Department of Health

1996 Report of the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Dd 3221150 C6 01/98

published by The Stationery Office as
ISBN 0 11 322115 0

19.50 [sterling]

Contents

About the Committees

This is the sixth 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 Committees 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 Chairmans 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 1987 as part of its consideration of the results of surveillance for chemicals in the UK diet. These considerations are published in the Food Surveillance Papers which report this surveillance work, rather than in the Committees annual reports.

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 industry 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, the Advisory Committee on Pesticides and the Steering Group on Chemical Aspects of Food Surveillance.

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

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

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

Annexes

Terms of Referenece

Declaration of Interests: a Code of Practice for Members

Glossary of Terms

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

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

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

Terms of Referenece

To advise at the request of:

Department of Health
Ministry of Agriculture, Fisheries and Food
Department of the Environment
Department of Trade and Industry
Department of Transport
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 Home and Health Department
Department of Agriculture and Fisheries for Scotland
Welsh Office
Department of Health and Social Services for Northern Ireland
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.

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

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

Plasticisers: Continuing Surveillance	30	27-32	1990
Di-2-ethylhexyl adipate		28-29	
Acetyl tributyl citrate		29-30	
Polymeric plasticisers		30	
Epoxidised soya bean oil		30-31	
Azelates		31	
Phthalates		31	
Baby foods		31	
Dioxins in Food	31	46-50	1992
Nitrate, Nitrite and N-Nitroso Compounds in Food: Second Report	32	39-43	1992
Veterinary Residues and Animal Products: 1986-90	33	54-59	1992
Stibines		54-55	
Growth-promoting hormones		55	
Sulphonamides		55	
Chloramphenicol		55	
Furazolidone		56	
Tetracyclines		56	
Other antimicrobial agents		56	
Clenbuterol		56	
Benzimidazoles		56-57	
Levamisole		57	
Nitroxynil		57	
Lasalocid		57-58	
Oxolinic acid		58	
Oxytetracycline		58	
Report of the Working Party on Pesticides Residues: 1988-90	34	83-88	1992
Mycotoxins: Third Report	36	59-63	1993
Aflatoxins		59	
Ochratoxin A		59-60	
Moniliformin		60	
Patulin		61	
Aluminium in Food	39	49	1993
Naturally occurring Toxicants in Food	42	50-54	1994
Aquatic biotoxins		50	

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

Professor H F Woods
(Chairman)
BSc BM BCh MRCP DPhil
FIFST FRCP(Lon & Edin)
FFOM
FFPM Hon FFOM

Preface

In my preface to the 1995 annual report I pointed out the changing pattern of advisory work carried out by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). The new pattern is now well established, as exemplified by the list of chemicals and other toxicological matters considered by the Committee during the year. In addition there has been a wider use of our expertise in relation to certain important, more general topics and I refer to these below.

During the year we considered new data concerning the use of chlorine and chlorine dioxide as flour treatment agents. This work reveals a good example of our working relationship with our sister committees; in this case the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment. It also demonstrates the complex chemical analyses upon which some of our work depends. A further example of chemical toxicology is our consideration of a new platinum-based fuel additive for diesel fuel. In this case, and in others, the COT were able to indicate additional studies which would be helpful to future toxicological assessments. Our expertise in this regard was sought by the Department of Health on its research strategy in chemical toxicology and the prioritisation of research topics in this area of work.

A subject of considerable public interest, namely the potential hazards of phthalates in infant formulae, was the subject of a review in the light of MAFF surveillance work on phthalates in food. The review led to a statement which was published and appears in full in this report. This general area of chemical contaminants and inherent toxicants in food is further considered in relation to the Food Surveillance Paper. The work undertaken by the COT revealed considerable problems, in particular concerning the data available on the human and animal toxicity of some inherent natural toxicants. It also led to a recommendation that one approach may be through a risk-benefit assessment which took into consideration the bioavailability of the toxicant together with possible benefits of the food(s) in which it occurred.

The COT has a close working relationship with the Advisory Committee on Novel Foods and Processes and the Food Advisory Committee (FAC). Examples of work linked to the former are the assessments of hemicellulase preparations for use in bread making and that of single cell protein. These examples demonstrate the key role of toxicological assessment in a consideration of the safety of genetically modified products. So far as the FAC is concerned, much of our work with them was unpublished and I am very pleased to see that now it is reported in the Food Safety Information Bulletin jointly published by MAFF and DH.

A new role for the COT is shown by the setting up of a Working Group to consider Peanut Allergy. The report of the group, which will include a comprehensive review of the subject together with advice to pregnant women, mothers and other carers will be published in early 1998. This work has utilised the committees considerable expertise in the field of

immunotoxicology. A further example is the establishment of a Working Group to consider the problem of food intolerance in relation to hyperactivity in children.

The Committee gratefully acknowledges the work of its secretariat and that of other officials from the Department of Health, MAFF and other government departments, who have given most excellent support and high quality information to facilitate its work during the year.

Frank Woods

Chlorine and Chlorine Dioxide as Flour Treatment Agents

1.1 During 1996 the COT considered the use of chlorine and chlorine dioxide as agents in the treatment of flour and agreed the following statements:

Chlorine

i. In 1993 the Committee had reviewed the results of a 90-day study in rats fed fractions of cake made from chlorinated flour. No adverse toxicological effects were reported in this study and larger safety margins were derived than from previous studies. However, the results indicated that chlorinated material was deposited in peri-renal fat samples and the predominant chlorinated compound was tentatively identified as 9,10-dichlorooctadecanoic acid (DCOA). The Committee advised at that time that it was necessary for Industry to confirm the presence, and to quantify the levels, of DCOA in these samples.

ii. The Committee has now reviewed the results of analytical studies carried out to address this request. These studies are of limited value due to problems with the methodology, the small number of samples analysed, and results which are difficult to interpret. They provide some further evidence that DCOA is a major constituent of chlorinated material in the fat pads from rats fed the lipid fraction of cake made from chlorinated flour but also indicate that other, unidentified materials may be present. These may also be important in toxicological terms. The Committee *considers* that further information is still required on the persistence and turnover of chlorinated compounds, particularly DCOA, in the body fat of rats fed fractions of cake made from chlorinated flour.

iii. The Committee is aware of literature data which indicate that chlorinated fatty acids can cross the placenta and be mobilised into milk in rats. Decreased weaning weights were reported in a very limited study in which lipids from chlorinated flour were fed to rats through three generations. It considers that further reassurance is required about the potential reproductive toxicity of chlorinated flour and *recommends* that a multigeneration study is carried out in rats. Since the 90-day study indicated that most chlorinated material was contained in the lipid fraction of cake made from chlorinated flour, and as this is the fraction most likely to be absorbed, it would be adequate for the multigeneration study to be carried out on this fraction alone. The results of this study should be submitted within 3 years.

iv. The COT has been advised by the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment that studies it has reviewed provide adequate reassurance as to the lack of any mutagenic potential resulting from the use of chlorine to treat flour used to bake cakes and that it requires no further work. Studies carried out in the 1970s in which animals were fed diets containing cake made from chlorinated flour showed no effects of concern, although the safety margins which can be derived from these studies are less than ideal because of the limitations on the amounts of cake which could be fed. These data, the lack of toxic effects seen in one study and the larger safety margins provided by this study, are sufficient to enable the Committee to *advise* that chlorine as a flour treatment agent remains temporarily acceptable pending receipt and evaluation of the studies described above.

Chlorine Dioxide

i. In 1990 the COM advised that both chlorine dioxide and its breakdown products should be regarded as potential mutagens. On the basis of this advice, the COT recommended that analytical studies should be carried out on the fate of chlorine dioxide following reaction with flour and on residues of chlorine dioxide itself, using a sensitive method of detection. It further recommended that these studies should ideally be done on both treated flour and on bread baked from it under commercial conditions. The need for further toxicity studies would be considered once

the results of the analytical studies were evaluated. The COT also recognised that the mutagenicity studies which the COM had asked for on chlorine would be relevant to the evaluation of chlorine dioxide.

ii. The COM is now in the process of reviewing the results of new mutagenicity studies on extracts of flour treated with chlorine dioxide. Initial advice is that the results are reassuring but the COM has asked for further details of the studies to be supplied before a final conclusion can be reached.

iii. The COT considers that analytical work is still required to determine whether potentially harmful reaction products and residues are present in flour following treatment with chlorine dioxide. Therefore, the Committee *reaffirms* its requirement for the analytical work described in paragraph 5. Further toxicity studies may be required following receipt of the results of the analytical work. The Committee *recommends* that the current temporary approval for the use chlorine dioxide as a flour treatment agent should be extended for a further 12 month period pending receipt and evaluation of this analytical work.

