Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

Preface

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 2010, the Committee's work focussed the development of a new approach to publishing guidance on assessment strategies and genotoxicity tests. The Committee completed its review of thresholds for mutagens and produced a consultation document on a revised strategy for testing and mutagen assessment.

The Committee also undertook a further review of the utility of the GADD45a GFP genotoxicity assay and initiated reviews on the development and validation of a mutation assay using the PIG-A gene.

The Committee heard two presentations. Professor David Kirkland (COM Member) gave a presentation entitled '*Which mammalian cell tests best complement the Ames test in terms of detecting rodent carcinogens and in vivo genotoxins*.' Dr Nabil Hajii (Imperial College, London) gave a presentation on the '*Cytokinesis-block (CBMN) assay for the measurement and comparison of carcinogenic and in vivo genotoxicity potency estimates*.' Dr Andrew Olaharski (Roche) gave a presentation on the *GADD45a 'Green screen' assay*

The COM also advised on a biomonitoring study undertaken as part of the Government funded research on organophosphate pesticides. Horizon scanning was discussed at the October 2010 meeting.

Professor P B Farmer Chair
MA DPhil CChem FRSC

COM evaluations

Organophosphates

2.1 In 1999 the COT published a report on organophosphates (OPs) which considered whether prolonged or repeated low level exposure to OPs or acute exposures to OPs at levels sufficient to cause overt toxicity, can cause long-term adverse health effects. In the report, the COT had drawn conclusions from the available data and made recommendations for further research to address issues relating to potential chronic ill health such neuropyschiatric, neuropsychological effects and evidence for the occurrence of sheep dippers flu.' The research had been funded jointly by a number of Government Departments with the Veterinary Medicines Directorate taking a coordinating role. The COT has considered research projects as they have been published. The most recent consideration was in September 2009.

2.2 One study conducted as part of the Government funded research was project VM02301, which examined evidence for genotoxic effects of OPs exposure in horticultural workers. Part of this study had recently been published as: Atherton K *et al*., 2009. Biomarkers, 14 (7), 443 – 451. The overall project aims were to investigate whether there is a causal relationship between chronic OP exposure and DNA damage. The project reported results of a bio-monitoring investigation which aimed to address the hypothesis that OPs can cause DNA damage in humans following low-level chronic exposure. It was noted that the COM had previously undertaken detailed reviews of the published literature on the evidence for genotoxicity of pesticides in pesticide applicators and on the factors influencing the background incidence of genotoxicity biomarkers. These additional papers were also provided for members' information. Members were asked for their views on the recently published study by Atherton K *et al*., 2009.

2.3 The COM noted that exposed workers in southern Spain had been compared with University workers in the North East of England. Members questioned the suitability of the control group as there could be differences between the two comparison groups which could affect the COMET assay such as, exposure to UV light, physical exercise/manual work and exposure to other chemicals.

2.4 The study design as reported was insufficient to allow any conclusions to be reached regarding the results reported and association with exposure to pesticides.

Publication of COM Guidance Statements on assessment strategies and genotoxicity tests

2.5 The COM has a general remit to advise on important general principles or new scientific discoveries in connection with mutagenic and genotoxic hazards (the inherent property of the substance) or risk (the likelihood of mutagenic or genotoxic effects occurring after a given exposure) and to present recommendations for genotoxicity testing. The committee agreed that the proposed approach to publishing Guidance Statements allowed for the development of statements on individual genotoxicity tests and the overall testing strategy and components of the testing strategy. One particular advantage was that Guidance Statements could be rapidly updated in the light of new scientific developments. The COM would continue to publish individual statement on chemicals or referrals for Government Departments and Agencies as requested.

2.6 Further information on the new structure for guidance statements can be found at http://www.iacom.org.uk/guidstate/index.htm

Thresholds for *in vivo* mutagens

2.7 The general advice of the COM when considering the risk assessment of chemicals which are mutagenic *in vivo* has been that it is prudent to assume a non threshold dose response. Thus it is assumed that any exposure to an *in vivo* mutagen is associated with some damage to DNA and consequently an increased risk of mutation leading to an increased risk of adverse health effects albeit that this may be small. In such instances the Committee has recommended that exposures be reduced to a low as is reasonably practicable. The Committee has previously considered specific chemicals, on a case-by-case basis, with regard to deviations from its general approach to *in vivo* mutagens (see COM 2001 statement on risk assessment of *in vivo* mutagens http://www.iacom.org.uk/statements/COM01S3.htm).

