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 2011, the Committee completed a strategy for testing and assessment for chemicals. This was a major task which involved a world-wide consultation with interested groups. The finalised strategy was published in the new Guidance series of statements on the COM Internet site. The COM also completed guidance for the assessment and testing of chemicals with inadequate genotoxicity data. The Committee completed a further review of genotoxicity data on Fumagillin Dicyclohexylamine.

The Committee has a number of ongoing generic reviews including the use of Quantitative Structure Activity Relationship (QSAR) approaches to mutagenicity evaluation, the genotoxicity testing and assessment of impurities, and a guidance document on the human health significance of mutagenicity. The Committee also considered the genotoxicity of nanomaterials and chlorophenols.

There was insufficient time to discuss the 2011 Horizon scanning paper which will be considered at the March 2012 meeting.

Professor P B Farmer Chair
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COM evaluations

Fumagillin Dicyclohexylamine

2.1 Fumagillin dicyclohexylamine (fumagillin DCHA) is an antibiotic 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 1). 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). The veterinary medicine Marketing Authorisation Holder (MAH) is CEVA Animal Health.

2.2 The COM recommended the following testing strategy for fumagillin DCHA in 2009.

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

2.3 The data holder submitted two additional studies:

i) Fumagillin DCHA was tested for its ability to induce chromosome aberrations in the bone marrow of male Crl:CD-1(ICR) mice when administered orally by gavage at 25, 50 and 75 mg/kg bw/day in a dose volume of 10ml/kg water-sugar syrup solution over 7 days.

ii) Fumagillin DCHA (suspended in a sugar/water 1:1 syrup) was administered orally to groups of 5-7 male and female OF1 mice on 8 consecutive days at dose levels of 25 mg/kg bw/day, 50 mg/kg bw/day and 75 mg/kg bw/day in the main study. The animals were killed 3-6 hours after the final dose and bone marrow isolated for micronucleus determination in polychromatic erythrocytes (male and females) and stomach for comet assay (males).

2.4 The COM reached the following conclusions;

i) The newly submitted bone marrow chromosome aberrations and micronucleus tests in mice adequately addressed the data requests

agreed by COM in 2009, and reported negative results. There were limitations in the conduct of the in vivo Comet assay in mice; overall a negative result was reported.

ii) Fumagillin DCHA should be considered as an *in vitro* mutagen.

iii) The weight of evidence available to the COM supports the conclusion that fumagillin DCHA is not an *in vivo* mutagen.

2.5 The full guidance statement can be found at http://www.iacom.org.uk/statements/documents/fumagillinfinalstatement2011_002.pdf

Guidance on a Strategy for Genotoxicity Testing of Chemical Substances

2.6 The COM has a remit to provide UK Government Departments and Agencies with advice on the most suitable approaches to testing chemical substances for genotoxicity. The COM published guidance in 1981, 1989 and again in 2000. A further review was completed during 2011 and the published strategy represents the most appropriate at the time of publication.

2.7 The COM recommended a staged approach to testing:

Stage 0 consists of preliminary considerations which include physico-chemical properties of the test chemical substance, Structure Activity Relationships (SAR), and information from screening tests. However, data from SAR and screening tests should not overrule test data from adequately designed and conducted genotoxicity tests.

Stage 1 consists of *in vitro* genotoxicity tests. The COM recommends a core-test battery of the Ames test combined with the *in vitro* micronucleus test. This combination provides information on three types of genetic damage for which data are required (namely, gene mutation, chromosomal damage and aneuploidy) and gives appropriate sensitivity to detect chemical mutagens. There is no need to independently replicate adequately designed and conducted core *in vitro* tests which are either clearly negative or clearly positive. The strategy document also considers the value which can be attributed to a number of non-core *in vitro* tests.

Stage 2 consists of *in vivo* genotoxicity tests. A case-by-case strategy should be developed to answer one or more of the following specific queries;

1) Investigation of mutagenic end point(s) identified in Stage 1,
2) Investigation of genotoxicity in tumour target tissue(s),
3) Investigation of potential for germ cell genotoxicity,
4) Investigation of *in vivo* mutagenicity for chemicals, which were negative in Stage 1 but where there is high or moderate and prolonged exposure,
5) Investigation of genotoxicity in site of contact tissues.

2.8 The core tests in Stage 2 are the rodent micronucleus/chromosome aberration assays for aneuploidy and clastogenicity, the transgenic rodent gene mutation assay and the rodent Comet assay for DNA damage. Usually negative results obtained in a carefully selected *in vivo* test (possibly studying more than one endpoint and tissue) will be sufficient to address positive results found *in vitro.* However, a further test(s) may be needed if some of the genotoxic effects seen in Stage 1 *in vitro* tests had not been adequately studied *in vivo* (e.g. the chemical affects multiple mutagenic end-points), or other aspects of the genotoxic potential of the chemical had not been fully resolved (e.g. in the case where an investigation of heritable effects was required). The strategy document also considers the value which can be attributed to a number of non-core *in vivo* tests. In most instances information from core *in vivo* tests is sufficient to evaluate the *in vivo* mutagenicity of chemical substances. A supplementary *in vivo* test strategy can provide additional information on a case-by-case basis, to investigate aspects such as further characterisation of germ cell genotoxicity, and DNA adduct data which can provide information to elucidate the mode of genotoxic action of carcinogenic chemicals.