Department of Health Research Strategy on Chemicals

1.2 In the course of the year the Committee, with its sister Committees on Carcinogenicity and Mutagenicity, provided advice to the Department on a research strategy appropriate to toxicological research on chemicals which might be present in food, consumer products and the environment. The advice included the prioritisation of research topics.

Hemicellulase Preparations for use in Bread Making

1.3 During 1996, the COT considered submissions from a number of companies on preparations of hemicellulase produced by different microorganisms. Two of the companies used genetically modified microorganisms (GMOs). Whereas the COT considers the toxicity of the enzyme preparation, the Advisory Committee on Novel Foods and Processes (ACNFP), a joint committee of the Department of Health and the Ministry of Agriculture, Fisheries and Food, considers the safety of the use of GMOs. The production of hemicellulase preparations is relatively simple, involving the fermentation of the microorganism, extraction of extracellular enzymes, removal of microbial debris, ultrafiltration and formulation into the final commercial product. All of the enzyme preparations were intended for use in the manufacture of bread. The submission on each preparation was considered with respect to the Committee's guidelines for the safety assessment of microbial enzyme preparations used in food. In three instances, the Committee reviewed additional data it had requested when it considered the original submission. The Committee was satisfied with the safety in use of these enzyme preparations and agreed to recommended full approval for use in bread making - in one case following clarification of a minor point. In the fourth instance, following review of the original submission, the Committee asked for clarification of two minor points, but this did not preclude it agreeing to recommend temporary approval for the use of this enzyme preparation in bread making.

Hyperactive Children's Support Group

1.4 At the request of the Parliamentary Secretary of the Ministry of Agriculture, Fisheries and Food the Committee met with a delegation from the Hyperactive Children's Support Group (HACSG). The Group had provided the Committee with a briefing paper and members of the delegation [Professor Brostoff, Consultant Physician and Reader in Clinical Immunology, Middlesex Medical School, London; Professor J Egger, Haunersches Kinderhospital, Munich, Germany; Dr Neil Ward, Senior Lecturer, Department of Chemistry, University of Surrey, Guildford; Dr Erik Millstone, Science Policy Research Unit, University of Sussex, Brighton; Mrs Sally Bunday, Founder/Director HACSG; Mrs I D Colquhoun, Hon. Chairman HACSG; Mrs O'Reilly (Allergy induced Autism)] gave presentations to the Committee. After the presentations the delegation were informed by the Chairman that the Committee would be setting up a Working Group to review the problem of food intolerance.

1.5 Subsequently, the Committee agreed that topics for discussion by the Working Group should include: epidemiological evidence; definitions of hyperactivity; comments on the validity of the data; consideration of an underlying mechanism; extrapolation from child to adult and consideration of socioeconomic factors.

Peanut Allergy

1.6 In 1996 the Committee established a Working Group to consider the issue of peanut allergy, and in particular whether early exposure was linked to the incidence of allergy later in life and whether any advice should be given to pregnant women. The Group will report back to the Committee when their consideration is complete.

Phthalates in Infant Formulae

1.7 During 1996 the Committee were asked to consider the potential hazards of phthalates found in a survey of infant formulae. They formulated the following statement:

i. The Committee was provided with details of the recently completed MAFF surveillance work on phthalates in food and infant formulae in the form of the published Food Surveillance Information Sheets Nos 82 and 83 dated March 1996. The Committee understands that the survey of individual phthalates in infant formulae is the first of its kind to be carried out in Europe and welcomes the new information it provides.

ii. Phthalates are common environmental contaminants and they have high solubility in fat. Their presence as contaminants in infant formulae and other fat-containing foods is not unexpected. The Committee noted that surveys have found phthalates not only in infant formulae but also in fresh cows' milk, meat, poultry, fish and eggs. The Committee is not aware of any information on phthalate levels in human breast milk but would anticipate from the nature of phthalates and the high fat content of breast milk that they are likely to be present in breast milk, as are other fat-soluble environmental contaminants.

iii. The Committee was informed that 59 samples from 15 brands of infant formulae were obtained and composite samples of each brand prepared for analysis. All brands tested are widely available in the UK. Although the analytical results only reflect the situation from purchases made at one point in time, they were likely to be generally representative of the UK market as a whole. The Committee noted that phthalates were found in all the infant formulae tested and that levels of total phthalates in the various formulae were similar, all being within one order of magnitude of each other. Levels of individual phthalates were also similar across the various formulae. Whilst the survey gives a good overall picture, there are insufficient data to assess whether any particular brand may have consistently higher phthalate levels than others.

iv. The Committee noted that the estimated intakes of individual phthalates were all below the relevant Tolerable Daily Intakes (TDIs) set by the EC Scientific Committee for Food. Looking at the overall picture, average intakes of total phthalates from infant formulae were estimated to range from 0.10 to 0.13 mg/kg bodyweight/day over the first 6 months of life. These exceed by 2-3 fold the temporary "group restriction" of 0.05 mg/kg bodyweight/day set by the SCF for those phthalates for which further toxicity testing is required. The Committee notes that TDIs are derived from doses which produce no effect in animal studies divided by a 100-fold safety factor. However the Committee was aware that new evidence on the reproductive effects of phthalates has been published since these TDIs were set. The Committee therefore gave separate and particular consideration to this aspect.

v. Earlier reproduction and teratology studies on phthalates have shown effects on the testis and on embryonic development only at very high doses and these effects were taken into account by the SCF in setting the TDIs. However in new work, two of the phthalates identified in infant formulae, butylbenzyl phthalate (BBP) and dibutyl phthalate (DBP), have been shown to have weak oestrogenic activity in sensitive *in vitro* assays.

vi. BBP has also been shown recently in an *in vivo* study by Sharpe *et al*. to have an effect on the developing rat testis in male offspring whose mothers were given BBP in their drinking water. In this study, a single concentration of 1 mg/l was given throughout pregnancy and lactation, resulting in an estimated maternal oral intake of 0.1-0.4 mg/kg bodyweight/day, but a dose-response relationship was not studied. Testis size in male offspring at 90 days of age was reduced by around 10% and sperm count reduced by around 20%. The mechanism(s) of these effects is unclear, as are the contributions of *in utero* compared with postnatal exposure. The Committee also notes that the critical period for testis development in both humans and rats begins prenatally and in rats extends for two weeks

postnatally, but in humans it may extend up until puberty. Thus it may not be satisfactory to use an experimental study on BBP in rats in which exposure has covered the entire prenatal and postnatal critical period to assess the risk of exposure of humans via infant formulae after birth.

vii. For DBP there are also recent reassuring data from a US National Toxicology Program (NTP) study on rats using dietary administration over a range of doses. In this study, male offspring were exposed to DBP via their mothers throughout pregnancy and lactation and subsequently directly exposed via the diet until they were adult. The study showed effects on adult testis weight and histopathological lesions at doses of 570 mg/kg bodyweight/day and above, with a no-effect level of 250 mg/kg bodyweight/day.

viii. Utilising these new but limited data, the highest estimated intake of BBP in infants on infant formula is 11.5 times lower than the dose said to cause minimal effects in rodents. The average estimated intake of BBP for all the formulae tested is 33 times lower than the dose said to cause minimal effects in rodents. The safety margins based on the data from Sharpe *et al*. are lower than the 100-fold safety margin we would usually wish to see. It should be noted however that the recent *in vitro* studies on BBP and DBP and the *in vivo* study on BBP were not designed for risk assessment purposes and caution should be exercised in extrapolating from these results to humans. In the case of DBP, a risk assessment can be made from the NTP study and even the highest estimated intake of DBP from infant formulae is over 17,000 times lower than the no-effect level found in that study.

ix. The Committee considers that further studies are required before a definitive overall risk assessment can be made. These studies would need to establish not only whether the putative oestrogenic or other activities of phthalates have a significant effect in the whole animal and, if so, at what dose they are without effect, but also should screen as yet untested phthalates. It would also be helpful to have data on phthalate levels in human breast milk.

x. In conclusion, the Committee considers that, on the basis of the evidence available to date, the levels of individual and total phthalates found in the recent survey of infant formulae are unlikely to pose any risk to the health of infants being fed on infant formulae, but it would be prudent to ensure there are adequate safety margins. The Committee therefore endorses the action which has already been taken to ask the manufacturers to trace the sources of phthalates present in infant formulae so that levels may be reduced. However, the Committee notes that given the widespead distribution of phthalates and their occurrence in ingredients of infant formulae, it is unlikely it will be possible to eliminate phthalates completely from infant formulae.

Platinum-based Fuel Catalyst for Diesel Fuel

1.8 At the request of the Department of Trade and Industry, the Committee was asked to advise on the implications of a new platinum-based fuel additive which had been designed to reduce the emissions of pollutants in diesel exhaust. Whilst the use of the platinum fuel catalyst would bring about a reduction in emission of particulates, the Committee was asked to consider whether the simultaneous increase in platinum emissions was of concern for public health. Specific points that the Committee were asked to take into account were: whether they were content that the platinum emissions were mostly in the form of metallic platinum, which is not associated with human toxicity; whether the small soluble fraction is unlikely to be in an allergenic form; and whether, even if the soluble fraction were allergenic, there was an adequate margin of safety below the lowest observed adverse effect level.

