1998 Annual Report of the Committees on Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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Department of Health

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About the Committees

This is the eighth joint annual report of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) and the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC). The aim of these reports is to provide the toxicological background to the Committees' decisions for the concerned professional. Those seeking further information on a particular subject can obtain relevant references from the Committee's administrative secretary.

Members of the COT, COM and COC are appointed by the Chief Medical Officer (CMO). The Committees advise the CMO and, through the CMO, the Government.

Committee members are appointed as independent scientific and medical experts on the basis of their special skills and knowledge. They are appointed for fixed time periods, generally three years, and are eligible for reappointment at the end of their terms. The terms of reference are at Annex 1.

The report also contains the commercial interests of committee members. Members are required to declare any commercial interests on appointment and, again, during meetings if a topic arises in which they have an interest. If a member declares a specific interest in a topic under discussion, he or she may, at the Chairman's discretion, be allowed to take part in the discussion, but they are excluded from decision making. Guidance on this is at Annex 2.

The report contains, at Annex 4, an alphabetical index to subjects and substances considered in previous reports. A second index, at Annex 5, contains details of the subjects on which the COT has given advice since 1988 as part of its consideration of the results of surveillance for chemicals in the UK diet. These considerations were published formerly in the Food Surveillance Papers which report this surveillance work, rather than in the Committee's annual reports.

The Committees are engaged in discussions on opening up their proceedings to enable better public scrutiny. This is in the light of the commitment of the Government to increase the openness of all advisory committees.

The usual way in which committee reviews are conducted is that the relevant Secretariat critically assesses all the relevant data and prepares papers for the committee. These normally consist of appendices giving detailed summaries of the studies reviewed – methodology and results – and a covering paper in which the available data are briefly summarised, the most important points highlighted and recommendations presented for discussion by the Committee. Although original study reports are not routinely circulated to members, they are made available on request and are circulated if the study is particularly complex. Definitive summaries are necessary because documentation on any one chemical can amount to many hundreds of pages. The Committees cannot undertake to review information provided by individuals, industry or other organisations that has not been forwarded through, or discussed with, the appropriate Secretariat.

Many of the reviews conducted by the Committees are done so at the request of other Government Departments and the Committee Secretariats liaise closely with colleagues in these Departments. The Committees offer advice independent of each other in their area of expertise but will, if need be, work closely together. This is helped by the close working relationship of the Secretariats. If, for example, during a review of a particular chemical by the COT, it becomes clear that there is need for expert advice on mutagenicity or carcinogenicity aspects, it will be referred to COM or COC as appropriate. These three Committees also provide expert advice to other advisory committees, such as the Advisory Committee on Novel Foods and Processes and the Food Advisory Committee. There are also links with the Veterinary Products Committee and the Advisory Committee on Pesticides.

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

Preface

1998 was a very busy year for the Committee as can be deduced from the contents page of this report. In previous reports I have pointed to the diverse nature of the toxicological topics considered by the COT and the text of this year's report demonstrates this. The topics considered have included those which combine epidemiology with toxicology (landfill sites and congenital anomalies), natural toxicants (Ochratoxin A and Sterigmatocystein) and topics relating to the quantitation of effects (Toxic Equivalency Factors for Dioxin analogues).

In June the Committee's report on peanut allergy was published. The report was written by a Working Group of the COT and contained members of the Committee itself together with co-opted experts. The central theme of this piece of work was immunotoxicity. The report, in addition to being a source of information about peanut allergy, included advice about early exposure to peanut allergens during pregnancy and lactation. The advice was based on a precautionary view despite uncertainties in the scientific evidence.

The membership of the Committee has changed during the year. The new appointments are such that the Committee retains a wide range of expertise in toxicology and related specialities. I particularly welcome the appointment of a lay (public interest) member.

As in previous years, we have been very well served by the Secretariat who have continued to ensure the smooth-running of Committee proceedings and have provided working documents of the highest quality.

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Chemicals in Contact with Water Supplies

1.1 The views of the Committee were sought on the health risk assessment procedures used by the Committee on Chemicals and Materials of Construction for use in Public Water Supply and Swimming Pools (CCM).

1.2 The CCM is a statutory committee designated to advise the Secretary of State for the Environment, Transport and the Regions and the Secretary of State for Wales on approval issues in relation to Regulation 25 of the Water Supply (Water Quality) Regulations 1989. Essentially, Regulation 25 requires statutory water companies to obtain approval for any substance or material which might be applied to or be in contact with water which was to be supplied for drinking, washing, cooking or food production purposes. Therefore substances as diverse as flocculants, disinfectants and epoxy resins are covered by the procedures as well as solid articles such as pipes, filters and fittings from which substances could leach into the water supply. The CCM had been in existence since 1966 and during 1998 was reconstituted as a non-departmental public body (NDPB). In the pursuit of increasing openness, the CCM was publishing additional guidance on its procedures, including the approaches for the health risk assessment of substances that could find their way into the water supply.

1.3 Members discussed the principles and approaches of how decisions for approval or rejection of chemicals and materials were reached by the CCM. As a result of comments received from the Committee, revisions were made to the guidance documents on CCM procedures. The Drinking Water Inspectorate published these in the autumn of 1998.[1]

Chlorinated Drinking Water and Reproductive Outcomes

1.4 The Committee produced the following statement as a response to the referral of a request from the Drinking Water Inspectorate:

(i) At the request of the Drinking Water Inspectorate, the Committee was asked to consider the evidence linking the consumption of chlorinated tapwater and adverse reproductive outcomes. The Committee has not previously considered the health effects of chlorinated water or the by-products of chlorination. However, the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) did review cancer epidemiology data in 1992. At that time the COC concluded that the 1986 opinion of CASW, the DH Committee on the Medical Aspects of Air, Soil and Water, (that there was no sound reason to conclude that the consumption of by-products of chlorination in drinking water increased the risk of cancer in humans) was adequately founded and that more recent studies did not alter that conclusion.[2] In 1998, the COC reviewed additional cancer epidemiological studies published since the 1992 evaluation, *vide infra*.

(ii) The request to the Committee followed the recent publication of two prospective epidemiological studies conducted in three geographic regions of California, USA. One study reported a weak to moderate association between high consumption of tapwater and the incidence of spontaneous abortion, albeit in only one of the three regions.[3] The second study, taking data from all three regions together, reported a weak to moderate association between high exposure to certain chlorination by-products in tapwater and spontaneous abortion (miscarriage).[4]

(iii) As most of the drinking water in the United Kingdom is chlorinated and similar levels of certain chlorination by-products could occur in UK tapwaters, the Committee was asked to comment on the relevance of these data for public health in the UK.

Epidemiological and toxicological data

(iv) The Committee considered available epidemiological information on the association of chlorination by-products in drinking water and a range of adverse reproductive outcomes. Of the seventeen reviewed studies concerned with consumption of drinking water, eight have paid attention to a potential association with chlorinated water or chlorination by-products in the tapwater. Of these, particular attention was focused upon the study from California[4] which the Committee considered to be a particularly well-designed and well-conducted study available for the evaluation of a possible association.

(v) This study, taking data from all three regions together, reported a weak to moderate association (adjusted odds ratio 3.0; 95% confidence interval 1.4-6.6) between high exposure to certain chlorination by-products in tapwater and spontaneous abortion. Nevertheless, the study did not exclude unidentified biases and other confounding factors and the findings, along with information from earlier studies, does not provide persuasive evidence of a causal association between exposure to chlorination by-products in drinking water and adverse reproductive outcomes.

(vi) In addition, the Committee examined available reproductive toxicity studies with some of the individual chlorination by-products. These data indicate that the levels of exposure to these substances in drinking water are about four orders of magnitude lower (ie 10,000 times lower) than levels at which adverse effects may occur in animals.

Conclusions

(vii) We consider that there is insufficient evidence to conclude that the presence of chlorination by-products in tapwater increases the risk of adverse reproductive outcomes.

(viii) We recommend, however, that the claimed associations between patterns of drinking-water intake and the incidence of adverse reproductive outcomes be investigated further, since any causal association would be of significant public health concern.

(ix) We therefore consider that efforts to minimise exposure to chlorination by-products by individuals and water authorities remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking water.

Di-isopropylnaphthalenes

1.5 Di-isopropylnaphthalenes (DIPNs) are used as a solvent for the colour former in carbonless copy-paper. Recycled paper used in making board may include carbonless copy paper. Not all of the DIPNs may be removed by the treatment of the recycled fibres and some may be present in the finished board and thus could migrate into the food. The Committee gave consideration to a JFSSG survey on DIPNs and agreed the following statement:

> The findings of this survey[5] were considered by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) at its meeting in February. The Committee were also provided with estimates of intake and were asked to consider the available toxicological data on di-isopropylnaphthalenes (DIPN). The COT concluded that, apart from one teratology study, the toxicological information on DIPN is inadequate and agreed that additional mutagenicity and long-term studies are needed. The Committee recommended that these studies should be submitted within 3 years and that, in the meantime, it would be prudent to ensure that the levels of DIPN in recycled paper and board food packaging should be kept as low as reasonably practicable to minimise migration into food.

1.6 Additional information was presented at the Committee meeting of October 1998 by representatives of the company involved in the manufacture of DIPN. There was discussion about the results from and procedures used in the carcinogenicity study. The Committee agreed that the presentation and the discussion had not altered its conclusions.

French Maritime Pine Bark Extract

1.7 At the request of the Advisory Committee on Novel Foods and Processes the Committee considered the available data on an extract of the bark of the French maritime pine. It produced the following statement. Subsequently additional data has been submitted to the Committee.

Introduction

(i) We were asked to consider a submission which had initially been made to the Advisory Committee on Novel Foods and Processes (ACNFP) seeking food safety clearance of an extract derived from the bark of the pine tree (*Pinus pinaster*). The French maritime pine bark extract is sold in the form of a dietary supplement. The ACNFP has advised that since the extract has been on sale in the UK and other European countries for a number of years, it would not be considered a novel food. However, the ACNFP did have some concerns regarding the toxicological data included in the submission. The submission was therefore referred to this Committee for advice on:

a) the significance of the findings reported in the toxicology studies on the extract;

b) what, if any, further toxicological data were required to support the safety-in-use of supplements containing this extract.

The extract

(ii) French maritime pine bark extract is a concentrate of a range of water-soluble flavonoids found in the bark of a special climatic species of pine, Pinus pinaster Solander. The extract contains phenolic acids, e.g. ferulic and gallic acids, and their glucopyranoside derivatives and, for example, the flavonoids catechin, taxifolin and procyanidins. The extract is reported to have a minimum polyphenol content of 90%. A specification for the extract was submitted. However, we consider the current specification to be inadequate and recommend that a more detailed specification be developed.

Toxicological data on the extract

(iii) A range of toxicological data had been submitted in support of the safety of the extract. These data included acute studies in rodents, as well as sub-chronic studies in rodents, beagle dogs and minipigs, and reproductive studies in rodents and rabbits. The quality of the studies submitted varied greatly and most had not been conducted in accordance with recognised test protocols. A range of *in vitro* and *in vivo* mutagenicity studies had also been submitted. These studies were conducted in accordance with OECD protocols and were compliant with Good Laboratory Practice (GLP). The mutagenicity studies were reviewed by the Secretariat of the Committee on Mutagenicity (COM).

(iv) The description of the test material used in the various toxicology and mutagenicity studies varied. The submission claimed that a range of names had, in the past, been used to describe the extract produced from pine bark and that all of the test materials used in studies were reported to be representative of the material on sale. However, in the absence of an adequate specification for these materials it is impossible to be sure that this is the case. As stated previously, a more detailed specification for French maritime pine bark extract must be developed before any further toxicological studies are conducted on the extract.

(v) The acute toxicity studies demonstrated that the extract was not acutely toxic, with LD50 values in the rat ranging from 1-5 grams per kilogram body weight(g/kg bw).[6,7] The subchronic studies included a 90-day study in the rat, a 26 week study in the guinea pig and a 6 month dog study conducted in 1975.[8] Evaluation of these studies was limited by the fact that full study reports were not available, only brief summaries of the studies were included in the submission. The rat and the guinea pig studies did not report any adverse effects. In the dog study animals receiving the top dose level of 500 milligrams per kilogram body weight per day (mg/kg bw/day), which was increased to 1000 mg/kg bw/day during the last six weeks of the dosing period, were reported to have reduced weight gain, increased serum triglyceride levels, decreased blood sugar levels and a "lowered heart frequency on EKG" compared to control animals. Examination of the brain from animals in this group revealed that the white substance of the brain had undergone changes, these changes are described as "minor disseminated demyelination". At the low and intermediate dose levels (60 and 150 mg/kg bw/day) blood sugar levels were also significantly decreased and serum triglyceride levels increased.

(vi) The results of two studies conducted in 1989 were also submitted.[9,10] In both studies the dosing period was 180 days and the test animals were rats and mini-pigs. The doses used in both studies were 1.77, 3.55 and 7.10 mg/kg bw/day. Full reports of these studies are available and although the conduct of the studies was not in accordance with recognized protocols for regulatory studies, they are considered to be acceptable. However, there was no histological examination of tissues in either study. No adverse effects were reported in either study. These studies do not provide any reassurance with regard to the brain lesions reported in the dog because the dose levels were much lower and there was no macroscopic or microscopic examination of the brain.

(vii) The reproductive studies consisted of 'embryo-foetal' studies in rats, mice and rabbits,[11] a fertility and teratogenesis study in mice[12] and a rabbit teratology study.[13] As with some of the sub-chronic studies, only brief summaries were available for 'embryo-foetal' studies. In the 'embryo-foetal' study in the rabbit a reduction in litter size was observed at the highest dose level (750 mg/kg bw/day for several days during the pregnancy) due to an increase in early and late resorptions and an increase in post-implantation loss. There was no evidence of maternal toxicity at this dose level. In the rabbit teratology study no adverse effects were reported in animals dosed for several days during the pregnancy with 3.55 or 7.10 mg/kg bw/day of the extract. No adverse effects were reported in the rat or mouse studies.

Mutagenicity studies

(viii) Five mutagenicity studies on pine bark extracts were submitted: two Ames tests, an *in vitro* cytogenetic assay in human lymphocytes, and two *in vivo* bone marrow micronucleus assays.[14–18] The COM Secretariat have reviewed these studies and have commented that generally these studies were conducted in accordance with GLP, but there were limitations with regard to the relevance of the test material used which need to be resolved before any of these tests can be accepted, i.e. the lack of a proper specification for the product. French maritime pine bark extract is a complex mixture of chemicals and therefore it is very difficult to judge whether chemicals in the mixture have structural alerts for mutagenic activity e.g. dimeric procyanidin contains six phenolic rings.

(ix) Both Ames tests were considered inadequate and therefore a repeat test would be required. The *in vitro* cytogenetics test was generally acceptable, although a further trial with an extended exposure time in the absence of activation by a liver S-9 fraction would normally be needed to complete the study. The *in vivo* tests were generally acceptable although the dose level used was probably slightly lower than could have been achieved. It is not possible to make any statement regarding the absorption and distribution of individual compounds present in French maritime pine bark extract and hence exposure of the bone marrow cannot be completely assessed, although it is noted that some evidence of slight toxicity was reported in the micronucleus studies, suggesting that dose levels were near to the maximum that could be tolerated. In addition, a third *in vitro* test in mammalian cells, such as the mouse lymphoma test, would normally be required. However, the COM Secretariat advise that this could be omitted for the extract provided the adequacy of the test material for the submitted studies can be resolved and a further Ames test is undertaken.

Human studies

(x) A number of human studies were cited in the submission on French maritime pine bark extract.[19–23] As with the animal studies, only brief summaries were available for some of the studies. Only one study reported adverse effects ('heart pain', stomach ache). However, it is not clear to what extent these studies were designed to detect and investigate adverse effects.

Recommendations

(xi) On the basis of the toxicological data available on French maritime pine bark extract it is not possible either to dismiss or to substantiate the effects reported on the brain in the 6-month dog study. It is therefore essential that further work be carried out in the dog to investigate whether these effects can be reproduced and, if necessary, to identify a dose at which no adverse effect occurs.

(xii) We note that the potential teratogenic activity of the extract was investigated using a maximum dose level of 7.10 mg/kg bw delivered on several days during the pregnancy in adequately conducted studies in mice and rabbits. However, without full study reports, which might provide sufficient reassurance, the results of the embryo-foetal studies in which there was no evidence of teratogenicity at much higher dose levels (720-750 mg/kg), cannot be relied upon. In view of this, and the fact that there is only a safety margin of approximately 5 between the recommended intakes of the extract (1.5 mg/kg bw/day) and the highest dose level tested in the teratology study (7.10 mg/kg bw/day), we recommend that a further teratology study be conducted with higher dose levels in order to adequately investigate the potential teratogenic/foetotoxic effects of the extract.

