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 2009, the Committee provided advice on a wide range of topics including genotoxicity of fumagillin, parachloroaniline and tobacco products. The COM finalised its statement on the mutagenicity of acrylamide.

The Committee undertook a review of thresholds for *in vivo* mutagens and reviewed recent data on the utility of the GADD45a GFP genotoxicity assay and the mouse lymphoma assay. A review of recent publications on the use of toxicogenomics in genotoxicology was undertaken.

We have also begun the process of reorganising the presentation of COM advice on the internet to produce guidance documents which can be updated when required in addition to formal statements on the mutagenicity of chemicals. This will be an important area of work for 2010.

Professor P B Farmer Chair
MA DPhil CChem FRSC

COM evaluations

Acrylamide

Background

2.1 Acrylamide is a small, simple molecule (Figure 1). It is an α,β-unsaturated carbonyl with electrophilic reactivity. This means it can react with nucleophilic groups (amines, carboxylates, sulphydryls etc) on biological molecules, such as proteins or DNA. *In vivo*, acrylamide may be metabolised to the reactive epoxide glycidamide, which is thought to have a role in acrylamide related toxicity.

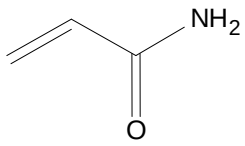

Figure 1: Acrylamide

2.2 In January 2007, the Health and Safefty Executive (HSE) requested that the Committee on Mutagenicity (COM) provide an opinion on the evidence regarding germ cell mutagenicity of acrylamide and the evidence regarding a threshold for germ cell mutagenicity with this chemical. The Committee was provided with a copy of the EU Risk Assessment Report and a submission from the Polyelectrolyte Producers Group (PPG), discussing the evidence regarding germ cell mutagenicity of acrylamide and the evidence for a threshold for germ cell mutagenicity with this chemical. A response was published in February 2007 (COM/07/S2) http://www.iacom.org.uk/statements/Acrylamide.htm. The COM was made aware of a response from the Polyelectrolyte Producers Group (PPG) to the chair (dated 8 May 2007) at the COM meeting of the 17 May 2007 http://www.iacom.org.uk/papers/documents/mut0716.pdf and agreed to a further evaluation of the genotoxicity data on acrylamide at the request of HSE. In view of the widespread dietary exposure to acrylamide, the Food Standards Agency requested that such a review should consider all available genotoxicity data on acrylamide.

2.3 The COM agreed that the EU risk assessment review completed by HSE (EU Risk Assessment report 2002) could be used as a basis for the review, and for this to be extended with a systematic review of the scientific literature available subsequent to 1995.

2.4 The Secretariat drafted an overview of the EU Risk Assessment of acrylamide and outlined a strategy for the review. The search strategy was devised in

order to identify all relevant studies that had not been cited in the EU Risk Assessment Report, and the last update to the search was performed on the 23rd September 2008. Details of this search strategy can be found in Annex A to the COM statement which is reproduced at the end of this annual report. Members reviewed the findings of the EU Risk Assessment Report and were content with the search strategy used for the COM review. Members were presented with a systematic review of data relating to the genotoxicity of acrylamide and glycidamide published after 1995, and other pre 1995 references that had not been included in the EU risk assessment report. This paper also provided an initial discussion of the acrylamide genotoxicity data, and this was extended to include the metabolite glycidamide.

2.5 The PPG were invited to give presentations at the October 2007 and February 2008 meetings and have submitted data and supporting references for Members to consider at several points during the review (as noted in relevant minutes and discussion papers). The PPG met with the Secretariat prior to each meeting in order to explain the Committee's procedures and to provide advice on the structure and content of the submissions, highlighting areas where more detail would be valuable to the Committee's deliberations. The PPG submitted comments on the second draft Working Paper and a relevant abstract from a recent scientific meeting (McDaniel *et al.,* 2008 Poster presentation at the 39th Annual Environmental Mutagen Society Meeting 2008, October 18-22). Both were tabled at the October 2008 meeting.

2.6 In January 2009, PPG submitted comments of the fourth draft working paper. They noted the absence of discussion of the *in vivo* potency of acrylamide, the relative contribution of putative mutagenic pathways, and the contribution of DNA repair. They also commented on the COM interpretation of the dose-response for micronuclei induction and the biological plausibility of applying linear or threshold models to the data; and suggested that the genotoxic risk be put in the context of human exposure with mention of a practical threshold in the conclusions. The COM noted PPG's proposals for a number of changes to the working paper and agreed to include a number of these proposals with some amendments.