1.9 The Committee considered the proposed usage and the projected emissions and noted that, if the majority of the emissions were in the form of the metal, there would be no risk to health. Concerns were expressed as to the extent to which allergenic platinum halides were present in the product and in the exhaust emissions. The Committee agreed that the soluble fraction of emitted platinum was unlikely to be allergenic provided it was not in a halide salt form.

1.10 After consideration of additional data requested by the Committee, members agreed that platinum emissions from the platinum based fuel catalyst were unlikely to be in an allergenic form. Additionally, the Committee expressed a wish to see the results of additional toxicity studies that they understood were planned for the material.

Scientific Committee for Food Guidelines on the Assessment of Novel Foods

1.11 The Committee was informed that the draft EU Regulation on Novel Foods referred to guidance from the Scientific Committee for Food (SCF) on the assessment of novel foods. The Committee was asked for its views on the latest draft of the Guidelines produced by the SCF, particularly the sections relating to toxicological testing requirements and allergenic potential. These views would be of value to the Secretariat of the ACNFP in their evaluations of novel foods under the EU regulation. They would be made available also to the UK members of the SCF.

Single Cell Protein

1.12 The COT was also asked to advise on the adequacy of the toxicological data included in a submission to the ACNFP seeking food safety clearance of a novel single cell protein. The protein, produced by bacterial fermentation, will initially be used as a raw material for the production of protein hydrolysates and autolysates.

1.13 The COT was not satisfied with the toxicological data submitted, which included 28-day and 90-day rat studies, an Ames test and an *in vivo* micronucleus test, and was of the view that the method of administration of the protein (as an addition to an already nutritionally adequate diet) may have resulted in a protein overload which would explain many of the effects seen in the 28- and 90-day studies. As a consequence of this probable protein overload, the studies were not a satisfactory basis for the evaluation of the food safety of the single cell protein. Additionally, the Committee was concerned that there were no data to provide reassurance that the novel protein did not have any adverse effects on reproductive performance.

1.14 The Committee recommended that a number of additional studies be conducted to address these concerns; they include:

i. a 90-day sub-acute toxicity study in the rat in which the novel protein is added as part of a synthetic diet; at the top dose the novel protein should provide the sole source of protein. The COT stressed that careful attention would need to be paid to the nutritional adequacy of the synthetic diets that are formulated, particularly in relation to mineral balance;

ii. a teratology study and a single generation fertility study, again using a synthetic diet to which the novel protein has been added as the protein source;

iii. further *in vitro* mutagenicity tests using both polar and non-polar extracts of the novel protein in order to determine any possible mutagenic effects of the non protein components present in the single cell protein without the complicating factor of the effects of the protein itself in such test systems and to increase the dose of the impurities tested;

iv. further investigation of the possible allergenic effects of the novel protein.

1.15 The COT noted that clearance had been sought for both nucleic acid reduced and non-nucleic acid reduced forms of the novel protein and recommended that the above studies be conducted on both materials.

Soluble Fibre Derived from Guar Gum

1.16 The COT was asked by the Advisory Committee on Novel Foods and Processes for its advice on the toxicology data submitted on a novel soluble fibre. The novel fibre is derived from guar gum and it is intended to be added to a range of processed liquid and solid foods to increase the fibre content. A limited amount of toxicology data had been submitted on the novel fibre itself, these included a 28-day rat study and an Ames test. However, the submission had cited the extensive toxicology data available on guar gum to support the safety-in-use of the fibre. A number of human volunteer studies had also been conducted on the novel fibre.

1.17 The COT advised that the novel fibre should be considered in its own right, as a substance distinct from guar gum. Therefore, the toxicology data cited on guar gum could not be used to support the safety-in-use of the novel fibre. In addition, the COT considered that the toxicology studies which had been carried out on novel fibre itself were inadequate and that no useful conclusions could be drawn from them. The COT advised that further data, including a 90-day rat study on the novel fibre, were required.

Food Surveillance Paper

1.18 In December 1996, the Ministry of Agriculture, Fisheries and Food's (MAFF) Steering Group on Chemical Aspects of Food Surveillance Working Party on Natural Toxicants published its fifty-first. The subject of this 51st report was 'Inherent Natural Toxicants in Food' but it also contained information on MAFF's 'Whole Food Approach' and the Naturally Occurring Toxicants Information System (NOTIS). The report was considered by the COT in 1996 and its advice is included in the Food Surveillance paper as an appendix. The full report is referenced and makes suggestions as to topics on which research would be welcome.

1.19 As part of the Committee's assessment, the advice of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) and/or the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) was sought as appropriate.

1.20 The Committee found the assessment of the risks of inherent natural toxicants in food to man to be particularly difficult. One reason was the paucity of animal and human data on the compounds under review. However, even if a full toxicological dataset had been available, the Committee considered that it would be incorrect to assess the potential hazards of the toxicant in isolation, as is normally done for chemicals occurring as contaminants or used in food manufacture. The toxicological hazard of an inherent toxicant depends on its bioavailability from the food matrix, and may also be influenced by other components of the food. The Committee considered that the potential hazard of a natural toxicant should be considered in the context of the nutritional or other benefits of the food(s) in which it occured. Review of these benefits was outside the Committee's remit but the Committee *recommended* that a risk-benefit assessment of this type should be made

before any advice is given which might cause the public to reduce its consumption of the food(s) concerned.

1.21 The Committee noted that the Working Party on Natural Toxicants has tried to take account of these factors in developing its 'Whole Food Approach' and that a case study on this was included in the report.

1.22 The Committee's advice on various classes of compounds is given below.

Glycoalkaloids

i. Glycoalkaloids are a group of natural toxicants which are present in potatoes. Concentrations are highest in the sprouts and peel and are greater when the potatoes are damaged. Removal of the sprouting and other damaged areas, together with peeling, substantially reduces their concentration in potatoes. Potatoes contain two major glycoalkaloids, a-solanine and a-chaconine, which together account for 95% of the total glycoalkaloid content. However, potatoes also contain a number of other inherent toxicants such as tropane alkaloids, terpenoids, saponins, protease inhibitors and lectins. Glycoalkaloids are heat stable and are, therefore, not removed in cooking, whereas lectins are heat labile and are likely to be of little toxicological consequence with regard to potato consumption.

ii. Glycoalkaloids are not acutely toxic by the oral route in laboratory animals even at very high doses (up to one gram per kilogram body weight (1g/kg bw)) in some species. This may be due in part to the hydrolysis of a-solanine in the gastro-intestinal tract to a less toxic metabolite, but most acute toxicity experiments have been carried out by the intraperitoneal route. Although glycoalkaloids are not acutely toxic by the oral route, they can cause gastric irritation and haemorrhagic damage following oral administration in the hamster but not in the rat. *in vitro*, glycoalkaloids have been shown to destabilize membranes and inhibit the enzyme acetylcholinesterase. *in vivo*, such effects have only been observed following parenteral administration. In cases of suspected potato poisoning in humans, individuals display gastrointestinal tract irritation, drowsiness and neurological disturbance and the similarity of these symptoms to those caused in some animals by glycoalkaloids has led to these toxicants being implicated as the toxic agent. However, there are no data on the plasma levels of glycoalkaloids in victims of suspected potato poisoning which might confirm this and, overall, the evidence implicating glycoalkaloids is not convincing. Although some cases of suspected potato poisoning have been serious, considering that potatoes are a staple dietary component, the number of cases of suspected potato poisoning is very small. Since human consumption of potatoes often involves removal of skin and other blemishes, it is likely that the intake of glycoalkaloids from potatoes is low, average intake for adults 2.4 mg/person/day, high level intake (97.5th percentile) 7.8 mg/person/day. Assuming an adult weighs 60 kg, then these intakes are equivalent to 0.04 mg/kg bw/day and 0.13 mg/kg bw/day respectively. However, the Committee were *concerned* about the increasing number of "skin-on" products which have become available but noted that the results of surveillance of "skin-on" products carried out by the Working Party were reassuring.

iii. The Committee considered data regarding the hypothesis that the ingestion of damaged potatoes in early pregnancy can result in spina bifida in the offspring and have observed that 13 investigations have failed to demonstrate such a link. The Committee *concluded* that the consumption of blighted potatoes in pregnancy was not associated with neural tube defects in offspring.

iv. With regard to the toxicity of glycoalkaloids, the Committee *concluded* that the risk to the population was low, provided that damaged, green and sprouting areas of potatoes were removed. If potatoes still taste bitter after removal of the green and/or sprouting parts, then they should not be eaten. The Committee *recommended* that further surveillance into this area should be given a low priority.

v. The Committee considered the 'Whole Food Approach' in the context of toxicants in potatoes. This has been developed to account for possible interactions between different food components and which may allow for the assessment of the risks of some constituents against the benefits of others. The Committee *concluded* that such an approach was useful and supported the intention to conduct studies in humans wherever possible. The Committee recognized the limitations of *in vitro* toxicity tests in the context of the 'Whole Food Approach'. *in vitro* data provide no information on bioavailability of the inherent toxicant from the gastro-intestinal tract or on its metabolism or availability in the target organ. Whilst *in vitro* tests can provide a quick screening process, such tests are not a substitute for *in vivo* studies and negative results *in vitro* are not evidence of safety *in vivo*. The Committee have been assured by MAFF that it will be kept informed of further developments of this approach and

it looks forward to reviewing further case studies in the validation of the 'Whole Food Approach'.

