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

Preface

The Committee on Mutagenicity provides advice on the 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 the expertise of the independent committee members is required to provide recommendations on potential hazards and risks and frequently suggestions for further studies.

During 2000, advice was provided on a diverse range of chemicals including food packaging contaminants, industrial chemicals and alcoholic beverages. Amongst the most complex evaluations of mutagenic potential are estimates of how conclusions may be extrapolated from *in vitro* to *in vivo* tests, and from somatic to germ cells. As part of an ongoing development of expertise in such extrapolations, the committee published statements on data extrapolation from somatic to germ cells for chemicals which disturb the fidelity of chromosome segregation which may thus induce aneuploidy.

The year 2000 saw the completion of a major committee project; the preparation of a guidance document on a strategy for the testing of chemicals for mutagenicity. This document provides guidance on both test method selection and their appropriate use in the assessment of the potential mutagenic activity of chemicals. The updated guidance provides advice on the application of a range of methods (such as the use of transgenic animals) that have been developed over the past 10 years and highlighted the importance of measuring the potential of aneuploidy induction by chemicals in mutagenic screening programmes. The development of the guidance document involved extensive discussions and meetings with a wide range of interested parties, including the Industrial Genotoxicology Group, the UK and the European Environmental Mutagen Societies. The completed guidance document is being widely distributed and current indications are that the strategy recommended will have a major influence on future European policy on chemical testing.

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Hydroquinone and phenol

2.1 At the request of HSE the Committee had provided advice on the interpretation of mutagenicity data on hydroquinone and phenol in 1994 and 1995. The COM had agreed that both hydroquinone and phenol should be regarded as somatic cell *in-vivo* mutagens. The Committee had been persuaded for these two compounds that by the oral route there was a potential for a threshold of activity. This conclusion was based on good evidence of protective mechanisms (namely rapid conjugation and detoxification via the glutathione pathway) that would substantially reduce systemic exposure to any active metabolites formed. However, members agreed that there was insufficient data regarding activity following inhalation and dermal exposures and it was not possible to assume a threshold existed for mutagenic activity when exposure was via the respiratory tract or via the skin. The Committee reviewed some additional data provided by industry in 1995 on the metabolism of hydroquinone and phenol in animals and humans. These data were useful but did not allow for an assessment of pre-systemic metabolism following either inhalation or dermal exposure. The Committee recommended that further studies were required which should include early sampling for free and conjugated hydroquinone or phenol in blood following administration of test substances to rats or dogs via a bronchoscope.

2.2 In 1999, further published data was provided to the Committee on the kinetics of hydroquinone metabolism in rats following intratracheal instillation and its percutaneous absorption, together with some *in-vitro* studies using rat skin and human stratum corneum. A number of additional *in-vivo* mutagenicity studies including an investigation of site of contact mutagenicity in skin and respiratory tract of Muta^{TM} mice using the LacZ transgene were also considered. The Committee considered these new data during 1999 and agreed a statement which was published on the COM Website in January 2000.

2.3 In summary the new toxicokinetic study involved giving rats a single intra-tracheal dose of ^{14}C-hydroquinone. Free hydroquinone in arterial blood was detected 5-10 seconds after dosing. This suggested a potential hazard of site-of- contact and systemic mutagenic effects following exposure by inhalation to hydroquinone. The Committee thus reaffirmed its previous conclusions on hydroquinone (and phenol) which are given in full in the statement at the end of this report.

3-Monochloropropane-1,2-diol (3-MCPD)

2.4 3-Monochloropropane-1,2-diol (3-MCPD) is a member of a group of chemicals present as contaminants known as chloropropanols. 3-MCPD can be present as a contaminant in epichlorhydrin/amine copolymers used as flocculants or coagulant aids in water treatment. These polyamine flocculants have been available for many years as approved products for use in water treatment and thus 3-MCPD may be present in drinking water arising from their use. The Committee was aware that 3-MCPD had been detected as a contaminant of several foods and food ingredients, including acid hydrolysed vegetable protein (acid-HVP). The COC was asked to evaluate and advise on the carcinogenicity of 3-MCPD by the Committee on Chemicals and Materials of Construction for use in Public Water Supply and Swimming Pools (CCM), a statutory committee which provides advice to the Secretary of State for the Environment, Transport and Regions on the approval of chemical substances in contact with public water supplies.

2.5 The COM had reviewed the available mutagenicity data on 3-MCPD in 1999 which suggested that 3-MCPD had mutagenic activity *in-vitro*. The Committee agreed that further negative results in an *in-vivo* mutagenicity test in a second tissue namely rat liver UDS were required in order to provide adequate reassurance that the activity seen *in-vitro* is not expressed *in vivo*. The Committee considered at its October 2000 meeting two new *in vivo* mutagenicity studies commissioned by the UK Drinking Water Inspectorate. These comprised a rat bone-marrow micronucleus test and a rat liver UDS assay, both of which are widely used to assess genotoxicity *in-vivo*.

2.6 The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to acceptable standards and were negative. Thus, the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in-vitro* was not expressed *in-vivo* had now been provided. The Committee agreed that ß-chlorolactic acid was the major urinary metabolite in rats formed by the oxidation of 3-MCPD and that the two new mutagenicity studies supported the view that reactive metabolites if formed did not produce genotoxicity *in-vivo* in the tissues assessed.

2.7 The Committee concluded that 3-MCPD can be regarded as having no significant genotoxic potential *in-vivo*. A copy of the revised COM statement on 3-MCPD can be found at the end of this report.

Mutagenicity of ethanol, acetaldehyde and alcoholic beverages

2.8 In 1995 the COM gave detailed consideration to the potential mutagenicity of ethanol, acetaldehyde and alcoholic beverages. This was to provide input to the Interdepartmental Working Group reviewing current advice on this topic. The Committee on Carcinogenicity (COC) also carried out a detailed review of the available data, mainly from epidemiology studies, on the carcinogenicity of alcoholic beverages. The advice from these Committees was considered by the Interdepartmental Working Group when drawing up their Report on Sensible Drinking published in December 1995. The main conclusions reached by COC and COM at that time were:

i) The COM agreed that the consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential.

ii) The COC concluded that the epidemiological evidence supported the view that drinking alcohol causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and for cancers of the oral cavity, pharynx, larynx and oesophagus.

2.9 The COM was asked to update its statement on the mutagenicity of ethanol, acetaldehyde and alcoholic beverages by the COC in order to provide additional information as part of a review of the evidence on the association between drinking alcohol and breast cancer.

2.10 The Committee reaffirmed its 1995 conclusion that consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential. A copy of the statement published on the COM Website can be found at the end of this report.