(xiii) Some components of the extract have structures suggestive of a relationship to phytoestrogens and it is possible that these components might be metabolised to compounds similar to equol, a compound reported to have anti-oestrogenic and weak oestrogenic activities. We therefore recommend that the possible endocrine/reproductive effects of the extract be investigated further.

(xiv) We endorse the recommendation of the Secretariat of the Committee on Mutagenicity that a further Ames test is required on French maritime pine bark extract.

(xv) Pine trees are known to contain allergenic proteins, we would therefore wish to see data on the protein content, if any, of the extract. If proteins are present in the extract then it must be determined whether these proteins have any homology with pine proteins known to cause allergenicity. It would also be advisable to carry out a drug interaction study on the extract at some stage to determine if there is any effect on cytochrome P450 isoenzymes.

(xvi) However, we would stress that none of these studies should be conducted until an adequate specification has been provided for the extract.

(xvii) Finally, in view of the concerns raised by the toxicology data on the extract we recommend that supplements containing French maritime pine bark extracts should not be consumed by children, pregnant women or nursing mothers until further data are available to support the safety-in-use of these supplements.

Immobilised Lipase from *Rhizopus niveus*

1.8 In 1994, the Committee was asked to consider the safety-in-use of an immobilised lipase (an enzyme) prepared by fermentation of the fungus *Rhizopus niveus*. The enzyme is intended for use in the production of interesterified fats for use as cocoa butter substitutes. Following this review the COT concluded that, whilst not fully satisfying the requirements of the guidelines,[24] the data provided sufficient evidence of safety and consistency. The Committee asked for the following data to be submitted: a more extensive specification, clarification of the variation in protease activity values, a test for pathogenicity of the production strain and an assay for chromosome aberrations on a concentrated extract of the enzyme. The Committee considered the additional data submitted during the year and agreed that the company had satisfied most of the requests but asked for further information on the specification of this enzyme.

Landfill Sites and Congenital Anomalies

1.9 In August 1998, The Lancet published the results of an epidemiological study called the EUROHAZCON study.[25] This was a case-control study which investigated the incidence of congenital anomalies (birth defects) around 21 landfill sites from 15 European centres, including ten sites (at six centres) in the UK. The combined results from the 21 sites suggested that women who lived within 3 km of a landfill site were more likely to have a malformed foetus than women living further away from the site. However, not all sites revealed an increased risk, indeed for four of the six UK centres, there was no statistically significant increased risk. However on combining the evidence from all sites, the authors reported a "fairly consistent decrease in risk with distance away from the site". Statistically significant increased risks were reported for neural tube defects, malformations of the cardiac septa and anomalies of the great arteries and veins. The Committee was informed that, in response to concerns raised about the findings of the EUROHAZCON study, Government Departments had commissioned a national epidemiological study of adverse health effects in relation to landfill sites in the UK from the Small Area Health Statistics Unit (SAHSU) at Imperial College.

1.10 Members were informed of another UK study,[26] in which the health outcomes in residents close to the Nant-y-Gwyddon landfill site in South Wales had been investigated. The main finding of concern was of an increased risk of births with congenital malformations for mothers living closest to the site – the "exposed" group – as compared with mothers living further away – the "unexposed" group. However, an increased risk for this "exposed" group was also evident over the 5 years **prior** to the opening of the site and, while the risk was increased in the 2 years after the site was opened, it declined to its previous, pre-site-opening, level over the subsequent six years of study. In addition, over the last five years of the study, a cluster of four cases of gastroschisis (a congenital defect of the abdominal wall) was found in the "exposed" group, compared with only one case in the much larger "non-exposed" group. The committee also reviewed the results of a number of older studies of adverse health effects around hazardous waste sites, which had been published in the scientific literature.

1.11 The Committee were asked to comment on the EUROHAZCON study and to advise on any additional factors which should be considered in finalising the protocol for the proposed SAHSU study.

1.12 Members considered that the EUROHAZCON study was well conducted, but agreed with the authors that "there is a need for further investigation of whether the association of raised risk of congenital anomaly and residence near landfill sites is a causal one".

1.13 The main difficulties with interpreting the findings of the study were considered to include the following:

exposure estimation: the study had used concentric circles to define exposure zones and assumed that these correlated with the level of exposure. This was a weakness that should be addressed in future studies. While inventories of wastes deposited in landfills were useful, although unlikely to be complete at older landfills in the UK, exposure estimates needed to consider what was emanating from these sites rather than what was deposited in them.

site heterogeneity: landfill sites could not be considered as a single toxicological entity. The differences in individual odds ratios seen across the sites investigated were not therefore surprising (ie the adjusted odds ratios for the 14 study areas ranged from 0.43 to 3.93).

confounding factors: it was not clear whether sufficient allowance had been made for confounding factors such as smoking habits, alcohol and socioeconomic status. It was noted that different approaches were used in different countries to measure socioeconomic aspects.

birth/congenital anomaly registers: it was pointed out that there was generally substantial under-reporting of congenital malformations and registers may not be complete or accurate. However, it was appreciated that the study areas in the EUROHAZCON study had been deliberately selected because these registers were in place.

cases/controls: there were some anomalies in the way cases and controls had been selected, but it was not possible to say what influence this would have upon the reported results.

1.14 Members also commented that the EUROHAZCON study should be viewed as a source of information which could provide clues upon which new hypotheses could be generated and tested in subsequent studies. For example, although the authors cautioned against overinterpretation of apparent differences between malformation subgroups, the types of anomalies identified with higher odds ratios could be selected for more detailed scrutiny, particularly in relation to the type of substances which might be released from landfill sites and which were known to cause similar birth defects.

1.15 In relation to the proposed study by SAHSU, Members were encouraged by the attention that had being given to the exposure assessment aspects of the study, which promised considerable improvement on the approach that had been taken in the EUROHAZCON investigation.

Moniliformin in Maize and Maize Products

1.16 Moniliformin is a naturally occurring toxicant produced by various species of moulds. The fungi that produce moniliformin have a widespread natural occurrence and many major cereal crops are susceptible to infection. The Committee has recommended periodic surveillance to check that levels remain low in maize products.

1.17 A survey of moniliformin in foodstuffs purchased in the UK between May 1997 and March 1998 was carried out by the Joint Food Safety and Standards Group of the Department of Health and the Ministry of Agriculture, Fisheries and Food. The Committee was asked to provide advice on the results. The Committee produced the following statement:

(i) The Committee have been informed of the results of a surveillance exercise conducted by the Joint Food Safety and Standards Group of the Ministry of Agriculture, Fisheries and Food and the Department of Health in which food samples collected between May 1997 and March 1998 were analysed for the presence of the mycotoxin.[27]

(ii) The Committee last considered this subject in 1992.[28] On the basis of available toxicological information and the surveillance data presented to us, we were unable to reach a definite conclusion as to the extent of the risk that this mycotoxin poses to the health of the UK population. However, we welcome the new data and are reassured by the low exposure indicated by the surveillance data. We reiterate our previous opinion that as few of the UK population are likely to consume unprocessed maize products other than on an occasional basis, the low levels of exposure found do not give particular cause for concern.

(iii) As there is a continuing possibility of sporadic instances of significant mycotoxin contamination occurring in foodstuffs, we recommend continued monitoring of the concentrations of moniliformin in foodstuffs in order to determine any trends in these concentrations.

(iv) Surveillance should continue for mycotoxins whose presence in the diet is likely to result in widespread exposure of the UK population and for those mycotoxins where toxicological data indicate a specific hazard. We consider that the available toxicological information on moniliformin does not allow us to make a full assessment of the possible risks to humans. We therefore recommend that studies of the long-term toxicity of moniliformin in two mammalian species should be undertaken to enable us to complete our risk assessment.

Multielement Surveys in Various Items of the Diet

1.18 The Committee was asked to consider the results of analyses for various elements in samples from the 1994 Total Diet Study, in dietary supplements (not including germanium supplements), in vegetables and milk collected near potential point sources of pollution and in marine fish and shellfish. The Committee prepared the following statement:

(i) We have considered estimates of intakes by adults in the United Kingdom of:

- antimony, barium, bismuth, germanium, gold, iridium, palladium, platinum, rhodium, ruthenium, strontium and thallium in the diet;[29]
- antimony, barium, bismuth, germanium, gold, strontium and thallium in certain dietary supplements (not including germanium supplements),[30] and in marine fish and shellfish;[31]
- antimony, indium and thallium in vegetables and milk collected near potential point sources of pollution.[32]

The estimates of intakes have been made available to us in draft Food Surveillance Information Sheets.[29-32]

(ii) We have been provided with the available information on the toxicology of these elements relevant to their oral administration or ingestion. In evaluating the implications for human health, we *note* the following assumptions and limitations:

a) the chemical forms of the elements in food are not known. The relevance of the available toxicity data is therefore uncertain;

b) the estimates of intake assume that, where an element has not been detected, it is present at the limit of detection. Intakes in these cases are therefore dependent on the limit of detection (or other limit) assigned and can be regarded as overestimates, possibly by a considerable margin;

c) the toxicity data available to us are inadequate for complete evaluation of any of these elements in the diet, particularly germanium, gold, indium, iridium, palladium, platinum, rhodium and ruthenium;

d) the data are insufficient to allow the identification of groups of individuals who might be particularly susceptible to any adverse health effects from dietary intakes of these elements. Consequently, our evaluation applies only to healthy adults.

(iii) Acknowledging these limitations, we have seen no evidence to suggest that any of the estimated intakes should be a cause for concern.

(iv) The estimated intakes of germanium from the dietary supplements tested (which did not include germanium supplements) were very small in comparison with intakes from germanium supplements. The longstanding advice from the Department of Health, that it is not safe to take any preparation containing germanium (ie germanium supplements), remains appropriate.

Nitrate Metabolism in Man

1.19 The European Commission (EC) has introduced a Regulation (EC Regulation No. 194/97) which sets maximum levels for nitrate in lettuce and spinach. This Regulation has been introduced to harmonise limits for nitrate in these vegetables and in response to the EC's Scientific Committee on Food's (SCF) consideration of the toxicity of nitrate. The UK and several other Member States are currently operating an optional derogation from the maximum levels for domestic produce.

1.20 Recently, the Committee considered a report of a study entitled 'Metabolism of dietary nitrate in the gastrointestinal tract in man', carried out by G. McKnight, L. Smith, M.N.H. Golden and N. Benjamin, which had been commissioned by the Ministry of Agriculture, Fisheries and Food (MAFF). The Committee was asked to give its view as to whether this study had implications for the determination of the Acceptable Daily Intake (ADI) for nitrate. The report and the Committee's views have been passed to the EC and the SCF.

The Study

1.21 The study investigated the relationship between ingested dietary nitrate in various forms and the production of nitric oxide in the stomach and whether the formation of nitric oxide might be an important mechanism in maintaining host defences against ingested pathogens, in the modification of platelet aggregation in the blood and in other aspects of physiology. An important objective of the study was to determine whether nitrate in an organic matrix ingested as part of a meal was metabolised differently from nitrate in a salt form as used in toxicological studies.

Conclusions

1.22 The Committee considered that the studies with nitrate salts, coupled with the work on isotopically-labelled lettuce, were relevant contributions to the study of nitrate metabolism and accepted that the administration of dietary nitrate gave rise to the generation of nitric oxide. Additionally, it noted a need to distinguish the source of the nitrate (i.e. whether from lettuce or salt) in any comments on nitric oxide generation.

1.23 As regards the role of nitric oxide in respect of the host animal's defence against ingested pathogens, the Committee regarded the proposal as speculative and not supported by the data. It also expressed its reservations about the proposed role of nitric oxide in the modification of platelet aggregation, a complex process which is not a specific indicator for the systemic activity of nitrate. The Committee considered that further studies were needed in order to fully assess the risk and benefit of dietary nitrate.

1.24 Although the Committee considered that the study by Professor Benjamin would not influence the ADI for nitrates and nitrites it agreed that the study report, with a statement of its views, should be submitted to the European Commission's Scientific Committee on Food to inform its deliberations.

Ochratoxin A

1.25 The Committee considered ochratoxin A (OA) in detail in 1992 when the Committee on Carcinogenicity advised that OA is a genotoxic carcinogen. The Committee concluded that levels in food should be reduced to the lowest level that is technologically achievable.[33] At that time, the Committee was informed of a proposed project to measure levels of OA in UK plasma samples, which would give an indication of the exposure of the UK population to this mycotoxin. The Committee welcomed this proposal and asked to be kept informed of the results.

1.26 In 1998, the Committee was asked to review the results of a preliminary project carried out to assess human exposure to OA.

1.27 The survey involved a duplicate diet study of 50 volunteers consuming a free choice of diet. Samples of duplicate diets, plasma and urine were collected from volunteers living in one area of the UK on a weekly basis. Aliquots of these samples were taken to provide a monthly composite sample. The samples were analysed for the presence of OA in order to investigate if there was a quantitative relationship between the dietary exposure to OA and plasma or urine OA concentrations.

1.28 The Committee welcomed the results of the pilot study and was reassured that the estimated dietary intake of OA of all volunteers was less than the 5 ng/kg bw per day recommended by the European Community's Scientific Committee for Food. The Committee agreed the results suggested that urine was a better marker of OA exposure, but pointed out that vegetarians had a higher intake of OA, which was not necessarily reflected in the plasma and urine levels. The Committee supported the continuation of this work but concluded that no definitive conclusions could be drawn from such a study in the absence of data on the bioavailability and excretion of this mycotoxin.

Organophosphorus esters

1.29 At the request of the Department of Health the Committee considered a draft report entitled "Organophosphorus Esters: A Review of Putative Neurotoxic Effects in Humans". This had been commissioned from the MRC Institute for Environment and Health and prepared by Dr David Ray of the MRC Toxicology Unit. The Department sought the view of the Committee on this report, in particular with regard to the possible long-term health effects arising from low-level exposure to organophosphates. It was agreed that the formation of a Working Group to consider this matter would be useful. The report of the Committee, drafted by the Working Group, has since been published.

Peanut Allergy

1.30 Following questions about the use of peanut butter during weaning, the Committee was asked to advise whether early exposure to peanuts and peanut products is a risk-factor for the development of peanut allergy later in life. The Committee was also asked what advice, if any, should be given about the consumption of peanuts and peanut products by pregnant and lactating women, infants and young children.

1.31 In the report, which was published in June,[34] the Committee identified atopy or atopic disease as a critical factor in the development of peanut allergy. They considered that there was insufficient evidence to determine conclusively whether early exposure to peanuts could be linked to the development of allergy to peanuts. Despite the uncertainties in the evidence, the COT took a precautionary view and advised that pregnant women or breast-feeding mothers who are themselves atopic, or where another first degree relative of the child is atopic, may wish to avoid eating peanuts and peanut products during pregnancy and lactation.

1.32 The Committee recommended that, in common with the advice given for all children, those infants with a parent or sibling with an atopic disease should, if possible, be breast-fed exclusively for four to six months. Also, during weaning of such infants, and until they are at least three years of age, they should not be exposed to peanuts and peanut products.

1.33 Refined peanut oils do not contain peanut allergens. The use of products containing these oils in food, ointments or creams will not result in allergic reactions.

1.34 The COT noted that exposure to peanut allergens may precipitate severe, even fatal, anaphylactic reactions and that rapid administration of adrenaline may reduce the risk of a fatal outcome. Advice on the benefits of prompt intramuscular injection of adrenaline and the use by patients of preloaded disposable syringes has been given previously.[35–37]

Phytoestrogens in Soya-based Infant Formulas

1.35 The Committee reviewed phytoestrogens in 1992 and in 1996. In 1992, the Committee recommended that more information should be obtained on the concentrations of phytoestrogens in soya-based milk products marketed for consumption by infants. This would enable more accurate exposure data to be calculated. In 1996, the Committee reviewed the possible effects on infants of consuming phytoestrogens in soya-based infant formulas and recommended that research should be undertaken as a matter of high priority to determine whether ingestion of soy-based formulas carries any risk for infants.[38]

1.36 During the year, the Committee was asked to comment on the results of a survey to determine the concentrations of isoflavonoid phytoestrogens in soya-based infant formulas available in the UK. Concentrations of isoflavone phytoestrogens in six different brands of soya-based infant formula ranged from 18-41 mg/isoflavone/litre formula as fed. Estimated average intakes ranged from 5 mg isoflavone/kg/bw/per day for 1 to 2 month old infants and 4.5 mg isoflavone/kg bw/per day for 4 to 6 month old infants.

