

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

OVERVIEW OF CHEMICAL SUBSTANCES PREVIOUSLY CONSIDERED BY COM 1998-2011

The following tabulation presents the key aspects of COM evaluations of chemical substances (excluding tobacco and metal wear debris from replacement hips). It is hoped these data will be informative for the COM in considering the most appropriate strategy for testing and assessing chemical substances with existing limited and/or inadequate data. It is not intended for COM to review these individual chemicals or to suggest further testing strategies for these compounds. The statements were reviewed to consider whether a tiered approach to assessment was used (i.e. consideration of Stage 0, then Stage 1 and finally Stage 2 data) and the strategy adopted for further testing and assessment of these compounds.

In general, the COM has sought to ensure adequate Stage 1 *in vitro* data (using all available data) before deriving conclusions on mutagenic potential. The strategy used for *in vivo* studies has changed over the period of this report moving away from specifying two *in vivo* assays (BMMN and rat liver UDS) towards a case-by case approach. Negative well conducted Stage 1 test results can overrule inconsistent Stage 2 data. However where there is evidence for mutagenicity *in vitro* and where Stage 2 data are inadequate, further Stage 2 testing is required. In some instances the appropriate carcinogenicity data has been used to provide reassurance regarding lack of *in vivo* genotoxicity.

In some instances potential, theoretical metabolism to genotoxic metabolites used to inform on strategy to be used.

| Chemical and date of evaluation | Key aspects | Comments |
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| Aclonifen (2008) | Data rich pesticide. Key question related to potential metabolism to genotoxic metabolite | COM evaluation focused on potential metabolism of Aclonifen and other diphenyl-ether compounds. |
| Acrylamide (2009) | Data rich chemical. Key question related to mutagenic hazard assessment of acrylamide and its metabolite glycidamide, the MoGAs for observed genotoxic effects, dose-response analysis and consideration of potential for threshold for mutagenicity | COM adopted a sequential approach considering a range of Stage 1 tests and MoGA data, and Stage 2 tests including germ cell tests and dose-response analysis. |
| Benzimidazoles (2007) | Extent of genotoxicity data for individual benzimidazoles was variable. | Tiered approach to assessment of data. COM attempted to rank potency where possible. Focus of evaluation was to consider |

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| | | the establishment of data needed to include benzimidazoles in a common mechanism group. |
| 2-Chlorobenzylidene malonitrile (CS and CS spray) (1999) | Negative Ames, Positive direct effect in V79 for gene mutation, MLA. Positive clastogen V79 and CHO. Evidence for aneugenicity <i>in vitro</i> also reported. Negative BMMN and peripheral blood MN. No data to show exposure of bone marrow. Considered for reassurance that data for site of contact required. Negative inhalation carcinogenicity bioassay considered sufficient data. | Sufficient from core and non core Stage 1 tests to assess <i>in vitro</i> mutagenicity. Inadequate Stage 2 data but results of inhalation carcinogenicity bioassays in rats and mice sufficient to provide reassurance. |
| Chromium Picolinate (2004) | Evidence suggested oxidative damage to cells. Conflicting <i>in vitro</i> mutagenicity data. Relevance of technical specification of test material possibly important. Positive <i>hprt</i> in CHO cells –S-9 (Uncertainty of test material purity). <i>In vivo</i> tests negative | Repeat testing needed to derive overall conclusion on <i>in vitro</i> mutagenicity. Repeat test using commercial grade material was negative. |
| Coumarin (1998) | Positive ST 100 +S-9 (rat and hamster S-9). Positive CA in CHO cells +S-9. Negative UDS human liver slices. Considered positive <i>in vitro</i> mutagen. Negative peripheral blood MN in rats (90d study) (limited to normochromatic erythrocytes?). COM recommended <i>in vivo</i> package Rodent BMMN and rat liver UDS. | Sufficient data to assess Stage 1. Inadequate Stage2 data. COM recommended default package of <i>in vivo</i> tests. |
| 1,3 Dichloropropan-2-ol (1,3,DCP) (2003) | Positive Ames TA1535/TA100 +/-S-9. Positive CAS mammalian cells. Positive MLA (2001) Negative <i>in vivo</i> studies rat BMMN and rat liver UDS submitted in 2003. | COM considered postulated genotoxic metabolites from 1,3DCP including epichlorohydrin, 1,3 dichloroacetone and 1,3 monochloropropane-1,2-diol but overall reassurance from Stage 2 studies was available. |
| 2,3 Dichloropropan-1-ol (2001,2004) | COM noted theoretical metabolism to epichlorohydrin and glycidol. Positive Ames TA1535/100 +/-S-9 (negative SCE noted). No <i>in vivo</i> genotoxicity tests. Negative <i>in vivo</i> studies rat BMMN and rat liver UDS submitted in 2004. | Stage 2 studies considered sufficient reassurance. |
| Dichlorvos (2002) | Data rich pesticide. <i>In vitro</i> | Tiered approach to |