Di-isopropylnaphthalene(s) in food packaging made from recycled paper and board: Conclusion on mutagenicity studies using the mouse lymphoma assay (MLA)

2.11 The Committee was asked by the COT to provide advice on the conduct and interpretation of *in-vitro* mutagenicity tests with di-isopropylnaphthalenes using the mouse lymphoma assay (MLA). The COM reviewed two separate tests, one at its May 1999 meeting and a further test at its February 2000 meeting. The COM concluded that the results of the two mouse lymphoma

assays were similar. The evidence suggested equivocal mutagenicity in the mouse lymphoma assay, therefore no conclusion based on the MLA studies could be drawn. A more detailed summary of the results from these two tests has been published on the COM Website.

2.12 However, the Committee noted that di-isopropylnaphthalene(s) contained no chemical groups which would be structurally alerting for potential mutagenicity. In addition there was no evidence for a mutagenic effect in other *in-vitro* mutagenicity tests or in an adequately performed *in-vivo* micronucleus assay in mice. The Committee agreed that no further mutagenicity testing was required.

Testing strategies and evaluation

2.13 The Committee completed a major piece of work during this year, namely the revision of its guidelines on an appropriate strategy for the testing of chemicals for mutagenicity. This involved contributions from members during 1999 whose terms of appointment ended in April 2000 and then from members of the new committee. The Committee's deliberations concentrated on the strategy itself, and members did not undertake any updating of the other aspects covered in the earlier guidelines. A draft document was issued for public consultation in March 2000. This was discussed at a meeting of the UKEMS's Industrial Genotoxicity Group in May 2000. It was agreed that the final document should be referred to as "Guidance on a strategy for testing of chemicals for mutagenicity" to emphasise the advisory nature of these recommendations.

2.14 The Committee also provided advice to the Advisory Committee on Pesticides on the evaluation of chemically induced aneuploidy and in particular the extrapolation of data from somatic cells to germ cells. This latter piece of work involved a detailed consideration of the conclusions reached by the European Commission's Group of Specialised Experts who were considering the classification and labelling of benomyl, carbendazim and thiophanate-methyl under the Dangerous Substances Directive 67/548/EEC. A report outlining the Committee's consideration of each of these items is given below and the statement by the Committee on the extrapolation of data on chemical induced aneuploidy is given at the end of this report.

Guidance on a strategy for testing of chemicals for mutagenicity.

2.15 The COM first published guidelines for testing of chemicals for mutagenicity in 1981. These provided guidance to the relevant government departments/agencies on the state of the art approach to testing at that time. The need for these to be periodically updated, to reflect advances in development and validation of methods, was recognised and revised guidelines were published in 1989. The new guidance which has been published as a separate document and on the COM Website continues this updating process. The strategy outlined is believed to be the most appropriate with regard to available methods and recognising the need to avoid the use of live animals where practical and validated alternative methods where available. It is recognised that, as with earlier guidelines, it will be some time before this strategy is reflected in the mandatory, regulatory guidelines of the various agencies, and it is not intended for this guidance to be applied retrospectively. The Committee believes that the approach outlined will remain valid for several years and will encourage steps to obtain international recognition of the newer tests being recommended for which there are, currently, no international harmonised guidelines.

2.16 An outline of the overall strategy in the revised guidance is given below. It is not possible to adequately cover all of the issues covered in the revised guidance document and the reader is encouraged to obtain a copy from the secretariat or to view the document on the COM Website.

2.17 The strategy being recommended, as in the Committee's earlier guidance, is based on three progressive stages.

2.18 Stage 1 (initial screening) is based on *in-vitro* tests. For most chemicals three tests are recommended, but for those where little or no human exposure is expected (eg industrial intermediates, some low production volume industrial chemicals) two tests may be appropriate, namely a bacterial assay for gene mutation and an *in-vitro* mammalian cell assay for clastogenicity and aneugenicity. The Committee believes that screening for both clastogenicity and aneugenicity is now possible in the initial (Stage 1) tests. The second test may be metaphase analysis, with consideration of hyperdiploidy, polyploidy and effects on mitotic indices as indicators of possible aneugenicity; if these suggest potential aneugenicity this needs to be confirmed by use of appropriate staining procedures, such as FISH and chromosome painting. Alternatively an *in-vitro* micronucleus test may be used. If a positive result is obtained, kinetochore or centromeric staining should be employed to ascertain the nature of the micronuclei induced (ie whether induction is due to clastogenicity or aneugenicity). The third recommended assay is an

additional gene mutation assay in mammalian cells, the mouse lymphoma assay being the current best choice. The three assays, if negative, will provide sufficient information for the assessment of most chemicals. However where high, or moderate and prolonged, levels of exposure are expected (eg most human medicines) an *in-vivo* assay is recommended to provide additional reassurance regarding lack of mutagenic activity. Decisions on the extent of testing appropriate for given exposure levels of specific chemicals need to be taken by the relevant regulatory authority on a case-by-case basis.

2.19 Stage 2 involves an assessment of whether genotoxic activity seen in any of the *in vitro* tests can be expressed in somatic cells *in vivo*. In addition, one appropriate *in-vivo* test is needed for all chemicals (which are negative in *in-vitro* assays) for which human exposure is expected to be high, or moderate and prolonged. A flexible approach is needed with consideration of the nature of the chemical, its metabolism and results obtained in the initial *in-vitro* tests. The most appropriate initial test will be a bone marrow micronucleus assay unless the initial considerations give an indication to the contrary. Techniques for the assessment of whole chromosomes are appropriate if there is evidence of aneugenicity. If negative results are obtained in this assay additional testing in other tissue(s) will be required for all compounds that are positive *in-vitro*, to provide adequate reassurance for the absence of activity *in vivo*. The type of study (or studies) needs to be considered on a case-by-case basis having regard to the available information on the compound including the results from earlier tests. Studies that may be appropriate include liver UDS assay, comet assay, ^{32}P-postlabelling assay, covalent binding to DNA and assays using transgenic animals; the reasons for the choice of assay in a specific given situation should be justified.

2.20 Stage 3 consists of assays in germ cells. The need for such studies requires careful consideration. In most cases chemicals that are recognised as *in-vivo* somatic cell mutagens will be assumed to be both potential genotoxic carcinogens and potential germ cell mutagens, and no further genotoxicity testing is necessary. However, in some cases germ cell studies may need to be undertaken to demonstrate that a somatic cell mutagen is not a germ cell mutagen. Information on whether a compound is genotoxic in germ cells may be obtained from a number of assays (eg metaphase analysis in spermatogonia or micronuclei induction in spermatocytes, the dominant lethal assay and mutation assays in transgenic animals). Information on the induction of DNA lesions in germ cells may be obtained using the various approaches listed for phase 2. Consideration of the type of mutation produced in earlier studies is important when selecting the appropriate assay. None of these assays provide conclusive information as to whether effects will be seen in future generations, and the only methods on which risk

estimates for the effects can currently be based are the heritable translocation test and the mouse specific locus test. These are not practical options in view of the very large number of animals needed. Currently there are no routine methods available for investigating the induction of aneuploidy in offspring following exposure of parental animals.