COM conclusions

2.7 The EU risk assessment report concluded that acrylamide is an *in vitro* mutagen, and *in vivo* somatic cell and germ cell mutagen. The predominant effect was clastogenicity with some evidence for aneugenicity. The published evidence available since 1995 extends the effects of acrylamide to include identifiable glycidamide DNA adducts and gene mutations, detectable in cultured mammalian cells and somatic cells *in vivo,* and with mutation spectra which are consistent with those adducts. An element of the mutagenic effect

of acrylamide, therefore, appears to be due to the formation of DNA adducts following metabolism to glycidamide.

(a) Assessment of the genotoxic potential of acrylamide is complicated by multiple potential mechanisms, which include extensive protein binding / enzyme inhibition, oxidative stress and DNA adduct formation. It is plausible that each of these mechanisms may contribute to the genotoxicity of acrylamide. These mechanisms are not mutually exclusive.

(b) Acrylamide is an *in vivo* mutagen. In experiments reviewed in this statement, genotoxic effects are generally only seen at relatively high acute doses (ca 50 mg/kg bw i.p. in mice). However genotoxic effects are also reported at much lower dose levels in repeat dose studies (ca 4 mg/kg bw, i.p.for 28 days in mice).

(c) The default assumption is that there is no level of exposure to this genotoxic carcinogen that is without some risk. In order to move away from this assumption, it will be necessary to identify evidence of a threshold with supporting mechanistic data for all of the potential genotoxic mechanisms of acrylamide in somatic cells and germ cells. Based on the currently available evidence, it should be considered that there is no level of exposure to acrylamide that is without some risk, although we acknowledge that the genotoxic effects of exposure to very low levels of acrylamide are likely to be pragmatically indistinguishable from background.

2.8 The COM agreed a statement can be found at: http://www.iacom.org.uk/statements/documents/COM09S1Acrylamide.pdf

Fumagillin

Background

2.9 Fumagillin dicyclohexylamine (fumagillin DCHA) is an antibiotic veterinary medicine authorised for use in honey bees for the prevention of infections caused by the *Nosema apis* parasite present in the gut of infected bees (Figure 2). Fumagillin DCHA is fed to the colony in winter over a period of several weeks in a medicated syrup as a supplementary food source to eradicate the parasites. The commercial formulation of fumagillin DCHA is a stabilised water-soluble preparation, Fumidil-B (CEVA Animal Health). Fumidil-B contains the excipient polysorbate 80, sodium phosphate (anhydrous) and sodium acid phosphate (anhydrous).

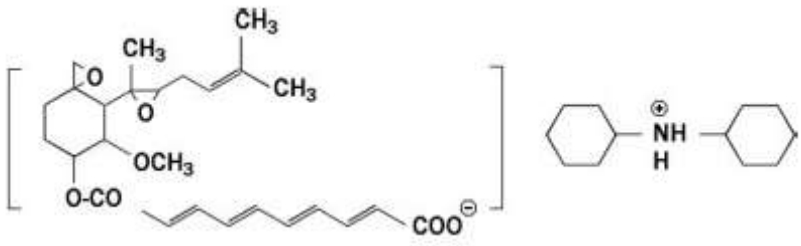

Figure 2. Structure of Fumagillin DCHA Salt

2.10 Fumagillin DCHA at the time of the COM evaluation during 2009 did not have a maximum residue level (MRL) status because the Committee on Veterinary Medicinal Products (CVMP) were unable to make a recommendation when the substance was evaluated in 1999. The main reasons given were that no ADI could be established because no overall NOEL was identified for repeated dose toxicity, reproductive toxicity or teratogenicity/fetotoxicity and no conclusions could be reached on the genotoxicity or carcinogenic potential of fumagillin. The veterinary medicine Marketing Authorisation Holder (MAH), CEVA Animal Health, indicated that they might make another MRL application to address the absence of the MRL. The MAH sought scientific advice from the CVMP and, in October 2006, the MAH stated that a 90-day repeated dose toxicity study was ongoing and that five mutagenicity tests had been performed. Reproductive toxicity studies have not been conducted. The COM was made aware of reports of genotoxic effects of fumagillin DCHA in cytogenetic tests both *in vitro* and *in vivo* published by the Stanimirovic group (see reference list at the end of the COM statement on Fumagillin at the end of this annual report). These reports were reviewed by VMD who recommended that an independent opinion should be sought on interpretation of the results, to establish if there is a potential risk to consumer safety. Subsequently, the MAH provided VMD with reports of six additional genotoxicity studies conducted between 2004 and 2007, together with an expert report on the genotoxic potential of fumagillin and a critique of the genotoxicity studies submitted by the MAH.