Furocoumarins

i. Furocoumarins occur in several food plants such as parsnip, celery and limes. The most commonly detected linear furocoumarins are 8-methoxypsoralen (8-MOP, xanthotoxin), 5 methoxypsoralen (5-MOP, bergapten) and psoralen itself. Our advice applies specifically to the usual dietary concentrations and intakes of furocoumarins as reported in this Food Surveillance Paper and to oral exposure only. The Committee noted that higher concentrations in foods have, on occasion, been reported after extreme fungal contamination. The Committee also commented on the potential risk from angelicin, a branched furocoumarin, in food. (The surveillance concentrated on linear furocoumarins since these were presumed to pose a greater risk).

ii. Most information on the toxic effects of 8-MOP and 5-MOP comes from studies in patients undergoing treatment for psoriasis and other skin diseases (PUVA therapy). Treatment involves an oral dose of 0.6 mg/kg bw 8-MOP or 1.2mg/kg bw 5-MOP followed by UVA exposure at 0.5 - 11 Joules/cm2. In comparison, the extreme UK dietary intake of furocoumarins has been estimated to be at least 30 times lower at 0.02 mg/kg bw. Daily UVA exposure can be around 1 Joule/cm2 in the UK. A small number of studies are available in PUVA-treated healthy volunteers. Unless specified otherwise, all studies cited used furocoumarins *and* UVA light. The main effects of 8-MOP at clinical doses are the effects on the skin and immune system and a longer term risk of skin cancer. The Committee considered whether these effects are likely to occur from consumption of furocoumarins from food sources and exposure to sunlight.

iii. The Committee considered that any risk arising from furocoumarins is substantially reduced at dietary intakes compared with the doses used in PUVA therapy. One reason is that the oral doses given in PUVA therapy give rise to high and variable blood concentrations of 8-MOP and 5-MOP. In contrast, in studies in 14 volunteers consuming large amounts of celery (300 - 700 g), blood concentrations of linear furocoumarins were below the detection limit and thus at least 75 fold lower than average blood concentrations seen in patients undergoing PUVA therapy. Absorption of 8-MOP and 5-MOP from a food matrix is probably reduced in rate and extent compared with absorption from a drug formulation. There is also extensive metabolism of 8-MOP in the liver before reaching the general circulation (first-pass metabolism). Saturation of this metabolism probably occurs at PUVA doses but does not after dietary intake and this could explain the high blood levels of 8-MOP found during PUVA therapy. Although the studies of absorption from food only involved celery, the Committee had no reason to believe the results would not apply to other food plants. The number of volunteers studied was small and the Committee *welcomed* the Working Party's funding of a study to further examine the bioavailability of furocoumarins from food.

iv. The mutagenicity and carcinogenicity of furocoumarins in the diet have been considered by the COM and COC and their opinions have been published. In summary, the Committee have been *advised* by the COM that, in the absence of UVA, the low levels of exposure arising from the diet are unlikely to be of concern with regard to systemic mutagenic effects and that the risk of a local photomutagenic effect on the skin from furocoumarins in the diet and exposure to UVA from sunlight is likely to be very small. The COC has *advised* that, although PUVA therapy with 8-MOP clearly causes an increase in squamous cell carcinomas of the skin in humans, any carcinogenic or photocarcinogenic risk arising from linear furocoumarins in the diet is likely to be very small.

v. PUVA therapy with 8-MOP or 5-MOP causes itching, pigmentation and, at UVA doses around 2 to 6 Joules/cm2, minimal erythema. There is great individual variability. Long term PUVA treatment with 8-MOP can cause persistent pigmented spots and other skin changes. The long term effects of PUVA therapy with 5-MOP are unknown. Erythema is the only effect which has been studied at doses (0.15 - 0.3 mg/kg bodyweight, 8-MOP) below clinical doses although the studies used small numbers and/or non-standard procedures. Erythema occurred at apparent blood concentrations down to 14 ng/ml, but the blood samples may have been taken after the peak blood concentration. In contrast, larger studies at higher doses suggested a threshold for erythema of 30 - 50 ng/ml. The Committee *considered* a threshold of 30 ng/ml the most appropriate estimate. This blood concentration is unlikely to be reached from dietary exposure. There have been 2 cases of phototoxic reactions after consumption of celery. Neither case was in the UK and both involved extreme intakes of celery and strong UVA

exposure.

vi. At therapeutic doses in psoriasis patients, PUVA with 8-MOP causes some signs of mild immunosuppression characterised by reduced delayed-type hypersensitivity, changes to immunocompetent cells in the skin and small reductions in the number and function of circulating T lymphocytes. Reductions in T cell number were also found in healthy volunteers receiving the same treatment. The effects of 5-MOP are unknown *in vivo* but *in vitro* are similar to those of 8-MOP. We *conclude* that therapeutic doses of PUVA with 8-MOP does have some impact on the integrity of the immune system but that, by analogy with the immune effects of UVB, this would not be expected at lower doses. Other adverse effects in patients receiving PUVA therapy such as liver damage or cataracts are extremely rare even at the high doses of 8-MOP and 5-MOP used clinically. Studies in rats of 8-MOP *without* UVA may have some relevance to toxicity to internal organs in man but effects were only seen at doses 50 fold higher than those used in PUVA therapy.

vii. There are no adequate absorption or metabolic data on psoralen or angelicin. Psoralen is of similar potency to 8-MOP in the skin after topical application in man but has less potency in the skin than 8-MOP after oral exposure in mice. It is prudent to assume it will have similar effects to 8-MOP. Angelicin does not cause erythema. Where studied in mice, angelicin has the same type of immune effects as 8-MOP. Although the effects are less well defined, there is no reason to suppose it is of greater toxicity than 8-MOP.

viii. Overall, the Committee *concluded* that the likelihood of any risk to health from dietary intakes of furocoumarins was very small. Further surveillance of levels of furocoumarins in food was therefore of low priority. However, the Committee *recommended* that new strains of furocoumarin-containing plants should be monitored to ensure the furocoumarin content was not significantly above current average levels.

Phytoestrogens

i. Phytoestrogens are widely distributed plant chemicals which can cause oestrogenic effects. Two of the main classes of phytoestrogens are the coumestans and the isoflavones. They are capable of binding to the oestrogen receptor but in many tests in vivo and *in vitro* are considerably less potent than endogenous oestrogens. Because of the differences in efficacy and in binding affinities to the oestrogen receptor and the dependence on the intrinsic oestrogenic state (ie prepubertal, premenopausal, postmenopausal) of the target tissue, either agonistic or antagonistic responses can be produced.

ii. Phytoestrogens have been shown to cause infertility in animals, the first cases being noted in ewes which became infertile, less sexually receptive and more aggressive. The infertility syndrome, named *Clover Disease*, was subsequently discovered to be caused by high concentrations of coumestrol (a coumestan) in the clover grazed by the sheep. In laboratory animals, exposure to coumestrol via dams' milk has also been shown to cause effects on oestrous cycling, LH response following oestrogen and progesterone priming, and behaviour which only became apparent as the animals reach sexual maturity. Furthermore, administration of phytoestrogens to these same species can cause androgenisation of females and feminisation of males, although the effects vary according to time and duration of intake.

iii. At present, there are only limited data on the intake of phytoestrogens in specific population groups in the UK. People consuming an Asian diet and vegetarians have high intakes of soy, a major source of isoflavones. The isoflavones found in plant material are mainly bound to sugar residues and are inactive. The active aglycones are released in the gastro-intestinal tract by gut microflora and are subsequently absorbed and metabolised. Known differences in the microflora between people consuming a Japanese diet and those consuming a Western diet could result in differences in the effects of isoflavones in these different population groups. Human studies have also indicated that there is inter-individual variation in the metabolism of daidzein, one of the isoflavones found in soy. Approximately 30% of the population is able to metabolise daidzein extensively to equol, while the majority of the population produce smaller amounts of this metabolite. Phytoestrogen excretion has also been reported in a small study in male infants fed either human milk, cows' milk formula or soy-based formula from birth to 4 months of age. Total urinary isoflavonoid concentration was approximately 20 microgram per litre (g/l) for the infants fed human milk, 100 g/l for the infants fed cows' milk formula and 600 g/l for the infants fed soy-based formula. Of the three isoflavones determined, only daidzein and genistein were detected, with the concentration of equol being minimal in all groups.

