from the OECD DRP number 31 and ECVAM pre-validation reports are not sufficient to recommend routine regulatory use of the SHE CTAs (pH 6.7 and pH 7.0) and BALB/c 3T3.

v) Further prospective validation of these assays using a wider range of carcinogens and non-carcinogens is required.

vi) The available evidence from the published literature does not support the routine use of the Bhas42 CTA in a regulatory setting. There is a need for further validation data, the development of an agreed protocol and photo-catalogue for the evaluation of morphologically transformed foci.

vii) There is a need to invoke quality control procedures for assessment of subjective morphology assessments of transformed colonies or foci. The use of peer review procedures should be considered.

viii) There is a need for more research to understand the molecular mechanism(s) of cell transformation. The available information on senescence by-pass is promising but no definite conclusions can be reached regarding the CTAs considered in this statement.

ix) An important aspect of the validation of CTAs is the development of objective biological markers of cell transformation. The available information on gene markers and use of infrared spectroscopy is promising.

2.15 The full statement can be found at http://www.iacom.org.uk/statements/documents/COM12S4-CellTransformationAssayStatementfinalforinternet_000.pdf

**Hormesis in Mutagenicity Dose-Response Relationships**

2.16 The COM considered evidence for hormesis in mutagenicity dose-response relationships. The COM and COC had previously considered proposals on hormesis in 2003. The COM acknowledged that a hormetic effect may be possible in genotoxicity, for example, via the induction of DNA repair at low doses. However, overall, the COM concluded that the data did not provide evidence for hormesis.

**Genotoxic Consequences of Exposure to Mixtures of Food-Derived Chemical Carcinogens**

2.17 The COM has previously expressed an interest in the evaluation of the mutagenicity of chemical mixtures. One important recommendation was to consider the possibility of mutagenic synergy and the implications of such a finding for risk assessment. The COM considered research funded by the Food Standards Agency (FSA) following a call for proposals *'to address the mixture effects of mixtures of genotoxic and non-genotoxic chemicals in food.'* As part of the external review process, the FSA sought the views of COM on the draft final report of the research project. This item was reserved business pending publication of the research.

**Horizon Scanning**

2.18 The horizon scanning exercise provides information which can be used by Government Departments/Regulatory Agencies to identify important areas for future work. The horizon scanning exercise for 2011 was delayed due to full agendas until the March 2012 meeting and the 2012 horizon scanning

exercise was undertaken in October 2012. Regarding progress on topics raised in the 2011 horizon scanning exercise, the COM was advised that a summary statement on photogenotoxicity and a review of swimming pool disinfection by-products were being taken forward.

2.19 The COM agreed that the main priorities in 2013 would be a review of the Pig-A assay and consideration of mutation spectra of genotoxic substances. The evaluation of the Comet assay by the Japanese Centre for the Validation of Alternative Methods was still considered to be important but of a lower priority while awaiting the outcome of a future review meeting on this topic in March 2013.

2.20 With regard to additional projects, the COM expressed an interest in topics associated with gene sequencing to understand the relation between mutation and cancer; human variability; environmental factors; and mutational changes in individual tumours. Also, a review of gene expression profiling of genotoxic and non-genotoxic carcinogens and consideration of alternative methods to the SHE assay were suggested. The COM also suggested a review of epigenetics and the role of genetic stability, however, it was noted that this was currently being reviewed by the COC. Another potential topic of interest related to the benchmark dose, *in vivo* genotoxic potency and the Margin of Exposure approach to genotoxicity risk assessment was suggested, however it was noted that this topic was currently being reviewed by the International Life Sciences Institute. The topics of human germ cell mutagenicity and germ cell testing were also suggested as future topics.