2.11 The COM was asked by the VMD for an opinion on the genotoxicity of fumagillin DCHA and in particular the interpretation of the published studies undertaken by the Stanimirovic group. The COM has not been asked in this review to advise on consumer risk assessment of consumption of fumagillin, fumagillin DCHA, or its breakdown products as potential contaminates of honey.

COM conclusions

2.12 The COM agreed that the Stanimirovic data were limited and no definite conclusions could be reached. There were several possible explanations for

the differences between the results obtained for *in vivo* genotoxicity studies undertaken the Stanimirovic group and the MAH. These included possible differences in absorption, metabolism of the administered test material, differences in stability of the test materials including storage, and different impurity profiles between test materials used by the research groups. The COM agreed that the genotoxicity data on fumagillin acid and dicyclohexylamine tested separately did not provide sufficient information to draw conclusions on the role of these substances in the potential mutagenicity of fumagillin DCHA. The COM agreed that a repeat of the Stanimirovic study in mice (using the same test protocol) with test material sourced by the MAH should be undertaken with appropriate measures of systemic absorption. The COM considered that a second *in vivo* tissue evaluation should be undertaken and suggested a site of contact comet formation in the gastrointestinal tract (with an appropriate positive control substance). Negative data from appropriately conducted tests (according to the Stanimirovic protocol) using two tissues in mice would be sufficient to refute Stanimirovic data. Equivocal or positive data from such tests would confirm that fumagillin DCHA should be considered an *in vivo* mutagen. The Committee also commented if any genotoxicity was observed with fumagillin DCHA, more genotoxicity data (*in vitro* chromosomal aberration test in human lymphocytes) should be provided on dicyclohexylamine to evaluate its potential role. (Any study should also include fumagillin DCHA for quantitative comparison). The COM considered that the differences in statistical reporting in the Stanimirovic group publications as highlighted by the MAH were not necessarily founded. Members agreed that further additional data on the influence of light/dark conditions on the genotoxicity of fumagillin DCHA were not necessary. Members agreed that the data on potential fungicidal mode of action submitted were not relevant to the potential genotoxicity mode of action of fumagillin DCHA.

2.13 The COM recommended the following testing strategy for fumagillin DCHA.

(a) A further *in-vivo* mutagenicity study using the same protocol used by Stanimirovic et al. (*Mutat Res* (2007) 628, 1-10.) to include sampling of bone marrow for MN and chromosomal aberrations.

(b) A site of contact comet assay using gastrointestinal (stomach) tissue. (The comet assay should also include an appropriate positive control substance).

(c) If any genotoxicity is observed with fumagillin DCHA, more genotoxicity data (*in vitro* chromosomal aberration test in human lymphocytes) should be provided on dicyclohexylamine to evaluate its potential role. (Any study should also include fumagillin DCHA for quantitative comparison).

2.14 The COM agreed a statement can be found at: http://www.iacom.org.uk/statements/documents/COM09S2FumagillinforVMD2.pdf

Parachloroaniline

Background

2.15 Parachloroaniline ((4- chloroaniline, 4-CA). see figure 3 below) is a potential human metabolite of the pesticide diflubenzuron. There is experimental evidence for urinary excretion of 4-CA in swine exposed to diflubenzuron, and for its presence as a metabolite in goats (liver) and in hens (liver and kidney). The Advisory Committee on Pesticides (ACP) asked the COM for a view on the available genotoxicity data on para-chloroaniline

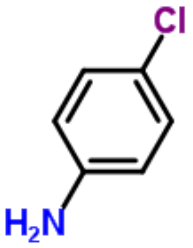

Figure 3 Parachloroaniline.