2.9 It is acknowledged that the field of genotoxicology and genotoxicity testing is rapidly developing. A short overview of possible future developments and techniques such as toxicogenomics was provided.

2.10 The full guidance statement can be found at http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL2.pdf

Guidance on Mutagenic Hazard Assessment and a Strategy for Genotoxicity Testing of Chemicals with Inadequate Genotoxicity Data

2.11 The purpose of the COM guidance was twofold: firstly, to provide guidance on deriving preliminary conclusions on mutagenic hazard in the absence of full genotoxicity data, and secondly to provide a framework which allows the integration of existing genotoxicity data on a chemical substance with a testing strategy designed to provide data sufficient for mutagenic hazard assessment to be completed, in compliance with the COM testing strategy (see paragraph 2.7 above). This guidance should therefore be read in conjunction with the published COM guidance on a strategy for genotoxicity testing.

2.12 The preliminary hazard assessment consists of a comprehensive literature search to identify available data relevant to genotoxicity, evaluation of the quality of the data and consideration of the completeness of the overall database. The assessment can include data from both core and non-core genotoxicity tests. (A listing of core and non-core tests is provided in the guidance statement). It is important to note that a case-by-case approach is needed using expert judgement to reach conclusions on mutagenic hazard assessment and on a testing strategy to complete the assessment. The COM agreed that genotoxicity tests not included in the guidance statement were not recommended for mutagenic hazard assessment, and considered that little or no weight of evidence should be attached to tests.

2.13 A stepwise approach to genotoxicity data assessment is presented in the guidance statement. Devising a strategy for genotoxicity testing of chemicals with inadequate genotoxicity data commences with the preliminary hazard assessment. If the available evidence is insufficient to reach conclusions on mutagenic hazard, identify key data gaps, taking into account the purpose of the evaluation, and derive a plan for each stage of the COM testing strategy as appropriate (see paragraph 2.7). This may include repeating specific genotoxicity tests from each stage of the COM testing strategy and/or undertaking additional studies from Stages 1 and 2 as appropriate.

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

Ongoing Work

Use of Quantitative Structure Activity Relationships (QSARs) for Mutagenicity

2.15 The COM considered a preliminary assessment of drinking water chemicals using CAESAR and Toxtree. The Committee were unable to reach definite conclusions based on the information provided. A further paper was submitted outlining more information on these two (Q)SAR models. Members agreed that where negative predictions were made, this would provide some reassurance regarding the absence of mutagenic potential. It was also noted that Toxtree, being a rule based system, gave the reasons for its predictions. CAESAR gave the results for six other structural analogues, so some confidence could be obtained in positive predictions if the results for analogues were considered reasonable. Overall the committee agreed that it would be useful for the COM to hear a presentation from a computational toxicologist with expertise in QSARs. A presentation is due to be heard at the March 2012 COM meeting.

Genotoxicity Testing of Impurities

2.16 The Committee considered a draft guidance statement at the March, June and October 2011 meetings. A further draft paper is due for consideration at the March 2012 meeting.

Human Health Significance of Chemical Induced Mutagenicity

2.17 The Committee considered a draft guidance statement at the March 2011 meeting. The document was considered to be a good draft with the target audience being the informed lay reader. There was need for inclusion of figures to explain the involvement of mutation in disease processes and hyperlinks to

explanations of terms used in the document. A revised draft is due for consideration at the March 2012 meeting.

Genotoxicity of Nanomaterials

2.18 A detailed review paper was considered at the October 2011 meeting. A statement is currently being drafted.

Chlorophenols

2.19 A detailed review paper was considered at the October 2011 meeting. A statement is currently being drafted

2011 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

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Dr D Benford BSc PhD	Joint Scientific Secretary – Food Standards Agency
Dr L Hetherington BSc PhD	Scientific – Health Protection Agency
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Ms S Kennedy	Administrative Secretary – Health Protection Agency

Declaration of COM members' interests during (2011) 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 Torotrak 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 AstraZeneca Diageo HBOS Marks & Spencer	Consultant Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Dr B M Elliott	Syngenta AstraZeneca Elliott GT Ltd Regulatory Science Associates	Pension 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 Hoffman-LaRoche Unilever CEFIC/ECETOC	Research Grant 2008 – 2010 Consultancy 2008 Research Grant 2008 - 2010 Honorarium 2008
Dr D Kirkland	Kirkland Consulting	Principal	NONE	NONE

Dr D P Lovell	National Grid plc	Shareholder	AstraZeneca National Grid plc	Spouse Shareholder Spouse 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 Takeda	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Consultant		