Thresholds for aneugens: Extrapolation of data from somatic cells to germ cells.

2.21 The safety evaluation of aneuploidy inducing chemicals (aneugens) acting by inhibition of microtubule formation is based on the identification of a threshold dose or NOEL below which aneuploidy is not induced. Benomyl, carbendazim and thiophanate-methyl belong to the methyl benzimadazole carbamate (MBCs) class of chemicals. The MBC class of chemicals are widely used in approved pesticide products as fungicides and also in veterinary medicines including anthelmintics in both food producing and companion animals. These chemicals act by interfering with microtubule formation during mitosis. The Committee was asked by the Advisory Committee on Pesticides (ACP) to advise on the applicability of extrapolating to germ cells evidence for thresholds for induced aneuploidy obtained in studies on somatic cells, and the relevance of these conclusions for the approach used by PSD to evaluate aneuploidy data in the risk assessment of agricultural pesticides, specifically in respect of MBCs.

2.22 The Specialised Experts concluded that "..current knowledge does not allow extrapolation to meiotic cells of the *in-vitro* finding of a threshold [for induced aneuploidy in somatic cells]. Meiosis I is fundamentally different from mitosis in the structures and processes involved in chromosome segregation. Due to the current lack of knowledge on the interaction of aneugens with these possible targets, the concept of a threshold for induced aneuploidy in germ cells is as yet a hypothetical one." The Committee undertook a detailed review of a study published by de Stoppelaar JM, *et al*, (1999) Mutagenesis, **14**, 621-631, which had been identified as the critical piece of evidence used by the Specialised Experts in reaching their conclusion.

2.23 The Committee concluded that the aneuploidy induced by methyl benzimadazole carbamates (specifically benomyl, carbendazim and thiophanate-methyl) which act by inhibiting spindle formation is a threshold-related effect. There is a sound scientific basis to assume that these chemicals have a threshold of action in both somatic and germ cells. The Committee did not agree with the interpretation reached by the European Commission's Group of Specialised Experts in fields of carcinogenicity, mutagenicity and

reprotoxicity at its meeting of the 1-2 September 1999 particularly with regard to the finding by de Stoppelaar *et al* (1999) of the induction of diploid sperm in rats in the absence of induction of micronuclei in peripheral erythrocytes. The Committee considered the finding of diploid sperm to be an expected effect of carbendazim on male germ cells undergoing meiosis and entirely consistent with the known effects of this chemical on microtubule formation.

2.24 A copy of the statement providing the detailed evaluation of the relevant data is given at the end of this report.

Ongoing Work

2.25 The Committee has agreed to consider Risk Assessment of mutagens other than aneugens with regard to thresholds during 2001.

Statements of the COM

Statement on the Mutagenicity of Hydroquinone and Phenol

Statement on Mutagenicity of 3-Monochloropropane 1,2- Diol (3-MCPD)

Statement on Alcoholic Beverages : Update on Information Published Between 1995-2000.

Statement on Thresholds for Aneugens : Extrapolation of Data from Somatic Cells to Germ Cells

STATEMENT ON THE MUTAGENICITY OF HYDROQUINONE AND PHENOL

Introduction

1. The Health and Safety Executive (HSE) asked for advice from the Committee during 1994 and 1995 and most recently in 1999 on the interpretation of the mutagenicity data on hydroquinone and phenol. The advice from COM was required by HSE as part of its regulatory reviews of occupational exposure limits to hydroquinone and phenol.

2. The principal use for hydroquinone is in the manufacture of black and white film developers. Other uses include the manufacture of antioxidants and polymerisation inhibitors; as a chemical intermediate in the manufacture of pharmaceutical, agrochemicals and dyes; in the production of cosmetics and topical creams; and as a laboratory reagent. Occupational exposure to hydroquinone in the UK is mainly via inhalation of airborne concentrations usually below 1 mg m^{-3} and averaging about 0.15 $mg.m^{3}$ [8 hour time weighted average (TWA)]. Dermal exposure to hydroquinone in the occupational setting is low. [The current UK occupational inhalation exposure limits for hydroquinone are 2 $mg.m^{-3}$ as an 8-hour TWA and 4 $mg.m^{-3}$ as a 15 minute short-term exposure limit (STEL).[1]]

3. Phenol is mostly used in the manufacture of phenolic resins, and is also used in the manufacture of disinfectants, some shampoos and in the preparation of soaps. The highest occupational exposures would be expected to occur in the paint stripping of aircraft, where exposures are controlled to below 8 $mg.m^{-3}$ (8 hour TWA). The other possible circumstances where high exposures may occur is in the use of phenolic resins in foundries. The resins contain small amounts of free phenol and whilst most exposures are very low, in some special cases exposures of up to 12 $mg.m^{-3}$ (8-hour TWA) may occur. There are no data available for occupational dermal exposure to phenol. However, as personal protective equipment is known to be extensively used, it is considered that exposure via the skin will be very low. [The current UK occupational inhalation exposure limits for phenol are 20 $mg.m^{-3}$ as an 8-hour TWA and 39 $mg.m^{-3}$ as a 15 minute STEL.[2]]

Overview of COM considerations.

4. A brief overview of the Committee's discussions held in 1994, 1995 and 1999 is given below. Full details of the Committee's considerations in 1994 and 1995 have been published in the Annual reports.[2,3]

5. In 1994, the COM agreed that both hydroquinone and phenol should be regarded as somatic cell *in-vivo* mutagens.[4-11] The Committee agreed that for exposure to these two compounds by the oral route there was potential for a threshold of activity as there was good evidence that two protective mechanisms (namely rapid conjugation and detoxification via the glutathione pathway) would substantially reduce systemic exposure to any active metabolites formed. However, Members agreed that there were insufficient data on inhalation and dermal exposure and it was not possible to assume that a threshold existed for activity when exposure was via the respiratory tract or the skin. The Committee noted the information from one published paper that when radiolabelled phenol was given intratracheally, initially all the radiolabel in the plasma was present as phenol.[12] These data suggested that there was little conjugation of phenol on the "first-pass" from airways to the circulation. The Committee recommended that appropriate toxicokinetic studies were needed.