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| | <p>direct acting positive in Ames test, mammalian cells, yeast. Negative in systemic BMMN tests. Positive in skin MN and liver (i.p. dosing) transgenic assay, suggesting site of contact mutagenicity</p> | <p>mutagenic hazard assessment used. COC noted limited evidence of carcinogenicity in oesophagus and forestomach (mouse).</p> |
| <p>Di-isopropyl naphthalene (2000)</p> | <p>Specific review of MLA. Negative in Ames (including <i>E.coli</i>). Inadequate CA in CHO and negative in mouse MN test. Equivocal in MLA, no conclusion reached. Repeat test requested.</p> | <p>Repeat test reported equivocal results. WoE assessment reached. Since compound had no structural alerts, no evidence from other <i>in vitro</i> tests and an adequate negative BMMN, no further data were requested.</p> |
| <p>Dimetrizadole (2002)</p> | <p>Positive in bacteria (TA 100) , including nitroreductase proficient bacteria. Positive in <i>Saccharomyces</i> D4. Inconsistent/uninterpretable MLA. Cytogenetics, and UDS <i>in vitro</i> could not be traced. Positive in Comet in human lymphocytes. Negative in <i>Drosophila</i> (no weight attached). Negative in adequate BMMN test in mice. Negative in rat liver UDS assay but study considered inadequate. Negative in DL in mice. Further rat liver UDS assay requested. Carcinogenicity data assessed. Mammary tumours but no conclusion on mechanism reached. Overall carcinogenicity data did not provide reassurance re genotoxicity.</p> | <p>COM requested a further <i>in vivo</i> rat liver UDS assay to complete Stage 2 testing.</p> |
| <p>Etaboxam (2007, 2 statements)</p> | <p>Data rich pesticide. COM asked for advice on a strategy to assess aneugenicity. Evidence for inhibition of mitosis seen in CA test. Small increase in BMMN in mice. Additional study showed induction of MN in PBLs COM requested further testing for <i>in vitro</i> MN with specialist staining for aneugenicity, investigation of dose-response and a further BMMN study (using i.p. administration) (Considered impractical to undertake site of contact aneugenicity) <i>In vitro</i> MN tests showed</p> | <p>Further testing designed to confirm results of initial <i>in vitro</i> and <i>in vivo</i> tests.</p> |

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| | NOEL for mutagenicity was 2-4 ug/ml. A small increase in BMMN seen at toxic doses in repeat test. COM could not discount a direct aneugenic effect. | |
| Flunixin meglumine (2003, 2005) | <p>Flunixin; limited evidence for mutagenicity in vitro. Negative in vivo but considered inadequate. For Flunixin meglumine salt, negative in old Ames, positive gene conversion <i>S.cerevisiae</i> Positive <i>E.coli</i> (repair deficient strain), limited evidence for mutagenicity in MLA, equivocal cytogenetics in CHO. Negative i.p BMMN in mice but concerns over adequacy of dose selection. Meglumine Negative in old Ames test. Inconsistent results in BMMN mouse tests.</p> <p>Negative carcinogenicity in mouse and rat with Flunixin meglumine considered informative of r Flunixin but not meglumine.</p> | <p>COM recommended Stage 1 tests for meglumine. Cas and MLA. Negative tests were submitted in 2005. Com concluded no need for further testing.</p> <p>Thus completion of an adequate Stage 1 strategy can overcome inconsistent results seen in Stage 2 test.</p> |
| Formaldehyde (2007) | Review focused on evidence for systemic mutagenicity. Direct acting in vitro mutagen in many test system. Secondary mechanism thought to be responsible for DL effects and comet assay results reported. Secondary mechanism for genotoxic effects seen in biomonitoring studies of peripheral blood lymphocytes. (Formaldehyde a normal intermediary metabolite and expected to | COM concluded that for occupational and environmental exposure o formaldehyde, the pattern of metabolism and distribution of formaldehyde indicated a threshold for <i>in vivo</i> systemic mutagenicity is likely. |
| Fumagillin Dicyclohexylamine (2009, 2011) | Data rich veterinary medicine. <i>In vitro</i> clastogen in mammalian cells. COM evaluation focussed on repeating the <i>in vivo</i> study where positive bone marrow clastogenicity had been reported. Strategy to include repeating BM CA, and in addition BMMN and stomach comet assay and toxicokinetic data on systemic exposure. | Repeat of critical part of Stae 2 strategy and further testing to provide reassurance. Negative results submitted 2011. |
| Halonitromethanes (2005) | In vitro Comet in CHO cells positive for a range of | Negative data for transgenic fish not considered relevant. |