1.37 The Committee was reassured that the survey indicated that the levels of phytoestrogens in soya infant formulas and intakes were comparable to those estimated by the Committee in 1996 and to data published from other countries. The Committee welcomed the publication of this information and concluded there was no need to amend the advice issued in 1996:[38] that it endorsed the advice of the Department of Health that breast milk and cows' milk are the preferred sources of nutrition for infants. However, parents who have been advised by their doctor or other health professionals to feed their baby soya-based infant formulae should continue to do so.

Sterigmatocystin

1.38 Sterigmatocystin is a mycotoxin produced by a wide range of fungi. Previous UK surveys indicate sterigmatocystin contamination of foods is infrequent but occasionally occurs in cereals and in cheese. The Committee have been advised by the Committees on Carcinogenicity and Mutagenicity that sterigmatocystin is an *in-vivo* mutagen, an animal carcinogen and a potential carcinogen for humans.[39] The Committee has previously advised that monitoring of food should continue for mycotoxins that are likely to pose a hazard to public health.[40] The Committee was asked to consider the results of the most recent survey and agreed the following statement:

(i) We have been informed of the results of a surveillance exercise conducted by the Joint Food Safety and Standards Group of the Ministry of Agriculture, Fisheries and Food and the Department of Health in which food samples collected between May 1997 and March 1998 were analysed for the presence of the mycotoxin, sterigmatocystin.[41]

(ii) We welcome the new data and are reassured that no sample contained detectable levels of sterigmatocystin using a sensitive reliable analytical procedure, (Limit of Detection 3µg/kg). In the absence of any detectable contamination of foodstuffs, there is no evidence of any risk to human health from sterigmatocystin.

(iii) We have been advised by the Committees on Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment that sterigmatocystin is an *in-vivo* mutagen, an animal carcinogen and a potential human carcinogen.[39]

(iv) We reiterate our earlier comments that, as there is a continuing possibility of sporadic instances of significant mycotoxin contamination occurring in foodstuffs, we recommend continued vigilance to ensure this mycotoxin does not contaminate the food.[42]

(v) Surveillance should continue for mycotoxins whose presence in the diet is likely to result in widespread exposure of the UK population and for those mycotoxins where toxicological data indicate a specific hazard.

Toxic Equivalency Factors for Dioxin Analogues

1.39 The Toxic Equivalency Factor (TEF) approach is used by regulatory agencies to facilitate risk assessment of the polychlorinated dibenzo-*p*-dioxins, dibenzofurans and and biphenyls (PCDDs, PCDFs and PCBs). The European Centre of Environmental Health of the World Health Organisation (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) initiated a programme to derive and evaluate TEFs for these chemicals.

1.40 A WHO-ECEH expert group met in June 1997 to consider new experimental data on the relative potencies of these chemicals. As a result of this meeting, several TEFs were revised, *e.g.* the TEF for 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin was raised from 0.5 to 1 and the TEFs for octachlorodibenzo-*p*-dioxin and octachlorodibenzofuran were reduced from 0.001 to 0.0001, a TEF value was established for PCB 81 (0.0001) and existing TEFs for di-ortho PCBs (*i.e.* PCBs 170 and 180) were withdrawn. The report of the consultation has subsequently been published.[43] These changes were endorsed at a WHO-ECEH/IPCS meeting held in Geneva in June 1998. The COT gave consideration to the experimental data considered by this expert group and agreed to endorse the proposed changes to the TEFs.

Vitamin B6

1.41 The Committee prepared a statement on the toxicity of vitamin B6 in 1997, subsequently a draft report from the United States National Academy of Sciences (NAS) was made public. This latter report reached substantially different conclusions on the toxicity and the Committee was asked to provide its views on the NAS report prior to an inquiry into the matter by the House of Commons' Select Committee on Agriculture. The Committee produced the following statement:

Purpose

(i) This note is intended to record the outcome of our preliminary consideration of a National Academy of Sciences (NAS) report on B vitamins. We were asked to give an initial consideration of the methods used and the recommendations for a Tolerable Upper Intake Level for vitamin B6 in the NAS report in order to inform the Chairman of the COT in advance of his appearance before the House of Commons Select Committee on Agriculture Inquiry into vitamin B6.

Introduction

(ii) The US National Academy of Sciences' Institute of Medicine are publishing a report which establishes a set of Dietary Reference Intakes for the B vitamins. The primary aim of this work was to consider the requirements of B vitamins necessary to maintain health. A Subcommittee on Upper Reference Levels of Nutrients (UL Subcommittee) considered the evidence and advised on the adverse effects of high intakes of B vitamins. The scope of the NAS report was therefore much wider than the work of the COT which has reviewed the toxicity of vitamin B6 on two occasions.

Methodology

(iii) We have been provided with the appropriate sections of a pre-publication, uncorrected proof version of the NAS report. Our own statement on the toxicity of vitamin B6 has been publicly available since July 1997.

(iv) Both the NAS and ourselves have based advice on reviews of the available literature on the safety of vitamin B6. Since a wholly adequate study of the safety of high dose supplementation of vitamin B6 in healthy individuals is not available, both assessments have been made on a series of clinical studies and experimental observations from which a tolerable or safe upper intake level has been derived.

(v) In our assessment, the Lowest Observed Effect Level of vitamin B6 in humans was identified as a daily dose of 50 mg, to which we applied an uncertainty factor of 5 to reach a recommended maximum daily intake from dietary supplements of 10 mg. The NAS considered that the No Observed Adverse Effect Level of vitamin B6 was 200 mg, to which they applied an uncertainty factor of 2 to derive a Tolerable Upper Intake Level of 100 mg.

Conclusions

(vi) As a result of our preliminary consideration of the NAS report on vitamin B6 our conclusions are:

a) We *note* the observation that there is no established benefit for healthy individuals if they consume nutrient intakes above the Recommended Daily Allowance or Adequate Intake;

b) We *agree* with the observation that, like all chemical agents, nutrients can produce adverse health effects if intakes are excessive and that there may be risks for subpopulations with extreme or distinct vulnerabilities;

c) We *note* that in deriving a Tolerable Upper Intake Limit for the B vitamins a Risk Assessment Model was used which employed smaller uncertainty factors than used for non-essential chemicals. This was justified on the grounds that "the consumption of balanced diets is consistent with the development and survival of humankind over many millennia". However, this is inconsistent with the observation made subsequently in the report that the effects of nutrients in fortified foods or supplements may differ from the effects of nutrients available in a balanced diet. It also ignores the fact that dietary supplements may be taken at levels far exceeding those available from a balanced diet. Moreover, the low uncertainty factor of 2 would (according to the model) be appropriate for high quality data, which are not available for vitamin B6;

d) We *note* that the study by Dalton and Dalton, which reported adverse effects at daily doses of 50 mg, was dismissed on grounds of methodological weaknesses. In our assessment of the toxicity of vitamin B6 we recognised these weaknesses but considered it would be unwise to ignore this evidence in the light of the supporting animal data; likewise, we also recognise weaknesses in those reports which have been used by the NAS for the determination of the No Observed Adverse Effect Level.

e) We *note* that there are no wholly adequate studies of the safety of high-dose dietary supplementation with vitamin B6, and that there are limitations with many of the studies. Nevertheless, we consider it important to weigh up all the evidence and to take a responsible approach for a substance with well established toxicity but a poorly defined long-term dose-response relationship. Against this background, we would not wish to change our earlier advice on vitamin B6.

1.42 As of March 2000 the United States National Academy of Sciences has not produced a final version of its report.

Topics Still Under Consideration

1.43 The following topics, which were discussed by the Committee at meetings held in 1998, are still under review:

Alitame
Bioprotein
CS Spray and MIBK
DIPN
Food Intolerance
Malachite Green
Survey of PCDDs, PCDFs and PCBs in Marine Fish
MMT and Manganese

1998 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor H F Woods BSc BM BCh DPhil FFPM FIFST HonFFOM FRCP(Lon & Edin)
Sir George Franklin Professor of Medicine
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Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor H F Woods (Chairman)	Halifax Bank	Shares	University of Sheffield, Faculty of Medicine Wide range of national & international food & chemical companies.	University of Sheffield, Faculty of Medicine. Has extensive activity in teaching and research in nutrition and toxicology and in topics related to and supported by many companies in the food and chemical industry. Trustee of Hallamshire Therapeutic Research Trust Ltd, Harry Bottom Charitable Trust and Special Trustee for the former United Sheffield Hospitals.
Professor P J Aggett	Nestec Wyeth Borax	Ad hoc Consultancy, Lecture and Chairing Meetings	Nestec FDF Abbot Unilever Meat and Livestock Commission	Departmental commissioned research and student placements
Professor N A Brown	Merck Glaxo Wellcome Searle Styrene Information Research Centre Du Pont	Consultancy Consultancy Consultancy Consultancy Consultancy	EC (DGXI and DGXII) Glaxo Wellcome US EPA	Research Support Research Support Research Support
Dr P Carthew	Provalis Unilever Cambridge Antibody Technology	Share Holder Share Holder Consultancy	NONE	NONE
Professor J K Chipman	Boots Healthcare International Quintiles	Consultancy Consultancy	Astra-Zeneca Glaxo-Wellcome Water Research Centre SmithKline & Beecham ICI	Research Support Research Support Research Support Research Support Research Support

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr M Joffe	Ilzro	Research grant	NONE	NONE
Dr I Kimber	British Airways British Petroleum-Amoco ICI Halifax AstraZeneca AstraZeneca	Share Holder Share Holder Share Holder Share Holder Share Holder Employee	Unilever plc	Grant for Research
Professor A G Renwick	International Sweeteners Association	Consultant	Hoffmann-La Roche Unilever SmithKline Beecham Pfizer Flavor and Extract Manufacturers Association (FEMA)	Research Support Research Support Research Support Research Support Research Support
Professor P A Routledge	Health Care Services Edinburgh	Fee	Paracetamol Information Centre	Member of Advisory Group
Professor I R Rowland	CNI Coulter Food Science Coca-Cola Unilever Biscuit Cocoa Chocolate & Confectionery Alliance (BCCA) Medici Pharmaceuticals (Germany) Kellogg's Kirin (Japan) Yakult (UK)	Consultancy Consultancy Consultancy Consultancy Consultancy Consultancy Consultancy Consultancy Consultancy	Coulter Food Science	Consultancy
Dr L Rushton	Institute of Petroleum Transport and General workers union	Consultancy, contracts and grants – completed Consultancy – completed	Concawe Eu	Contract to Institute for Environment and Health – Now completed Contract to Institute for Environment and Health
Ms J Salfield	Alliance & Leicester Halifax Woolwich Northern Rock	Shares Shares Shares Shares	NONE	NONE
Dr A Smith	Abbey National British Telecom Halifax Bank	Share Holder Share Holder Share Holder	Rhône Poulenc	Research Support
Professor S Strobel	NONE	NONE	NONE	NONE
Dr A Thomas	NONE	NONE	NONE	NONE
Professor J A Timbrell	Shook, Hardy & Bacon (Law firm) Sorex Ltd	Occasional Fee Occasional Fee	Glaxo Wellcome Taisho Pharmaceutical Co	Research Support Research Support
Dr M Tucker	Zeneca	Pension	NONE	NONE

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

Preface

The COM evaluates the potential mutagenic activity of chemicals at the request of the Department of Health and other Government Departments. During 1998 the Committee evaluated the available information on chemicals as diverse as food flavourings (Coumarin), air pollutants (e.g. benzene and 1,3-butadiene), chemical incapacitants (CS spray) and a metabolite of a veterinary growth hormone (zearalenone).

An increasing feature of the evaluations being undertaken by the COM is a requirement to consider data obtained from what might be described as "non-standard" methodologies for measuring mutagenic activity. Many of these methods involve the detection of damage to the genetic material the DNA, rather than the production of genetic changes i.e. mutations. The availability of this increasing range of information on chemical activity has both increased the complexity of providing advice and the need for the independent Committee members to keep well abreast of developments in their areas of expertise. As each new method is used the Committee provides advice on the method itself and the relevance of the data obtained. This advice is then made available to the "user" Departments of Government who undertake regular evaluations of chemical safety data sets made available by manufacturers.

The Committee and its Secretariat have spent a considerable amount of its time developing new procedures for greater openness and transparency in its work. Significant changes to working practices which will be introduced in 1999, include publication of the agenda via the Internet prior to each meeting and publication after each meeting of the minutes and completed statements and conclusions. Special arrangements will be needed when information is submitted to the Committee on an "in-confidence basis". 1999 will also see the appointment of a "lay member" to the Committee to supplement the advice of the independent experts.

Professor J M Parry (Chairman)
BSc PhD DSc

Zearalenone

2.1 Zearalenone is a non-steroidal, oestrogenic mycotoxin. It is the major metabolite of zearanol, which is used in some countries as a veterinary anabolic (growth hormone) but not within the EU where such use is not permitted. The COM considered zearalenone in 1997 as one of a number of compounds, which had been identified by the COC as mouse-specific carcinogens. The COM agreed in 1997 that Zearalenone should be provisionally considered as a potential genotoxic carcinogen.

2.2 The Committee recalled that there was no evidence for mutagenicity or clastogenic activity from the available mutagenicity screening studies but evidence of DNA adducts had been reported in two studies undertaken by one research group from France.[44,45] Members agreed that although there was some reservation regarding the significance of minor adducts, the data clearly demonstrated that zearalenone induced DNA adducts in the liver and kidneys of female mice with more limited evidence for DNA adduct formation in the ovaries. No evidence of DNA adducts had been reported in male mice or in male or female rats. The pattern of DNA adducts reported by this group was similar to the sex and species specificity of the carcinogenic effects of zearalenone which was limited to the female mouse liver. Members felt that there was a need for confirmatory data from a separate research group before definite conclusions could be drawn. The Committee confirmed the conclusions reached during 1997 which are reproduced below;

(i) Negative results have consistently been obtained when zearalenone has been investigated for its ability to induce gene mutation in bacteria. Negative results were also obtained when the compound was investigated in the yeast *Saccharomyces cerevisiae* D3.

(ii) Only one limited metaphase study has been carried out using zearalenone and this provided no convincing evidence for clastogenicity. There was some evidence that zearalenone could induce SCEs but only in the absence of S-9 and at very high concentrations (above 10,000 µg/ml). Overall negative results were obtained when using the mouse lymphoma assay.

(iii) One group has demonstrated DNA adduct formation using ^{32}-P-post labelling techniques in the liver and to a lesser extent in the kidney and ovaries of female mice.[44,45] Further data are desirable on the reproducibility of this study, and to define any sex/species specificity. At present it would be appropriate to provisionally regard zearalenone as being potentially genotoxic *in-vivo*.

2.3 The Committee agreed that the data on zearalenone raised a number of issues of potential relevance to mutagenicity testing strategies. These included the use of alternative exogenous metabolising fractions for *in-vitro* tests and the use of *in-vivo* DNA adduct studies in addition to the current recommended testing strategy published in the Guidelines for Testing of Chemicals for Mutagenicity (Department of Health Report on Health and Social Subjects, No 35, 1989, HMSO). Members agreed that consideration of such studies might be appropriate when the proposed uses of compounds were likely to result in high human exposures. The Committee agreed that the subject of testing strategies would be discussed in detail during the forthcoming revision of the COM guidelines.

Coumarin

2.4 Coumarin is a component of natural flavourings and is under active consideration by the EU's Scientific Committee on Food (SCF) and the Council of Europe's Expert Committee on Flavourings (CEFS). It had been shown to have carcinogenic effects in rats, in particular inducing hepatocellular carcinomas and chlolangiocarcinomas, although there is uncertainty about its mechanism of carcinogenic action in the rat. The COM was asked to advise on the available mutagenicity data and in particular whether further studies were needed. The Committee concluded that coumarin was clearly genotoxic *in vitro* using standard OECD test systems and that further data were necessary to provide reassurance that it was not genotoxic *in vivo* (bone marrow micronucleus and rat liver UDS assays conducted to current OECD guidelines). The COC were also asked to comment on the

adequacy and results of the available animal carcinogenicity bioassays. The joint COM/COC statement on coumarin was agreed in August 1998 and was submitted to CEFS for its meeting in October 1998 and also made available on the COM Internet site (see preface to this report for address) The evaluation of the mutagenicity data is reproduced below (see section 3.1 of the COC report for the remaining sections of the statement summarising the carcinogenicity studies). CEFS agreed with the COM/COC opinion that further *in-vivo* mutagenicity studies with coumarin were required to clarify the potential activity of coumarin.

2.5 The Committee made the following statement for Council of Europe on mutagenicity of coumarin:

Background to request

(i) The Committees on Mutagenicity (COM) and Carcinogenicity (COC) of Chemicals in Food, Consumer Products and the Environment are independent advisory committees, which report to the Chief Medical Officer. Their terms of reference include, at the request of UK Government Departments, assessing and advising on the mutagenic and carcinogenic risks to man of substances, which are used or proposed to be used as food additives, or which may be present in food as contaminants.