6. In 1995, a submission from industry to the HSE provided some additional studies on the metabolism of hydroquinone and phenolic derivatives in the lung and skin, HSE requested the Committee's assessment of these new data.[13-20]

7. The Committee agreed that the new data on the metabolism of hydroquinone and phenol in animals and in humans were valuable but appropriate studies to determine the extent of pre-systemic metabolism following either inhalation or dermal exposure had not been undertaken. It was agreed that the following studies were needed to answer this question:

   i) Further *in-vivo* studies in rats or dogs using administration of hydroquinone or phenol via a bronchoscope with very early sampling for free and conjugated test substance in the blood.

   ii) It was essential that the method be sensitive enough to measure both free and conjugated substance.

   iii) Additional investigations in volunteers following dermal administration would also be useful but should be undertaken using higher doses of hydroquinone and early sampling times. (Members acknowledged that the skin irritancy of phenol would limit the dose level of this compound that could be studied.)

8. In 1999, further data from published papers on the kinetics of hydroquinone in rats following intratracheal instillation and on its percutaneous absorption in *in-vitro* studies using rat skin and human stratum corneum were provided to the Committee.[21,22] A number of additional *in-vivo* mutagenicity studies

including an investigation of site of contact mutagenicity in skin and respiratory tract of Muta^{TM} mice using the LacZ transgene were also considered.[23-25]

9. Regarding the new data on hydroquinone,[23] the Committee agreed that a positive result had been obtained in a new bone-marrow micronucleus assay and that these results were consistent with previous studies considered in 1994. The new toxicokinetic study in which rats were given a single intratracheal dose of ^{14}C-hydroquinone showed detectable free hydroquinone in arterial blood within 5-10 seconds after dosing.[21] This new information suggested a potential risk of site-of- contact and systemic mutagenic effects following inhalation exposure to hydroquinone.

10. Regarding the new data on phenol, the new bone-marrow micronucleus studies showed that a small but consistent positive result with phenol could be identified in studies conducted according to OECD guidelines at intraperitoneal dose levels of around 100-160 mg/kg.[23,24]

11. The Committee considered the new transgenic mutagenicity test with phenol using the LacZ transgene in Muta^{TM} mice.[25] Animals were given either dermal doses of 100 mg/kg bw or exposed for a period of 2 hours to a vapour containing 100 ppm phenol (390 mg.m^{-3}) on five consecutive days. Samples of tissues (liver, bone marrow, and blood, and also, for inhalation exposure, nasal epithelia and lung) were taken at a number of time points after dosing and the DNA extracted and packaged for analysis of LacZ mutants. Members noted that a positive control chemical (benzo(a)pyrene) had been used for the dermal studies but no positive control had been used for the inhalation studies presumably because of the potential hazards involved in handling and controlling exposures to test animals. Members acknowledged that there would be an observable degree of inter-animal variation in results for *in-vivo* mutation assays such as LacZ, which complicates the assessment of data but agreed that the results reported for the study concerned could not be assessed in view of the failure to obtain acceptable levels of DNA packaging in many of the trials. The Committee considered that inhalation exposure to phenol followed by assessment of mutation frequency in nasal tissue were critical to the identification of site-of-contact mutagenicity and felt that a further study with acceptable levels of DNA packaging would be needed before any conclusions on site-of-contact mutagenicity could be reached.

Overall conclusions

12. The Committee reached the following conclusions based on all the available information.

Hydroquinone

a. Hydroquinone is an *in-vivo* mutagen in somatic cells,[4-11] but there is no convincing evidence for effects in germ cells *in vivo*.[26-28] Any risk to human health by ingestion would be likely to be greatly reduced by rapid conjugation and detoxification via the glutathione pathway. Furthermore, mutagenicity appeared to be positively related to peroxidase activity while catalase could also have a protective role.[29] Actual systemic exposure levels in humans would be very much lower than levels at which positive results had been achieved in studies in animals.

b. The Committee concluded that by the oral route there was potential for a threshold of activity based on the protective mechanisms outlined at (a).

c. However, there is insufficient evidence to support a threshold approach to risk assessment for inhalation or dermal exposure to hydroquinone.

d. The Committee concluded that the available data showed that occupational exposure to hydroquinone was associated with a mutagenic hazard but it was not possible to quantify the risk.

Phenol

a. *In-vitro* mutagenicity data on phenol were of poor quality and results difficult to interpret, but *in-vivo* data show phenol to be a somatic cell mutagen following intraperitoneal doses of approximately 100-160 mg/kg [5,8,23,24] No conclusions can be drawn from the one available study in transgenic animals (Muta^{TM} mice) on site-of-contact mutagenicity following dermal or inhalation exposure.[25] The Committee felt that a further study in transgenic animals, with acceptable levels of DNA packaging, would be helpful before any conclusions on site-of-contact mutagenicity could be reached. Data from germ cell studies *in vivo* were inadequate to allow any definite conclusions to be drawn.[30,31]

b. Any risk to human health by ingestion would be likely to be greatly reduced by rapid conjugation and detoxification via the glutathione pathway. Furthermore mutagenicity also appeared to be positively related to peroxidase activity while catalase could also have a protective role. Actual systemic exposure levels in humans would be very much lower than levels at which positive results had been achieved in studies in animals.

c. The Committee concluded that by the oral route there was potential for a threshold of activity based on the protective mechanism outlined at (b).

d. However, there is insufficient evidence to support a threshold approach to risk assessment for inhalation or dermal exposure to phenol.

e. The Committee concluded that the available data showed that occupational exposure to phenol was associated with a mutagenic hazard but it was not possible to quantify the risk.

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MUTAGENICITY OF 3-MONOCHLORO PROPANE-1,2-DIOL (3-MCPD)

Introduction

1. 3-Monochloropropane-1,2-diol (3-MCPD) can be present as a contaminant in epichlorhydrin/amine copolymers used as flocculants or coagulent aids in water treatment. These polyamine flocculants have been available for many years as approved products for use in water treatment and thus 3-MCPD may be present in drinking water from their use. 3-MCPD is a member of a group of contaminants known as chloropropanols. This group includes some known genotoxic carcinogens in animals such as 1,3-dichloropropan-2-ol. The COM was asked in 1999 to evaluate the available mutagenicity data on 3-MCPD and to provide conclusions for the Committee on Carcinogenicity (COC) who had been asked to consider the carcinogenicity data on 3-MCPD. The COM was aware that 3-MCPD had been detected as a contaminant of several foods and food ingredients, including acid hydrolysed vegetable protein (acid-HVP) and that the EU Scientific Committee for Food had published an opinion in 1994 where it was agreed that 3-MCPD should be regarded as a genotoxic carcinogen.[1]

2. In 1999 the COC noted that in a carcinogenicity study undertaken by Sunahara *et al* (1993) 1,3-MCPD was administered via drinking water to groups of 50 male and 50 female (aged 6 weeks at start) F344 rats for a period of 104 weeks.[2] Statistically significant increases in leydig cell adenomas (intermediate and high dose level) and mammary gland fibroadenomas (high dose level) had been noted in males and a statistically significant increase in kidney tumours had been noted in females at the high dose level. The COC had noted in 1999 that the high dose level had exceeded the Maximum Tolerated Dose. The COC therefore asked the COM for an assessment of the mutagenicity data on 3-MCPD as part of its evaluation of the mechanism for the carcinogenic effects seen in rats.