2.8 The Committee considered draft guidance statements at its June and October meetings in 2009 and its March 2010 meeting.

2.9 The Committee agreed that evidence for a plausible threshold mode of action for genotoxicity was a prerequisite before conducting studies to identify threshold doses. Mutagenic effects that have been reported only at dose levels inducing a high level of toxicity or mortality should not be included in any evaluation for threshold dose levels, as the observed genotoxicity may not reflect a true mutagenic mode of action for the chemical under consideration. The biological significance of high dose positive *in vivo* mutagenic effects needs to be assessed on a case-by-case basis.

2.10 The COM reached the following conclusions.

(i) The COM reaffirmed the default position that for *in vivo* mutagens, in the absence of mechanistic data to infer a threshold, it is prudent to assume that there is no threshold for mutagenicity.

(ii) If there is good reason to consider that a threshold mode of action is appropriate, then it is necessary to investigate the biologically meaningful threshold for all genotoxic effects that have been reported.

(iii) An appropriate strategy should be devised for each chemical under consideration to identify threshold dose levels or NOELs for all potential thresholded modes of action of genotoxicity, which may include either *in vitro* or *in vivo* studies.

2.11 A copy of the link to the full statement is appended at the end of this annual report.

GADD45a GFP 'Green Screen' assay

2.12 At the COM meeting in October 2009, members evaluated reports on the development of the GADD45a-GFP genotoxicity assay. It was generally agreed that the assay may be useful as a high throughput pre-screening tool similar to QSAR using DEREK, but that it could not be used as part of a regulatory mutagenicity testing strategy at present. It was felt that further analysis of the low sensitivity reported by the study by Olaharski et al., 2009, (Mutation Research, 672, 10-16, 2009) for Roche proprietary compounds would provide a better understanding of the performance of this assay. Previously, members had noted the presentation of the data could have affected the reported results. The COM also noted that not all genotoxic substances are carcinogenic. Dr Olaharski (Roche) had provided a presentation on an analysis for the sensitivity, specificity and concordance of the GADD45a-GFP genotoxicity assay with *in vitro* genotoxicity and rodent carcinogenicity bioassay data for 91 Roche compounds. Out of the 91 compounds, 50 had been tested in a two year carcinogenicity assays, with 33 identified as rodent carcinogens and 17 as non-carcinogens. The reported sensitivity and specificity using the GADD45a-GFP 'GreenScreen HC' genotoxicity assay for genotoxicity (based on combined Ames and *in vitro* MN data) was 30% and 97% respectively (17/57 and 33/34) when a GFP induction of 1.5-fold was used as the criterion for a positive result. Its sensitivity and specificity for rodent carcinogenicity prediction was 30% and 80% respectively (10/33 and 15/17). The available data suggested a high concordance between laboratories indicating that the assay was both robust and reproducible.

2.13 Subsequently, Dr Olaharski agreed to make a presentation providing more detail regarding the genotoxicity data for the 41 proprietary Roche compounds that were used in the analysis. (An overview of the main points raised by Dr Olaharski can be found at http://www.iacom.org.uk/meetings/documents/COMminsMarch2010finalforinternet_000.pdf

2.14 Members concurred that Dr Olaharski had provided a valuable assessment of the 41 Roche proprietary compounds. However more information would be needed to assess the mode of action for the positive results in the *in vitro* genotoxicity assays conducted with these compounds (e.g. the degree of cytotoxicity in the MN assays).