(ii) The Joint Food Safety and Standards Group of the Department of Health and the Ministry of Agriculture Fisheries and Food has requested advice on the interpretation of the available carcinogenicity and mutagenicity data on coumarin and whether the substance should be regarded as a genotoxic carcinogen. The toxicology data are under consideration by the Council of Europe's Committee of Experts on Flavouring Substances (CEFS) as part of its review of flavouring source materials and this committee, of which the UK is a member, has asked for advice from national experts on the genotoxic potential of coumarin.

Introduction

(iii) Coumarin (2H-1-benzopyran-2-one) is present as a major constituent in plants such as tonka beans and as a minor constituent in certain edible fruits such as strawberries, cherries and apricots.[46,47] It also occurs in natural flavouring source materials such as cinnamon and is also used in fragrances. Limits on coumarin levels in food as a result of its presence in natural flavouring sources are set out in Directive 88/388/EEC.[48] These limits, which were recommended by the EU Scientific Committee for Food (SCF), were based on those previously set by the CEFS in its review of natural flavouring source materials.[49] An extensive review of the toxicology of coumarin was subsequently carried out by the SCF in 1994 which concluded that coumarin was carcinogenic in rats via the oral route and possibly in mice. The SCF considered that the genotoxicity of coumarin could not be ruled out and that the general permitted level in foods and beverages should be reduced from 2 mg/kg to the currently achievable limit of detection (0.5 mg/kg) and action be taken to reduce levels permitted in other specific products.[47] The CEFS is currently considering coumarin again as part of a further toxicological review of natural flavouring source materials and it is for current discussions at this committee that the opinions of the expert committees, the COM and COC, have been sought.

(iv) The COM and COC have primarily considered the adequacy of the available mutagenicity data but have also commented on the significance of the data for human health and the possible mechanisms for carcinogenicity documented in rodents.

Metabolism

(v) There is a substantial amount of data on the pharmacokinetics and metabolism of coumarin, which has recently been reviewed.[50] Coumarin is rapidly absorbed from the gastrointestinal tract and undergoes extensive metabolism in the liver. In most species, including humans, coumarin is predominantly excreted as metabolites in the urine whereas significant biliary excretion and enterohepatic recirculation takes place in the rat. The major inter-species differences in metabolism have been known for some years. Essentially, the major pathway in the rat involves hydroxylation at position 3 of the pyrone ring giving rise via a number of metabolic steps, to *o*-hydroxyphenyl acetic acid which is excreted in the urine. In baboons and humans, the predominant route involves hydroxylation of the phenyl ring giving rise to 7'-hydroxycoumarin which is then excreted in the urine in a conjugated form, mainly as a glucuronide. However, the metabolism of coumarin in humans has been shown to be very variable and complex. In addition the rate and extent of 7'-hydroxylation of coumarin depends on a variety of factors including diet, smoking, liver disease, impaired renal function and treatment with certain medicines. There is also some evidence for genetic polymorphism in humans in the metabolism of coumarin.[5]

(vi) A number of research groups have examined whether there is any correlation between species differences in coumarin metabolism and the documented inter-species differences in coumarin hepatotoxicity.[51,52,53,54] Initial studies in rats suggested that 3'-hydroxylation might lead to the formation of a reactive intermediate (3,4-epoxide) which might be important in the mechanism of hepatotoxicity.[51,52,53] However there is no satisfactory correlation between 3'-hydroxylation in experimental animals and the occurrence of hepatotoxicity[54]. Thus 3'-hydroxylation occurs in the Syrian hamster, but this species is insensitive to the hepatotoxic effects of coumarin.

(vii) The relevant metabolic pathways and specific P450 isozymes involved in the hepatotoxicity and carcinogenicity in rodents have not been elucidated and thus no definite conclusions on the significance of the available metabolism data for human health assessment can be drawn at present. It is therefore not possible to discount the relevance for human health of the findings from carcinogenicity bioassays on the basis of metabolism data, particularly in view of the evidence for variation in coumarin metabolism in humans.

Mutagenicity

(viii) The COM assessed the available mutagenicity data and reached the following conclusions.

a) Coumarin has been fairly extensively investigated for its genotoxicity *in vitro*[55,56,57,58,59,60,61,62,63,64,65,66,67,68,69]. Positive results were obtained in the Salmonella assay with strain TA100 in the presence of rat or hamster S9.[66] Coumarin has also been shown to produce chromosome aberrations in a metaphase analysis study in CHO cells in the presence of rat S9.[66] There is also some evidence for the induction of SCE in CHO cells. Negative results were recently obtained in an assay for UDS using human liver slices.[42] The *in vitro* data indicate that coumarin has mutagenic potential.

b) Negative results were obtained when coumarin was investigated in the sex-linked recessive lethal assay in *Drosophila melanogaster*; the compound was administered either in the diet or by injection.[66]

c) Negative results were obtained in inadequately conducted bone marrow micronucleus tests using non-standard protocols.[70,71] Negative results were also obtained in a peripheral blood micronucleus assay in mice following sub-chronic (90 day) exposure as part of an NTP study.[66] However, this study was limited to examination of normochromatic erythrocytes of peripheral blood only and no investigation of the bone marrow had been undertaken.

d) The Committee concluded that coumarin was clearly genotoxic *in vitro* and that further data were necessary to provide reassurance that it was not genotoxic *in vivo* (rodent bone marrow micronucleus and rat liver UDS assays conducted to OECD guideline standards).

(ix) The COM asked for advice from the COC on the mechanisms of tumorigenesis particularly with respect to the occurrence of cholangiocarcinomas documented in a carcinogenicity bioassay in Sprague-Dawley rats.[72,73] A brief summary of the carcinogenicity bioassays is given in section 3.1 of this report.

Consideration of Evidence for a Threshold for Benzene Induced Carcinogenicity

Introduction

2.6 Benzene is recognised as a human carcinogen that acts by a genotoxic mechanism. There is widespread exposure to low levels in the environment. Exposure standards are set on the assumption that there is no threshold to the effects (ie there is some increase in health detriment, albeit this may be very small, at any exposure level). Arguments have been made by some groups that there is now sufficient evidence to conclude that there is a threshold for the induction, by benzene, of leukaemia in humans. In particular, a recent document from CONCAWE* has been published that recommends a non-linear (threshold) approach to risk assessment of benzene. The COM was asked to comment on this document and to advise on whether such an approach could be recommended.

Mutagenicity of benzene

2.7 A comprehensive review of the health effects of benzene is provided in the recent IPCS Environmental Health Criteria document (No 150) published in 1993.[74] There is *in-vivo* evidence to demonstrate both clastogenicity and gene mutations with benzene.[74,75] In addition more recent data are consistent with this view. Of particular note in this regard is the recent publication of a transgenic assay in mice using the bacterial *lac1* gene as a marker which shows benzene induces gene mutations in lung and spleen.[75]

2.8 There is now much evidence to indicate that the primary metabolic pathway of benzene in rodents (and probably in humans) is mediated by hepatic cytochrome P450 2E1 to benzene oxide.[76,77,78,79,80,81] This is converted via various pathways to other electrophilic metabolites. Benzene oxide is, however, relatively stable (t½ rat blood 7–9 minutes).[77] This is long enough to ensure that benzene oxide is distributed throughout the body.

* Conservation of Clean Air and Water Europe. The Oil companies European organisation for environment, health and safety. The CONCAWE document is entitled "Benzene risk characterisation" and was prepared under contract for a European industry group comprising the CEFIC Aromatic Producers Association, CONCAWE and EUROPIA.

COM conclusions on CONCAWE document

2.9 The Committee concluded there were a number of reasons why the arguments proposed by CONCAWE could not be accepted.

(i) The CONCAWE document provided only circumstantial evidence for a threshold effect for benzene-induced leukaemogenesis. There was no evidence that the initial steps in the carcinogenic mechanism were threshold related.

(ii) Benzene clearly produced both point mutations and chromosome damage *in-vivo*. There is increasing evidence that the critical metabolite formed in the liver is benzene oxide and that the half-life of this metabolite is long enough to ensure systemic distribution and subsequent effects. The CONCAWE document largely ignores consideration of this metabolite.

(iii) There is no evidence to support the claim in the CONCAWE document that clastogenicity is subject to a threshold.

(iv) There is thus no data to suggest that benzene should be treated any differently from other *in-vivo* mutagens, i.e. it should be treated as if there is no threshold.

Overall conclusion

2.10 The COM concluded that it is prudent to continue to assume a linear (non-threshold) dose response for benzene carcinogenicity.

1,3-Butadiene

Introduction

2.11 1,3-Butadiene is a ubiquitous air pollutant produced in cigarette smoke, vehicle exhausts and incineration products from fossil fuels. The COM last reviewed the available mutagenicity data on 1,3-butadiene in 1992 when it was concluded that the chemical was clearly mutagenic *in vivo*, giving a positive result in a bone marrow micronucleus test in mice following exposure by inhalation.[82] No data were available, at that time, on the effects in germ cells, but the Committee had agreed that it would be prudent to assume that 1,3-butadiene had the potential for producing heritable mutations. A considerable amount of new data have recently been published on germ cell mutagenicity of this compound as part of the EU project on 'Multi-endpoint analysis of genetic damage by 1,3-butadiene and its major metabolites in somatic and germ cells in mice, rats and man.[83] The Committee therefore considered these new data and the proposed risk assessment for potential human heritable genetic damage published by these authors using a modelling approach referred to as the "parallelogram method".[83]

Conclusions on 1,3-butadiene

2.12 The Committee agreed that the new data demonstrated that 1,3-butadiene was an *in-vivo* germ cell mutagen in mice.[83] Members concluded that these data confirmed the COM's view that this chemical should be regarded as a potential heritable mutagen in humans. The Committee agreed that it would be prudent to reduce exposures to 1,3-butadiene to as low as is reasonably practical.

Conclusions on proposed risk assessment

2.13 The Committee agreed that the estimated human risk of heritable mutations due to 1,3-butadiene reported in the EU project publication[83] was unreliable. In particular the selection of the mouse as the species to model for human risk assessment was not appropriate. There was substantial uncertainty in the mouse data, which could only be used to indicate orders of magnitude of risk. The Committee was not convinced of quantitative risk estimates of heritable damage using the parallelogram approach even with a compound with as much data as 1,3-butadiene. Thus the COM agreed that the proposed risk estimate for heritable genetic damage proposed by Pacchierotti et al could not be used for deriving guidance values for 1,3-butadiene.

2-Chlorobenzylidene Malonitrile (CS)

2.14 The Department of Health asked the COT to advise on the health effects of CS spray when used as a chemical incapacitant. The COT considerations have not, at the time of writing, been finalised but will include a contribution from its sister committees, the COM and COC, on the mutagenic and carcinogenic potential of CS. Such sprays have now been used for some time by police forces in England and Wales and CS has not been subjected to scrutiny by an independent expert advisory committee. It was for this reason, and the potential public health concerns, that the Department of Health considered that such a referral was appropriate at this time. The Committee was aware that any policy decisions on the use of CS spray as a chemical incapacitant were a matter for the Home Office and individual Chief Police Officers, who will need to take account of the COT advice when weighing up the risks and benefits of CS spray.

2.15 The COM observed that CS was mutagenic *in vitro* inducing both aneugenic and clastogenic effects in mammalian cells. Negative results were consistently obtained in bone marrow assays for micronuclei induction using high dose levels and oral and intraperitoneal routes. It was noted that no data were available to indicate if adequate amounts of CS or short-lived reactive metabolites reached the target organ. Data from DNA binding studies in the liver and kidney did not help in this regard as no relevant analyses of tissues of initial contact (i.e. skin or nasal mucosa) were undertaken. The Committee was aware that carcinogenicity bioassays conducted as part of the US National Toxicology Programme, which used inhalation exposure in both rats, and mice had yielded negative results. Such information provided reassurance that the *in vitro* effects seen with CS do not occur *in-vivo* at a site of initial contact in animals. The COM asked the Committee on Carcinogenicity (COC) to advise on the adequacy of the NTP carcinogenicity bioassays (see section 3.15 of this report).

2.16 A more extensive report of the COM conclusions and references cited in respect of CS will be included in the COT statement which will be published when completed.

Test Strategies and Evaluation

2.17 The Committee initiated a review of its guidelines for the testing of chemicals for mutagenicity (Report on Health and Social Subjects No 35, published HMSO 1989). The document was now 10 years old and there had been considerable advances in genetic toxicology over the intervening period. The Committee agreed that it was important to consult as widely as possible with colleagues engaged in the testing of chemicals for mutagenicity regarding any revisions to the current mutagenicity testing strategy.

In-vitro micronucleus test

2.18 The Committee commented on a draft protocol for the use of the *in-vitro* micronucleus test which had been prepared by a subcommittee of the European Environmental Mutagen Society (EEMS) and was intended to be submitted to the OECD for consideration as a regulatory test guideline.

The Comet Assay

2.19 The Committee considered further published information on the use of the COMET assay in mutagenicity testing strategies.[84,85,86]

Introduction

(i) The Comet (single cell gel electrophoresis) assay had been proposed by several research groups as a rapid *in-vivo* assay for detecting genotoxicity at multiple sites. In 1995 the Committee assessed all the available published literature on the use of this assay to detect chemical mutagens. The Committee agreed, in 1995, that the methods used in the 'Comet assay' represented an interesting research area but further research work was required before the significance of results from this assay could be interpreted.

Conclusions

a) Although much work has been carried out on the development of the Comet assay since 1995, further work on optimising methodology, and then on validation (to assess robustness and reliability) are needed before the method can routinely be used for regulatory purposes.

b) Although work on optimising methodology can be based on the *in vitro* assay, the main value of this assay for genotoxicity testing is the potential to provide a simple *in vivo* assay that may be used in a range of tissues including non-dividing cells.

c) The method does have the potential to provide useful supplementary data on a case-by-case basis to complement the *in vitro* and *in vivo* package of tests. In particular such studies may provide valuable information on direct and indirect mechanisms of DNA damage in target tissues.

Joint COC/COM Symposium on Genetic Susceptibility to Cancer, Department of Health, London, UK, October 1998

2.20 The joint symposium was held on 19 October 1998 at Skipton House, Elephant and Castle, London, UK. The meeting was attended by members of COM and COC and the Committee on Medical Aspects of Radiation in the Environment (COMARE), representatives from the National Radiological Protection Board (NRPB), the Chairman of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), invited speakers, and delegates from Government Departments. A report of this meeting was published in *Mutagenesis* Vol. 14 no. 5 pp.521-525, 1999.

Topics Under Consideration

2.21 The following topics were discussed by the Committee at meetings held in 1998 are still under review:

COM Guidelines for the Testing of Chemicals for Mutagenicity

Mutagenicity of Ozone and the use of Historical Control Data to Evaluate Results from Mutagenicity Studies.

