

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

**HORIZON SCANNING PAPER 2009**

**Introduction**

1. Members will wish to consider horizon scanning topics identified by the secretariat. A literature search using PUBMED indicated several thousand publications from August 2008 up to June 2009 which might be potentially relevant. The search strategy was subsequently focused into a number of areas. Retrieved references and number of references scanned and abstracts obtained were as follows; Mutagens (1510 identified, 69 abstracts retrieved), Chemical Mutagenesis (457 identified, a further 10 retrieved), High Potency Mutagens (8 identified, none selected). Potency of genotoxins (27 identified, a further 1 reference where abstract was obtained), Mutagens in the environment (140 identified, a further 5 abstracts retrieved), and Genotoxicity Biomonitoring (16 identified, a further 4 abstracts retrieved).

2. The abstracts 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 information which it is hoped is valuable to members and advisers from Government Departments/Regulatory agencies. The paper has been subdivided to assist members' discussion. A number of pragmatic priorities for future COM work are outlined in the discussion section.

**Progress on topics raised during 2008 Horizon Scanning exercise.**

3. A large amount of Committee time was spent undertaking the reviews of Aclonifen, Fumagillin and Tobacco Products. Progress has been made on Thresholds (and a guidance paper on risk assessment of *in vivo* mutagens has been drafted), a draft outline proposal for a testing strategy is due for the March 2010 meeting. No progress was made on mutational fingerprints or mitochondrial mutagenicity.

**Overview of identified published papers**

*Chemicals, substances and exposures*

4. A number of papers relating to genotoxicity of individual chemicals and substances were retrieved for members' information. There seems to have been quite a few publications relating to investigations of genotoxicity of nanomaterials.

## Studies investigating nanomaterials

Reference	Material tested	Comment
Asha Rani PV, ACS Nano, 3, 279-290, 2009	Ag-NP	Positive genotoxicity in human fibroblast cells using comet and CBMN (oxidative DNA damage reported).
DiSotto A, Toxicol lett, 184, 192-7, 2009	Multiwalled-CNT	Negative in Ames and E.coli +/- S-9
Yoshida, J Tox Sci, 34, 119-22, 2009	ZnO NP	Negative in Ames & E.coli +/- S-9.
Yang, H	CNT and ZnONP	CNT induced DNA damage in primary mouse embryo fibroblasts. Negative in ZnO.
Wirnitzer, TOx Lett, 186, 160-5, 2009	Baytube (agglomerates of multiwalled CNT)	Negative for CAs in V79 cells +/- S-9, and negative in Ames test, particle size data available.
Lindberg, HK, Tox lett, 186, 166-73, 2009	CNTs and graphite nanotubes.	Positive genotoxicity in bronchial epithelial BEAS 2B cells using comet and CBMN.
Sharm, Tox Lett, 185, 211-8, 2009	ZnO NPs	Positive genotoxicity in human epidermal cell line A431 using comet assay.
Balasbramanyam, A Mutagenesis, 24, 245-51, 2009	Aluminium oxide NMs	Positive genotoxicity in peripheral blood cells using CBMN and comet assay. Data on particle characterisation available.
Doak SH Mutagenesis e-pub Apr7 2009	Review of data	Confounding experimental interactions with colourimetric and fluorometric dyes.

## Exposures

### Cigarette smoking

Reference	Materials tested	Comments
Lou J Mutat Res, e pub May 3 2009	Cigarette smoke condensate	T cell receptor, TCR, gene mutation in peripheral blood lymphocytes
Shia HJ Fd Chem Tox, 47, 192-7, 2009	TPM cigarette smoke from non filter/filtered 2R4F	Mutagenicity of TPM in Ames was 30-40% lower in non filter on a TPM basis but was higher in the non filter cigarette when expressed on a per cigarette basis

### Soil

Reference	Materials tested	Comments
Mattsson A Env Mol Mut, 50, 337-48, 2009	Extracts from soils (6 industrial settings in Sweden)	DNA damage signals (Western blotting/ immunochemistry) in HepG2 cells. Authors report signals varied between samples in unpredictable manner with oxy-PAHs possibly contributing to reported results.
Hua G, Env Poll, 157, 916-921, 2009	DNA extracted from soil samples incubated with BaP for 540 days	DNA adducts can be identified from soils. Authors suggest this approach is of value in assessing remediation.