2.15 Professor Walmsley (Genotrix Ltd) provided some additional analyses of the GADD45a 'Green Screen' assay data and in particular the 41 Roche proprietary compounds reported by Olaharski et al 2009. This was supplied as a manuscript to be submitted for publication entitled 'Interpretation of correlations between data sets from different *in vitro* genotoxicity tests.' In respect of the new data presented by Professor Walmsley, members commented that it was critical to assess the *in vitro* genotoxicity data on the 41 Roche compounds and in particular the mode of action for compounds with reported positive results in genotoxicity tests.

2.16 The COM also considered the paper by L Birrel *et al* (Mutation Research 695, 87 – 95, 2010) which assessed the 'Green Screen HC' assay results for a list of chemicals recommended by ECVAM. This study concluded that the GADD45a GFP 'Green Screen' assay demonstrated sensitivity for genotoxins comparable with other *in vitro* mammalian cell assays with a high specificity. This paper had been previously reviewed by the COM and members agreed that there were still only limited information regarding compounds which required exogenous metabolic activation.

2.17 The COM was also asked to consider a paper from the US EPA ToxCast programme by A Knight et al (Regulatory Toxicology and Pharmacology, 55, 188-199, 2009). This study was part of the US EPA ToxCast programme and reported data from three high-throughput screening (HTS) genotoxicity assays including the GADD45a GFP 'Green Screen' assays. Around 320 compounds with a large number of pesticides had been included in this initial part of this research programme. The sensitivity of the GADD45a GFP 'Green Screen' assay for a variety of end points including Ames positive, and rodent carcinogens was 11.6%-22.4% whilst specificity was reported to be 90-94.4%. Members noted that the GADD45a GFP 'Green Screen' assays had been conducted without exogenous metabolic activation. Thus, it was assumed that a proportion of the compounds that were negative in the 'Green Screen HC'

assay were pro-carcinogens that would require metabolic activation to produce a positive result in an *in vitro* genotoxicity test. This would reduce the sensitivity substantially. The authors of the ToxCast study also suggested the possibility that the limited concentration used (maximum test concentration of 200 µM) could have reduced the sensitivity. This research examined HTS assays in general and not the 'Green Screen HC' assay specifically and therefore may not have been sufficiently detailed to critically evaluate the performance of this assay.

2.18 Overall the committee agreed that the GADD45a-GFP genotoxicity assay was most suited as part of a battery of high throughput screening and noted it would still be useful in this respect even if sensitivity was low, as long as specificity was high.

In vivo PIG-A mutagenicity assay

2.19 The PIG-A gene codes for one subunit of a glycosylphosphatidyl inositol (GPI) anchor protein. Mutation of GPI (+) to GPI (-) results in loss of protein anchorage which can be evaluated using immunohistochemical approaches. The PIG-A assay has been shown to work in a number of experimental animals using a variety of blood cells and splenocytes. The method is easily adapted to flow cytometry approaches. It can potentially be used as an adjunct investigation in conventional rodent toxicology studies and could potentially be developed for use in biomonitoring investigations.

2.20 The potential role that this assay might play in a genotoxicity testing strategy needed to be better defined before further validation. There were already transgenic assays that also detected gene mutation *in vivo*, which had been extensively validated and could be used to assess mutagenicity in a wide range of tissues. Furthermore, the haematopoietic system was already a target tissue in the bone marrow MN *in vivo* assay. However, the *in vivo* PIG-A assay had potential to be an alternative to transgenic *in vivo* gene mutation assays in the future as it had the advantage of easy access; simpler method; a relative quick response time; and potentially could be used for more species and standard animal strains

2.21 The Committee was aware of a Health and Environmental Sciences Institute (HESI) presentation on the ongoing approaches to validation of the PIG-A assay
http://www.hesiglobal.org/files/public/2010%20Annual%20Meeting/Presentations/IVGT_Sessioin/1_jkim_intro.pdf

2.22 The PIG-A assay was an *in vivo* mutation assay using easily accessible sampling of blood which might potentially be incorporated into routine

toxicology studies. Participants in the HESI validation study were initially investigating the dynamics of PIG-A response with known *in vivo* mutagens. There was evidence to suggest that the mutagenic response in the assay accumulated with repeated exposure to *in vivo* mutagens and that inter-laboratory response was good. A number of participants in the HESI project were considering investigation of PIG-A response in the liver and gastrointestinal tract. One possible approach would be to undertake immunohistochemistry of tissue slices.