1998 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor J M Parry BSc PhD DSc
Professor of Genetics. School of Biological Sciences, University of Wales, Swansea

Members

Professor J Ashby BSc PhD CChem FRCS
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P Bryant BSc MSc PhD DSc
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Professor C Cooper BSc PhD DSc
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Professor D S Davies BSc PhD CChem FRCS FRCPath FRCP
Section of Bio-Chemical Pharmacology, Hammersmith Hospital

Professor M H L Green BSc PhD
MRC Cell Mutation Unit, University of Sussex

Professor R F Newbold BSc PhD CBiol FIBiol
Professor of Cancer Genetics, Dean of Faculty of Science and Head of Department of Biology and Biochemistry, Brunel University

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Secretariat

R J Fielder BSc PhD Dip RCPath *(Scientific)*

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)	Albright and Wilson Compass Catering JIB Insurance National Power Powergen Smith Kline Beecham	Share Holder Share Holder Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Welsh Water Glaxo Wellcome BAT Astra	Grant Grant Grant Grant Grant Grant
Prof J Ashby	Zeneca ML Labs Phytopharm	Employee, Salary, Share option Share Holder Share Holder	NONE	NONE
Dr P Bryant	British Gas Centrica Abbey National	Share Holder Share Holder Share Holder	NONE	NONE
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE
Prof D S Davies	ICI plc ML Laboratories plc Schering Plough, USA Zeneca plc	Share Holder Non Executive Director and Shareholder Consultantcy Share Holder	Astra Draco Bayer Plc Bristol Myers Squibb Eisai Pharmaceuticals Glaxo Wellcome Hoechst Marion Roussel Invicta Knoll Pharmaceuticals Leo Pharmaceutical Lilly Research E Merck Merck Sharpe & Dohme ML Laboratories Napp Laboratories Orion-Farmos Parke Davis Perkin Elmer Pfizer Procter & Gamble Rhone-Poulenc Rorer Roche Sanofi Winthrop Servier SmithKline Beecham Solvay Zeneca	Fellowship Research & Meeting support Research & Meeting support Support for meeting Advisor Meeting support Research Advisor Research Assistant Studentships Research Meeting support Research & Meeting support Meeting support Research Research & advisor Research & Meeting Support Research & Fellowship Research Research Advisor Support for meeting Meeting support Commissioned research Meeting support Advisor Advisor Research Support for meeting Research Meeting Support Research Studentship Advisor Research Meeting support Research

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof M Green	Dixons Foreign and Colonial Eurotrust HSBC Holdings Marks & Spencer National Westminister Second Alliance Investment Trust Shell Transport TR Property Investment Trust 3I	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	MRC Pension Scheme	Trustee Director
Prof R F Newbold	NONE	NONE	NONE	NONE
Dr D J Tweats	Glaxo Wellcome Halifax Co-operative Bank plc	Salary Employee Share Option Holder Share Holder Share Holder PEP Holder	NONE	NONE
Dr S Venitt	Abbey National plc Northern Rock	Share Holder Share Holder	NONE	NONE

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

Preface

The COC evaluates chemicals for their human carcinogenic potential at the request of the Department of Health and other Government Departments. There have been several major pieces of work undertaken by the Committee this year. These include the statements on coumarin (a component of natural flavourings), 2,3,7,8-TCDD (dioxin), and three substitute-materials (polyvinyl alcohol fibres (PVA), p-aramid fibres and cellulose fibres) intended as replacement products for chrysotile. I am particularly grateful for the efforts and diligence shown by members in respect of the evaluation of chrysotile-substitute fibres where members attended an ad-hoc working group meeting at short notice and reached final conclusions on the subject within a matter of approximately 6-8 weeks. The COC statement on chrysotile-substitutes was influential regarding changes to UK legislation and European policy on chrysotile, a known human carcinogen, which resulted in greater restrictions on its use.

The Committee has also spent a considerable amount of time developing new procedures for greater openness of its work. Significant changes to working practices to be introduced in full during 1999, include the publication of the agenda via the Internet of prior to each meeting, and publication after each meeting of minutes and completed statements and conclusions. Special arrangements will be needed when information is submitted on an in-confidence basis. One outcome is that much of the work referred to in this report has already been published via the Internet and increasing rapidity of publication will become more evident over the next few years.

Professor P G Blain (Chairman)
BMedSci MB PhD FRCP(Lond) FRCP(Edin) FFOM CBiol FIBiol

Coumarin

3.1 Coumarin is a component of natural flavourings and is under active consideration by the EU's Scientific Committee on Food (SCF) and the Council of Europe's Expert Committee on Flavourings (CEFS). It had been shown to have carcinogenic effects in rats, in particular inducing hepatocellular carcinomas and chloangiocarcinomas, although there is uncertainty about its mechanism of carcinogenic action in the rat. The COM concluded that coumarin was clearly genotoxic *in vitro* using standard OECD test systems and that further data were necessary to provide reassurance that it was not genotoxic *in vivo* (bone marrow micronucleus and rat liver UDS assays conducted to current OECD guidelines). The COC were asked to comment on the adequacy and results of the available animal carcinogenicity bioassays. The joint COM/COC statement on coumarin was agreed in August 1998 and was submitted to CEFS for its meeting in October 1998 and also made available on the COC Internet site (see preface to this report for address). The evaluation of the carcinogenicity data is reproduced below (see section of the COM report for the remaining sections of the statement summarising the mutagenicity studies). CEFS agreed with the COM/COC opinion that further *in-vivo* mutagenicity studies with coumarin were required to clarify the potential activity of coumarin. The COC considered the available carcinogenicity bioassays. A brief summary of the critical data is given below:

Carcinogenicity

Rat

(i) Coumarin was fed to groups of 50 male and 50 female Sprague-Dawley rats in the diet at 333, 1000, 2000, 3000 or 5000 ppm for 104 weeks (males) and 110 weeks (females). Rats receiving 333, 1000 or 2000 ppm were also exposed to these dietary levels during gestation and lactation. The maximum tolerated dose (MTD) was exceeded at dietary levels of 2000 ppm and above which may limit the value of these results for human health assessment. A statistically significant increase in cholangiocarcinoma was documented in males and females fed 5000 ppm (approximately 230 mg/kg bw/day in males and 280 mg/kg bw/day in females). A single cholangiocarcinoma was documented in a male rat fed 3000 ppm. Statistically significant increases in hepatocellular adenomas and hepatocellular carcinomas were also documented at 5000 ppm. Increased incidence of non-neoplastic liver pathology was documented in male and female animals at 5000 ppm and, to a lesser extent, at 3000 ppm which included cholangiofibrosis, cystic bile ducts and hepatocellular degeneration.[87,88]

(ii) Coumarin was fed to groups of 40 male and 40 female Sprague-Dawley rats in the diet at 200, 600, 1800 or 5400 ppm for 137 weeks. Satellite groups of 10-40 animals of each sex per dose level were also included in the study to investigate clinical chemical effects and to conduct interim histology. The MTD was clearly exceeded at the top dose level which equated to dose levels of approximately 300-340 mg/kg bw/day. An increase in the incidence of malignant hepatoma was reported in male and female rats at the top dose level.[89,90] Many of the animals fed 5400 ppm were reported to have proliferative cholangiofibrosis of the liver.[89,90] [We understand that a number of these lesions have been subsequently described as cholangiocarcinomas in a peer review of slides].

(iii) Groups of 50 male and 50 female F344 rats were given oral doses of 25, 50 or 100 mg/kg bw/day of coumarin in corn oil for 5 days per week for up to 103 weeks as part of the NTP testing programme. A significant increase in mortality due to nephropathy was seen in males and females at 50 and 100 mg/kg bw/day and only 2 animals in these dose groups survived to termination. An increased incidence of renal tubule adenomas was noted at all dose levels in males, but the increase was only statistically significant at the mid-dose level. A slight increase in renal tubule adenomas was also reported in high dose females. There was no evidence for an increase in the incidence of liver or bile duct tumours in this study although an increase in the incidence of bile duct hyperplasia, and coagulative necrosis/fibrosis and cytologic alterations of liver parenchyma were reported in males and females at the top dose level.[91]

Mouse

(iv) Coumarin was fed to groups of 52 male and 52 female CD1 mice in the diet at 300, 1000 or 3000 ppm for 137 weeks. The intake of coumarin at the top dose level equated to approximately 270-280 mg/kg bw/day. Body weight gain was reduced by 18% and 10% at 3000 ppm and 1000 ppm respectively during the first 52 weeks only. Food utilisation was marginally reduced in the top dose male animals. These data suggest that the MTD was achieved in this study (and may have been exceeded at the high dose level). There was no evidence reported of a treatment related carcinogenic effect in this study.[88]

(v) Groups of 50 male and 50 female $B6C3F_1$ mice were given oral doses of 50, 100 or 200 mg/kg bw/day of coumarin in corn oil for 5 days per week for up to 103 weeks as part of the NTP testing programme. Body weights were approximately 3-10% below controls in top dose males between week 10-81. Mean body weights in females were 3-18% below controls during weeks 11-49 and about 12% lower at the end of the study. These data suggest that the MTD was achieved in this study. Statistically significant increases in the incidence of alveolar/bronchiolar adenomas were reported in top dose male and female animals and alveolar/bronchiolar carcinomas in top dose females. A statistically significant increase in the incidence of hepatocellular adenoma was reported in low and mid dose level females. A marginal increase in the incidence of forestomach papillomas was reported in low dose male and female animals.[91]

3.2 The COC agreed that the finding of lung and forestomach tumours in mice probably represented high dose species-specific effects and were unlikely to be of significance for human health assessment following exposure to low levels of coumarin in the diet. Thus most emphasis was placed on the finding of liver, bile duct and kidney tumours in rats. Members agreed that the renal adenomas seen in F344 rats may have been induced by coumarin. The finding of significant non-neoplastic pathology including nephropathy in male and female animals and renal tubule hyperplasia in male animals suggested that non-genotoxic mechanisms may have played a role in the aetiology of these tumours. Members agreed that the hepatocellular carcinomas and cholangiocarcinomas reported in Sprague-Dawley rats were induced by coumarin, but occurred at dose levels which exceeded the MTD. It was noted that treatment related non-neoplastic pathology including bile duct cysts and hyperplasia and degenerative changes and necrosis of the liver parenchyma, had been documented in Sprague-Dawley rats given the high dose level, suggesting non-genotoxic mechanisms may have played a role.

3.3 However, Members were aware that cholangiocarcinomas had been reported in rats in bioassays where both genotoxic and non-genotoxic chemical carcinogens had been tested and agreed it was not possible to draw any definite conclusions regarding the mechanism of coumarin carcinogenicity in the absence of adequate mutagenicity data on coumarin. It was noted that hepatotoxicity had been reported at a very low incidence (ca 0.2-0.4%) among patients treated with coumarin in respect of cancer or chronic infections.[92,93] The hepatotoxicity in humans may involve an idiosyncratic reaction, but it is not possible to discount the involvement of toxic metabolites. Thus these data strengthened the need for adequate *in vivo* mutagenicity data on coumarin.

Conclusion

3.4 The COM and COC agreed that there were insufficient data to draw any definite conclusions regarding the mechanism of coumarin-induced carcinogenicity in the rat. It was agreed that in view of the evidence for mutagenic effects *in vitro*, the first step should be the provision of adequate *in vivo* mutagenicity data. In this respect adequate rodent bone marrow micronucleus and rat liver UDS assays conducted to the current OECD guidelines were required.

Review of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin: Consideration of IARC Monograph Published in 1997

Introduction

3.5 The COC considered the available epidemiological and experimental data on 2,3,7,8-tetrachlorodibenzo[*b,e*][1,4]dioxin (2,3,7,8-TCDD or TCDD) in 1993 when the Committee concluded "...that there was insufficient evidence for a causal link, but it would be prudent at present to regard TCDD as a possible human carcinogen." This was a similar conclusion to that reached by the IARC in 1987 where TCDD was classified in group 2B (ie possibly carcinogenic to humans). The IARC have undertaken a further review of the literature (and have now concluded that TCDD should be considered as a definite human carcinogen (ie group 1 carcinogen).[94] The conclusion reached by the IARC Working Group may have potential public health implications with respect to the hazard and risk assessment of TCDD and also with respect to other polychlorinated dibenzo dioxins (PCDDs) and polychlorinated dibenzo furans (PCDFs) which are widely dispersed environmental contaminants.

3.6 It was therefore important for the Committee to reconsider its previous conclusion. The Committee reviewed the IARC monograph and specifically the critical epidemiology studies on TCDD cited in the monograph, ie those investigations which considered individuals whose exposure to TCDD occurred under industrial situations and was documented to be substantially higher than background exposures from environmental sources of TCDD.[95,96,97,98,99,100,101,102,103,104,105,106,107,108] The Committee also considered the literature on animal studies and investigations of the carcinogenic mechanism of TCDD in animals as cited in the monograph and a number of recent papers on the toxicological mechansisms of TCDD.[109,110,111,112,113,114,115,116,117,118,119]

3.7 The Committee reached the following conclusions:

Epidemiological data

3.8 There is limited epidemiological evidence in humans for carcinogenicity of 2,3,7,8-TCDD. The Committee reviewed the available epidemiological studies published since the previous COC review completed in 1993. It was noted that the new studies were predominantly comprised of updates of cohort studies previously reviewed in 1993. The Committee was also aware of a publication reporting the most recent results from the IARC multi-country study.[105] Members agreed that all of the limitations previously noted in 1993 applied to the current studies namely;

a) mixed chemical exposures which included some known carcinogens,

b) exposure to PCDDs was due to their presence as low level contaminants of other chemicals such as chlorophenoxy acid herbicides to which the cohorts had much greater exposure and,

c) the lack of data to infer an association with any specific cancer.

3.9 The Committee agreed that the approach used by the IARC Working group to evaluate the epidemiological data on TCDD was satisfactory and the monograph clearly identified the critical studies which all involved the most highly exposed cohorts. The IARC working group calculated a relative risk estimate for total cancer of 1.4 (95% CI 1.2-1.6). The Committee agreed that since its previous evaluation, there was considerably more epidemiological data now available and this was consistent with an increase in overall cancer mortality but concluded that, since no consistent significant association between TCDD and any specific cancer was evident, the epidemiological data should be considered as indicating limited evidence of cancer in humans, ie the same conclusion as that reached by the IARC Working Group.

Animal studies and data on mechanism(s)

3.10 There is sufficient evidence for carcinogenicity of 2,3,7,8-TCDD in animals. The mechanism of carcinogenicity in animals has not been established for individual tumour sites but the available evidence suggests that this might involve tumour promotion. TCDD induced gene expression in laboratory animals is mediated through binding to the Ah receptor protein and this includes the induction of genes involved with control of cell replication but there is no convincing evidence to associate these particular effects with the induction of specific tumours. The COM is to review mutagenicity data on TCDD.

3.11 The IARC working group considered three supporting pieces of evidence when considering their final recommendation that TCDD should be regarded as a definite human carcinogen (ie category 1). The Committee agreed the following response regarding the supporting statements;

Statement 1.

TCDD is a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor.

With regard to statement 1, the Committee agreed that TCDD is a multi-species carcinogen in laboratory animals. The Committee concluded that the available evidence was not sufficient to draw any definite conclusions with regard to the mechanism of carcinogenicity in laboratory animals and it was not possible to comment on the role of the Ah receptor in this regard.

Statement 2.

This receptor is highly conserved in an evolutionary sense and functions in the same way in humans as in experimental animals.

With regard to statement 2, the Committee agreed that there was considerable sequence homology between Ah receptor proteins isolated from laboratory animals and humans. However, there was no adequate information with which to compare Ah induced gene expression in laboratory animals and humans or to identify all of the genes induced in humans. It was therefore not possible to draw any definite conclusions on the potential significance for carcinogenesis of Ah receptor-mediated gene induction in humans.

Statement 3.

Tissue concentrations are similar both in heavily exposed human populations in which an overall increased cancer risk was observed and in rats exposed to carcinogenic dosage regimens in bioassays.

With regard to statement 3, the Committee considered that the comparison of TCDD tissue concentrations using data from rat cancer bioassays and human populations with heavy occupational exposures to PCDD mixtures was inappropriate. Members agreed that such comparisons took insufficient account of the relative differences in toxicokinetics of TCDD between laboratory animals and humans or the different exposure regimes under which the data were obtained. Members agreed that the comparison of life-time exposure in rodents with high level occupational exposure which occurred for varying and proportionately shorter periods in the IARC analysis was not appropriate.

Conclusion

3.12 Members considered that TCDD was a potent carcinogen in laboratory animals. However, the information from the most heavily occupationally exposed cohorts suggested there was at most, only a weak carcinogenic effect in these individuals. The Committee concluded that there were are insufficient epidemiological and toxicological data on TCDD to conclude a causal link with cancer in humans, but it would be prudent to consider TCDD as a 'probable weak human carcinogen'.

Carcinogenic Risks of Three Chrysotile-Substitutes

3.13 Chrysotile is recognised as a known human carcinogen and HSE place a high priority on further restricting the marketing and use of this material. However an interim opinion of the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE) questioned the safety of specific substitute materials and this needs to be resolved. As a way forward HSE commissioned the Institute for Environment and Health to produce a critical evaluation of chrysotile and its substitutes and asked the COC for advice on the conclusions reached by the authors of this review.

3.14 The following statement was agreed in July 1998 and placed on the COC Internet site:

Introduction

(i) The COC has been asked by HSE to provide advice on the relative carcinogenic risks of three chrysotile-substitutes namely, polyvinyl alcohol (PVA) fibres*, p-aramid fibres, and cellulose fibres. The specific question referred by HSE asks whether these three materials pose less of a carcinogenic risk than chrysotile with respect to occupational and consumer health. In view of the urgency of providing DETR Ministers with advice on this issue of chrysotile-substitutes a subgroup of the COC gave initial consideration to the question posed by HSE at a meeting on the 22 May 1998. The subgroup considered four reports provided by HSE along with a number of published scientific investigations. These reports are listed below:

a) Chrysotile and its substitutes: A critical evaluation. An Institute for Environment and Health unpublished report for the Health and Safety Executive, 6 April 1998.

b) A final report by ERM, Oxford for Directorate-General III of the European Commission, entitled "Recent Assessments of the Hazards and Risks Posed by Asbestos and Substitute Fibres, and Recent Regulation of Fibres Worldwide", November 1997.

c) A paper described as a constructive commentary on the June 1997 draft of the ERM report written by Gibbs, Davis, Dunnigan and Nolan, for the Quebec Ministry of Natural Resources, Department of Natural Resources, Canada and the Asbestos Institute, September 1997.

d) The opinion of the DGXXIV SCTEE, on a study commissioned by DG III of the European Commission on recent assessments of the hazards and risks posed by asbestos and substitute fibres prepared by ERM, February 1998.