2.16 The Committee was aware that NTP (U.S. National Toxicology Program) bioassays for potential carcinogenicity have been undertaken in rats and mice. There was clear evidence of carcinogenicity in male rats (splenic sarcoma and osteosarcoma associated with fibrosis of the spleen, and phaeochromocytoma of the adrenal gland). There was equivocal evidence for tumours of the spleen in female rats. There was some evidence for liver tumours in male mice and no evidence for carcinogenicity in female mice. It is notable that increased haemangiosarcomas were seen in both rats and mice (in spleen and/or liver).

COM conclusions

2.17 The COM reached a number of conclusions;

(a) The COM concluded that 4-CA was an *in vitro* mutagen.
(b) No definite conclusions on the *in vivo* mutagenicity could be drawn on the information reviewed.
(c) A further *in vivo* genotoxicity testing strategy was agreed. This comprised two studies. Study ii) should be undertaken if the results of Study i) were negative or equivocal.

Study i) a repeat MN test in mice conducted to internationally acceptable standards to include sampling of the bone marrow and peripheral blood for reticulocytes

Study ii) The second study should be a rat liver UDS assay with a concurrent rat comet assay to investigate DNA damage in the spleen, liver and other tissue (not considered to be a rat tumour target organ).

2.18 The COM agreed a statement which can be found at: http://www.iacom.org.uk/statements/documents/ParachloroanilineforcomintersitetDec09.pdf

Tobacco products

2.19 The Department of Health (DH) had specifically requested an update of the 2004 joint COM/COC/COT statement. Dossiers on the toxicological testing of tobacco product ingredients in their burnt and unburnt forms are submitted to DH. As there are no internationally agreed approaches to the hazard assessment of these products, scientific advice was sought from the COM on the suitability of mutagenicity tests for the evaluation of these products. Another reason for the DH request was that there are a number of new products purporting to reduce harm to users (i.e. by reducing exposure to harmful chemicals), for which the Department had no means of evaluating toxicity. New tobacco products that potentially reduce exposure to harmful chemicals, such as electrically heated tobacco products, are known as PREPS. There was a contention that existing tests do not give sufficient information to draw meaningful conclusions.

2.20 The Committee considered a draft discussion paper on the genotoxicity of tobacco products. This was provided in conjunction with a short discussion paper on the regulatory aspects relating to the toxicity testing of tobacco products and a scoping paper on the toxicology of tobacco products. The documents provided to COM followed-up the 2004 joint COM/COC/COT statement on the toxicological testing of tobacco products. Members were also provided with a copy of a letter to the Secretariat from British American Tobacco (BAT), outlining their approach to the toxicology of tobacco products and an additional paper on whole smoke exposure of human pulmonary carcinoma cells. Members also had access to a submission from Philip Morris. Additionally, members were provided with an email from an independent expert on smoking behaviour on compensatory smoking. The overall objective was to produce an update statement from COM/COC/COT.

2.21 As an initial comment, members agreed with the statements abstracted from WHO Technical Series Report 945 that the rate limiting steps in the

mechanistic pathways leading to tobacco product induced disease were not understood and hence this limited the value which could be attributed to the available data on biomarkers. The available data on biomarkers of mutagenicity would inform on overall exposure to mutagens. Members commented that the available test strategies for evaluation of mutagenicity of tobacco products had been largely dependent on the practicality of deriving cigarette smoke condensate (CSC) or total particulate matter (TPM) which could be easily obtained, stored frozen and transported between laboratories and the observation that there was a correlation between potency in skin painting bioassays of tumourigenicity in mice and potency in *Salmonella typhimurium* mutagenicity tests.

2.22 COM members encouraged the development of whole smoke exposure procedures which were likely to provide more relevant data on mutagenic activity of tobacco smoke, but noted none of the test systems had been adequately validated and there was no agreement on what reference material would be used for comparative purposes.

2.23 The COM made a number of comments on the draft discussion paper on the genotoxicity of tobacco products.

Validity of genotoxicity tests

2.24 Members reaffirmed that any ranking of mutagenicity of tobacco products could not be extrapolated to *in vivo* exposure to chemicals in tobacco smoke. Thus tobacco smoke was a multi-site carcinogen in humans and it was not possible to evaluate which exposures were relevant for each of the fifteen different target organ cancers induced by tobacco smoke. Members commented that data on appropriate reference materials were needed for comparative data on whole smoke methods currently under development, and that the smoking regimes used did not necessarily reflect human exposure to tobacco smoke. It was also noted that mutagenic effects *in vivo* would also be influenced by target organ inflammation.