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| | <p>halonitromethanes (HNMs) (rank order established). In vitro positive for a range of HNMs (including strains expressing glutathione transferase theta) but rank order was not established. COM agreed both direct activity and metabolic activation occurred. COM agreed in vitro mutagens and thus potential in vivo mutagens. COM agreed Stage 2 strategy of rat liver UDS followed by site of contact comet assay.</p> | <p>Negative BMMN for one HNM (trichloronitromethane)</p> |
| <p>Hydroquinone and Phenol (2000) and Phenol (2008)</p> | <p>In 2000, Hydroquinone and phenol considered to be in vivo somatic cell mutagens (BMMN in mice) but risk from oral ingestion considered much reduced by rapid detoxification and rapid conjugation. Insufficient evidence to support threshold for risk assessment for inhalation/dermal exposure. In 2008 Further data to show in vivo somatic cell mutation with phenol (BMMN Mice). Concurrent hypothermia reported, but COM considered evidence insufficient to move away from non-threshold approach to RA.</p> | <p>Convincing data for MoGA being threshold related not provided.</p> |
| <p>Malathion (2003)</p> | <p>Data rish pesticide. Malathion was mutagenic <i>in vitro</i> due to malathion, its metabolites and impurities. N evidence for technical grade malathion in bacteria but positive +/-S-9 in mammalian cell cytogenetics and positive hprt in lymphocytes. Positive in MLA +/-S-9. Negative in vitro UDS but dose level was cytotoxic (and assessment of impurities was therefore not possible). The metabolite malaoxon was mutagenic in MLA.</p> <p>A lot of in vivo published studies reported positive results but COM could not reach conclusions as purity and specification of test material was not provided and it was known that impurities influence in vitro</p> | <p>COM agreed a repeat of the dermal <i>in-vivo</i> MN assay in rats should be undertaken. Noted all studies in rats were negative and positive findings were only reported in mice.</p> <p>Company argued rat liver US was negative, but COM felt a repeat study should be undertaken.</p> <p>COC agreed that nasal tumours in rats were due to a non-genotoxic mechanism.</p> |

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| | <p>mutagenic activity. Limited evidence for CAs in bone marrow in mice using technical material synthesised in India. Overall COM considered that lack of carcinogenicity seen in mice overruled this result. A positive MN by dermal application in mice (technical grade manufactured in Argentina) was reported. Positive results by i.p. dosing in a number of studies. Negative oral rat liver UDS reported but COM felt response in two animals indicated a positive response.</p> | |
| Malachite Green (1999, 2004) | <p>In 1999 positive in TA 98 +S-9, some evidence for clastogenicity in CHL cells considered an <i>in vitro</i> mutagen. Poor rodent BMMN. Recent ³²P-postlabelling indicated DNA adducts in liver. COM concluded prudent to assume MG is an <i>in vivo</i> mutagen. Update in 2004, more evidence for mutagenicity in bacteria. Further evidence for DNA adducts <i>in vivo</i>. Negative MN in peripheral blood lymphocytes and <i>hprt</i> in spleen, but COM considered studies suboptimal. Conclusion MG reaffirmed.</p> | <p>NTP carcinogenicity bioassay with MG, considered equivocal evidence for liver tumours in F344 rats, negative in mice.</p> |
| Leucomalachite green (1999, 2004) (metabolite of malachite green) | <p>In 1999 no adequate Stage 1 data. Evidence for ³²P-postlabelling indicated LMG may also be an <i>in vivo</i> mutagen. In 2004 negative results in limited <i>Salmonella</i>, CHO <i>hprt</i> and Comet assays. COM concluded <i>in vitro</i> studies too poor to reach conclusions. Positive transgenic mutation (<i>cII</i>) in rat liver. LMG considered an <i>in vivo</i> mutagen.</p> | <p>NTP bioassays positive for liver tumours in mice and rats.</p> <p>COC considered not possible to exclude genotoxic mechanism for liver tumours and therefore prudent to consider LMG a genotoxic carcinogen.</p> |
| 3-Monochloropropane,1,2-diol (3MCPD) (1999 and 2000) | <p>3MCPD positive in bacteria – S-9. Positive in yeast. Negative in BMMN but no evidence of tissue exposure. No conclusions could be reached on colonic MN and DL assays. COM asked for</p> | <p>In 1999 COC noted evidence for Leydig cell adenomas and mammary gland fibroadenomas in male rats and kidney tumours in female rats. COC considered tumours non-genotoxic.</p> |