2.23 Members agreed there were many aspects of the PIG-A assay which needed investigation including identification of the optimum GPI-linked protein to use and the need for confirmatory DNA sequencing to confirm mutations.

2.24 Overall members felt that the PIG-A mutagenicity assay was an interesting development in genotoxicity testing, but that further work would be required before validation and there was a need to identify its role within a genotoxicity testing strategy.

Presentations to COM

'*Which mammalian cell tests best complement the Ames test in terms of detecting rodent carcinogens and in vivo genotoxins*.'
Professor David Kirkland (COM Member)

2.25 Professor Kirkland had provided a short paper for the March 2010 meeting entitled 'Is an *in vitro* battery of Ames plus micronucleus sufficient?' Subsequently, Professor Kirkland agreed to make a presentation to the COM to update the committee with additional analyses of rodent carcinogens and *in vivo* genotoxins.

2.26 Professor Kirkland outlined that most genotoxicity test guidelines recommended three *in vitro* genotoxicity tests i.e. gene mutations in bacteria; a test for induction of gene mutations in mammalian cells (usually the mouse lymphoma assay (MLA); and chromosomal aberration (CA) or micronucleus test.

2.27 It was agreed in various genotoxicity guidelines that there was a requirement for bacterial and mammalian tests and that the endpoints of gene mutation; chromosomal damage; and aneuploidy needed to be investigated. However, Professor Kirkland examined whether two mammalian cell tests were necessary to achieve this aim i.e. whether both bacterial and mammalian cell tests were required to investigate the endpoint of gene mutation. It was

suggested that the *in vitro* micronucleus test (MNvit) included in a test battery would be sufficient to detect both chromosomal aberrations and aneuploidy.

2.28 Published carcinogenicity data were analysed to address two questions: 1) Are there Ames negative rodent carcinogens that are not positive in the mouse lymphoma assay (MLA) that are not detected in the MNvit or CA assays?; and
2) Are there rodent carcinogens that are not detected in either Ames or MNvit, but might be uniquely positive in the MLA?

2.29 These two questions were addressed using a database of 756 rodent carcinogens and 461 *in vivo* genotoxins. A detailed paper summarising the evaluation has been published on the COM internet site http://www.iacom.org.uk/papers/documents/POSTMTGNOTEKirklandRevisedproposalfor2testbatterymut201014_000.pdf

2.30 Professor Kirkland reached a number of conclusions.

(i) From the available data, no genotoxic rodent carcinogens would be "missed" by using an *in vitro* battery consisting of Ames and *in vitro* micronucleus tests.

(ii) Thus, a 2-test battery consisting of Ames + MNvit is comparable to a 3-test battery consisting of Ames + MLA + MNvit in terms of detecting genotoxic rodent carcinogens as positive.

(iii) There is no notable advantage achieved by adding MLA to a battery consisting of Ames + MNvit. Based on the above analysis there are no examples of *in vivo* genotoxins for which it is essential to include the MLA in addition to Ames plus MNvit in order to detect genotoxic potential.

(iv) Thus, a 2-test battery consisting of Ames + MNvit is comparable to a 3-test battery consisting of Ames + MLA + MNvit in terms of detecting *in vivo* genotoxins as positive. There is no notable advantage achieved by adding MLA to a battery consisting of Ames + MNvit.

Cytokinesis-block (CBMN) assay for the measurement and comparison of carcinogenic and in vivo genotoxicity potency estimates.'