2.25 Members noted that potency rankings were also dependent on the smoking regime used. Members commented that there were advantages to reporting mutagenicity data on a per cigarette basis and on a per mg nicotine basis. The former gave an easy measure on mutagenic potency per unit consumed whereas reporting data in terms of mutagenicity/mg nicotine more closely reflected the behaviour of smokers who adjusted cigarette consumption to maintain nicotine exposure. The COM agreed that there was a need for international harmonisation to reach a consensus on mutagenicity test procedures, use of reference materials, and cigarette smoke generation regimes.

Potential effects of ingredients, additives and flavours

2.26 Members felt that the mutagenicity evaluation of ingredients, additives and flavours by adding test materials to tobacco products pyrolysing and then testing CSC in *in vitro* mutagenicity tests with *Salmonella typhimurium* would not provide any useful information on the mutagenic properties of the pyrolysed ingredients, additives and flavours.

Biomarkers of effect

2.27 Members agreed that biomonitoring of urinary mutagenicity using *Salmonella typhimurium* TA98 and TA 100 in the presence of exogenous metabolic activation using rat liver S-9 from Aroclor 1254 treated rats might inform on potential risks of bladder carcinogenesis but not for other tobacco related cancer target organs. Thus urinary mutagenicity was essentially a biomarker of exposure to absorbed mutagens that were sensitive to the mutagenicity testing regime used. Members noted the standard deviation for urinary mutagenicity in the paper submitted (Mendes P et al *Regulatory Toxicology and Pharmacology*, 51, 295-305, 2008) suggested there were large inter individual differences in absorbed mutagens. There was discussion relating to the possibility of developing biomarkers for potential inflammation induced by tobacco smoke.

Available information on PREPS

2.28 The available data on mutagenicity suggested significant reductions in both *in vitro* mutagenic activity in *Salmonella typhimurium* TA98 and TA 100 in the presence of exogenous metabolic activation using rat liver S-9 from Aroclor 1254 treated rats and urinary mutagenicity in biomonitoring studies using a number of acceptable study designs. These data indicated that mutagenicity was not reduced to background levels. The data were consistent with a substantial reduction in exposure to mutagenic effects of aromatic amines in tobacco smoke. Overall the COM agreed the data supported the approaches used to reduce exposure to mutagens, notwithstanding the primary advice not to smoke tobacco products, but cautioned that the association between chemical mixtures present in tobacco smoke and disease outcomes was very complex and no conclusions regarding risk of mutagenicity could be reached from the available data.

Horizon Scanning

2.29 The horizon scanning exercise provides information which can be used by Government Departments/Regulatory Agencies to identify important areas for future work. Regarding progress on topics raised in the 2008 horizon scanning exercise, members were informed that a large amount of committee time had been spent undertaking reviews of aclonifen, fumagillin and tobacco products. Progress had been made on thresholds (and a draft guidance document on the risk assessment of *in vivo* mutagens), toxicogenomics and a draft outline proposal for a testing strategy. No progress had been made on mutational finger prints or mitochondrial mutagenicity.

2.30 The committee agreed that the main priority for COM work in 2010 would be to consider a revision of the mutagenicity testing strategy. Members agreed that a review of the mutagenicity of nanomaterials would be important and that the consideration of mutational spectra to investigate the role of chemicals in mutagenicity and carcinogenesis could be useful. Regarding mutagenicity testing, the COM made some suggestions, which included consideration of the PIG A assay, the potential integration of genotoxicity tests into standard toxicity studies; measures for cytotoxicity in genotoxicity tests; top doses; reliability of cell types; and the use of oncogene/tumour suppressor gene arrays. Members also suggested epigenetics as a potentially important topic.

2.31 The Committee agreed that the highest priority should be to review COM guidance on testing strategy and undertake a specific review of nanomaterial genotoxicity testing. One member noted that his group had recently published a review of nanomaterial genotoxicity and this would be a useful starting point for any review.