* Throughout this statement the term "fibre" has been used to refer to airborne material derived from fibrous materials which may be spun woven or felted and used commercially. This is a much broader definition than the term "regulated fibre" as used by WHO or HSE for counting purposes which refers to particles of length >5μm in length, ≤ 3 μm in actual diameter, with an aspect ratio (length to diameter) of ≥ 3. Throughout this statement the terms "regulated fibre " and "respirable fibre" can be used interchangeably.

(ii) The subgroup reviewed all four documents but placed most emphasis on the IEH report.[120] In addition, Dr L Levy, one of the authors of this report made a short presentation of the IEH findings to the subgroup.

(iii) Members of the subgroup considered all the submitted papers and agreed that it was not possible to undertake a full review of the literature in the time available. However, the subgroup agreed that it was possible to undertake a comparative risk assessment of the carcinogenicity of chrysotile-substitutes using available data on their physical properties (eg dimensions and potential for fragmentation) and information which indicated that exposures to respirable fibres would be significantly lower than the control limit for chrysotile of 0.5 f/ml. The subgroup also identified further information which should be provided to the COC in order for conclusions to be reached on the relative carcinogenic risks of the three materials under consideration compared to chrysotile.

(iv) The COC considered the minutes of the subgroup meeting, the report prepared by IEH for HSE and additional data identified by the subgroup at its meeting on 25 June 1998. The Committee also reviewed some additional written comments from IEH. Further confirmatory information on exposures to chrysotile-substitutes was forwarded to the Chairman. This statement reports the conclusions reached by the COC. A summary table of the evidence considered is appended as table 1, annex 1 of this statement.

Background

(v) The World Health Organisation's International Agency for Research on cancer (IARC) has classified chrysotile as a definite human carcinogen (group 1).[120,121] The IARC working group agreed that chrysotile induced lung cancer and pulmonary mesothelioma in humans and also in experimental animals following inhalation exposure.121 The European Union (EU), has classified chrysotile as a definite human carcinogen (ie category 1) and regulatory controls are set out in the Asbestos Directive (91/382/EEC) to limit its uses and control exposure. These measures have been enacted in the UK by the Control of Asbestos at Work Regulations (1987) as amended by the Control of Asbestos at Work Amendment Regulations (1992). These regulations set a control limit of 0.5 chrysotile fibres/ml of air averaged over any continuous period of 4 hours or 1.5 fibres/ml of air averaged over any continuous period of 10 minutes.[122] The Asbestos (Prohibitions) Regulations (1992) ban the import, supply and use of amphibole forms of asbestos, including crocidolite and amosite asbestos. These regulations also ban some specific uses of chrysotile. However chrysotile is now the only type of asbestos that can be supplied and used within the EU. We understand that currently eight out of fifteen Member States have already instituted either total bans or restrictions on chrysotile which exceed the requirement of the current EU Directive. Research undertaken by Peto J et al has suggested that annual male mesothelioma deaths due to exposure to asbestos fibres (eg crocidolite and chrysotile) will increase to around 2,700–3,300 by the year 2020.[123] This prediction is based on an analysis of death certificates and the known long latency period involved in asbestos fibre related disease. The majority of individuals at risk would have probably been occupationally exposed to asbestos prior to the 1960s. HSE report that occupational categories with intermittent exposure to asbestos such as plumbers, carpenters and electricians may be at increased risk of asbestos related disease.[124] There remain many hundreds of thousands of tonnes of asbestos installed in buildings and workplaces across the UK. This material will be progressively removed, presenting considerable management and control problems. Continuing new use of chrysotile, in building products in particular, compounds the overall management problem.

(vi) We consider that any comparative assessment of the carcinogenic risks posed by chrysotile-substitutes and chrysotile cannot be based predominantly on an epidemiological assessment since the substitute fibres have only been used for periods of up to approximately 20 years and occupational hygiene controls have been placed on these materials. Hence exposures to these materials have been of shorter duration and lower intensity compared to exposures to chrysotile which occurred prior to establish-

ing modern hygiene controls on the use of asbestos. Thus we have placed most emphasis on a review of data on fibre dimensions and potential for fragmentation, evidence of biological effects and biopersistence in experimental animal systems and information on likely occupational exposures in use and compared these data to that on chrysotile.

Carcinogenicity of fibres

(vii) General perspectives on the properties of hazardous fibres have been reviewed in the IEH report.[120] A comprehensive review of fibre toxicology has also been published by HSE.[125] We consider that the definition used by the WHO and HSE to identify "regulated fibres" can be used to assess the potential hazard associated with chrysotile-substitutes. Thus a potential carcinogenic hazard may exist with fibres of length > 5μm, diameter ≤ 3μm and with an aspect ratio of ≥3:1. The length of regulated fibres known to be capable of inducing lung cancer are >10 μm and >8-10 μm for the induction of mesothelioma.[125] The potential for fragmentation (ie fibrillation producing potentially hazardous fibrils) also needs to be considered. We note that the predominant physical determinant of deposition in the lung is fibre diameter. For inhaled mineral fibres, the maximum alveolar deposition occurs with fibres of diameters of about 1 mm, whereas fibres of diameter >3μm are essentially non-respirable. Such fibres would not be expected to present a carcinogenic hazard to the lung or induce pleural mesothelioma. We therefore agree that consideration of information on fibre dimensions and information on evidence from animal studies should provide a basis for carcinogenic hazard assessment.

(viii) In order to characterise potential carcinogenic risks of fibres, information on actual exposures and the biopersistence of fibres are also required. We consider biopersistence to be dependent on (i) the mechanical clearance of fibres (ie mucociliary removal up the trachea), (ii) the solubility and fragmentation of deposited fibres and (iii) the biological removal of fibres by lung macrophages.[125] A low biopersistence suggests that the fibre is cleared from the lung and is thus likely to present a lower carcinogenic risk than a fibre of similar carcinogenic potential but which shows evidence of biopersistence.

Consideration of individual chrysotile substitutes

(ix) We have used the criteria outlined in the above section to consider the relevant data identified in table 1 (Annex 1) to produce a short summary of the potential carcinogenic hazard and risk for each of the three chrysotile-substitutes. (All information has been derived from the IEH report, unless otherwise stated).

Polyvinyl alcohol (PVA) fibres

(x) The respirable fraction of PVA fibres is likely to be very small. There is no evidence that PVA will fibrillate. The aspect ratio of the vast majority of airborne PVA fibres is likely to be below 3 suggesting that these fibres will have no potential for the induction of lung cancer or mesothelioma. Although no appropriate animal carcinogenicity bioassays are available, the information on PVA suggests a low carcinogenic hazard. However any PVA fibres deposited in the lung may degrade at a slow rate. The evidence suggests a lower carcinogenic risk than chrysotile. We are also reassured to note that exposures to respirable PVA fibres are likely to be very much below 0.5 f/ml.[126]

p-Aramid fibres

(xi) The respirable fraction of p-aramid fibres is likely to be very small, but limited fibrillation may occur under certain conditions. The aspect ratio for airborne fibres is predominantly above 3.[127] The formation of proliferative keratinising cysts (PKC) of the lung had been documented in a long term inhalation study in the rat, but these authors had used extreme abrasion of p-aramid fibres to produce a large number of respirable fibrils.[128] Thus we consider exposure conditions in this study were unrealistic. The biological behaviour of PKC lung lesions is uncertain and a definitive diagnosis as to

whether they are neoplasms cannot be ascertained at present.[129,130] However, they have only been documented in rats at high exposures where the normal clearance mechanisms of the lung are overloaded and thus we consider them to be of no significance for human health assessment. The reports of the induction of a low level of peritoneal mesotheliomas in rats following intraperitoneal administration of p-aramid fibrous dusts in saline is not considered relevant to hazard assessment. In particular the sample of p-aramid administered to rats had been specially treated (which included drying, milling and ultrasonication) in order to prepare an aqueous saline suspension which could be administered to animals; despite this, the effect obtained was substantially lower than that seen with chrysotile.[131,132] Thus, although there is some evidence of adverse biological effects with p-aramid there is no convincing evidence to suggest a carcinogenic hazard. The rate of degradation of p-aramid fibres in the rat lung has been documented to be faster than chrysotile fibres.[133] The evidence suggests a lower carcinogenic risk than chrysotile. We are also reassured to note that exposures to p-aramid fibres are likely to be below 0.5 f/ml.[126]

Cellulose fibres

(xii) The respirable fraction of cellulose fibres is likely to be very small. It is possible that cellulose fibres could fibrillate, but in practice this appears to be extremely limited.[134] The aspect ratio of airborne cellulose is likely to be variable according to industry and uses. No appropriate animal carcinogenicity bioassays are available. A recent investigation with cellulose fibres had documented evidence of a long biopersistence in the rat lung.[135] However the COC agreed that this study was not relevant to consideration of the question posed by HSE. In particular, the investigators used excessively high doses of respirable cellulose fibres which resulted in overloading of the normal clearance mechanisms of the lung. Information on exposures in the IEH report indicate that respirable fibre counts are consistently below 0.05 f/ml, although occasional levels up to 0.2 f/ml had been documented. The COC undertook a review of the readily available epidemiological investigations considered in one review[136] cited in the IEH report. Members concluded that these studies were inadequate and that it was unlikely that they could identify a carcinogenic response attributable to cellulose fibres.[137,138,139,140]

Discussion and conclusions

(xiii) Our approach has been to undertake a comparative risk assessment based on the information presented to us. The key assumptions in our deliberation are that;

a) carcinogenicity associated with exposure to chrysotile has been clearly demonstrated,

b) the physical properties (ie dimensions and potential for fragmentation) of chrysotile substitutes can be used to indicate potential hazard,

c) adequate epidemiological data are unlikely to become available,

d) occupational exposures to respirable PVA, p-aramid and cellulose fibres will be below the control limit for chrysotile of 0.5 f/ml (4h Time Weighted Average).

(xiv) We therefore conclude:

"The evidence presented to the Committee on fibre dimensions, studies in animals including that of biopersistence in the lung, indicate that the carcinogenic risk posed by PVA fibres, p-aramid fibres or cellulose fibres is likely to be less than that posed by chrysotile. Additional reassurance can be gleaned by noting that these materials are unlikely to form significant amounts of respirable fibres under normal working conditions and that occupational exposures to these materials will be below the control limit for chrysotile."

Comparison of fibre risks

Table 1 Annex 1

FIBRE	HAZARD					EXPOSURE		COMPARATIVE RISK TO CHRYSOTILE			
	FIBRE DIMENSIONS			Animal study	Aspect ratio	Biopersistence	Actual	Potential		Epidemiology	
Risk Factors	Length <5 NC 8-10 LC 10-15 F	Diameter ≤3u *	Fibrillation	Effects reported.	>3:1**	Biopesistence in lung	Actual levels of respirable fibres.!!	Lung cancer	Mesothelioma	Lung cancer	Mesothelioma
CROCIDOLITE	>5u	<1u particularly from fibrillation	++++ depends on processing	F, LC,ME	>3:1	+++ Accumulate	< 0.2 f/ml (control limit 4 h TWA).!!!!!	Human carcinogen	Human carcinogen	+++	++++
CHRYSOTILE	>5u	<1u particularly from fibrillation	+++	F, LC,ME	>3:1	+++ Accumulate	< 0.5 f/ml (control limit 4 h TWA).!!!!!	Human carcinogen	Human carcinogen	++	++ (Role of amosite unclear)
PVA	>5u!	10-16u	Evidence suggests that PVA will not fibrillate.	No relevant study	<3:1	No relevant study. Respired fibres might degrade slowly	< 0.05 f/ml	Lower relative to chysotile	Lower relative to chysotile	No relevant study	No relevant study
ARAMID	>5u!	10-12u	Fibrils may be formed. Need extreme abrasion to produce many.	PKC at 100 & 400 f/ml.!!!	>3:1 (predominantly)	Faster bio-degradation than chrysotile	< 0.5 f/ml	Lower relative to chysotile	Lower relative to chysotile	No relevant study	No relevant study
CELLULOSE	>5u!	12-40u	Possible but available exposure data suggests very limited in practice	No relevant study.	Variable according to industry	No relevant study. !!!!	< 0.5 f/ml	Lower relative to chysotile	Lower relative to chrysotile	No evidence after many years of use, but available studies inadequate for evaluation.	

KEY

Shaded areas refer to data on two forms of asbestos fibres, crocidolite and chrysotile.

The number "+"s have been used to indicate potential for adverse effects.

* Mean actual diameters (respirable limit can be up to 7µm for low density fibres)** Aspect ratio of predominant airborne fibres.

! Data from Asbestos information Centre, Widnes, Cheshire. Fibre lengths; Aramid 3-12 mm(composits), 38 mm (textiles). PVA 4-6 mm, Cellulose 90%> 0.5 mm

NC= No concern. F= Fibrosis, LC = lung cancer, ME = mesothelioma, PKC = proliferating Kertinsising cysts.

!! Data refers to levels of respirable fibres (ie >5µm in length, ≤ 3µm in actual diameter, with an aspect ratio of ≥ 3)

!!! The finding of a very slight increased incidence of mesothelioma in one study using intraperitoneal administration was considered irrelevant by the Committee. (see statement text for details).

!!!! The finding of a high biopersistence in the rat lung in one study was considered irrelevant by the Committee. (see statement text for details).

!!!!! Historically and with poor control exposures were much higher.

2-Chlorobenzylidene Malonitrile (CS).

3.15 The Department of Health asked the COT to advise on the health effects of CS spray when used as a chemical incapacitant. The COT considerations have not, at the time of writing, been finalised but will include an input from its sister committees, the COM and COC with regard to the mutagenic and carcinogenic potential of CS. Such sprays had now been used for some time by police forces in England and Wales and CS had not been subjected to scrutiny by an independent expert advisory committee. It was for this reason, and the potential public health concerns, that the Department of Health considered that such a referral was appropriate at this time. The Committee was aware that any policy decisions on the use of CS spray as a chemical incapacitant were a matter for the Home Office and individual Chief Police Officers, who will need to take account of the COT advice when weighing up the risks and benefits of CS spray.

3.16 The COC was asked by the COM (see Section 2.14 of this report) to specifically consider the adequacy of the NTP carcinogenicity bioassays. Confirmation that these bioassays were adequately conducted and the results were negative is important with regard to finalising the advice on mutagenicity and carcinogenicity.

3.17 The Committee concluded:

i) The NTP carcinogenicity studies provide no evidence that CS had any carcinogenic effects in adequately conduced inhalation bioassays in rats or in mice following 2 year exposure at up to 0.75 mg/m^3 and 1.5 mg/m^3 respectively. These data provide reassurance that CS does not have mutagenic activity *in-vivo* at site of contact tissues, a concern raised by the COM. No further work relating to CS is therefore needed in this area.

Test Strategies and Evaluation

3.18 The Committee has continued to provide advice on strategies for assessing carcinogenic potential in respect of the ongoing discussions of the Genotoxicity Working Party of the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The Committee assessed the available information in respect of the neonatal rodent short-term bioassay for carcinogenicity.

ICH Guidelines: Consideration of Neonatal Rodent Bioassay

3.19 The following conclusions were agreed at the May 1998 COC meeting:

(i) The Committee reviewed the available published information[141,142,143,144] on the neonatal rat and mouse bioassays in the context of the proposal by the ICH for human medicines that these tests could be used to supplement information from pharmacokinetic, mutagenicity and one long-term rodent (usually rat) carcinogenicity bioassay.

(ii) Neonatal mouse and rat carcinogenicity bioassays have been shown to identify genotoxic carcinogens. The Committee agreed that there were very limited validation data on the neonatal mouse bioassay and even fewer data regarding the neonatal rat bioassay. The available information showed tumour yields with genotoxic carcinogens were highly dependent on the strain of animal, age at start of treatment, and treatment protocol.

(iii) Some published information highlighted particular problems with regard to misclassification of carcinogens and non-carcinogens. There was also evidence for considerable animal mortality during studies which raised animal welfare issues. Members also noted that there were potential difficulties in extrapolating from results in neonatal animals derived from parenteral dosing of the test material to other potential routes of human exposure.

(iv) The Committee agreed that there were no validation data regarding the use of short-term neonatal rodent bioassays for the identification of non-genotoxic carcinogens. Furthermore, the use of appropriate mutagenicity testing in addition to one carcinogenicity bioassay in a rodent species would be expected to identify genotoxic carcinogens. Overall, the Committee concluded that there was no current evidence to support the use of the neonatal mouse or rat bioassays as apart of the regulatory testing strategy for human medicines.

