

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

**HORIZON SCANNING PAPER 2011**

**Introduction**

1. Members will wish to consider horizon scanning topics identified by the secretariat. A literature search using PUBMED which indicated several thousand publications in 2010/11 period which might be potentially relevant. The search strategy (July2010-August 2011) was subsequently focused into a number of areas. The references which have been scanned are shown in parenthesis. Mutagenicity (461), Genotoxicity (869), and Chemical Mutagens (281). The literature searches were briefly scanned to highlight potential chemicals, exposures and generic areas of mutagenicity evaluation which might be of interest to members. A brief overview has been produced below. It is acknowledged that a more extensive literature search and wider selection of papers could have been undertaken but the objective is to provide areas of interest for discussion rather than a complete literature scan. The horizon scanning exercise provides an opportunity for members and advisers from Government Departments/Regulatory agencies to discuss and suggest topics for further work. The paper has been subdivided to assist members discussion.

**Progress during 2011**

2. Progress has been made in a number of generic topics of genotoxicity testing and evaluation previously identified by COM for review.

- Initial review of testing and evaluation of impurities (Interim advice to be completed)
- Draft of human health significance of mutagenicity (see MUT/2011/15)
- Draft of use of QSAR approaches to mutagenicity evaluation (Secretariat currently seeking a speaker for the March 2012 meeting)
- Completion of strategy for genotoxicity testing
- Draft of strategy for genotoxicity testing and mutagenic hazard assessment of chemicals with inadequate genotoxicity data. (see MUT/2011/11)
- Genotoxicity of nanomaterials (see MUT/2011/12)

**Items identified from literature searches**

3. The title and abstract for selected articles were screened and information tabulated in Annex 1. A number of topics were considered in more detail in the following sections of this draft discussion paper. Members may wish to identify any papers in addition to those provided along with this draft discussion paper they would like to see in full. (Papers appended as

Annex 4). The topics identified in this horizon scanning paper could be considered at more than one COM meeting, should there be insufficient time at the October 2011 meeting.

### Photogenotoxicity

4. This topic has not been previously considered by COM. There is interest from HSE CRD in obtaining COM views. A very useful review of the topic based on discussions held at the 5<sup>th</sup> IWGT meeting in Basel Switzerland (August 17-19, 2009) has been published. (Lynch AM et al Mutation Research, 723, 91-100, 2011). Some brief comments on this publication are provided below.

5. The appended review paper focuses on pharmaceutical testing but there are many generic principles considered which would be pertinent to the consideration of photogenotoxicity in general. Consideration of the need for photomutagenicity testing is included in the updated EU data requirements for pesticides (soon to be published). CRD (HSE) would therefore welcome COM advice on testing for photogenotoxicity as this topic is not currently included in COM testing strategy guidance. (An excerpt from the revised Pesticides Directive is appended as Annex 2)

- The triggers for photogenotoxicity testing are relatively crude (absorption of light between 270-790 nm, and evidence for presence of compound/metabolites in the skin)
- The Expert Panel recommended that the molar extinction/absorption coefficient of  $< 1000 \text{ Lmol}^{-1} \text{ cm}^{-1}$  (MEC) should be used as a trigger for testing. This contrasts with the proposed revision of the Pesticides Directive where an MEC of  $< 10 \text{ Lmol}^{-1} \text{ cm}^{-1}$  is proposed.
- Photochemical reactivity assays can provide a good predictor of phototoxic liability and might be a better trigger than absorption of light between 290 nm-700 nm.
- There are significant inconsistencies between *in vitro* phototoxicity and *in vitro* photogenotoxicity studies despite the observation that the underlying mechanisms are the same for both assays (i.e. photochemical activation leading to generation of free radicals and/or active oxygen species. This may in due to the occurrence of pseudophotogenotoxicity. The Expert Panel questioned reliability of the *in vitro* photoclastogenicity assay.
- There are no validated *in vivo* photogenotoxicity assays. Some initial results have been reported for *in vivo* photocomet and photomicronucleus assays. It was also suggested that that human 3D reconstructed skin could be considered an alternative to *in vitro* phototoxicity (3T3 NRU assay).
- The Expert Panel considered the positioning of photogenotoxicity within photosafety testing. It was considered that mammalian cell tests for photogenotoxicity were unreliable and should not be used. Thus if there were a need to undertake photosafety assessment, an *in vitro* phototoxicity test should be undertaken. Negative data from such an *in vitro* test would not require further testing. Positive data might trigger *in vivo* testing (negative data should overrule *in vitro* positive data).