Evaluation: 1999

3. The Committee was aware that 3-MCPD had been detected as a contaminant of savoury food ingredients, including acid hydrolysed vegetable protein (acid-HVP) and that the EU Scientific Committee for Food had published an opinion in 1994 where it was agreed that 3-MCPD should be regarded as a genotoxic carcinogen.[1] The Committee also had access to published mutagenicity data on 3-MCPD, a safety evaluation prepared by CanTox Inc (Ontario, Canada) for the International Hydrolysed Protein Council,[3] a review

document published by the Institute of Toxicology, National Food Agency of Denmark,[4] and one *in-vivo* mutagenicity submitted in an in-confidence basis.[5] In reviewing these documents, members commented that the available metabolism data on 3-MCPD were relatively old and focused on metabolic pathways following intraperitoneal administration. There was no oral mass balance investigation available. The Committee considered the proposal by CanTox Inc regarding the formation of bacterial-specific mutagens and agreed that there was no evidence to support this speculation.

4. The Committee reached the following conclusions on the mutagenicity data available in 1999.

   i) 3-MCPD was mutagenic in Salmonella typhimurium in the absence of exogenous metabolic activation.[6-9] The addition of S-9 mix did not increase the mutagenic response observed.

   ii) Positive results have also been reported in the mouse lymphoma assay in the presence of metabolic activation,[4] but the full report of the study was not available to the Committee. Positive results were also reported in tests in yeast (Schizosaccharomyces pombe)[10] and in tests for Sister Chromatid Exchange in mammalian cells.[4]

   iii) The Committee concluded that 3-MCPD had mutagenic activity *in-vitro*.

   iv) Negative results have been reported from a bone marrow micronucleus assay in mice using a single oral dose of up to 120 mg/kg bw and sampling of bone marrow at 24, 48 or 72 hours post administration. The authors stated that higher doses would result in significant weight loss and mortality. The Committee noted that there was no evidence for a reduction in the ratio of polychromatic to normochromatic erythrocytes (ie ratio of PCE/NCE) and thus there was no evidence to show exposure of the bone marrow to the test material and its metabolites had occurred.[5]

   v) The Committee agreed that no conclusions could be drawn from the investigation of colonic micronuclei in mice[5] in view of the limited database available for this assay or from the inadequately reported dominant lethal assays.[11,12]

   vi) The Committee agreed that further negative results in an *in-vivo* mutagenicity test in a second tissue, namely rat liver UDS, were required in order to provide adequate reassurance that the activity seen *in vitro* is not expressed *in vivo*.

Evaluation: 2000

5. The Committee considered two new *in-vivo* mutagenicity studies commissioned by the UK Drinking Water Inspectorate at its October 2000 meeting. These comprised a rat bone-marrow micronucleus test and a rat liver UDS assay, both of which are widely used to assess genotoxicity *in vivo*.

Rat in-vivo bone-marrow micronucleus test[13]

6. The assay protocol conformed to OECD 474. A single sex [male (Crl: HanWist BR): group size 6] was used as there was no substantial sex differences in toxicity. The top dose was selected from a range-finding study in which single oral doses of between 20-100 mg/kg bw were administered once daily for two consecutive days to groups of male and female rats. Dose levels of 60 mg/kg bw resulted in severe toxicity and some deaths. In the main study, doses of 15, 30 and 60 mg/kg bw were given for two consecutive days. Signs of toxicity were seen at the top dose level (piloerection) which was also associated with a clear reduction in polychromatic erythrocytes to normochromatic erythrocytes, indicating bone marrow cytotoxicity (and hence that 3-MCPD and/ or its metabolites reached the bone-marrow).

7. There was no increase in the number of micronucleated PCEs at any dose level in 3-MCPD treated animals (2000 polychromatic erythrocytes scored/animal). The positive control, cyclophosphamide produced a clear increase in micronuclei.

Rat Liver UDS assay[14]

8. The UDS assay protocol conformed to OECD protocol 486. The top dose, a single oral dose of 100 mg/kg bw, was chosen on the basis of a sighting toxicity study which had shown severe toxicity at oral doses of 150 mg/kg bw. In the main study single oral doses of 40 mg/kg bw or 100 mg/kg bw were administered to male rats (Han Wistar) and hepatocytes recovered for UDS analysis using autoradiography after 12-24 hours (4 animals/dose level) and 2-4 hours (5 animals/dose level). No signs of toxicity were seen at either dose level. There was no evidence for any increase in UDS at either dose level or time point. The two positive control substances (2-AAF and DMN) both gave clear positive results.

Discussion

9. Members agreed that 3-MCPD has a chemical structure which suggests that it may be metabolised to genotoxic intermediates (particularly glycidol). 3-MCPD was clearly mutagenic *in vitro* in the salmonella assay and in the mouse lymphoma assay in the presence of metabolic activation.

10. The committee noted that the predominant urinary metabolite in rats fed or given intraperitoneal doses of 3-MCPD was ß-chlorolactic acid[15] ie by a pathway not producing glycidol or other genotoxic intermediates. A degradation product of ß-chlorolactic acid, namely oxalic acid, has been documented to induce the nephrotoxic effects seen with 3-MCPD.[5,16] One study has also shown that 3-MCPD may be metabolised by a minor pathway and undergo conjugation with glutathione to ultimately form a mercapturic acid in urine of rats [N-acetyl-S-(2,3-dihydroxypropyl) cysteine],[17] suggesting the formation of a reactive metabolite, glycidol, at low levels that are subsequently inactivated. The COM considered that the metabolism of the 3-MCPD in rats had not been fully examined, but agreed that evidence from the two new *in-vivo* mutagenicity studies supported the view that reactive metabolites were not produced in the tissues where genotoxicity was assessed. Thus the Committee reached the following conclusions.

Conclusion

11. The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to an acceptable standard and were negative. Thus the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in vitro* was not expressed *in vivo* had now been provided.

12. The Committee agreed that the major urinary metabolite ß-chlorolactic acid in rats was formed by oxidation of 3-MCPD and that the two new mutagenicity studies supported the view that reactive metabolites if formed did not produce genotoxicity *in vivo* in the tissues assessed.