iv. Consideration of phytoestrogen toxicity and subsequent risk to certain groups of the population is complex since their actions are tissue and end-point specific and depend on the developmental and maturational context in which they are assessed. The complexity is further compounded by differences in metabolism as outlined above.

v. In preliminary studies in premenopausal women normally consuming a Western diet, ingestion of soy protein (60 g per day for one month, equivalent to ca 0.73 mg isoflavones/kg bw/day) has been shown to suppress mid-cycle peaks of LH and FSH and significantly increase the duration of the follicular phase. The effects lasted for up to 3 months following the termination of soy consumption. There are reports that phytoestrogens can reduce blood cholesterol, and that they may protect against osteoporosis and reduce flushing in postmenopausal women. Epidemiological evidence from adult populations which habitually ingest high quantities of soy (eg. Chinese and Japanese) suggest that these individuals have a lower incidence of some types of cancer. However, it is difficult to resolve the effects and consequences of other dietary variables such as fibre, vitamins, fruit, vegetables and meat when considering the validity of this observation. The subject of dietary constituents and cancer is presently under review by a Working Group of the Committee on Medical Aspects of Food Policy.

vi. The potential for phytoestrogens, including isoflavones, to affect adversely infants is of particular concern since it is possible that a hormonal imbalance in early life can permanently affect sexual development and fertility. Such effects have been observed in a number of animal species. The Committee was not aware of any reports which suggest that populations which habitually ingest high quantities of soy (eg. Chinese, Japanese) have impaired fertility or altered sexual development. Limited data indicate that the estimated intake of isoflavones by infants fed soy-based formulae is in the region of 4 mg/kg bw/day. This is higher than the intake reported to cause hormonal effects in premenopausal women (approximately 0.7 mg/kg bw/day). Since the Committee did not have data specifically relating to the potential effects of soy phytoestrogens in human infants, particularly in those whose mothers normally consume a western diet, the Committee *recommended* that research should be undertaken as a matter of high priority to determine whether ingestion of soy based formulae carries any risk for infants. As a result of further research, it may be necessary to consider the potential risk of soy products to other sectors of the population. The Committee *endorsed* the advice of the Department of Health that breast milk and cows' milk formulae are the preferred sources of nutrition for infants. However, women who have been advised by their doctor or other health professionals to feed their baby soy-based formulae should continue to do so. The Committee on Medical Aspects of Food Policy has published a more detailed report of the preferred sources of nutrition for infants.

Hydrazine Derivatives

i. Naturally occurring hydrazines, such as agaritine, are found in edible mushrooms, and the Committee were informed that human consumption of the commercially grown edible mushroom *Agaricus bisporus* is increasing. The Committee was *advised* by the COC and COM that the chemical structure of agaritine and its putative metabolites (N acetyl 4 (hydroxymethyl) phenylhydrazine and 4 (hydroxymethyl) benzene diazonium ion) give rise to *concern* about mutagenic and carcinogenic potential, a concern strengthened by the activity of agaritine in bacterial mutation assays. The data concerning the mutagenic potential of agaritine in mammalian cells *in vivo* and *in vitro* are limited and insufficient to allow any firm conclusions to be reached. The stabilised forms of the putative metabolites of agaritine showed evidence of carcinogenicity in long term testing in Swiss mice, although the studies were of unusual design. N-acetyl-4-(hydroxymethyl)phenylhydrazine induced an increased incidence of tumours of the lung and blood vessels; 4-(hydroxymethyl)benzene diazonium ion tetrafluoroborate induced an increase in glandular stomach adenocarcinomas. There was evidence that agaritine itself was carcinogenic in laboratory animals, but further testing would be necessary to confirm this.

ii. The Committee *agreed* with the concerns of the COC and COM that the structure and mutagenic potential of agaritine suggested that future research was needed. In order to proceed with this assessment, information on absorption, distribution, metabolism and possible detoxification of this compound by mammals was needed. The data available indicate that there are other toxins present in A. *bisporus* and that toxic effects in laboratory animals fed mushrooms cannot be directly attributed to agaritine. The Committee recommended that more knowledge was required on the nature, bioavailability, and toxicology, including mutagenic potential, of the other toxins present in A. *bisporus*, before a definitive conclusion could be reached and *welcome* the research that MAFF was funding to address these questions. The Committee *agreed* with the COC and COM that the data currently available were not of sufficient concern to require the need for measures to reduce agaritine intake by the population.

Bracken Fern Mutagens

i. In 1988, the COC, was asked by MAFF to consider the carcinogenicity of bracken and its human health risks, following reports that bracken may constitute a human health risk and the possible occurrence of bracken "toxins" in the food supply. There were, however, few data available at that time about the extent to which farm animals grazed on bracken and the occurrence of bracken constituents in dairy products.

ii. Human epidemiological data are limited and the COC *concluded* that evidence of carcinogenicity was inconclusive. Carcinogenicity studies in laboratory animals in which whole bracken had been administered in the diet, in some cases accounting for up to one third of the total diet, were flawed in their experimental design, execution and interpretation. However, despite these limitations, the COC *concluded* that these studies had demonstrated a clear trend for the increased incidence of benign and malignant tumours of the small and/or large intestine and/or urinary bladder and that there was a need for properly conducted carcinogenicity bioassays in rats and mice. The COC also considered the active constituents of bracken and *concluded* that ptaquiloside, an inherent constituent of bracken, had been shown to be capable to reproducing some the carcinogenic effects of whole bracken. Following the conclusions of the COC, MAFF commissioned work to investigate whether mutagenic components in bracken could be passed into the milk of bracken-fed goats and thus enter the food chain. Goats rather than cows were used in this study since cows do not usually eat bracken and are reluctant to graze it.

iii. In 1993, the COM reviewed the mutagenic potential of solvent extracts of bracken and *agreed* that such solvent extracts showed mutagenic activity in bacterial assays *in vitro*. There was evidence that most of the mutagenic activity appeared to be due to ptaquiloside, but the COM *concluded* that other potentially mutagenic compounds might be present. There was evidence that mutagenic activity was expressed in mammalian cells *in vitro* and there was limited evidence that this activity might also be expressed *in vivo*. The COM also reviewed the results of the work referred to in previous paragraph, concerning the possibility of bracken mutagens being transmitted to the milk of bracken-fed goats. It *concluded* that very little, if any, mutagenic activity was present in the milk of the goats which were exposed to UK bracken for short time periods of up to one month. The COM *recommended* that no further work should be carried out provided that milk was bulked and centrally processed.

iv. The Committee *accepted* the advice of the COC and COM, and *agreed* that the risk to the population was very low and that further research need not be undertaken on bracken fern mutagens.

Topics Still Under Consideration

1.23 The following topics, which were discussed by the COT at meetings held in 1996, are still under review:

Various aspects of the toxicity of the polychlorinated biphenyls (PCBs) which are a family of widely-dispersed environmental contaminants, some of which have toxicological similarities to the polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans.

Under referral from the Advisory Committee on Novel Foods and Processes; consideration of a synthetic structured triglyceride intended to act as a low calorie fat replacer.

1996 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor H F Woods BSc BM BCh DPhil FIFST FRCP(Lon & Edin) FFOM FFPM Hon FFOM
Dean of the Faculty of Medicine. University of Sheffield

Members

Professor P J Aggett MB ChB FRCP MSc DCH FRCPCH
Assistant Director. Institute of Food Research, Norwich and Honorary Consultant Paediatrician, Norfolk and Norwich Hospital

P N Bennett MB ChB MD FRCP *Reader in Clinical Pharmacology. University of Bath and Deputy Head of the School of Postgraduate Medicine*

V Beral MB BS FRCP *Epidemiologist. Gibson Laboratories, Radcliffe Infirmary*

N A Brown BSc PhD Senior Scientist, Medical Research Council Experimental Embryology and Teratology Unit and Honorary Senior Lecturer. St *Georges Hospital Medical School, University of London*

J K Chipman BSc PhD CBiol FIBiol MRCP
Reader in Cell Toxicology. School of Biochemistry, University of Birmingham

Professor A D Dayan BSc MD FRCP FRCPath FFOM FFPM CBiol FIBiol
Director of DH Toxicology Laboratory, St Bartholomews and the Royal London School of Medicine and Dentistry

Professor G G Gibson BSc PhD DSc FRCPath
Professor of Molecular Toxicology, University of Surrey