Topics Under Consideration

3.20 The following topics, which were discusses by the Committee at meetings held in 1998, are still under review:

Alcohol and Breast Cancer
Certain Organochlorine Pesticides and Breast Cancer
Drinking Water and Cancer.

1998 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor P G Blain BMedSci MB PhD FRCP(Lond) FRCP(Edin) FFOM CBiol FIBiol
Head of Department of Environmental and Occupational Medicine, University of Newcastle

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Head of Molecular Carcinogenesis Section, Institute of Cancer Research, Haddow Laboratories

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Director of DH Toxicology Laboratory, St Bartholomew's Hospital Medical College

Professor P B Farmer MA DPhil CChem FRSC
Professor of Chemistry, MRC Toxicology Unit, University of Leicester

Professor D Forman BA PhD Hon MFPHM
Professor of Cancer Epidemiology, Centre for Cancer Research, University of Leeds

Sandra Jane Kennedy BSc PhD FRCPath CBiol FIBiol
Scientific Director, Quintiles England Limited.

Professor R F Newbold BSc PhD CBiol FIBiol
Professor of Cancer Genetics, Dean of Faculty of Science and Head of Department of Biology and Biochemistry. Brunel University

Professor J M Parry BSc PhD DSc
Professor of Genetics, School of Biological Sciences, University of Wales, Swansea

I F H Purchase OBE BVSc MRCVS PhD FRCPath CBiol FBiol
Director Zeneca Central Toxicology Laboratory until August 1998. Visiting Professor at the School of Biological Sciences, University of Manchester from September 1998.

Professor A G Renwick BSc PhD DSc
Professor of Biochemical Pharmacology, University of Southampton

S Venitt BSc PhD
Institute of Cancer Research, Haddow Laboratories

Professor G T Williams BSc MD FRCP FRCPath
Department of Pathology, University of Wales College of Medicine

Secretariat

J M Battershill BSc MSc *(Scientific)*

K N Mistry *(Administrative)*

R J Fielder BSc PhD Dip RCPath

Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P G Blain (Chairman)	NONE	NONE	Unilever plc	Research Studentship
Prof C Chilvers	ICI plc Tomkins Morgan Crucible Lloyds TSB British Telecom UTD Assurance Group Hardy's & Hanson Glaxo-Wellcome Shell Cadbury Schweppes SmithKline Beecham	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE
Prof A D Dayan	British Petroleum British Gas Research & Technology Dow Agrosciences Glaxo-Wellcome Schering Plough SmithKline Beecham TI	Share Holder Consultant Consultant Pension Consultant Consultant 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
Prof D Forman	Halifax Woolwich	Share Holder Share Holder	NONE	NONE
Dr S J Kennedy	Unilever Quintiles	Share Holder Share Holder	NONE	NONE
Prof R F Newbold	NONE	NONE	NONE	NONE
Prof J M Parry	Albright and Wilson Compass Catering JIB Insurance National Power Powergen SmithKline Beecham	Share Holder Share Holder Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Welsh Water Glaxo/Wellcome BAT Astra	Grant Grant Grant Grant Grant Grant
Dr I F H Purchase	Zeneca Celltec AstraZeneca	Shareholder Shareholder Consultant/Shareholder	NONE	NONE
Prof A G Renwick	International Sweeteners Association	Consultant	Hoffmann-La Roche Unilever Smith Kline Beecham Pfizer FEMA	Research Support Research Support Research Support Grant Grant
Dr S Venitt	Abbey National plc Northern Rock	Share Holder Share Holder	NONE	NONE
Prof G T Williams	Abbey Nationalplc Norwich Union plc AMP Ltd Aphton Corporation	Share Holder Share Holder Share Holder Consultant	NONE	NONE

Annexes

Annex 1

Terms of Reference

To advise at the request of:

Department of Health
Ministry of Agriculture, Fisheries and Food
Department of the Environment, Transport and the Regions
Department of Trade and Industry
Health and Safety Executive
Medicines Control Agency: Section 4 Committees and the Licensing Authority
Committee on the Medical Aspects of Food Policy
Home Office
Scottish Executive
National Assembly for Wales
Northern Ireland Assembly
Other Government Departments

1. To assess and advise on the toxic risk to man of substances which are:

   a. used or proposed to be used as food additives, or used in such a way that they might contaminate food through their use or natural occurrence in agriculture, including horticulture and veterinary practice or in the distribution, storage, preparation, processing or packaging of food;

   b. used or proposed to be used or manufactured or produced in industry, agriculture, food storage or any other workplace;

   c. used or proposed to be used as household goods or toilet goods and preparations;

   d. used or proposed to be used as drugs, when advice is requested by the Medicines Control Agency, Section 4 Committee or the Licensing Authority;

   e. used or proposed to be used or disposed of in such a way as to result in pollution of the environment.

2. To advise on important general principles or new scientific discoveries in connection with toxic risks, to co-ordinate with other bodies concerned with the assessment of toxic risks and to present recommendations for toxicity testing.

Annex 2

Declaration of Interests: a Code of Practice for Members

Introduction

1. This Code of Practice is intended to act as a guide for members of these three Committees as to the circumstances in which they should declare an interest in the chemical industry.

2. The advice of these Committees concerns matters which are connected with the chemical industry and it is therefore desirable that members should have a good understanding of the work of the industry. It is also desirable that some members should have practical experience of the scientific problems of product development. To avoid any public concern that commercial interests might affect the advice of the Committees, it has been decided that the arrangements which govern relationships between members and the chemical industry and information on significant and relevant interests should be on public record.

Definitions

3. In this code, 'the chemical industry' means

   a. companies, partnerships or individuals who are involved with the manufacture, sale or supply of products subject to the following legislation:

      The Food Safety Act 1990
      The Medicines Acts 1968 and 1971
      The Food and Environmental Protection Act 1985
      The Consumer Protection Act 1987
      The Cosmetic (Safety) (Amendment) Regulations 1987
      The Notification of New Substances Regulations 1982

   b. trade associations representing companies involved with such products,

   c. companies, partnerships or individuals who are directly concerned with research, development or marketing of a product which is being considered by the Committees on Toxicity, Mutagenicity or Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

4. In this code 'the Secretariat' means the Secretariat of the relevant Committee.

Different types of interest

5. There are a number of different types of interests and the following is intended only as a guide.

   A **personal interest** involves payment to the member personally. The main examples are:

   Consultancies: any consultancy, directorship, position in or work for the chemical industry, which attracts regular or occasional payments in cash or kind.

   Fee-Paid Work: any work commissioned by the chemical industry for which the member is paid in cash or kind.

Shareholdings: any shareholding in or other beneficial interest in shares of the chemical industry. This does not include shareholdings through unit trusts or similar arrangements where the member has no financial management.

A **non-personal interest** involves payment which benefits a department for which a member is responsible, but is not received by the member personally. The main examples are:

Fellowships: the holding of a fellowship endowed by the chemical industry.

Support by industry: any payment, other support or sponsorship by the chemical industry which does not convey any pecuniary or material benefit to a member personally but which does benefit their position or department, for example:

i. a grant from a company for the running of a unit or department for which a member is - responsible;

ii. a grant or fellowship or other payment to sponsor a post or a member of staff in the unit for which a member is responsible. This does not include financial assistance for students;

iii. the commissioning of research or other work by, or advice from, staff who work in a unit for which the member is responsible.

Trusteeship: where a member is a trustee of a charity with investments in the chemical industry, the Secretariat can agree with the member a general declaration to cover this interest rather than draw up a detailed portfolio.

6. Members are under no obligation to seek out knowledge of work done for or on behalf of the chemical industry within departments for which they are responsible if they would not normally expect to be informed.

7. Members should inform the Department in writing when they are appointed of their *current personal and non-personal* interests. Only the name of the company and the nature of the interest is required; the amount of any salary, fee, shareholding, grant etc need not be disclosed to the Department. An interest is current if the member has an on-going financial involvement with the chemical industry, eg if he or she holds shares in a chemical industry, has a consultancy contract, or if the member or the department for which he or she is responsible is in the process of carrying out work for the chemical industry. Members are asked to inform the Department at any time of any change in their *personal* interests, and will be invited to complete a declaration form once a year. It would be sufficient if changes in *non-personal* interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1000 from a particular company in the previous year need not be declared to the Department.)

8. Members are required to declare relevant interests at Committee meetings, and to state whether they are personal or non-personal interests and whether they are specific to the product under consideration or non-specific.

a. A member must declare a *personal specific* interest if he or she has *at any time* worked on the product under consideration and has personally received payment for that work, in any form, from the chemical industry. If the interest is no longer current, the member may declare it as a *lapsed personal specific* interest. The member may then only take part in the proceedings at the Chairman's discretion.

b. A member must declare a *personal non-specific* interest if he or she has a **current** personal interest in the company concerned which does not relate specifically to the product under discussion. The member may then only take part in the proceedings at the Chairman's discretion.

c. A member must declare a *non-personal specific* interest if he or she is aware that the department for which he or she is responsible has at any time worked on the product but the member has not personally received payment in any form from the industry for the work done. The member may then take part in the proceedings unless the Chairman should decide otherwise.

d. A member must declare a *non-personal non-specific* interest if he or she is aware that the department for which he or she is responsible is *currently* receiving payment from the company concerned which does not relate specifically to the product under discussion. The member may then take part in the proceedings unless the Chairman should decide otherwise.

9. If a member is aware that a product under consideration is or may become a competitor of a product manufactured, sold or supplied by a company in which the member has a *current personal* interest, he or she should declare the interest in the company marketing the rival product.

10. A member who is in any doubt during a meeting as to whether he or she has an interest which should be declared, or whether to take part in the proceedings, should ask the Chairman for guidance. The Chairman has the power to determine whether or not a member with an interest shall take part in the proceedings.

11. If the Chairman should declare an interest of any kind he or she should stand down from the chair for that item and the meeting should be conducted by the Deputy Chairman.

Record of interests

12. A record is kept in the Department of names of members who have declared interests to the Department on appointment, as the interest first arises or through the annual declaration, and the nature of the interest.

13. It is the responsibility of individual members to declare all relevant interests. The Secretariat does not check whether members have done so. However, members can seek advice from the Secretariat if they have any doubts as to whether or not an interest should be declared.

Annex 3

Glossary of Terms

ACUTE Describes a disease of rapid onset, severe symptoms and brief duration.

ADDUCT A chemical grouping which is covalently bound (strong bond formed by the sharing of a pair of electrons) to a large molecule such as DNA (qv) or protein.

ADENOMA (see tumour).

ADI Acceptable daily intake, defined as 'An estimate of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk'.

Ah RECEPTOR The Ah (Aromatic hydrocarbon) receptor protein regulates gene expression. The identity of the natural endogenous chemical which bind to the Ah receptor are unknown. A range of chemical such as chlorinated dibenzodioxins and polychlorinated biphenyls bind to Ah receptor. The available research suggests that binding to the Ah receptor is an intrical part of the toxicological mechanism of these compounds.

ALIQUOT A small quantity.

AMES TEST *in vitro* (qv) assay for bacterial gene mutations (qv) using strains of Salmonella typhimurium developed by Ames and his colleagues.

ANEUGENIC Inducing aneuploidy (qv).

ANEUPLOIDY The circumstances in which the total number of chromosomes within a cell is not an exact multiple of the normal haploid (see 'polyploidy') number. Chromosomes may be lost or gained during cell division.

APOPTOSIS "Programmed" cell death, which is part of normal cell growth and differentiation.

ASSAY A procedure for measurement or identification.

ATOPY Predisposition to IgE production associated with allergy to several common allergens.

BIAS An inference which at any stage of an epidemiological investigation tends to produce results that depart systemically from the true values (to be distinguished from random error). The term does not necessarily carry an imputation of prejudice or any other subjective factor such as the experimenter's desire for a particular outcome.

BIOAVAILABILITY A term referring to the proportion of a substance which reaches the systemic circulation unchanged after a particular route of administration.

CARCINOGENICITY BIOASSAY Tests carried out in laboratory animals, usually rats and mice, to determine whether a substance is carcinogenic. The test material is given, usually in the diet, throughout life to groups of animals, at different dose levels.

CARCINOGENIC POTENCY The magnitude, with respect to dose, of the carcinogenic activity of a chemical in the species under consideration. Inherent in this definition are a dose descriptor (defined here as a dose needed to induce tumours) and other elements bearing on carcinogenic potency including dose-response relationships, site/species/strain/gender activity and degree of malignancy, mechanisms including genotoxicity, other mechanistic information relevant to humans and toxicokinetic properties of the substance.

CARCINOGENESIS The origin, causation and development of tumours. The term applies to all forms of tumours, benign as well as malignant (see 'tumour') and not just to carcinomas (qv).

CARCINOGENS The causal agents which induce tumours. They include external factors (chemicals, physical agents, viruses) and internal factors such as hormones. Chemical carcinogens are structurally diverse and include naturally-occurring substances as well as synthetic compounds. An important distinction can be drawn between *genotoxic* (qv) carcinogens which have been shown to react directly with and mutate DNA, and non-genotoxic carcinogens which act through other mechanisms. The activity of genotoxic carcinogens can often be predicted from their chemical structure – either of the parent compound or of activated metabolites (qv). Most chemical carcinogens exert their effects after prolonged exposure, show a dose-response relationship and tend to act on a limited range of susceptible target tissues. Carcinogens are sometimes species- or sex-specific and the term should be qualified by the appropriate descriptive adjectives to aid clarity. Several different chemical and other carcinogens may interact, and constitutional factors (genetic susceptibility, hormonal status) may also contribute, emphasising the multifactorial nature of the carcinogenic process.

CARCINOMA Malignant tumour arising from epithelial cells lining, for example, the alimentary, respiratory and urogenital tracts and from epidermis, also from solid viscera such as the liver, pancreas, kidneys and some endocrine glands. (See also 'tumour').

CASE-CONTROL STUDY (Synonyms – case comparison study, case referent study) A study that starts with the identification of persons with the disease of interest and a suitable control group of persons without the disease. The relationship of some attribute to the disease (such as occupational exposure to a carcinogen) is examined by comparing the disease and nondiseased with regard to how frequently the attribute is implicated in each of the groups.

CELL TRANSFORMATION ASSAY See Transformation.

CHROMOSOME ABERRATION Collective term of particular types of chromosome damage induced after exposure to exogenous chemical or physical agents which damage the DNA. (see clastogen).

CHRONIC Describing a disease of long duration involving very slow changes. Such disease is often of gradual onset. The term does not imply anything about the severity of the disease.

CLASTOGEN An agent that produces chromosome breaks and other structural aberrations such as translocations (qv). Clastogens may be viruses or physical agents as well as chemicals. Clastogenic events play an important part in the development of some tumours.

COHORT A defined population.

COHORT STUDY (Synonyms – follow-up, longitudinal, prospective study) The method of epidemiological study in which subsets of a defined population can be identified who may be exposed to a factor or factors hypothesized to influence the probability of occurrence of a given disease. An essential feature of the method is observation of the population for a sufficient number of person-years to generate reliable incidence or mortality rates in the population subsets. This generally implies study of a large population and/or study for a prolonged period of time.

COVALENT The type of binding formed by the sharing of an electron pair between two atoms. Molecules are combinations of atoms bound together by covalent bonds.

CYTOCHROME Haem proteins that catalyse electron transfer reactions. Cytochrome P450 is a collective term for an extensive family of haem proteins involved in enzymic oxidation of a wide range of substances and their conversion to forms that are more easily excreted. In some cases the metabolites produced may be reactive and may have carcinogenic potential.

CYTOGENETIC Concerning chromosomes, their origin, structure and function.

CYTOGENETICS ASSAY An assay for detecting damage to the genome of a cell.

DNA (DEOXYRIBONUCLEIC ACID) The carrier of genetic information for all living organisms except the group of RNA viruses. Each of the 46 chromosomes in normal human cells consists of 2 strands of DNA containing up to 100,000 nucleotides, specific sequences of which make up genes (qv). DNA itself is composed of two interwound chains of linked nucleotides, each nucleotide consisting of 3 elements: a pentose sugar, a phosphate group and a nitrogenous base derived from either purine (adenine, guanine) or pyrimidine (cytosine, thymine).

DNA GYRASE A bacterial enzyme which contributes to the maintenance of the 3-dimensional structure of DNA.

DOMINANT LETHAL ASSAY See Dominant Lethal mutation.

DOMINANT LETHAL MUTATION A dominant mutation that causes death of an early embryo.

EMULSIFIER A compound or preparation that facilitates the formation of emulsions.