Test Strategies and Evaluation

Mouse Lymphoma (MUT/09/10)

2.32 The committee was provided with a paper (Wang J et al., Toxicological Sciences 2009, 109 (1), 96-105) which reported data on detailed genetic alterations in L5178Y $TK^{+/-}$ mutants with either small or large colony growth characteristics from studies investigating the mutagenicity of 3'- azido-3-deoxythymidine (AZT), mitomycin C (clastogens) and taxol (aneugen). Colonies that exhibited significant loss of heterozygosity in chromosome 11 were selected for further investigation. The increased mutation frequency in studies ensured that a high proportion of the mutants selected were due to chemical treatment. TK gene dosage, G-banding analysis for chromosomal changes, and FISH for detection of chromosome 11 numerical changes were

undertaken. The results showed complex genetic changes with all three test substances, with evidence for deletion, recombination and aneuploidy. The absence of a functional P53 gene in L5178Y $TK^{+/-}$ cells was in part responsible for survival of cells with larger scale DNA damage. The authors suggested that these new data provide evidence for the utility of the mouse lymphoma assay (MLA) in a mechanistically based genotoxicity hazard identification battery. The COM strategy suggests that the MLA is suitable for regulatory use for the detection of gene mutations and provides complimentary rather than equivalent data to metaphase analysis.

2.33 The COM was aware that it recommended the MLA in its guidance on a strategy for mutagenicity testing (or an alternative of equivalent statistical power) as the third *in vitro* test in stage 1. More recently the COM has seen data to suggest that the MLA can detect clastogens and in some instances aneugens (the latter only at high doses resulting in cytotoxicity). The committee was asked its views on the proposed use of the MLA as part of a mechanistically based genotoxicity hazard identification battery.

2.34 Members agreed that this was an interesting paper. However, it was felt that the method outlined in the paper is dependent on a selective growth mechanism and thus will only detect the loss of chromosome 11 TK^{+}. This meant that only a limited analysis could be conducted and that non-disjunction could not be detected. The test might be useful as an indicator of aneuploidy, but would not permit exact measurement. It was noted that chromosomal aberration studies had indicated that cells with structural chromosomal aberrations and aneuploidy do not survive cell division when changes represented a balanced event and genetic gain is better tolerated than loss and thus would not go on to form a colony. Colonies that were found would represent potentially toxicological relevant events. Most colonies analysed had acquired a duplicate chromosome 11. This may indicate that cells with a loss of chromosome 11 do not survive. Members also noted the importance of the lack of p53 gene in L5178Y $TK^{+/-}$ cells leading to genomic instability. The COM agreed that small and large colonies related to different mutagenic events, but that this was not demonstrated by the data presented. Members also felt that it was probably better to use the micronucleus test to analyse for aneuploidy. Overall, the committee agreed that MLA was a useful assay when used as part as of a battery of tests for mutagenicity, but could not be used in isolation of other tests.

GADD 45a GFP assay

2.35 The committee had been introduced to the TK6 GADD 45a assay in 2007 when Professor Walmsley (Gentronix Ltd) had given a comprehensive talk on this newly developed high-throughput *in vitro* genotoxicity assay. The assay

utilises GADD45a, a gene considered to play a role in DNA repair, cell cycle control and apoptosis in response to genotoxicity. Induction of GADD45a has been identified in early gene expression in microarray experiments in response to a wide range of genotoxins (e.g. direct DNA damaging, topoisomerase inhibitors, nucleotide synthesis inhibitors, aneugens and generators of reactive oxygen species) in various cell types. The increase in GADD45a gene expression suggested that it could be used as a marker for genotoxic stress. In the test system GADD45a is fused to a green fluorescent protein (GFP) gene. The plasmid construct is transfected into P53 proficient human lymphoblastoid cell line (TK6) and the assay is conducted in microplates. After incubation with test compounds GFP reporter fluorescence and cell culture absorbance are measured.

2.36 Since the original presentation, a number of significant studies have been conducted to further validate the assay and introduce modifications. Most importantly, a protocol using metabolic activation and a higher throughput schedule had been outlined. A written overview detailing these developments by Professor Walmsley was submitted to the COM. A number of peer reviewed published papers were also made available to members covering areas such as: inter-laboratory validation; metabolic activation; further general validation; a trial of ECVAM recommended chemicals as part of a project to reduce the number of false positives; and a higher throughput protocol. Generally, the assay appeared to perform robustly and had been shown to have high specificity (correct identification of negatives) and sensitivity (correct identification of positives. However, one study by Olaharski A *et al* (Mutation Research, 672, 10-16, 2009) suggested a lower sensitivity.