Professor G Hawksworth BSc PhD
Professor of Molecular Toxicology. Department of Medicine and Therapeutics, University of Aberdeen
M Joffe MD MScEcon FRCP FFPHM
Reader in Epidemiology. Imperial College of Medicine

I Kimber BSc MSc PhD FIBMS CBiol MIBiol
Head of Research. Zeneca Central Toxicology Laboratory

D E Prentice MA VetMB MVSc FRCPath MRCVS
Independent consultant. Animal toxicopathologist

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

A G Smith BSc PhD CChem FRSC
Molecular Toxicologist, Medical Research Council Toxicology Unit in Leicester

F M Sullivan BSc
Senior Lecturer in Pharmacology, UMDS, St Thomas Hospital

A Thomas MB ChB PhD FRCP
Consultant Physician with special responsibility for the elderly, Plymouth Hospital NHS Trust

Dr M Tucker BSc PhD FRCPath
Independent pathologist and animal histopathologist

Professor D Walker BVSc FRCVS CBiol FIBiol
Independent consultant. APT Consultancy

Professor R Walker PhD FRSC CChem FIFST
Professor of Food Science, University of Surrey

Secretariat

Miss F D Pollitt MA DipRCPath (Scientific - until October 1996)
A Wadge BSc PhD CBiol MIBiol (Scientific - from November 1996)
Mrs D Davey (Administrative)

D E Jaeger BSc PhD CBiol MIBiol
J Shavila BSc MSc PhD
H A Walton BSc DPhil (Until September 1996)
Miss C Mulholland BSc (From October 1996)
Ms P Tew (February 1996)
Ms J Sorensen (From April until July 1996)
Mrs C Steed BSc MSc (from October 1996)

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

	Personal Interest		Non-Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Prof H F Woods (Chairman)	NONE	NONE	Wide range of national & international food & Chemical Companies	Dean of the Faculty of Medicine, University of Sheffield, which 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
Prof P J Aggett	NONE	NONE	Nutrica Milupa SMA Nutrition Unilever Nestl	Research Support Research Support Research Support Institute intellectual agreement Meeting Support
Dr P N Bennett	Glaxo Wellcome Grand Metropolitan Hanson Marks and Spencer Tomkins Shell	Share holder Share holder Share holder Share holder Share holder Share holder	Research Institute for the Care of the Elderly plc Bath Clinical Trials Ltd	Director Director
Dr V Beral	NONE	NONE	NONE	NONE
Dr N A Brown	Glaxo Shook,Hardy & Bacon Styrene Information Research Control Du Pont	Consultancy Consultancy Consultancy Consultancy	EC (DGXII) Glaxo Welcome Trust US EPA	Research Support Research Support Fellowship & Research Support Research Support
Dr J K Chipman	Boots	Consultancy	Astra Glaxo-Wellcome STD Pharmaceutical Ltd Water Research Centre Zeneca, Safety of Medicine Zeneca, Central Tox. Lab.	MRC collaborative Research Award Research Support Research Support Research Support CASE Research Award MRC Collaborative Research Award
Prof A D Dayan	British Petroleum Glaxo-Wellcome Schering Plough Smith Kline Beecham	Consultant Pension Consultant Consultant Share Holder	NONE	NONE

	TI			
Prof G G Gibson	NONE	NONE	NONE	NONE
Prof G Hawksworth	NONE	NONE	Glaxo-Wellcome Pfizer Central Research Servier Research & Dev Smith Kline Beecham Solvay Pharma Zeneca	Research Support Research Support Research Support Research Support Research Support Research Support
Dr M Joffe	Ilzro Woolwich Abbey National	Research Grant Share Holder Share Holder	NONE	NONE
Dr I Kimber	British Airways British Petroleum ICI ICI Zeneca Zeneca	Share Holder Share Holder Share Holder Consultant Share Holder Employee	Unilever	Grant for Research
Dr D E Prentice	Athena Neurosciences Eisai Limited Gensia Gilead Sciences Hoechst Veterinr Hoffman-La Roche Nestl Novartis Crop Protection Novartis Pharma Rhne-Poulenc Schering-Plough Synthlabo (LERS) Wyeth-Ayerst	Occasional fee Consultant Occasional Fee Occasional Fee Occasional Fee Occasional Fee Occasional Fee Consultant Consultant Consultant Consultant Consultant	NONE	NONE
Prof A G Renwick	International Sweeteners Association	Consultant	Hoffmann-La Roche Unilever Smith Kline Beecham	Research Support Research Support Research Support
Dr A Smith	British Telecom	Share Holder	Rhone Poulenc	Research Support
Mr F Sullivan	Borax Ltd Cocensys Frauenhofer Research Rank Hovis Mc Dougle	Consultant Occasional Fee Consultant Consultant	NONE	NONE
Dr A Thomas	NONE	NONE	NONE	NONE
Dr M Tucker	Zeneca	Pension	NONE	NONE
Prof D Walker	Amersham Bayer British Petroleum Grampain Phamaceuticals Rhon Mrieux Sandoz Solvay Duphar	Share Holder Occasional Fee Share Holder Occasional Fee Occasional Fee Occasional Fee Occasional Fee	NONE	NONE
Prof D Walker	Barlow, Lyde & Gilbert Cadbury Beverages Europe Coffee Science Info Centre Collodes Naturals International, France Food Safety Advisory Centre IDV Ltd	Occasional Fee Occasional Fee Consultant Consultant Consultant Consultant Occasional Fee	Nestl	Research Studentship

Nutricia

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

Professor J M Parry (Chairman) BSc PhD DSc

Preface

The Committee on Mutagenicity provides advice to Government Departments and Agencies on both the interpretation of studies investigating the mutagenic activity of chemicals and methods and strategies for the testing of new and/or pre-existing chemicals. Within each of the general areas the independent members of the Advisory Committee are supported by the dedicated work of the DOH Secretariat who produce comprehensive documentation upon which the Committees discussions and advice can be based.

During the year the Committee provided advice on the mutagenic activity included chemicals as diverse as Agaratine (derived from mushrooms) bisphenol A diglycidyl ether (component of can linings), chlorine dioxide (used in flour treatment), chlorinated solvents and benzimidazole fungicides and anthelmintics. Advice on testing methodologies covered; transgenic animal assays, *in vitro* methods for measuring the induction of micronuclei and chromosome non-disjunction and cell transformation assays. In both areas the Committee was able to provide advice based upon currently available data and to also provide recommendations for further studies when information available was inadequate. As a general aid to those evaluating regulatory submissions the Committee was also able to provide an ongoing set of notes and advice on the conduct and interpretation of mutagenicity data sets.

On a sad note, the year saw the retirement of Professor H. John Evans CBE and Dr. Jane Cole. Both members have served the Committee with dedication and distinction. Their expertise and contributions to the Committee will be considerably missed by the members.

James Parry

Agaratine

2.1 Agaratine occurs in appreciable quantities in the commercially grown edible mushroom *Agaricus bisporus*

2.2 The COM had recommended that *in vivo* studies were required in order to complete the assessment of the mutagenic hazard of agaratine. New data were now available, in particular a study in transgenic mice (Big Blue) in which the authors claimed a weak positive result following the administration of agaratine. These authors had also suggested that the transgenic mouse assay could be of potential use in estimating carcinogenic potency. The Committees advice on the assessment of agaratine and use of data from transgenic assays to estimate carcinogenic potency was requested.

2.3 The Committee concurred with its previous conclusion that there was insufficient concern based upon the current information to warrant introducing any extensive measures to reduce intakes of agaratine in the diet. The following conclusions regarding agaratine were agreed.

i. The chemical structure of agaratine and its putative metabolite (N-acetyl-4-(hydroymethyl) phenylhydrazine and 4-(hydroxymethyl) benzene diazonium ion give rise to concern about mutagenic potential, a concern which is strengthened by the activity of agaratine and its metabolites in bacterial mutation assays. The *in vitro* data in mammalian cells are inadequate to draw any definite conclusions.

ii. Alcoholic extracts of mushrooms demonstrate direct acting mutagenic activity in the Salmonella assay, but this was principally due to components other than agaratine. The activity profile seen across strains of *Salmonella typhimurium* with mushroom extracts differs from that seen with agaratine itself and the response to metabolic activation differs.

iii. The only available *in vivo* data are from a recent study in the lac I transgenic mouse assay (Big Blue mice - Stratagene) using a 15 week dosing regime. The Committee agreed that this study had been inadequately conducted and was inappropriate to draw any conclusions regarding the *in vivo* mutagenicity of agaratine.

iv. These data do not warrant any changes in the Committees earlier overall conclusion that there was insufficient concern arising from the current information to warrant advising that any extensive measures should be taken to reduce agaratine intakes.