- The most significant conclusion of the Expert Panel was that photogenotoxicity testing should not be part of the standard approach to photosafeting testing.

6. Given the recommendations from the IWGT Expert Panel, can the COM reach any generic conclusions on the triggers for photogenotoxicity testing? and also the need for specific photogenotoxicity tests?

7. Should any more detailed literature work on photogenotoxicity testing be undertaken or is there sufficient information available for COM to draft advice?

8. A number of additional questions have been received from HSE CRD.

- When is such an investigation necessary? Hence what is suitable molar extinction value? Do the results of standard genotox assays have any impact on whether an investigation of photogenotox is necessary?

- What type of tests should be conducted, and what methods (no OECD photogenotox method)?

- Does COM wish to add anything to the main conclusions and consensus statements (section 7 of paper) of the IWGT paper? Are these conclusions relevant to all types of chemicals?

- What does COM think about the current wording in the pesticide data requirements (see Annex 2)?

#### Can Genotoxicity information be used in the development of Integrated Testing Strategies (ITS) for Skin Sensitisation

9. A number of papers were retrieved which put forward the proposal that genotoxicity data can be used as part of an integrated approach to skin sensitisation hazard assessment. The background to this is the need to reduce animal testing in accordance with the principles set out in REACH and in response to the Cosmetics Directive (which bans repeat dose testing from 2013). Mckenyan O and colleagues compared mechanisms of DNA reactivity and protein reactivity and concluded there is a great deal of overlap. Do members agree with the proposal that mutagenicity information could be used as part of ITS for skin sensitisation? (copy of paper appended in Annex 4, with apologies for poor reproduction of paper)

10. If COM agree this approach, it might be useful to alert COT.

#### What's in the Pool? Disinfection by-products in swimming pools and mutagenicity assessment

11. A copy of a paper by Richardson SD et al (Env Health Perspectives, 118, 1523-1530, 2010) is appended (in Annex 4). The publication reports a systematic approach to the identification potentially mutagenic DBPs in a

number of swimming pools and mutagenicity of testing of swimming pool samples. It is interesting to note that the authors report that many DBPs identified had not been previously reported in swimming pools or drinking water. These data are based on sampling from two swimming pools in Barcelona (one using chlorine (sodium hypochlorite) and the other bromine (1-bromo-3-chloro-5,5-dimethyl-2,4-imidazolidinedione)

12. Kogevinas M et al (Env Health Perspectives, 118, 1531-1537, 2010) measured exhalation of DBPs before and after swimming and investigated MN formation in lymphocytes and urothelial cells. (50 subjects who swam in a chlorinated pool in Barcelona were selected for study). Total THMs in exhaled breath increased seven times after swimming (40 mins). A correlation between exhaled DBPs (brominated THMs) and MN in lymphocytes was reported. Urine mutagenicity increased significantly after swimming, in association with increased exhalation of bromoform. There were no changes in MN in urothelial cells.

13. What are members views on these results? Some general information on chemical hazards in swimming pools can be found in the WHO Guidelines for Safe Recreational Water Environments (Volume 2; Swimming Pools and Similar Environments. These indicate that well managed pools achieve THM levels below drinking water guidelines (See Annex 4). It is noted that there is a need to balance any potential risk resulting from genotoxicity of DBPs in swimming pools with the need to have adequate disinfection to prevent the spread of infections.

#### Evidence for hormesis in mutagenicity dose-response relationships?

14. The COC considered proposals from Calabrese EJ and colleagues for the occurrence of hormesis in toxicological responses during the 2003 horizon scanning exercise. COC members concluded

*Members considered that other topics which might usefully be considered included dose-response at low dose levels. Members noted the appended paper to CC/03/22 from Calabrese EJ and Baldwin LA (Nature, vol 421, 691-692, 2003.) provided an argument for the occurrence of hormesis (ie the occurrence of a "U" shaped dose-response curve at low dose level). However, although it was stated that there were up to 5000 examples of the hormetic effect in the published literature, it was not possible to assess this claim on the evidence available. It was noted that in the few studies on genotoxic carcinogens using group sizes sufficient to detect effects at 1% levels or below, the evidence was generally consistent with the absence of a threshold. Also, DNA adduct formation with genotoxic carcinogens was linear down to the lowest measurable dose levels. The dose-response data for non-genotoxic carcinogens was consistent with the occurrence of a threshold. Members considered that there was no evidence available to justify the use of a hormetic approach to risk assessment for chemical carcinogens. It was agreed that the evidence for DNA repair following exposure to very low doses of genotoxic carcinogens warranted further review. Overall members felt that the arguments presented by Calabrese and Baldwin should be considered further in the future. It was agreed that there might theoretically be a point of*