2.37 The COM agreed that there was a lot of new data conducted to acceptable standards that provided evidence of a high degree of sensitivity and specificity. Regarding the inter-laboratory trial by Billinton N *et al*., (Mutation Research, 653, 23-33, 2008), members noted that this study had included a number of genotoxic and non-genotoxic compounds, but none of the genotoxic chemicals required metabolic activation. The GADD45a assay had been adapted to use S9 exogenous metabolic activation by Jagger C *et al*., (Mutagenesis, 24, 35-50, 2009) but it was felt that this aspect of the assay was less well validated. Overall, members felt that the assay was as good as any other *in vitro* genotoxicity test without metabolic activation. However, they wished to see more data with metabolic activation. Members felt that the evaluation of the results for the Roche proprietary compounds was important to deriving the overall estimate of sensitivity in this study. In answer to a question from the chair, Professor Walmsley reported that Gentronix did not have access to the identity of the Roche proprietary compounds. The secretariat was asked to obtain any information Roche were willing to provide on an in-confidence basis for COM members only.

2.38 The COM agreed that the GADD45a-GFP assay might be useful as a pre-screening tool similar to DEREK, but that it could not be used in a regulatory genotoxicity testing strategy at present. More data on the use of the GADD45a assay with metabolic activation and further analysis of the low sensitivity reported by the study by Olaharski *et al*., 2009 would be required before the committee could produce a statement on the use of this assay.

Ongoing Reviews

Thresholds for *in vivo* mutagens

2.39 The Committee considered a draft discussion paper on studies investigating thresholds in mutagenicity published since the COM 2001 statement. Members also heard a presentation from Dr Gareth Jenkins (University of Swansea) at the February 2009 meeting which included an outline of the various definitions used to describe thresholds for genotoxic effects. Dr Jenkins concluded that some genotoxins have demonstrated a threshold for mutagenicity both *in vitro* and *in vivo*. However, it is not possible to generalise to other chemicals that have not been tested to the same extent. There is a need to consider chemicals on a case-by-case basis and to have confidence in the mechanism for a threshold and the dataset. To date the evidence was most convincing for ethyl methanesulphonate (EMS).

2.40 The COM considered pre-publication studies undertaken by Roche to investigate the threshold for genotoxicity of EMS. In brief, during 2007, several thousand HIV patients had ingested Viracept (Nelfinavir mesylate) tablets as an HIV protease inhibitor, which contained relatively high levels of the impurity EMS. The available *in vitro* mutagenicity and toxicity data for EMS did not allow a full risk assessment of this incident to be undertaken. This led the manufacturer Roche, to undertake *in vivo* mutagenicity studies in mice (i.e.bone marrow (BM) micronucleus (MN), *lac*Z gene mutation in BM, liver and small intestine). Roche employed a novel statistical analysis of the data andundertook investigations to allow a risk assessment based on toxicokinetic data. Overall, the COM agreed that threshold had been demonstrated for EMS mutagenicity and that there was an adequate MOE between the NOEL for mutagenicity and the likely maximum exposures in patients who ingested the EMS contaminated Viracept tablets. Members briefly discussed the hypothetical argument of the one-hit hypothesis of mutagenicity and noted this still applied even though there was a great deal of redundancy in DNA.

2.41 The COM considered a draft guidance documents at the June 2009 and October 2009 meetings. It was agreed that the draft Guidance document on

the risk assessment of *in vivo* mutagens could be split into two sections i.e. a section on thresholds for mutagenicity and a section on the risk assessment of *in vivo* mutagens. This would be consistent with the proposal to produce guidance documents which could be rapidly updated when required.

Toxicogenomics

2.42 The COT/COC/COM intend to update their joint statement on toxicogenomics published in 2004. To contribute to this process a literature search had been conducted and studies most relevant to the COM was considered at the October 2008 and February 2009 meetings. This included a number of studies that for the first time had made comparisons between transcriptomics and proteomics for the same mutagen using identical culture conditions except that transcriptomics was measured 4h post exposure and proteomics 12h post exposure. Members noted that although there was some comparability between the two toxicogenomic approaches for both MNNG and BPDE regarding overall functions affected by treatment, there was very little comparability at the individual gene level. Overall, members considered that there was no evidence from these studies for a good correlation between transcriptomics and proteomic approaches. It was also noted that changes seen at the mRNA level did not necessarily mean there would be a change at the protein level and vice versa. An International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) trial of inter-laboratory variation in transcriptomic studies for genotoxicity has been published and should be considered during 2010.