daSilva Junior FM, Mutat Res, 673, 116-23, 2009	Salmonella mutagenicity tests with various strains to identify dispersion routes of mutagens from coal wastes contaminated soils.	Data interpreted to suggest frameshift mutagens dispersed by run off and leaching, while base-pair dispersed mainly by atmosphere. A wide number of substances could mediate the mutagenic activity observed (aliphatic hydrocarbons, metals)
Park J Env Tox Contam, 27, 2207-15, 2008	Reverse mutation of E.coli strains sensitive to ROS. Bioassay directed fractionation of soil.	ROS DNA damage mediated by H <sub>2</sub> O <sub>2</sub> which was decreased in deasphalted whole extracts.

### Water disinfection by products

Reference	Materials Tested	Comments
Liviac D Env Res, 109, 232-8, 2009	Genotoxicity assessment of two Halonitromethanes (trichloronitromethane, and bromonitromethane) in human cells (PBLs, TK6 cells)	Positive genotoxicity in comet assay but not MN assay. Oxidised DNA bases contribute high proportion of DNA damage (50%-70%). Authors indicate lack of fixed genetic damage minimises potential risk associated with HNM exposure.
Schenck, KM J TOx Env health, 72, 461-7, 2009	Information on genotoxicity of water from 5 full scale water treatment plants (in USA).	Highest correlation of mutagenicity was for total organic halide concentrations in treated samples.

### Selected data on chemicals of interest

References	Materials tested	Comments
Pandey AK Mutat Res, 661, 57-70, 2009	In-vitro genotoxicity (comet/CBMN, CA) of benzene, p-benzoquinone, hydroquinone, catechol, 1,2,4-benznetriol, trans trans muconic acid.	Rank order potency values and molecular docking studies with topoisomerase undertaken. Authors suggest that mutagenicity of benzene in mammalian cells is mainly due to inhibition of topoisomerase by the metabolites.
Jiang W, Toxicology, 252, 113-7, 2008	Previous results showed positive genotoxicity in biomonitoring studies of vincristine occupational exposure (comet, CBMN, house keeping genes), additional investigations in-vitro using PBLs	In addition to expected genotoxicity in these assays, evidence for the formation of nucleoplasmic bridges (biomarker for DNA misrepair) and nuclear buds (a biomarker of elimination of amplified DNA and/or DNA repair complexes) and evidence of increased (T cell receptor) TCR, mutation was reported.
Shinmura K, J Pathol, 216, 365-74, 2008	Investigations of effects of BaPDE on numerical integrity of centrosomes in lung cancer cells lacking p53	BaPDE induced centrosome amplification and consequent chromosome destabilisation. Evidence of multiple genotoxic effects contributing to neoplasia.

## Mutational Spectra and other aspects of mutagenicity.

5. As with previous horizon scans, there are a small number of papers retrieved using mutation spectra to investigate the role of chemicals in mutagenicity and carcinogenesis.

### Mutation Spectra

References	Materials Tested	Comments
Page V Mutat Res, 656, 55-61, 2008	Comparison of mutation spectra in TP53 in normal human fibroblasts and hepatocellular carcinoma related to exposure to aflatoxin B1	Authors suggest hotspot at codon 249 (G>T transversion in HCC is not found in normal fibroblasts where hotspot is G>A transitions at codon 245. Authors comment that codon 249 mutation in HCC is a result of selection bias during carcinogenesis.
Fang H and Taylor JS Nucleic Acids Res, 36, 6004-12, 2008	Authors report undertook serial analysis of mutation spectra to investigate sequence context on mutagenesis	Data presented for tetrahydrofuran abasic site model to show DNA damage induced by DNA damage bypass polymerases
Gu J et al Cancer Epi Biomark prev, 17, 2445-50, 2008	Authors hypothesised that aberrations of chromosome 9p21 is a molecular target for BPDE mutagenicity in bladder cancer. A case control study was undertaken investigating mutation spectrum of chromosome 21 following in-vitro exposure to BPDE	9p21 BPDE sensitivity was associated with bladder cancer (OR 5.29 (95% CI 3.26-8.59)
Nedelko T et al Int J Cancer, 124, 987-90,2009	Mutation spectra of tumours from Balkan endemic nephropathy patients compared to aristolochic acid exposed cultures	Comparability of mutation spectra between tumours and cultures was suggested to infer a role for aristolochic acid in BEN-associated cancer.