*departure in the dose-response for a genotoxic carcinogen but it was not possible to identify any potential threshold with methods available. Members considered it prudent to reaffirm that for practical purposes genotoxic carcinogens should be presumed to have no threshold.*

14. Calabrese EJ and colleagues have now published a systematic review of mutagenicity data for *Salmonella typhimurium* (ST) tests, and have reported evidence for a hormetic dose-response for a number of tests using ST TA100. What are members views of this publication? (Appended in Annex 4)

15. The COM considered genotoxic effects of mutagens at low dose and the evidence for induced DNA repair in 2004. No evidence for a 'U' shaped dose-response curve was reported. The Committee noted *'Members agreed that bacteria would most likely demonstrate the most sensitivity to low doses of mutagens and had reservations as to whether mammalian cell systems would have sufficient sensitivity to detect evidence for an effect of DNA repair induction on the dose-response relationship for mutation. The Committee considered that natural variability would make the detection of any 'U' shaped curve very difficult.'*

### **Developing a Forward work plan for COM for 2012**

16. The first priority suggested would be to complete the COM guidance statements currently in draft form, namely;

generic guidance on significance of human health of chemically induced mutagenesis,  
interim guidance on impurities,  
strategy for chemicals with inadequate genotoxicity data

17. The next priority would be to undertake work with respect to individual genotoxicity tests identified in the guidance statement framework. It was anticipated that the guidance statements produced would focus on data interpretation and assessment rather than test methods. The work would be aided by seeking examples of data interpretation from Government Departments and Agencies. Priorities would be set following advice from Government Departments and agencies.

18. Other projects previously identified by COM which have been previously considered, or have not been completed or have only recently been identified during completion of COM strategy for genotoxicity testing

Review of approaches to Expanded Simple Tandem Repeats  
High throughput assays for Germ line mutations  
Toxicogenomics  
Emerging assays/technologies  
Mutagenic spectra

18. In addition is there anything identified from the current horizon scanning exercise which should be subject to further assessment.
19. The COM is asked to advise on priorities for future COM work.

**Secretariat September 2011**

- Annex 1 Overview of papers identified during horizon scan.
- Annex 2 Extract from draft Pesticides Directive
- Annex 3 Extract from executive summary of WHO guidance on swimming pools.
- Annex 4 Published papers

## Tabulated comments on selected papers from literature search.

### Annex1 to MUT/2011/14

#### Dose-Response

Reference	Comments
Calabrese EJ et al <i>Mutation Res</i> epub April 2011	Authors claim evidence for hormesis in bacterial mutagenicity in TA100. See further comment in discussion paper
Elhajouji A et al <i>Mutagenesis</i> , 26, 199-204, 2011	Review of approaches to identification of thresholds in MN test with focus on clastogens

#### QSAR

Reference	Comments
Hillbrecht A et al <i>Chem Res Toxicol</i> , 24, 843-854, 2011	Comparison of DEREK, Toxtree, MC4PC and Leadscope MA for public and proprietary compounds. Lower sensitivity for proprietary compounds reported.
Naven RT et al, <i>Expert Opinion Drug Metab Toxicol</i> , 6, 797-807, 2010	Review of developments of application of QSARs to mutagenicity.. Current systems perform well with publicly-derived data, but performance outside domain of applicability considerably lower. Access to high quality proprietary data needed to enhance models.
Devillers J et al SAR and QSAR in environmental research, 21, 753-769 and 771-783, 2010	Application of OECD Toolbox and Toxtree to aromatic amines and alpha-beta unsaturated aliphatic aldehydes.

#### In vitro genotoxicity tests

Reference	Comments
Hovhannisyan GG <i>Mol Cytogenet</i> , 3, 17-, 2010	FISH in combination with the comet assay and MN test. (review article)
Kawaguchi S, <i>J Nucleic Acids</i> , Nov 1 2010, 541050, 2010	Comparative study investigating sensitivity of Comet assay and MN test in human lymphoblastoid cells with a range of direct acting mutagens. Overall sensitivity was similar. Sensitivity of Comet assay can be enhanced by using DNA resynthesis inhibitors.
Kirsch-Volders M <i>Mutagenesis</i> , 26, 177-84, 2011.	Review of use of MN in cell lines, lymphocytes and 3D skin models.