Development of guidance documents on COM internet site

2.43 The structure of the proposed Guidance section of the COM website. Is under consideration and will be presented to the COM for comment in 2010. It is intended that the Guidance notes should be divided into areas that could be updated more quickly. It was suggested that the draft Guidance document on the risk assessment of *in vivo* mutagens could be split into two sections i.e. a section on thresholds for mutagenicity and a section on the risk assessment of *in vivo* mutagens.

2009 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

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Professor David Kirkland BSc (Hons), PhD (from 1 April 2009)
Principal, Kirkland Consulting (from 1 July 2009), until 1 July 2009, Vice President of Scientific and Regulatory Consulting, Covance Laboratories Europe

Dr David P Lovell BSc PhD CStat FSS CBiol FIBiol
Reader in Medical Statistics, Postgraduate Medical School, University of Surrey

Dr Anthony Lynch BSc (Joint Honours) PhD (from 1 April 2009)
Manager Investigative Studies & New Screening Technologies, Genetic Toxicology, GlaxoSmithKline

Dr Ian Mitchell BA PhD (upto 31 March 2009)
Consultant in Genetic and Molecular Toxicology, Kelvin Associates and Chilfrome Enterprises Ltd

Dr Elizabeth M Parry BSc DPhil
Part-time Senior Research Fellow, School of Medicine, 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 FBTS	Joint Scientific Secretary – Food Standards Agency
Dr L Hetherington BSc PhD	Scientific – Health Protection Agency
Mr S Robjohns BSc MSc	Scientific – Health Protection Agency
Ms S Kennedy	Administrative Secretary – Health Protection Agency

Declaration of COM members' interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P B Farmer (Chairman)	Santander Bradford & Bingley Foreign & Colonial Friends Provident Health Effects Institute Torotrak ILSI HESI	Shareholder Shareholder Shareholder Shareholder Research Committee Member Shareholder Committee Member	American Chemistry Council	Research support and conference attendance expenses.
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 J Clements (to 31 March 2009)	Covance	Salary Share Option Shareholder	NONE	NONE
Dr B M Elliott	Syngenta AstraZeneca	Salary Share Option Holder Shareholder	NONE	NONE
Dr D Gatehouse	Covance GlaxoSmithKline	Salary Independent Consultant Shareholder Pension Share Option Holder Shareholder	NONE	NONE

Mrs R Glazebrook	BT Group Lloyds TSB National Grid	Shareholder Shareholder Shareholder	NONE	NONE
Professor N J Gooderham (to 31 March 2009)	Banco Santander CENES Silence Therapeutics Hargreaves Lansdown Proctor & Gamble	Shareholder Shareholder Shareholder Shareholder Consultant	FSA GlaxoSmith Kline FEMA (USA)	Research contract CASE studentship Research support
Dr G Jenkins (from 1 April 2009)	NONE	NONE	Hoffman-LaRoche Hoffman-LaRoche Unilever CEFIC/ECE TOC	Research Grant 2008 – 2010 Consultancy 2008 Research Grant 2008 - 2010 Honorarium 2008
Dr D Kirkland (from 1 April 2009)	Covance Covance Kirkland Consulting	Salary & Consultant (until June 2009) Stock Options Principal	NONE	NONE
Dr D P Lovell	National Grid Transco Pfizer	Shareholder Shareholder Share Options Pension	AstraZeneca National Grid Transco	Spouse Shareholder Spouse Shareholder
Dr A Lynch (from 1 April 2009)	GlaxoSmithKline	Salary Shareholder	NONE	NONE
Dr I Mitchell (to 31 March 2009)	Kelvin Associates IM Enterprises Chilfrome Enterprises GlaxoSmithKline	Associate Consultant Director/Creditor Director Pensioner Option and Shareholder	NONE	NONE

	Allergy Therapeutics BG Cadbury Schweppes GEC GSK ICH Mitchell & Butler Pfizer Real Good Food Renishaw Royal Dutch Shell RTZ Unilever Vedanta BP Centrica Green King Scottish & Southern	Consultant Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder PEP Holder PEP Holder PEP Holder PEP Holder		
Dr E M Parry	Invesco Fleming Legal & General Quintiles	PEP Holder PEP Holder PEP Holder Consultancy	NONE	NONE
Prof D H Phillips	Aviva Banco Santander BG Group Bradford & Bingley Centrica National Grid ECETOC Servier Butler Jeffries (solicitors)	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Honorarium Honorarium Honorarium	NONE	NONE