EPIDEMIOLOGY Study of the distribution and, in some instances, the causal factors of disease in communities and populations.

EPITHELIUM The tissue covering the outer surface of the body, the mucous membranes and cavities of the body.

EPOXIDE A compound containing an epoxy group (an oxygen atom bound to two other atoms, usually carbon) as part of its chemical structure. Epoxy groups are usually reactive and an epoxy group and/or the ability to form an epoxy group is considered an "alerting" structure for genotoxic activity (ie interaction with DNA).

EQUOL A naturally occurring compound with oestrogen-like properties.

ERYTHROCYTE Red blood cell.

ESTER Compound formed by the reaction of an alcohol with an acid.

EXOGENOUS Arising outside the body.

F1 First filial generation – offspring resulting from the (specified) parental generation.

FIBROSIS: A thickening and scarring of connective tissue most often the consequence of inflammation or injury.

FOETOTOXIC Causing toxic, potentially lethal effects to the developing foetus.

FORESTOMACH (See glandular stomach).

GASTROSCHISIS A congenital malformation of the abdominal wall.

GENE The functional unit of inheritance: a specific sequence of nucleotides along the DNA molecule, forming part of a chromosome.

GENOTOXIC The ability of a substance to cause DNA damage, either directly or after metabolic activation (see also 'carcinogens).

GERM CELL Reproductive cell eg spermatid.

HEPATIC Pertaining to the liver (see hepatocellular).

HEPATOCELLULAR Relating to the cells of the liver.

HEPATOTOXIC Causing damage to the liver.

HISTOLOGY The study of the minute structure of tissues.

HOMOLOGY Having a similar structure.

HYPERPLASIA An increase in the size of organs and tissues due to an increase in the total numbers of the normal cell constituents.

IgE One of the five main classes of human immunoglobulin. IgE is involved in allergy and anaphylaxis as well as protecting against intestinal parasites. IgE-mediated hypersensitivity is characterised by the speedy release of mediators such as histamine.

INTERESTERIFIED A chemical process by which a chemical group (ester) is added to a molecule.

INTRAPERITONEAL Within the abdominal cavity.

IN VITRO A Latin term used to describe effects in biological material outside the living animal.

IN VIVO A Latin term used to describe effects in living animals.

IPCS The World Health Organization's International Programme on Chemical Safety.

ISOFLAVONOID PHYTOESTROGEN A particular type of phytoestrogen.

LEUKAEMIA A group of neoplastic disorders (see tumour) affecting blood-forming elements in the bone marrow, characterised by uncontrolled proliferation and disordered differentiation (qv) or maturation (stage which forms final cell types). Examples include the lymphocytic leukaemias which develop from lymphoid (qv) cells and the myeloid leukaemias which are derived from myeloid cells (producing red blood cells, mainly in bone marrow).

LD50 The dose of a toxic compound that causes death in 50% of a group of experimental animals to which it is administered. It can be used to assess the acute toxicity of a compound.

LIGAND A molecule which binds to a receptor.

LOWEST OBSERVED ADVERSE EFFECT LEVEL (LOAEL) The lowest administered dose at which a toxic effect has been observed.

LYMPHOCYTE Type of white blood cell.

LYMPHOMA Malignant tumours arising from lymphoid tissues. They are usually multifocal, involving lymph nodes, spleen, thymus and sometimes bone marrow and other sites outside the anatomically defined lymphoid system. (See also 'tumour').

MACROPHAGE Scavenging cells found in tissues, such as the lung, and in circulating blood (where they are known as monocytes). They ingest foreign material such as bacteria and form part of the normal defence system of the body.

MALIGNANCY See 'tumour'.

MAXIMUM TOLERATED DOSE Usually the highest dose used in a carcinogenicity bioassay (qv). Commonly chosen from a preliminary 90 day study and set at the highest dose at which there is no organ toxicity or gross functional effect. If there is no specific toxicity the dose which will cause a 10% reduction in weight gain over the life span of the animals is conventionally used.

MESOTHELIOMA A rare tumour, usually malignant (see tumour), which develops from the thin, flattened (mesothelial) cells which line the lung, heart and abdominal cavities. The commonest cause of mesothelioma is asbestos.

METABOLIC ACTIVATION Conversion by enzymes of a chemical from one state to another, for example by chemical reactions such as hydroxylation, epoxidation or conjugation. The term is used in a more narrow sense to describe the addition of a mammalian cell free preparation from livers of rats pre-treated with a substance which stimulates production of metabolising enzymes. These preparations are added to *in vitro* short-term tests to mimic the metabolic activation typical of mammals.

METABOLISM Changes made to a compound by biological systems to modify it's properties.

METABOLITE Product formed from the original compound by enzymic reactions in the body/cell.

METAPHASE Stage of cell division (mitosis and meiosis) during which the chromosomes are arranged on the equator of the nuclear spindle (the collection of microtubule filaments which are responsible for the movement of chromosomes during cell division). As the chromosomes are most easily examined in metaphase, cells are arrested at this stage for microscopical examination for chromosome aberrations (qv) – known as metaphase analysis.

MICRONUCLEI Isolated or broken chromosome fragments which are not expelled when the nucleus is lost during cell division, but remain in the body of the cell forming micronuclei. Centromere positive micronuclei contain DNA and/or protein material derived from the centromere (qv). The presence of centromere positive micronuclei following exposure to chemicals can be used to evaluate the aneugenic (qv) potential of chemicals.

MICRONUCLEUS TEST See Micronuclei.

MITOSIS The type of cell division which occurs in somatic cells when they proliferate. Each daughter cell has the same complement as the parent cell.

MOUSE LYMPHOMA ASSAY An *in vitro* assay for gene mutation in mammalian cells using a mouse lymphoma cell line L5178Y, which is heterozygous for the gene (carries only one functional gene rather than a pair) for the enzyme thymidine kinase ($TK^{+/-}$). Mutation of that single gene is measured by resistance to toxic trifluorothymidine. Mutant cells produce two forms of colony – large, which represent mutations within the gene and small, which represent large genetic changes in the chromosome such as chromosome aberrations. Thus this assay can provide additional information about the type of mutation which has occurred if colony size is scored.

MOUSE SPOT TEST An *in vivo* test for mutation, in which pregnant mice are dosed with the test compound and mutations are detected by changes (spots) in coat colour of the offspring. Mutations in the melanocytes (skin pigment cells) of the developing foetus are measured.

MRC Medical Research Council.

MUTATION A permanent change in the amount or structure of the genetic material in an organism which can result in a change in the characteristics of the organism. The alternation may involve a single gene, a block of genes, or a whole chromosome. Mutations involving single genes may be a consequence of effects on single DNA bases (point mutations) or of large changes, including deletions, within the gene. Changes involving whole chromosomes may be numerical or structural. A mutation in the germ cells of sexually reproducing organisms may be transmitted to the offspring, whereas a mutation that occurs in somatic cells may be transferred only to descendent daughter cells.

MYCOTOXIN Toxic compound produced by a fungus.

NEOPLASM See 'tumour'.

NEUROTOXIC EFFECTS Harmful effects on nerve cells.

NON-GENOTOXIC See 'carcinogens'.

NON-HODGKIN'S LYMPHOMA (NHL) Lymphomas are classified as Hodgkins or Non-Hodgkins based on their histological appearance (the structure of the tissues). Hodgkins Disease is characterized by destruction of the normal architecture of the lymph nodes and replacement with giant cells. Non-Hodgkins lymphomas lack this distinctive feature.

NO OBSERVED ADVERSE EFFECT LEVEL (NOAEL) The highest administered dose at which no toxic effect has been observed.

ODDS RATIO (OR) A measure of association which is interpreted similarly to the Relative Risk (see Relative Risk); it is similar in magnitude to the Relative Risk in the case of rare diseases.

OECD Organization for Economic Cooperation and Development.

OESTROGEN Is the hormone which develops and maintain female bodily characteristics.

ORGANOPHOSPHATES A group of chemical compounds used as pesticides and medicines.

PARENCHYMA The functional part of an organ, as opposed to the support tissue.

PARENTERAL The word applied to the administration of substances to an animal or to man by any route other than by the mouth or by the bowel.

PATHOGENICITY Particular type of adverse effect caused by an infectious external micro organism.

PHENOLIC RINGS Six carbon rings with a hydroxyl (-OH) group attached.

PERITONEAL MESOTHELIOMA A rare tumour, usually malignant (see 'tumour') which develops from the thin, flattened (mesothial) cells which line the abdominal cavity.

PEROXISOME PROLIFERATION Increased formation of the certain organelles in the cell.

PHYTOESTROGEN Phytoestrogens are plant chemicals that are similar to the human female hormone oestrogen but are much less potent (10,000–140,000 times less potent in animal models).

PLASTICISER A substance which increases the flexibility of certain plastics.

POLYMERIC A form of chemical structure, e.g. a plastic, made up of joined monomers (qv).

POLYMORPHISM A condition in which a genetic character occurs in more than one form.

POLYPLOIDY Having three or more times the haploid (single set of unpaired chromosomes as found in germ cells) number of chromosomes. Somatic cells from animals generally contain a diploid set of chromosomes, with pairs of equivalent chromosomes, so that twice the haploid number are present.

POLYURETHANE A thermoplastic polymer produced from the condensation of a polyisocyanate and a hydroxyl containing material. Polyurethane coatings have excellent hardness, flexibility and abrasion resistance.

POSTNATAL The period after birth.

RECEPTOR A small, discrete area on the cell membrane or within the cell with which specific molecules interact to initiate a change in the working of a cell.

RELATIVE RISK A measure of the association between exposure and outcome. The rate of disease in the exposed population divided by the rate of disease among the unexposed population in a cohort study. A RR of 2 means that the exposed group has twice the disease risk compared to the unexposed group.

RENAL Relating to the kidney.

RESORPTION A concept which, having been implanted in the uterus, subsequently died and is being, or has been, resorbed.

SARCOMA Malignant tumour arising from connective tissues such as fat, cartilage or bone. (See also 'tumour').

SCF The European Commission's Scientific Committee on Food.

SERUM The fluid remaining after blood has clotted.

SISTER CHROMATID EXCHANGE (SCE) Exchange of genetic material between two sub-units of a replicated chromosome.

SOMATIC Occurring in cells of the body other than germ cells (see mutation).

TERATOGENIC ACTIVITY Ability to cause developmental abnormalities in the foetus.

TERATOGENESIS STUDY A study of developmental abnormalities in the foetus and their causes.

TERATOLOGY The study of development abnormalities and their causes.

THRESHOLD The lowest dose which will produce a toxic effect and below which no toxicity is observed.

TOXIC EQUIVALENCY FACTOR (TEF) A measure of relative toxicological potency of a chemical compared to a well characterised reference compound. TEFs can be used to sum the toxicological potency of a mixture of chemicals which are all members of the same chemical class, having common structural, toxicological and biochemical properties. Systems have been published for chlorinated dibenzodioxins and dibenzofurans and for polycylic aromatic hydrocarbons.

TOXICOKINETICS The description of the fate of chemicals in the body, including a mathematical account of their absorption, distribution, metabolism and excretion.

TRANSFORMATION The process by which a normal cell acquires the capacity for neoplastic growth. Complete transformation occurs in several stages both *in vitro* and *in vivo*. One step which has been identified *in vitro* is 'immortalisation' by which a cell acquires the ability to divide indefinitely in culture. Such cells do not have the capacity to form tumours in animals, but can be induced to do so by extended passage *in vitro*, by treatment with chemicals, or by transfection with oncogene DNA. The transformed phenotype so generated is usually, but not always, associated with the ability of the cells to grow in soft agar and to form tumours when transplanted into animals. It should be noted that each of these stages of transformation can involve multiple events which may or may not be genetic. The order in which these events take place, if they occur at all, *in vivo* is not known.

TRANSGENIC Genetically modified to contain genetic material from another species (see also genetically modified organism).

TRIGLYCERIDES Esters formed from one molecule of glycerol and three fatty acid molecules, found in fat.

TUMOUR (Synonym – neoplasm) A mass of abnormal, disorganised cells, arising from pre-existing tissue, which are characterised by excessive and uncoordinated proliferation and by abnormal differentiation (qv). BENIGN tumours show a close morphological resemblance to their tissue of origin; grow in a slow expansile fashion; and form circumscribed and (usually) encapsulated masses. They may stop growing and they may regress. Benign tumours do not infiltrate through local tissues and they do not metastasise (qv). They are rarely fatal. MALIGNANT tumours (synonym – cancer) resemble their parent tissues less closely and are composed of increasingly abnormal cells in terms of their form and function. Well differentiated examples still retain recognizable features of their tissue of origin but these characteristics are progressively lost in moderately and poorly differentiated malignancies: undifferentiated or anaplastic tumours are composed of cells which resemble no known normal tissue. Most malignant tumours grow rapidly, spread progressively through adjacent tissues and metastasise to distant sites. Tumours are conventionally classified according to the anatomical site of the primary tumour and its microscopical appearance, rather than by cause. Some common examples of nomenclature are as follows:

> Tumours arising from epithelia (qv): *benign* – adenomas, papillomas; *malignant* – adenocarcinomas, papillary carcinomas.
>
> Tumours arising from connective tissues such as fat, cartilage or bone: *benign* – lipomas, chondromas, osteomas; *malignant* – fibrosarcomas, liposarcomas, chondrosarcomas, osteosarcomas. Tumours arising from lymphoid tissues are malignant and are called lymphomas (qv); they are often multifocal. Malignant proliferations of bone marrow cells are called leukaemias. Benign tumours may evolve to the corresponding malignant tumours; examples involve the adenoma -> carcinoma sequence in the large bowel in humans, and the papilloma -> carcinoma sequence in mouse skin.

TUMOUR PROMOTION An increasingly confusing term, originally used, like 'tumour initiation', to describe events in multistage carcinogenesis in experimental animals. In that context, promotion is regarded as the protracted process whereby initiated cells undergo clonal expansion to form overt tumours. The mechanisms of clonal expansion are diverse, but include direct stimulation of cell proliferation, repeated cycles of cell damage and cell regeneration and release of cells from normal growth-controlling mechanisms. Initiating and promoting agents were originally regarded as separate categories, but the distinction between them is becoming increasingly hard to sustain. The various modes of promotion are non-genotoxic, but it is incorrect to conclude that 'non-genotoxic carcinogen' and 'promoter' are synonymous.

TUMOUR SUPPRESSOR GENE (Synonym – anti-oncogene, recessive oncogene). A gene whose continued expression is thought to be essential for normal growth and differentiation of cells. Many tumour suppressor genes probably exist, deletion or suppression of which appears to be a critical event in tumour development.

UDS See Unscheduled DNA Synthesis.

UNSCHEDULED DNA SYNTHESIS (UDS) DNA synthesis that occurs at some stage in the cell cycle other than the S period (the normal or 'scheduled' DNA synthesis period) in response to DNA damage. It is usually associated with DNA repair.

UMU TEST The *umu* test (essentially similar to the SOS-chromotest) is based on *Salmonella typhimurium* TA 1535/pSK1002 and measuring induction of the *umu* C operon by DNA damage using â- galactosidase activity as an indicator. The *umu* test has not been considered by the OECD and has not been validated for regulatory purposes and is not suited to the mutagenicity assessment of complex mixtures of chemicals in water samples.

WHO-ECEH/IPCS The World Health Organization's European Centre for Environment and Health and the WHO's International Programme on Chemical Safety.

XENOBIOTIC A chemical foreign to the biologic system.

Annex 4

Index to Subjects and Substances considered in previous Annual Reports of the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Annex 5

Index of Considerations by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment of MAFF Food Surveillance Papers

Subject	FSP No.	Page	Year
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Annex 6

Publications Produced by the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

1991 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321529 0 Price £9.50.

1992 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321604-1 Price £11.70.

1993 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321808-7 Price £11.95.

1994 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321912-1 Price £12.50.

1995 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321988-1 Price £18.50.

1996 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. The Stationery Office ISBN 0 11 322115-0 Price £19.50.

1997 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Department of Health.

Guidelines for the Testing of Chemicals for Toxicity. DHSS Report on Health and Social Subjects 27. HMSO ISBN 0 11 320815 4 Price £4.30.

Guidelines for the Evaluation of Chemicals for Carcinogenicity. DH Report on Health and Social Subjects 42. HMSO ISBN 0 11 321453 7 Price £7.30.

Guidelines for the Testing of Chemicals for Mutagenicity. DH Report on Health and Social Subjects 35. HMSO ISBN 0 11 321222 4 Price £6.80.

Guidelines for the Preparation of Summaries of Data on Chemicals in Food, Consumer Products and the Environment submitted to DHSS Report on Health and Social Subjects 30. HMSO ISBN 0 11 321063 9 Price £2.70.

Annex 7

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