	Author comments on possibility of combined 3D skin primary human whole blood culture. Need to adapt assay for nanomaterials noted.
Sakai A et al <i>Mutation Res</i> , 702, 100-22, 2010	Evaluation of Bhas 42 cell transformation assay on 98 chemicals. (Two protocols used, initiation and promotion). Assay reported to identify Ames negative carcinogens.
Shibai-Ogata A <i>Mutagenesis</i> Jul 10, 2011.	Report of an 96-well automated high-content MN assay for use in drug discovery.

### *In vivo* genotoxicity tests

Reference	Comments
Coffing S et al <i>Env Mol Mutagen</i> , 52, 269-79, 2011	Initial studies of rat gut MN assay. Evidence from limited number of mutagens suggested this assay would be useful for compounds with high concentrations in the gastrointestinal tract with little systemic exposure.
Dertiger SD <i>Mutat Res</i> , 721, 163-70, 2011	Immunomagnetic separation facilitates rapid determination of Pig-a mutations. (seen as part of COM strategy for testing)
Dobrovolsky VN et al <i>Env Mol Mutagen</i> , 51, 825-35, 2010.	Review article, induced mutant frequencies after 2 weeks in reticulocytes and in total red blood cells after 2 months exposure. (seen as part of COM strategy for testing)
Mughal et al <i>Mutat Res</i> , 700, 86-94, 2010.	Kinetics of MN formation and Comet response in peripheral blood erythrocytes in juvenile rats for a range of mutagens was reported to be similar with peak 36-48h post single dose treatment.
Rothfuss A et al <i>Mutat Res</i> , 723, 108-20, 2011	Report of IWGT (5th) group. Combination of acute <i>in vivo</i> MN and comet technically feasible.. Proposals for protocols reported.
Rothfuss A et al <i>Mutat Res</i> , 702, 40-69, 2010	Collaborative study of 15 compounds in rat liver Comet assay integrated into 2-or 4- week repeat dose studies. High concordance with acute (1-3 dose) studies.
Shwed PS et al <i>Mutagenesis</i> , 25, 609-16, 2010	Authors report characterisation of transgene rearrangements in the

	MutaMouse animal model and suggest an approach for investigating clastogenic/aneugenic effects.
Trivedi PP et al <i>Mutat Res</i> , 703, 115-21, 2010	Strong correlation reported for sperm head morphology and sperm comet assay for doxorubicin

### Photogenotoxicity

Reference	Comments
Ringeissen S et al <i>Toxicology In-vitro</i> , 25, 324-334, 2011	SAR model for predicting phototoxicity/photodegradation. (based on limited number of 56 compounds)
Lynch AM et al <i>Regulatory Toxicology and Pharmacology</i> , 58, 219-223, 2010	Evidence from 14 compounds suggests that photochemical analysis is a good predictor of <i>in vitro</i> photogenotoxicity toxicity. See draft discussion paper and Annex 4
Lynch AM <i>Mutation Research</i> , 723, 91-100, 2011.	Report of outcome of 5 <sup>th</sup> IWGT consideration of photochemical genotoxicity. Paper appended and more comments provided in cover paper. See draft discussion paper and Annex 4

Prediction of Skin sensitiation: see draft discussion paper and appendix 4

Genotoxins in Swimming pools, see draft discussion paper and Annex 4.

## Excerpts from draft Pesticides Directive

### 5.2.7. Phototoxicity

The study will provide information on the potential of certain active substances to induce cytotoxicity in combination with light, e.g. active substances that are phototoxic in vivo after systemic exposure and distribution to the skin, as well as active substances that acts as photoirritants after dermal application to the skin. A positive result should be taken into account when considering potential human exposure.

#### *Circumstances in which required*

The in vitro study is be required where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution.

Before biological testing is considered, an Ultraviolet/Visible (UV/Vis) absorption spectrum of the active substance shall be determined. If the molar extinction/absorption coefficient is less than  $10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ , the chemical is probably not photoreactive and may not need to be tested

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### 5.4. Genotoxicity testing

These studies are of value in:

- the prediction of genotoxic potential,
- the early identification of genotoxic carcinogens,
- the elucidation of the mechanism of action of some carcinogens.

Appropriate dose levels, depending on the test requirements, shall be used in either in vitro or in vivo assays. It is important that a tiered approach is adopted, with selection of higher tier tests being dependent upon interpretation of results at each stage. Special testing requirements in relation to photomutagenicity may be indicated by the structure of a molecule. Before biological testing is considered, a UV/Vis absorption spectrum of the active substance and its major metabolites shall be determined. If the molar extinction / absorption coefficient is less than  $10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$  the chemical is probably not photoreactive and therefore need not be tested for photomutagenicity.