13. The Committee concluded that 3-MCPD can be regarded as having no significant genotoxic potential *in vivo*.

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COM/00/S4

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STATEMENT ON ALCOHOLIC BEVERAGES: UPDATE ON INFORMATION PUBLISHED BETWEEN 1995-2000

Introduction

1. In 1995 the COM gave detailed consideration to the mutagenicity of ethanol, acetaldehyde and alcoholic beverages. This was to provide input to the Government Interdepartmental Working Group reviewing overall advice on this topic. The Committee on Carcinogenicity (COC) also carried out a detailed review of the available data, mainly from epidemiology studies on the carcinogenicity of alcoholic beverages. The advice from these Committees was considered by the Interdepartmental Working Group when drawing up their Report on Sensible Drinking published in December 1995.[1] The main conclusions reached were:

   i) The COC concluded that the epidemiological evidence supported the view that drinking alcohol causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and for cancers of the oral cavity, pharynx, larynx and oesophagus.

   ii) The COM agreed that the consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential.

2. With regard to the COC conclusions on the particularly important topic of the association between alcohol and breast cancer, the Committee felt that while there was no decisive evidence that breast cancer is causally related to drinking alcohol, the potential significance for public health of a weak causal association between alcohol and breast cancer was such that they recommended that this matter be kept under review.

3. The COC has recently finalised its review of the published literature from 1995-1999 on alcohol and breast cancer (*www.doh.gov.uk/coc.htm*). The COC concluded:

   i) There is an association between drinking alcoholic beverages and increased risk of breast cancer. It is difficult to resolve whether this is causal. The magnitude of the observed association is small (ie the relative risk is modest and, even for heavy drinkers, rarely in excess of 3) and within the range where it is difficult to exclude bias and/or confounding as explanations for the observed results in epidemiological studies. It is difficult to derive a quantitative relationship from the dose-response data available in the literature.

ii) Further epidemiological studies have been published since 1995. There is a need for further systematic review of the epidemiological literature to assess fully the influence of bias, confounding and effect modification. This will contribute to a conclusion on causality and population attributable risk associated with drinking alcoholic beverages.

iii) Studies of possible mechanisms provide evidence for a plausible basis for the causation of breast cancer by consumption of alcohol. Alcohol increases blood levels of oestrogens and in particular oestradiol in both premenopausal and postmenopausal women. These data suggested a similar mechanism to other known breast cancer risk factors.

iv) The COM should be asked to update its opinion of 1995 on the mutagenicity data on alcohol.

4. This statement details the conclusions reached by the COM with regard to the published information on ethanol, acetaldehyde and alcoholic beverages from 1995 to February 2000, and whether there was any need to modify the conclusions drawn in 1995. The Committee recalled that alcoholic beverages contain small amounts of a significant number of volatile and non-volatile organic compounds formed during production, storage and maturation. The Committee reaffirmed its view that it was not essential nor practical to review these constituents individually for their mutagenic potential.

5. The conclusions reached with regard to the mutagenic potential of ethanol, acetaldehyde and alcoholic beverages are given below. A discussion of one recent hypothesis[1] that alcohol might induce breast cancer via the production of reactive oxygen species (ROS) is also included.[2]

Mutagenicity of ethanol

6. The Committee noted that there were no new *in-vitro* mutagenicity studies with ethanol. No conclusions could be drawn regarding the *in-vitro* investigations of effects of ethanol in the pre-implantation development of mouse oocytes injected with spermatozoa stored in 70% ethanol.[3]

7. The Committee reaffirmed its previous conclusions with regard to the mutagenicity data on ethanol, namely: negative results have been obtained in a wide range of *in-vitro* tests and in *in-vivo* tests including those for effects on germ cells; it was concluded that there was no evidence that ethanol induces germ cell mutation *in vivo.*

Mutagenicity of acetaldehyde

8. The committee agreed that the most recent experiments using human lymphoma cells had confirmed earlier studies that acetaldehyde induces protein-DNA cross links, but only at concentrations which resulted in cell death. In addition acetaldehyde induced HPRT mutations in human T cells.[5] Members agreed that no conclusions could be drawn from the finding of acetaldehyde DNA adducts in peripheral white blood cells of alcoholics in view of lack of control for the effects of smoking by alcoholics in the study group and the well known abnormalities in metabolism in alcoholics.[6]

9. The Committee reaffirmed its previous conclusions with regard to acetaldehyde. The available data show that acetaldehyde induces chromosome aberrations in mammalian cells in the absence of an exogenous metabolising fraction. There is some evidence to show that covalent binding (DNA-protein cross links) in the nasal mucosa of rats exposed to high levels of acetaldehyde by inhalation.

10. The mutagenic profile of acetaldehyde is very similar to that of formaldehyde. The compound has direct acting mutagenic potential *in vitro*, but would only be expected to have the potential of *in-vivo* activity at sites where it is not rapidly metabolised to acetic acid. The COC has concluded that the observation of tumours in animals exposed to high inhalation doses of acetaldehyde is not relevant to drinking alcohol.[1]

Mutagenicity of Alcoholic Beverages

11. The Committee recalled that in 1995, considerable weight had been attached to one study from the Medical Research Council's Cell Mutation Unit, who has examined hprt mutant frequency in circulating T-lymphocytes of normal adults and the relationship with alcohol intake.[7] The study showed that alcohol intake in 143 people over the range of 0-56 units/week (1 unit – 8g ethanol) had no effects on hprt mutant frequency. Less weight had been placed on studies which examined the mutagenicity of concentrated extracts of wines and sprits in bacteria,[1] and the significance of such data was felt to be questionable. There were no adequate *in-vivo* mutagenicity studies of alcoholic beverages available in 1995 or for the current review.

12. Since 1995 two further studies of the relationship between hprt mutant frequency in lymphocytes obtained from individuals for whom information on drinking patterns were available.[8,9] There was no association between hprt mutant frequency and alcohol ingestion in these studies, thus confirming the results of the earlier MRC investigation.

The Reactive Oxygen Species hypothesis of alcohol induced breast cancer

13. Members reviewed the hypothesis published by Wright *et al*.[2] Wright and colleagues had noted the finding that alcohol metabolism is known to produce reactive oxygen species (ROS) and mammary tissue contains the necessary metabolising enzymes to produce ROS from alcohol. In addition, Wright and colleagues noted two further observations which supported their hypothesis, namely that breast cancer is associated with higher levels of hydroxyl modified DNA and iron, which has been proposed to catalyse the formation of ROS, accumulated with time in breast tissue. Members agreed that there was evidence that ethanol and its metabolites induced the formation of free radicals *in vitro*, but the evidence *in vivo* was conflicting. Members commented that the observations reported by Wright and colleagues might be a result of tumour progression rather than an initiator of cancer. In addition it was noted that co-administration of iron and alcohol to rats in the initiation phase of a two stage model for hepatocarcinogenesis[10] did not result in any genotoxic effects. Overall, the COM concluded that there was insufficient evidence to support the Wright *et al* hypothesis regarding breast cancer.