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| | <p>second tissue rat liver UDS. In 2000 rat BMMN and rat liver UDS submitted considered adequate and negative.</p> | <p>COM noted theoretical metabolised to glycidol. ADME data did not indicate glycidol formation. Negative results for two Stage 2 tests considered sufficient.</p> |
| Ozone (1999) | <p>COC noted <i>k-ras</i> mutations in lung neoplasms from ozone treated mice and asked for COM advice.</p> <p>COM concluded ozone reacts with most biological macromolecules including DNA. COM concluded positive in TA 102 +/-S-9. No adequate clastogenicity studies in mammalian cells but SCEs reported. Ozone considered to have <i>in vitro</i> mutagenic potential. Negative in limited bone marrow/peripheral blood in hamsters, mice, and rats. Negative limited germ cell assay.</p> <p>In addition to <i>k-ras</i> mutational spectra, including A-T transversions in codon 61, which appeared specific to ozone. Prudent to assume ozone has <i>in vivo</i> mutagenic potential.</p> | <p>Com considered additional work on A-T transversion <i>in vitro</i> and mutation signatures of inert particles such TiO₂ in mouse lung tumours</p> <p>COM considered default was to assume no threshold.</p> |
| Para-chloroaniline (2009) | <p>Positive TA98 +S-9. Evidence DNA damage PolA+/PolA-. Data from yeast considered of limited value. Positive CA +/-S-9 CHO cells. Positive MLA +/-S-9 (but assays did not meet GEF standard). Negative DNA strand breaks in MLA cells. Inconsistent results for UDS in rat hepatocytes. COM concluded <i>in vitro</i> mutagen. <i>In vivo</i> positive in <i>Drosophila</i> not considered relevant. No evidence DNA binding (radiolabel) but significant binding to haemoglobin reported. Positive <i>in vivo</i> Comet (Sasaki) but no weight attached to this study. Positive BMMN using regime that may have induced significant toxicity and biological significance uncertain. COM asked for the following Stage 2 strategy. 1) repeat</p> | <p>Para-chloroaniline was carcinogenic in rats inducing splenic sarcoma, osteosarcoma. Some evidence for liver tumours in male mice. Haemangiosarcoma induced in rats and mice (in spleen/liver).</p> |

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| | bone marrow and peripheral blood MN in mice. (If negative, then) 2) rat liver UDS with concurrent rat comet assay in spleen, liver and one other tissue (not considered to be a rat tumour target organ). | |
| Phosphine (2001) | Update of 1997 statement. Phosphine some limited evidence to suggest <i>in vivo</i> mutagen. Phosphine negative in bacteria, but positive for CAs and in MLA. Elevated levels of chromosome damage in biomonitoring studies of grain workers. Overall negative in bone marrow/peripheral blood assays for clastogenicity. Negative in rat liver UDS and in a DL assay in mice (limited quality). COM recommended further biomonitoring under UK use conditions. Further data submitted in 2001. Negative inhalation carcinogenicity bioassay in rats. Overall COM reassured that phosphine (a highly reactive compound inducing oxidative DNA damage). | COM conclusion in 2001 was not with regard to the mutagenic hazard assessment of phosphine but a further consideration as to whether biomonitoring under UK conditions was required. Overall COM agreed that taking all data into account and the very low potential exposures under UK conditions that a biomonitoring study was no longer necessary. |
| Proquinazid (2005) | ACP considering mechanism for cholangiocarcinoma seen with this data rich pesticide. Ames test adequate and negative. COM asked about adequacy and conduct of <i>in vitro</i> CA and <i>hprt</i> gene mutation assays in mammalian cells. Negative in two bone marrow MN assay in mice. COM agreed extended exposure CA assay and MLA (<i>hprt</i> considered insensitive). | COM strategy was that adequate Stage 1 was required even though negative bone marrow MN assays. Company undertook MLA assay which was considered adequate and negative. COM agreed that extended exposure CA not now necessary. |
| Terephthalic acid (2001, 2007) | 2001. negative <i>in vitro</i> <i>S.typhimurium</i> . negative <i>in vivo</i> BMMN in mice (no data on exposure, but signs of toxicity reported). COM considered <i>in vitro</i> CA was required. Data submitted in 2007 included negative <i>in vitro</i> CA | COM considered bladder tumours seen a rat carcinogenicity were induced via a non-genotoxic mechanism. |

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| | and negative rat liver UDS | |
| 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD (1987 conclusions updated 1999) | COM considered largely negative results in a range of tests for DNA damage, gene mutations, SCEs and cell transformation. Positive/equivocal results seen when assays were either non-standard or suboptimal. COM recommended repeat of a MN study in human lymphocytes (CBMN assay) as positive result had been obtained in a non-standard assay which needed repetition. | IARC conclusion 1997, TCDD category 1 human carcinogen. TCDD considered a non-genotoxic substance. |