2.4 The Committee would continue to monitor any future studies on agaratine. The Committee also agreed that further consideration should be given to the development of transgenic animal models for use in mutagenicity testing although at present there was insufficient published work to validate the proposed methods for mutagenicity testing and it was not possible at the present time to draw any conclusions regarding its use to estimate carcinogenic potency (see section 2.28 below).

Bisphenol A diglycidyl ether (BADGE)

2.5 Bisphenol A diglycidyl ether (BADGE) is used in the production of some food contact plastic materials and can linings. BADGE can be used in some food contact plastics as one of the basic building blocks of the plastic. It is also used as an intermediate in the manufacture of epoxy resins used to line some food cans. Neither of these uses was considered likely to lead to significant migration of BADGE into food unless there was incomplete incorporation of BADGE into the respective polymers. BADGE is also used as a heat stabilising additive in flexible (Organosol) coatings for easy open cans such as now used in some cases for fish. Recent analyses from laboratories, particularly in Switzerland suggested that migration could occur from Oragnosol coatings in cans. There is potential for greater migration of BADGE from Organosol coatings because it is not incorporated into the polymer backbone of the coating.

2.6 The COM had previously advised on the mutagenicity of BADGE in 1986 in the context of its use for relining water mains as well the use of epoxy resins in food contact materials. The Committee had concluded that BADGE was a clear *in vitro* mutagen and had proposed that the possibility of a local genotoxic effect on the gastrointestinal tract could not be excluded. The available information at that time suggested that exposures resulting from relining of water mains were low and of a very short duration whilst there was no detectable migration of BADGE from food contact materials. The Department of Health requested the COM to update its advice on BADGE particularly in view of the recent results from Switzerland suggesting migration could occur into the oil used in certain canned fish products which might subsequently be ingested. There were also new carcinogenicity bioassays in animals using the dermal route and data from studies of DNA binding in the skin available. The Committee noted that the European Unions Scientific Committee for Food (SCF) had recently published an opinion (dated 7 June 1996) which requested additional *in vivo* mutagenicity studies on BADGE.

2.7 The Committee agreed the following conclusions:

i. BADGE is a direct acting alkylating agent and is clearly mutagenic in in vitro tests causing point mutations in bacteria, gene mutations and structural chromosome aberrations in cultured mammalian cells, and mitotic gene conversion in yeasts.

ii. Negative results were obtained in in vivo bone marrow assay for clastogenicity. Data are also available which indicate the absence of genotoxic effects in the liver (using an alkaline elution assay) and in the male germ cells of mice using cytogenetics and a dominant lethal assay, by oral exposure. The compound is rapidly metabolised by detoxifying enzymes in the liver, and the available evidence suggests that very little systemic exposure would be expected following oral administration.

iii. Data from a number of dermal carcinogenicity bioassays indicate that BADGE does not produce skin tumours. Only very low level DNA binding occurred in the skin due to a glycidaldehyde adduct. These data suggest that detoxication via epoxide hydrolase is very rapid, and prevents any significant DNA adduct formation at this local site of application. The relevance of this to the gastric mucosa following ingestion of BADGE was questionable. It was noted that there were no data available from carcinogenicity bioassays using the oral route performed to an acceptable standard.

iv. The Committee agreed that further work was needed before conclusions could be drawn regarding genotoxic effects (and hence potential carcinogenicity) in the gastrointestinal tract following ingestion. Data were needed on DNA binding in the gastric mucosa following ingestion and it was recommended that studies with (14C-) BADGE radiolabelled in the side chain be carried out to investigate covalent binding to DNA in the rat gastric and intestinal mucosa following gavage of a solution of BADGE in vegetable oil. Studies should be carried out both in the presence and absence of an inhibitor of epoxide hydrolase. If these data indicated significant binding to DNA, the Committee would wish to consider this area further.

Chlorine Dioxide as a flour treatment agent

2.8 Chlorine dioxide is a permitted flour treatment agent used in a wide range of flour and bread, except for whole meal. It is used in the gaseous state to enhance the performance of flour during baking. Like other flour treatment agents its improving action is believed to be due to oxidation of sulphydril groups in flour protein, altering the physical properties of the dough and allowing the production of high volume loaves. The gas is completely reduced to chloride and hypochlorite by reacting with flour and there is no unreacted chlorine dioxide in the final product. The COM advised on the mutagenicity of chlorine dioxide during a wider review of the toxicity of all flour treatment agents undertaken over the period 1989-91. The Committee agreed that the available data indicated that chlorine dioxide had the ability to produce gene mutations in bacteria and chromosome damage in mice (micronucleus test) following intraperitoneal but not oral administration. The Committee felt that there was a potential for the formation of chlorinated products with mutagenic potential in treated flour and requested appropriate studies to address this concern.

2.9 The Committee reviewed a submission from the Flour Milling and Baking Research Association (FMBRA) which consisted of *in vitro* mutagenicity studies using 3 extracts from treated flour and comparing the results with those obtained using similar extracts prepared from untreated flour. These were; a predominantly lipid fraction (cyclohexane: ethanol extract), an amylolytic digest of delipidated flour, and the remaining insoluble residue. Each fraction was examined for genotoxicity using bacterial assays [forward mutation in *Salmonella* BA13 (ARA assay) and the Pol assay for DNA damage

in *E.coli* and also in the mouse lymphoma L5178Y TK+/-assay]. The Committee reviewed a similar set of studies using extracts derived from chlorine treated flour in 1993 and agreed that these genotoxicity assays were appropriate for investigating activity in extracts contaminated with amino acids which might be difficult to remove.

2.10 Members agreed that the current assays were of a satisfactory standard, although it was felt that the ARA assay was relatively insensitive and that sub-optimal dose levels were used in the mouse lymphoma assays.

2.11 The Committee concluded that the data obtained were reassuring and did not indicate that there was any difference in mutagenic potential between the chlorine dioxide treated and control flour. It was agreed that no further testing of the mutagenic potential of flour treated with chlorine dioxide was necessary.

Tetrachloroethylene

2.12 Tetrachloroethylene is a high production volume chemical used in a wide range of industrial applications. It is widely dispersed in the food chain and is present predominantly in fatty foods as a contaminant. The COC gave advice regarding the potential carcinogenic risk of food contamination in 1993 and concluded that the data on residues of tetrachloroethylene in butter and lard did not give rise to concern with regard to human health effects. More recently the IARC published an update monograph (volume 63, 1995) in which it was concluded that tetrachloroethylene should be regarded as a probable human carcinogen, ie a significantly different view from that reached by the COC and hence the Department of Health asked the COC to update their conclusions. The decision by IARC to upgrade their view was apparently based largely on the conclusion that the epidemiology data now provided greater evidence to support a link between tetrachloroethylene and cancer. The COC noted that the weight of evidence suggested that tetrachloroethylene was not an *in vivo* genotoxin but were aware of recent positive results in an *in vitro* micronucleus test specifically designed to differentiate between clastogenic and aneugenic effects by the use of specialised stains using a protocol designed to detect whole chromosomes. The COC asked the COM for advice on this aspect of the mutagenicity of tetrachloroethylene. [See also section 3.30 - 3.33 of COC report for additional background information]

2.13 The Committee noted that the *in vitro* micronucleus assay used the cytocholasin B technique to block cell division in three genetically engineered cell lines (namely AHH-1, MCL-5 and H2E1) which contained human metabolising enzymes. Members agreed that there was some evidence for an aneugenic effect of trichloroethylene in AHH-1 cells but it was not possible to draw any definite conclusions regarding the relevance of these results in the absence of complete mechanistic information. The Committee also reviewed the available mutagenicity data on tetrachloroethylene.

2.14 The Committee agreed the following conclusions.

i. Tetrachloroethylene has been recently tested in the cytocholasin B-blocked micronucleus assay utilising 3 genetically engineered cell lines, namely AHH-1, MCL-5 and H2E1. The induction of kinetochore positive micronuclei was demonstrated at the highest dose of tetrachloroethylene tested in AHH-1 cells. At the present time there is no satisfactory mechanistic explanation for these results and thus no conclusions can be drawn regarding the significance of these experiments.

ii. The COM reviewed the available *in-vitro* (in mammalian cells) and *in-vivo* tests. Although there were deficiencies in the conduct and/or reporting of many of these studies, the weight of evidence suggested that tetrachloroethylene was not an *in-vivo* genotoxin. The Committee agreed that it would be desirable to have an adequate *in-vivo* bone marrow micronucleus assay to provide additional reassurance.