Overall Conclusion

13. The Committee reaffirmed its 1995 conclusion that consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential.

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STATEMENT ON THRESHOLDS FOR ANEUGENS: EXTRAPOLATION OF DATA FROM SOMATIC CELLS TO GERM CELLS

Consideration of Summary record of European Commission group of specialised experts in fields of carcinogenicity, mutagenicity and reprotoxicity meeting 1-2 September 1999

Introduction

Risk Assessment of benomyl, carbendazim and thiophanate-methyl

1. Benomyl, carbendazim and thiophanate-methyl belong to the methyl benzimadazole carbamate (MBCs) class of chemicals. These are widely used in approved pesticide products as fungicides and also in veterinary medicines, in particular as anthelmintics, in both food producing and companion animals. These chemicals act by interfering with microtubule formation during mitosis. The COM has provided advice to the UK regulatory Authorities namely the Pesticides Safety Directorate (PSD) and the Veterinary Medicines Directorate (VMD) of the Ministry of Agriculture, Fisheries and Food on the most appropriate approach for the risk assessment of MBCs. [1-3]

2. In 1993 the COM agreed that it was reasonable to assume that aneuploidy inducing chemicals (particularly those that function by interfering with the spindle apparatus of cell division) have a threshold of action.[1] The safety evaluation of aneuploidy inducing chemicals (aneugens) acting by inhibition of microtubule formation is based on the identification of a threshold dose below which aneuploidy does not occur. The Committee provided advice on methodology for identifying thresholds in 1993, namely appropriate *in-vitro* experiments in human lymphocytes using the detection and quantification of non-disjunction, chromosome loss and centromere positive micronuclei using FISH (Fluorescent in-situ hybridisation) analysis of selected chromosomes for centromeric DNA. This advice was used by PSD and VMD when requesting data from approval/licence holders of products containing MBCs. In 1996, the Committee considered the results of experiments undertaken with benomyl and carbendazim and concluded that the studies had been satisfactorily conducted and the data indicated No Observed Effect Levels (NOELs) for these two chemicals.[4-6] It was noted that that it would be difficult to define

precise thresholds for activity from these data and the mathematical models that had been used for their analysis. Appropriate studies which provided evidence for a threshold effect have also been undertaken with thiophanate-methyl.[7]

3. The UK Advisory Committee on Pesticides (ACP) considered that the available *in vitro* aneuploidy data were consistent with a threshold for MBC-induced aneuploidy. The ACP considered that *in vitro* aneuploidy threshold studies should be regarded as providing data that underpinned the regulatory decision rather than providing critical NOELS for direct use in setting Acceptable Daily Intakes (ADIs) and Acceptable Operator Exposure Level (AOEL) values. The conclusions of the UK review have been passed to the rapporteur for the ongoing EC review (under Directive 91/414/EEC) of the use of MBCs as agricultural pesticides.

4. In the case of consumer safety for veterinary medicines, ADIs and Maximum Residue Limits (MRLs) in edible tissues are set on a substance specific basis by the EU Committee for Veterinary Medicinal Products (CVMP). All currently authorised veterinary medicines have been assessed on the basis of the concept that aneuploidy induced by spindle inhibitors is a threshold effect.

Background to current review

5. The Committee was asked by the ACP to consider the conclusions reached by the European Commission's Group of Specialised Experts who were considering the classification and labelling of benomyl, carbendazim and thiophanate-methyl under the Dangerous Substances Directive 67/548/EEC. The Committee considered a draft summary record of the meeting of the Specialised Experts held on the 1-2 September 1999.[8] The Committee was asked by the ACP to advice on the applicability of extrapolating to germ cells, evidence for thresholds for induced aneuploidy obtained in studies on somatic cells, and the relevance of those conclusions for the approach used by PSD to evaluate aneuploidy data in the risk assessment of agricultural pesticides. The Committee has not been asked to comment on the proposals for classification and labelling of these MBCs.

Conclusion reached by Specialised Experts

6. The Specialised Experts concluded that "..current knowledge does not allow extrapolation to meiotic cells of the *in-vitro* finding of a threshold [for induced aneuploidy in somatic cells]. Meiosis I is fundamentally different from mitosis in the structures and processes involved in chromosome

segregation. Due to the current lack of knowledge on the interaction of aneugens with these possible targets, the concept of a threshold for induced aneuploidy in germ cells is as yet a hypothetical one."

7. The COM considered that the critical piece of evidence used to reach this conclusion came from the publication by de Stoppelaar *et al* (1999) which reported diploidy (ie polyploidy but not aneuploidy) in sperm of rats exposed to carbendazim.[9] de Stoppelaar *et al* concluded that their findings suggested that diploidy in sperm is induced at a lower dose level than micronuclei in peripheral blood erythrocytes (no micronuclei were seen in this study). The Committee reaffirmed that there was adequate information available on the mechanism of interaction of MBCs with microtubules to assess the effects of these chemicals in both somatic and germ cells. The Committee agreed it therefore important to review the results obtained by de Stoppelaar *et al* in detail in order to comment on the conclusions reached by the Specialised Experts.

Consideration of de Stoppelaar *et al* Mutagenesis, 14, 621-631, 1999

8. Groups of 5 Wistar (Unilever) rats aged 13-14 week were given a single oral dose of carbendazim (50, 150, 450 or 800 mg/kg bw) in corn oil. The control group received corn oil only. A further group of 5 rats were given an intraperitoneal dose of 150 mg/kg bw carbendazim in corn oil. The animals were killed at thirty-one or 50 days (at 450 mg/kg bw only) and epididymal sperm isolated. In a second experiment, groups of three rats received a single oral dose of carbendazim (2.5, 5, 10, 20, 30, 40, 50, 100, 150, 450, 800 mg/kg bw) in corn oil. A group of 4 rats received 3 mg/kg bw mitomycin C (intraperitoneal) and the negative control received corn oil only. One day before and 48 hours and 72 hours after treatment peripheral blood samples were collected from the tail vein for assessment of micronucleated erythrocytes. The animals in the second experiment were killed thirty-one days after treatment and epididymal sperm isolated. Fluorescence in situ hybridisation was carried out using DNA probes specific for rat chromosomes 4 and Y (and 19 in the second experiment). Additional analyses were undertaken using some animals from the first experiment for chromosome 4 and 19 to confirm the presence of diploid sperm. Five thousand sperm were scored on two slides per animal (ie 10,000 sperm per animal). Only one slide was scored for rats treated intraperitioneally or in trials where rats were killed at 50 days after treatment.