## Annex 3 to MUT/2011/04

### Excerpt for WHO Guidelines for Swimming pools: Executive Summary for Chemical Hazards.

#### Chemical hazards

Chemicals found in swimming pool water can be derived from a number of sources, namely the source water, deliberate additions such as disinfectants and pool users themselves (these include sweat, urine, soap residues, cosmetics and suntan oil). There are three main routes of exposure to chemicals in swimming pools and similar environments: direct ingestion of the water, inhalation of volatile or aerosolized solutes and dermal contact and absorption through the skin. The amount of water ingested by swimmers and bathers will depend upon a range of factors, including experience, age, skill and type of activity. Experimental evidence suggests that water intake varies according to age and sex, with adult women ingesting the least and male children ingesting the most. Swimmers inhale from the atmosphere just above the water's surface, and the volume of air inhaled is a function of the intensity of effort and time. Inhalation exposure will be largely associated with volatile substances that are lost from the water surface, but will also include some inhalation of aerosols, within a hot tub (for example) or where there is significant splashing. Dermal exposure depends upon the period of contact with the water, water temperature and the concentration of the chemical.

The principal management-derived chemicals are disinfectants, added to minimize the risk to pool users from microbial contaminants. Coagulants may be added as part of the water treatment process to enhance the removal of dissolved, colloidal or suspended material. Acids and alkalis may also be added to the water in order to maintain an appropriate pH for optimal water treatment and also the comfort of bathers. The chemical disinfectants that are used most frequently include chlorine (as a gas, hypochlorite or, generally for outdoor pools, chlorinated isocyanurates), chlorine dioxide, bromochlorodimethylhydantoin (BCDMH), ozone and ultraviolet (UV) radiation (with ozone and UV usually being used in combination with a chlorine or bromine-based disinfectant). Practice varies widely around the world, as do the levels of chemicals that are currently considered to be acceptable in order to achieve adequate disinfection while minimizing user discomfort. It is recommended that acceptable levels of free chlorine continue to be set at the local level, but in public and semi-public pools these should not exceed 3 mg/l and in public and semi-public hot tubs should not exceed 5 mg/l. It is recommended that total bromine does not exceed 4 mg/l in public and semi-public pools and 5 mg/l in hot tubs. Where chlorinated isocyanurates are used, levels of cyanuric acid in pool water should not exceed 100 mg/l. Where ozone is used, an air quality guideline of 0.12 mg/m<sup>3</sup> is recommended in order to protect bathers and staff working in the pool building.

A number of disinfectants can react with other chemicals in the water to give rise to unwanted by-products, known as disinfection by-products. Most is known about the by-products that result from the reaction of chlorine with humic and fulvic acids, but there is evidence from model studies with amino acids that other organic substances will also give rise to a similar range of by-products. Although there is potentially a large number of by-products, the substances produced in the greatest quantities are trihalomethanes, of which chloroform is generally present in the greatest concentrations, and the haloacetic acids, of which di- and trichloroacetic acid are generally present in the greatest concentrations. Both chlorine and bromine will react with ammonia in the water (resulting from the presence of urine) to form chloramines (monochloramine, dichloramine and nitrogen trichloride) and bromamines.

Trihalomethanes have been considered more than other chlorination by-products, reflecting the level of available information. Concentrations vary as a consequence of the concentration of precursor compounds, chlorine dose, temperature and pH. Trihalomethanes are volatile in nature and can be lost from the surface of the water, so they are also found in the air above the pool.

The guideline values in the WHO *Guidelines for Drinking-water Quality* can be used to screen for potential risks arising from swimming pools and similar environments, while making appropriate allowance for the much lower quantities of water ingested, shorter exposure periods and non-ingestion exposure. Although there are data to indicate that the concentrations of chlorination by-products in swimming pools and similar environments may exceed the concentrations proposed by WHO for drinking-water, the evidence indicates that for reasonably well managed pools, concentrations less than the drinking-water guideline values can be consistently achieved. The risks from exposure to chlorination by-products in reasonably well managed swimming pools would be considered to be small and must be set against the benefits of aerobic exercise and the risks of microbial disease in the absence of disinfection. Nevertheless, competitive swimmers and pool attendants can experience substantial exposure to volatile disinfection by-products via inhalation and dermal absorption. The chloramines and bromamines, particularly nitrogen trichloride and nitrogen tribromide, which are both volatile, can give rise to significant eye and respiratory irritation in swimmers and pool attendants. The provisional guideline value for chlorine species, expressed as nitrogen trichloride, in the atmosphere of swimming pools and similar environments is 0.5 mg/m<sup>3</sup>.