2.31 Dr Nabil Hajji from Imperial College, London gave a presentation on developing a generic approach to ranking *in vivo* mutagens where there is no carcinogenicity data. An approach using only a single end point from an *in vivo* genotoxicity test was suggested to be preferable as this would be relatively simple and readily comparable. An approach to ranking *in vivo* mutagens, which did not have carcinogenicity data, using the lowest effective dose (LED) had already been developed by Sanner and Dybing 2005 (Basic & Clinical Pharmacology & Toxicology 2005,96, 131 – 139)**).**

2.32 Dr Hajji had identified three potentially useful database sources. A published evaluation of the rodent MN tests undertaken as part of the US EPA Gene Tox program during the 1980s and 1990s was suggested as a useful source of information. Under this program, 506 chemicals had been assessed, but not all the current data were available in the public domain or readily accessible for the derivation of LED values. Another potentially useful data source was the 6th Collaborative Study Group on the Micronucleus Test (CSGMT) available from the Japanese Environmental Mutagen Society (JMS). This identified approximately 100 mouse MN assays predominantly undertaken by using the intraperitoneal dosing. A third suggested data source was the International Programme on Chemical Safety (IPCS) INCHEM database.

2.33 It was proposed that the data sources would be used to obtain *in vivo* genotoxicity potency estimates such as the LED or the Benchmark Dose (BMD) (i.e. where there were at least 3 dose-response data points). These could then be compared with available carcinogenicity potency estimates such as the TD50 (the chronic daily dose that will give rise to 50% of the test animals having tumours above background at a specific site).

2.34 The Committee recommended that Dr Hajji contact other research groups who were already undertaking similar work and that it may be useful to liaise with them. For example, RIVM and ILSI/HESI Members noted that the ILSI/HESI group were looking at extrapolating from *in vitro* genotoxicity potency to *in vivo* potency. Whereas the RIVM group, were considered to be mainly looking at *in vivo* data for prioritising mutagens and to examine what could be learned about carcinogenic potential without carcinogenicity data.

2.35 The committee agreed that where possible, the use of BMD would be preferable to the LED, and that it would be important to define the biologically significant response level e.g. 1% or 10% above the control response

Horizon Scanning

2.36 A horizon scanning exercise is conducted every year, where new and emerging topics in the field of genotoxicity are identified that may require review. The horizon scanning process provides an opportunity for members and advisors from Government Departments and regulatory agencies to suggest topics for further work. This year, most of the committee's work had involved updating the current COM guidance and resources had not been available to undertake all of the projects identified in the 2009 horizon scanning exercise. Some topics raised during the 2009 horizon scanning exercise were considered as part of the drafting of the revised genotoxicity testing strategy and generation of new guidance documents. These included:

- Does the mouse lymphoma assay detect aneugens?
- Which mammalian cell test best complements the Ames test in terms of detecting rodent carcinogens and *in vivo* genotoxins?
- An evaluation of the GADD45a-GFP 'GreenScreen HC' genotoxicity assay.

2.37 Additionally, the COM agreed a format for separating the guidance into separate statements. Progress was also made on consideration of the validation of the mutation assay using the PIG-A gene. A review of expanded simple tandem repeat (ESTR) mutation had been initiated, but not completed.

2.38 Topics not addressed in 2010 included a review of the mutagenicity of nanomaterials; mutational spectra in the investigation of chemical mutagenesis; the role of epigenetics in mutagenesis; and mitochondrial mutagenesis. The genotoxicity of nanomaterials was suggested as a priority topic. The Chair noted that the secretariat would have to complete a review of the significance of chemical induced mutation for human health and a review of genotoxicity testing of impurities which had been identified as priorities during the consideration of a revised strategy for testing for genotoxicity.

2.39 The committee was also informed that some funding was available to the HPA for research projects relevant to public health. This included initial funding for a one year project of around 25K as well as larger two to three year projects of up to a maximum of 250K per year. There was also scope to fund PhD's. Members were asked for suggestions for suitable projects which could also involve collaborative work between different organisations.

2.40 Some initial suggestions included research into low dose genotoxicity effects compared with higher doses; non-DNA targets; systems biology approach to key gene suppression and expression; and the sequencing of whole genomes for different cancers.

Ongoing work

Consultation on a strategy for genotoxicity testing and mutagenic Hazard assessment of chemical substances

2.41 The draft paper on Guidance on a strategy for Genotoxicity testing and mutagenic hazard assessment of chemical substances was issued for consultation in early December 2010, together with associated diagrams and a copy of the consultation list. These are available on the Publications page of the COM internet site. Comments were invited by 12th February 2011. The finalisation of COM guidance on a strategy for testing and assessment of

mutagenicity of chemical substances will be a major item for completion during 2011.