### Polymorphisms

Ryk C *et al* (Environ Mol Mut, 49, 669-75, 2008) undertook a small study investigating MMS induced DNA damage (comet) in peripheral blood lymphocytes of 52 healthy individuals and undertook genotyping for a range of DNA repair genes. Evidence for greater damage in individuals with variant alleles of APEX1, XRCC3 and NBS1 genes.

### DNA repair

Kisby GE *et al* (DNA repair (Amst), 8, 400-12, 2009) reported differential DNA damage in neuronal cultures exposed to a range of alkylating agents from wild type mice compare to cultures from mice deficient in alkyl guanine DNA glycosylase or methylguanine methyltransferase.

## Mitochondrial mutagenicity

Partridge MA *et al* (J Toxicol Environ health A, 72, 301-4, 2009) reported that Arsenic, Asbestos and UV radiation all induced increased the number of rare transversions in mitochondrial DNA.

## Mutagenicity testing strategy

6. A number of relevant references were identified. Of note with regard to *in vitro* testing is the development of an AmesII test, the spectrum of potential response in the *in vitro* LacZ mutation test, and the development of EpiDerm to investigate MN formation as a possible alternative to *in vivo* testing under circumstances where no *in vivo* test can be included in the testing strategy. With regard to *in vivo* testing the use of prebleeding to increase sensitivity of peripheral blood MN tests in rats and the development of MN liver assay in young rats were noted. A number of references on the utility of the comet assays and PigA assay were identified. Of particular interest was a review of the use of the comet assay in germ cell genotoxicity assays. A couple of references which report on tests using fish have been included. There has been a number of suggestions that tests in fish could be used as part of hazard screening for mutagens. It is hoped to further review some of these papers when a draft revised test strategy is brought to the COM.

## QSAR/*In vitro* metabolism

Reference(s)	Aspect of strategy considered	Comments
Benigni R, Bossa C Mutat Res, 659, 248-61, 2008	<b>QSAR.</b> Overview of ToxTree 1.50 (available from European Chemicals Bureau). For SA for Salmonella mutagenicity and potential applications	Authors stress the role of mechanistic knowledge for integration of QSAR in regulatory approaches.
Langham JJ, Jain AN J Chem Inf Model, 48, 1833-9, 2008	<b>QSAR.</b> Novel classification model developed.	Authors consider method compares favourably with literature data and can be expanded to other areas of toxicology
Tak YK et al Anal Biochem, 380, 91-8, 2008	<b>Gene Screen.</b> Studies were undertaken exposing target gene (AcGFP1) in absence of plasmid backbone	Authors suggested approach eliminated false-negative arising from target gene expression that arose from BPDE adducts in plasmid backbone. Authors consider this to be efficient use of GFP as biosensor for mutation detection
Obach RS, Dobo Env Mol Mut, 49, 631-41, 2008	<b><i>In vitro</i> metabolism.</b> Metabolite profile compared for 16 common drugs using liver S-9 from Aroclor 1254 treated rats and pooled human liver S-9.	Differences contrasted. Value of human S-9 data noted for interpreting results of genotoxicity tests.

## *In vitro* tests

Reference	Aspect of strategy	Comment
Kamber M et al Mutagenesis,	<b>AmesII test</b> using fluctuation	For 71 compounds 84 %

May 15 2006 epub	procedure using TAMix for base pair and TA98 for frameshift mutations	agreement, equivalent to the 87% intra-interlaboratory reproducibility for the traditional test.
Laingam S et al Mutat Res, 656, 19-26, 2008	<b>In vitro micronucleus</b> tests using L5178Y and WIL2-NS cells.	Authors comment that one approach to reduce apoptosis is to use cells resistant to apoptosis
Stang A, Witte I Mutat Res, 675, 5-10, 2009	<b>In vitro comet assay</b> A multichamber plate approach described	Authors suggest approach is suitable for early screening of chemicals.
Mahabir AG et al Mutat Res, 666, 50-56, 2009	<b>In vitro LacZ</b> in mouse embryo fibroblasts using MMC, BLM, BaP, N-aac-AAF, ENU.	Authors suggest both mutagenic and clasotgenic effects can be determined
Mun GC Mutat Res, 673, 92-9, 2009	<b>In vitro MN assay using EpiDerm</b> Authors report on development of micronucleus assay using three dimensional human skin reconstruct (EpiDerm)	Authors report 7 genotoxins and 5 non genotoxins correctly identified.
Hu T et al Mutat res, 673, 100-8, 2009.	<b>In vitro MN assay using EpiDerm</b> Authors report results of tests of 7 chemicals (3 genotoxins, 4 non genotoxins) in three laboratories	Authors report good interlaboratory agreement.