9. The main finding of experiment one was a small but dose-related increase in the absolute frequency of diploid sperm (0.03% to 0.22%) following oral dosing and analysis of sperm at 31 days after treatment. An increase in sperm classified as diploid was only seen in one of five animals following intraperitoneal treatment with carbendazim at 150 mg/kg bw. No increase in 'diploid' sperm was seen in animals killed 50 days after treatment with an oral dose of 450 mg/kg bw carbendazim. The Committee noted that a smaller increased frequency of diploid sperm was reported in the second experiment in animals given an oral dose of 800 mg/kg bw carbendazim which may have resulted from sub-optimal exposure conditions in the experiment. No micronuclei were induced in peripheral blood erythrocytes following oral treatment of up to 800 mg/kg bw after sampling peripheral blood at 24 or 48 hours post treatment.

10. The Committee noted that the mechanism of action of carbendazim involved interference with the formation of polar microtubules. This effect combined with differences in the type of nuclear organising centres (NOC's) for germ cells in first meiotic division in males (a single pole) and in females (multiple poles) would result in carbendazim inducing polyploidy in sperm and aneuploidy in oocytes. The Committee agreed that the finding of diploid but not aneuploid sperm by de Stoppelaar *et al* was to be expected. The Committee noted the finding of aneuploid oocytes in hamsters given a single oral dose of 1000 mg/kg bw carbendazim was also an expected finding.[10] The Committee considered that in the case of MBCs such as benomyl, carbendazim and thiophanate-methyl that affect the formation of spindles it was scientifically plausible for such compounds to be aneugenic in both somatic and germ cells. There is currently no evidence to suggest that MBCs are capable of modifying meiosis I specific events such as chromosome pairing. The Committee agreed that it would be expected that MBCs had a threshold of activity in somatic and germ cells.

11. The Committee considered that the results obtained by de Stoppelaar *et al* did not provide evidence for a lower threshold for aneuploidy in germ cells compared to somatic cells. The Committee felt that the analysis of peripheral blood samples for micronuclei in rats undertaken by de Stoppelaar *et al* was suboptimal in that a 24 hour sampling time point should have been used as micronucleated erythrocytes may have been efficiently removed by the spleen in rats and, as noted in paragraph 9 above, exposure conditions used may not have been optimal for the production of micronuclei. The Committee agreed that a more appropriate study in somatic cells for comparison with germ cells in the rat would be an investigation of the dose-response for the formation of micronuclei containing aneuploid chromosomes in polychromatic erythrocytes obtained in bone marrow smears from rats treated using a similar protocol to that used by de Stoppelaar *et al*. The Committee noted that such

data would be informative with regard to any differences between aneuploidy in somatic and germ cells and agreed to review the subject when appropriate studies had been undertaken. Even if it could be established that the effects on sperm occur at lower doses than for somatic cells, this would not invalidate the concept of a threshold effect.

Conclusions

12. The Committee agreed the following conclusions:

i) The aneuploidy induced by methyl benzimadazole carbamates (specifically benomyl, carbendazim and thiophanate-methyl) which act by inhibiting spindle formation is a threshold related effect. There is a sound scientific basis to assume that these chemicals have a threshold of action in both somatic and germ cells. The Committee did not agree with the interpretation reached by the European Commission's Group of Specialised Experts in fields of carcinogenicity, mutagenicity and reprotoxicity at its meeting of the 1-2 September 1999 particularly with regard to the finding by de Stoppelaar *et al*[8] of diploid sperm in rats. The Committee considered the finding of diploid sperm to be an expected effect of carbendazim on male germ cells undergoing meiosis and entirely consistent with the known effects of this chemical on microtubule formation.

ii) The Committee concluded that de Stoppelaar *et al*[9] had not adequately demonstrated a lower threshold for aneuploidy in male germ cells of the rat compared to somatic cells. The Committee agreed that a more appropriate study in somatic cells for comparison with germ cells in the rat would be an investigation of the dose-response for the formation of micronuclei containing aneuploid chromosomes in polychromatic erythrocytes obtained in bone marrow smears from rats using a similar treatment protocol to that used by de Stoppelaar *et al*. The Committee agreed to review the subject when appropriate studies had been undertaken.

iii) The Committee agreed that the approach used for risk assessment of MBCs by regulatory authorities for pesticides and veterinary medicines and the strategy outlined in the Pesticide Safety Directorate position paper on the role of aneuploidy in the risk assessment of agricultural pesticides were acceptable but would need to be reviewed should a marked difference in sensitivity to aneuploidy induced by these chemicals be reported between germ cells and somatic cells.

iv) The Committee agreed that these conclusions were only relevant to aneuploidy inducing chemicals acting by spindle inhibition. The risk assessment (ie consideration of thresholds in somatic and germ cells) of aneuploidy inducing chemicals acting via other mechanisms needed to be considered on a case by case basis.

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COM/00/S2

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K N Mistry (Administrative)
J M Battershill BSc MSc

Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof J M Parry (Chairman)	Compass Catering JIB Insurance National Power SmithKline Beecham Quintiles	Share Holder Share Holder Share Holder Consultant Consultant	Astra BAT Boehringer Glaxo/Wellcome Pfizer Welsh Water	Grant Grant Grant Grant Grant Grant
Prof J Ashby	Zeneca ML Labs Phytopharm	Employee, Salary, Share option Share Holder Share Holder	NONE	NONE
Dr J Clements	Covance	Employee, Salary, Share Option, Share Holder	NONE	NONE
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE
Prof P B Farmer	Chiroscience Abbey National plc Woolwich Alliance & Leicester	Share Holder Share Holder Share Holder Share Holder	Glaxo Scotia Zeneca	Research Studentship & Research Support Research Support Research Support
Dr N Gooderham	Abbey National plc Albert Fisher Bula resources ML Laboratories plc Northern Rock Protherics Sunderland AFC	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	SmithKline Beecham	BBSRC Collaborative Studentship
Ms M Langley	NONE	NONE	NONE	NONE
Prof D Phillips	Abbey National plc BG Group Bradford & Bingley Centrica CGNU Lattice Group National Grid	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr I Mitchell	Kelvin Associates	Associate/ Consultant	NONE	NONE
	IM Enterprises	Director/ Creditor		
	Glaxo Smithkline	Pensioner, Option and Share Holder, Consultant		
	Bass	Share Holder		
	Cable & Wireless	Share Holder		
	Cadbury Schweppes	Share Holder		
	Marconi	Share Holder		
	Nokia	Share Holder		
	Pfizer	Share Holder		
	RTZ	Share Holder		
	Shell	Share Holder		
	Unilever	Share Holder		
	Vodafone	Share Holder		
	Whitbread	Share Holder		
	British Telecom	PEP Holder		
	Centrica	PEP Holder		
	Scottish Power	PEP Holder		
	Shire	PEP Holder		
Prof D J Tweats	Glaxo Wellcome	Salary Employee Share Option Holder Share Holder	NONE	NONE
	Halifax	Share Holder		
	Co-operative Bank plc	PEP Holder		