Published Statement
http://www.iacom.org.uk/guidstate/documents/Thresholdsforinternetfinal.pdf

2010 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

CHAIR

Professor Peter B Farmer MA DPhil CChem FRSC
Professor of Biochemistry, Cancer Studies and Molecular Medicine,
Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester

MEMBERS

Dr Carolyn Allen BSc MSc PhD
Non-specialist Member

Dr Brian Burlinson CBiol MIBiol PhD
Director of Safety Assessment, Huntingdon Life Sciences

Dr Gillian Clare BSc PhD
Cytogeneticist, Covance

Dr Barry M Elliott BSc MSc PhD
Independent Consultant in Genetic Toxicology

Dr David Gatehouse BSc PhD CIBiol FIBiol FRCPath
Consultant in Genetic Toxicology

Mrs Rosie Glazebrook MA
Non-specialist member

Dr Gareth Jenkins BSc, MSc, PhD
Reader, Institute of Life Science, Swansea School of Medicine,
Honorary Non-clinical Senior Lecturer, Swansea NHS Trust.

Professor David Kirkland BSc (Hons), PhD
Principal, Kirkland Consulting

Dr David P Lovell BSc PhD CStat FSS CBiol FIBiol
Reader in Medical Statistics, Division of Biomedical Sciences,
St George's University of London

Professor Anthony Lynch BSc (Joint Honours), PhD
Manager, Investigative Studies & New Screening Technologies, Genetic Toxicology, GlaxoSmithKline

Dr Elizabeth M Parry BSc DPhil
Senior Research Fellow, School of Biological Sciences, University of Wales

Professor David H Phillips BA PhD DSc FRCPath
Professor of Environmental Carcinogenesis, Institute of Cancer Research

SECRETARIAT

Mr J Battershill BSc MSc	Joint Scientific Secretary – Health Protection Agency
Dr D Benford BSc PhD	Joint Scientific Secretary – Food Standards Agency
Dr L Hetherington BSc PhD	Scientific – Health Protection Agency
Ms F Pollitt MA DipRCPath	Scientific– Health Protection Agency
Ms S Kennedy	Administrative Secretary – Health Protection Agency

Declaration of COM members' interests during the period of this report
(an up-to-date version can be found on the COM website)

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P B Farmer (Chairman)	Santander Foreign & Colonial Friends Provident Tototrak ILSI HESI EFSA	Shareholder Shareholder Shareholder Shareholder Committee Member Member of Scientific Panel	Van Geest Foundation	Research support
Dr C Allen	NONE	NONE	NONE	NONE
Dr B Burlinson	Huntingdon Life Sciences	Salary Employee Share Option Holder	NONE	NONE
Dr G Clare	Covance Allied Domecq AstraZeneca Diageo HBOS Marks & Spencer	Consultant Shareholder Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Dr B M Elliott	Syngenta AstraZeneca Elliott GT Ltd Regulatory Science Associates	Pension & Shareholder Shareholder Director Associate	NONE	NONE
Dr D Gatehouse	GlaxoSmithKline	Pension Share Option Holder Shareholder	NONE	NONE
Mrs R Glazebrook	BT Group Lloyds TSB National Grid	Shareholder Shareholder Shareholder	NONE	NONE
Dr G Jenkins	NONE	NONE	Hoffman-LaRoche Unilever Hoffman-LaRoche CEFIC/ECETOC	Research Grant 2008 – 2010 Consultancy 2008 Honorarium 2008
Dr D Kirkland	Kirkland Consulting	Principal	NONE	NONE

Dr D P Lovell	National Grid Plc Pfizer	Shareholder Pension	AstraZeneca National Grid Plc	Spouse is Shareholder
Dr A Lynch	GlaxoSmithKline	Salary & Shareholder	NONE	NONE
Dr E M Parry	F & C M & S Compass BP	Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Prof D H Phillips	Aviva Banco Santander BG Group Bradford & Bingley Centrica National Grid	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder	AstraZeneca	Research Support