### *In-vivo* tests

Reference	Aspect of Strategy	Comments
Vikram A et al Regul Toxicol Pharmacol, 52, 147-57, 2008	<b>In vivo MN assay in rats</b> Authors investigated effect of prebleeding on MN formation using a number of mutagens	Significant increase in sensitivity reported for PBMN The most suitable time was collection 36h post a bleeding time was 0, 2, 6 post dose.
Japanese Environmental Mutagen Society, Mutagenesis, 24, 9-16, 2009	<b>In vivo MN assay in liver in rats.</b> Authors investigated autonomous proliferation of hepatocytes in rats.	Authors report positive results with a number of rat hepatocarcinogens (2AAF, 2,4-DAT, MMC, 1,2-DMH, cyclophosphamide, 2,4 DNT.
Oshida K et al J Toxicol Sci, 33, 515-24, 2008	<b>In vivo comet in liver kidneys and bone marrow undertaken with concomitant haematology.</b> Authors investigated MMS and acetaminophen	Authors considered concurrent haematology and blood chemistry markers of nephrotoxicity could be used to differentiate between genotoxicity (MMS) and cytotoxicity (acetaminophen) positives in comet assay.
Glei M et al Mutat Res, 681, 33-43, 2009	<b>In vivo comet FISH</b> Review of the approach	Authors propose useful tool in investigating site-specific DNA damage
Spivak, G et al Mutat Res, 681, 44-50, 2009	<b>In vivo comet FISH</b> Authors report assessment of Transcription-coupled repair can be undertaken in conjunction with comet-FISH	Authors identify preferential repair in the nuclear matrix of comets as evidence of TCR.
Miura D Mutat Res, epub 3 June 2009	<b>In vivo Pig-A assay</b> Authors examined accumulation and persistence of Pig-A mutant RBCs in rats treated with	Authors reported sampling up to 26 weeks after the initiation of treatment. Maximum response was

	ENU using single or 4 weekly i.p doses of ENU	reported after 6 weeks which persisted to the end of the sampling time. The authors suggest the characteristics of the Pig A assay could be important for detecting weak mutagens by repeated dosing subchronic/chronic dosing protocols.
Speit G et al Mutat Res, 681, 3-12, 2009	<b>In vivo Germ cell genotoxicity.</b> Review of application of comet assay to germ cell genotoxicity	Authors conclude that studies in rodents using germ cells were promising but there was a need to standardise and validate methods. With regard to biomonitoring studies using sperm, it was noted background levels of DNA damage were highly variable and there was a need for standardisation and validation of the comet assay in sperm. It was considered premature to use comet biomonitoring studies in sperm for hazard identification.

### *Studies in fish*

Reference	Aspect of Strategy	Comments
Cavas T, Konen S, Aquat Toxicol, 90, 154-9, 2008	Micronucleus and comet assay undertaken on peripheral blood erythrocytes following intracoelomic injections with domoic acid and EMS (positive control)	Positive results with both Domoic acid and EMS. (Domoic acid is produced by some diatoms and has been previously associated with a potential for neurotoxicity)
Yin D et al Environ Toxicol Chem, 28, 603-8, 2009	Investigation of 2,4,6-trichlorophenol p53 mutation in liver of zebrafish exposed for 10 days.	Positive results. Authors suggest that mutation may be involved in mechanism of carcinogenesis of TCP.

### **Biomonitoring**

7. A number of references on generic aspects of biomonitoring were identified. Of interest is the evidence for a wide diversity of life-style factors affecting background MN frequency in PBLs and the evidence for sources of variation in the performance of comet assays. A number of examples of application of genotoxicity biomonitoring are noted for information.

#### *Generic aspects of genotoxicity biomonitoring*

Reference	Aspect of Biomonitoring considered	Comments
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Decordier I Mutagenesis, 24, 85-93,2009	The authors describe an automated system for scoring Giemsa-stained in-vitro CBMN slides, discriminating between mono-bi-polynucleated cells.	The false-positive MN rate was 0.5-1.5% and thus a visual-interactive validation was introduced. Authors suggest approach could be used for biomonitoring studies.
Huang P et al Prev Med, epub Jan 22, 2009	Study of background variation in PBL MN using 208 healthy adult Japanese hard metal workers. A Total Life-Score quality was derived (for 8 life-style aspects).	Nutritional imbalance, lack of regular exercise, insufficient sleep, overtime working contributed to increased MN frequency. Mental stress, eating breakfast, alcohol drinking had no effect on MN frequency.
Weng H and Morimoto K, Mutat Res, 672, 1-9, 2009	Authors review studies of MN, SCE, comet in CD4+ (T), CD8+ (T), B-cells and NK-cells.	Authors suggest a reference of target cells for response to mutagens was developed
Valverde M and Rojas E, Mutat Res, 681, 93-109, 2009	Review of literature on comet assay used in biomonitoring reveals the importance of the need to establish standard methodological conditions that affect unwinding and electrophoresis times and tail values (length, moment, tail DNA)	Authors suggest comet assay is susceptible to subtle artefacts of manipulation depending on the type and timing of sampling performed. The context of how DNA damage was created needs to be reported.
Lindberg HK et al Mutagenesis, 23, 371-6, 2008	Characterisation of Centromeric and Telomeric status of binucleate lymphocytes from four individuals.	Most C+ T+ MN had one centromere and two or one telomeric signals (suggesting most involved single chromatids than both sister chromatids. Among C- MN telomeric signals were found in 91% men, 79% women showing most MN fragments were terminal. Additionally most C- T+ had one telomeric signal indicating chromatid fragments.
Fenech M et al Mutagenesis, 24, 199-201, 2009	Report of Buccal MN assay	Agreement reached on need for method collection of databases, writing of protocol, inter laboratory slide scoring exercise. Follow up at 10 <sup>th</sup> Int Conf on Environmental Mutagens in Florence in 2009.

### *Biomonitoring- some recent examples*

<b>Reference</b>	<b>Study</b>	<b>Comments</b>
Arsy NS et al Mutagenesis, May 7, 2009.	Investigation of MN induction in monocytes (24, 48 72 h after start of culture) and in binucleate PBLs (72h after start of culture) in 22 patients on cytostatic drugs (cf 13 health controls)	Marginal increase in MN in monocytes and clear statistically significant increase in binucleate PBLs indicating a large proportion of damage occurred during ex-vivo proliferation. A high proportion of MN exhibited centromeric signals.

Catalan J Environ Mol Mut, 50, 304-16, 2009	Investigation of CA in PBLs and genotype in 48 railroad workers compared to 39 referents	Increased CA frequency modified by genotype (decreased by MTHFR wild type, XRCC3 codon 241 variant) and increased by other polymorphisms (NAT2, ERCC2 exon10, XRCC1 codon 194). Small study indicating polymorphisms modify genotoxic effect or baseline CA levels.
El-Setouhy M et al Mutat Res, 655, 36-40, 2008	MN in oral exfoliated cells from water-pipe smokers evaluated	Increased MN frequency (2 fold) reported as first evidence to associate water-pipe smoking with cytogenetic damage.
Varella SD, J Occup Health, 50, 415-22, 2008	Urinary mutagenicity in laboratory workers exposed to solvents.	Increased urinary mutagenicity in ST YG1024 (+S-9) in lab workers.

## Discussion

8. The main priority for COM work in 2010 is to consider a revised testing strategy. There have been competing priorities which have prevented a first draft from being submitted to the October 2009 COM meeting.

9. It is hoped to complete the guidance note on risk assessment of *in vivo* mutagens in the near future and to set up a section on the COM internet site dealing with guidance notes.

10. With regard to items previously identified, it is hoped to initiate the reviews of mitochondrial mutation and mutational spectra.

11. With regard to potential items identified from this current scan of the peer reviewed published literature, members may wish to consider whether a targeted review of nanomaterials would be informative on the development of mutagenicity test methods for these novel materials.

12. Thus the proposed priorities for future work are

- i) Complete guidance note on risk assessment of *in vivo* mutagens.
- ii) Develop revised COM testing strategy.
- iii) Initiate reviews of mitochondrial mutagenicity and mutational spectra.
- iv) Undertake targeted work on nanomaterials focussing on mutagenicity method development.

**Secretariat July 2009.**