Trichloroethylene

2.15 Trichloroethylene is a high volume production chemical used in a wide range of industrial applications. It is widely dispersed in the food chain where it may be predominantly found in fatty foods as a contaminant. Recently the International Agency for Research on Cancer (IARC) published an update monograph (volume 63, 1995) in which it was concluded that trichloroethylene should be regarded as a probable human carcinogen (ie category 2A) rather than as a possible human carcinogen (category 2B) as previously agreed. This revised view differed significantly from that reached by the COC, when it was last reviewed this compound in 1988, ie that there was insufficient evidence to classify trichloroethylene as a human carcinogen and the Department of Health therefore asked the COC to update its advice on this compound. In their most recent evaluation, the IARC Working Group concluded that there was sufficient evidence of carcinogenicity in animals and limited evidence in humans. The conclusion reached by the IARC with respect to animal data was heavily influenced by the results of the NTP bioassays published after the most recent COC consideration in 1988 and 1990 and also information from 3 epidemiological cohort studies which considered occupational exposure in individuals with biological monitoring data and investigations of workers involved in general manufacturing industries. The COC asked for advice from the COM on the available mutagenicity data before finalising its conclusions

2.16 The Committee agreed the following conclusions.

i. Trichloroethylene has been extensively studied in vitro. In many cases limited protocols were used and inadequate information was provided on the purity of the test material or on the presence of potentially mutagenic stabilisers, and in these cases no conclusions could be drawn regarding the genotoxicity of pure trichloroethylene.

ii. However, epoxide free trichloroethylene has been shown to covalently bind to DNA in vitro, and to induce gene mutations in Salmonella in some studies when tested in the vapour phase. It has also given a weak positive result in the mouse lymphoma assay (epoxide free trichloroethylene). These studies indicate that trichloroethylene has mutagenic potential in the presence of S-9 mix.

iii. Conflicting results have been reported in *in-vivo* bone marrow assays for clastogenicity, with evidence of micronuclei induction (but not chromosome damage) in male CD rats when exposed by inhalation to trichloroethylene , but negative results were obtained in mice using the intraperitoneal route (in both cases the test material was > 99% pure). Negative results were consistently obtained in the in vivo liver UDS assay (purity unspecified).

iv. Covalent binding studies using radiolabelled material (>99% pure) do not indicate that trichloroethylene has any significant genotoxic effects in liver or kidney of mice. The significance of the increase in single strand DNA breaks seen at high dose levels in mice using the alkaline unwinding technique is questionable and no conclusions can be drawn from these studies regarding the potential mutagenicity of trichloroethylene (test material > 99.5% pure).

v. A weak positive result was reported in the mouse spot test using intraperitoneal administration of 350 mg/kg bw (>99.55% pure) to pregnant mice on day 11 of gestation. The relevance of these results to the mutagenicity of trichloroethylene is unclear .

vi. The ability of trichloroethylene to induce mutations in germ cells in vivo has been investigated using the inhalation route in a dominant lethal assay and in an assay to investigate micronuclei induction in spermatids. Negative results were obtained in both cases.

vii. In summary, in-vitro studies indicate that trichloroethylene has some mutagenic potential. Regarding the *in-vivo* data, negative results were obtained in the liver UDS and germ cell assays, whereas conflicting results were obtained in bone marrow assays. The situation should be clarified by carrying out an *in-vivo* bone marrow micronucleus test using appropriate staining (to distinguish whole chromosomes and chromosome fragments and

hence to identify any aneugenic and clastogenic potential) in male CD rats. The inhalation route (6 hour exposure time) should be used. Negative results in this assay would allow the conclusion to be drawn that trichloroethylene does not have mutagenic potential of significance for human health.

Thiabendazole (TBZ)

2.17 Thiabendazole (TBZ) is a benzimidazole derivative used as a fungicide in agriculture, as a food additive to prevent the growth of mould on the skin of citrus fruit and bananas and as an anthelminthic in human and veterinary medicines. The COT reviewed a submission of new data regarding the food additive use of thiabendazole in September 1995 and recommended an extension of the temporary ADI of 0.05mg/kg bw until 1 January 1997 pending further data on the mechanism of thyroid tumours seen in rats and advice from the COM in respect of new mutagenicity data. The COT asked for advice from the COM on whether this compound should be regarded as a potential *in-vivo* aneugen and if a threshold could be identified. The Committee reviewed all the available studies and drew the following conclusions.

i. Thiabendazole does not produce gene mutations or structural damage to chromosomes in-vitro, but the results of tests in yeast and mammalian cell systems provide consistent evidence of aneugenicity in-vitro.

ii. All the *in-vivo* mutagenicity assays using the oral route were considered negative in both somatic and germ cells.

iii. There are a number of reports of aneuploidy in bone marrow cells *in-vivo* following intraperitoneal administration of thiabendazole to mice and in one study in mice where the route of administration was not specified. The conduct and standard of reporting of these studies were considered inadequate by contemporary standards and no conclusions were drawn from these data with regard to the mutagenic hazard to humans consuming thiabendazole residues in food.

iv. Since no effects were seen by the oral route, it was concluded that dietary ingestion of thiabendazole at current levels in the diet does not give rise to concern with respect to mutagenic hazard.

Oxibendazole and Significance of polyploidy

2.18 In 1995, the Committee reviewed its advice on the health significance of chemicals that induce polyploidy whilst considering the available mutagenicity data on oxibendazole, a benzimidazole anthelmintic used in veterinary medicines in cattle, sheep and pigs. The Committee provided advice on a possible testing strategy to assess the significance of residues of oxibendazole which had been forwarded to a Working Group of European Unions Committee on Veterinary Medicinal Products. The Committee had also proposed that an assessment of the relationship between polyploidy and the results of dominant lethal studies of structurally related benzimadazoles would assist in identifying further testing requirements for oxibendazole.

2.19 As a result of the discussions within Europe, the use of oxibendazole was restricted to anthelmintic application to suckling pigs; which are not consumed to any extent in the UK. There was thus no longer a requirement for detailed testing. The Committee did however, undertake a review of the available dominant lethal studies on the structurally related benzimadazoles, mebendazole and thiabendazole with regard to the general question of whether such compounds affected germ cell development. The Committee concluded that mebendazole and thiabendazole should be considered as giving negative results in the dominant lethal assay. These results supported the view that there was no need to investigate compounds similar in structure to oxibendazole in germ cell assays.

2.20 The Committee reviewed its policy on the significance of chemicals that induce polyploidy after further consideration of the discussions on oxibendazole in Europe and in the light of the review of dominant lethal data on structurally related benzimadazoles. It was agreed that there were insufficient data available to draw conclusions with regard to the carcinogenic potential of chemicals that induced polyploidy and aneugenicity. The Committees conclusions on the health significance of polyploidy are given below.

i. Chemicals that specifically induce polyploidy do not give rise to concern with regard to heritable effects via germ cells, if the polyploidy is confined to later stage germ cells, because polyploid humans embryos are not normally viable. However, polyploid induction in germ cell precursors could give rise to later production of chromosomally

unbalanced gametes.

ii. The possibility of teratogenic effects following exposure to such compounds *in utero* is of concern; there is a clear potential for adverse effects in this regard.

iii. The significance of chemically induced polyploidy and indeed aneugenicity with regard to carcinogenic potential cannot be addressed at the present, due to a lack of information. Such information is urgently required.

Thresholds for aneuploidy inducing chemicals: Studies on Benomyl and Carbendazim.

2.21 The Committee agreed, in 1993, that it was reasonable to assume that aneuploidy inducing chemicals (particularly those that function by damaging the cell division apparatus) have a threshold of action. It would therefore be important, in relation to the safety evaluation of aneuploidy inducing chemicals (aneugens) to identify the threshold level below which aneuploidy does not occur. (See section 2.20 regarding the potential significance to human health of chemically induced polyploidy and aneuploidy.)

2.22 The Committee provided advice on methodology for identifying thresholds for aneugens acting by spindle inhibition in 1993. This advice was used by the Pesticides Safety Directorate (PSD) and the Veterinary Medicines Directorate (VMD) when requesting data from approval/licence holders of products containing such compounds. Some further advice regarding the number of positive control substances required for validation of assays and when testing compounds in validated assays was given in 1995. Pesticides Safety Directorate (PSD) asked the COM for an evaluation of the two study reports documenting data generated on benomyl and carbendazim. Specifically, PSD asked whether a No Observed Effect Level (NOEL) could be demonstrated from these experiments for aneuploidy induced by exposure in-vitro to benomyl or carbendazim, and if NOELs derived from these studies could be extrapolated to the *in-vivo* situation.

2.23 The Committee considered the two reports submitted to PSD and a recent publication reporting *in-vitro* data for carbendazim in human peripheral lymphocytes using different methodology.

2.24 The Committee agreed the following conclusions.

i. The COM considered recently conducted in vitro experiments using benomyl and carbendazim, which had been commissioned by the manufacturers at the request of the Pesticides Safety Directorate (PSD). These experiments were designed to investigate a potential threshold for aneuploidy and determine a No Observed Effect Level (NOEL) in human lymphocytes (whole blood cultures). The methods involved the detection and quantification of non-disjunction, chromosome loss and centromere positive micronuclei using FISH analysis of 6 separate chromosome probes for centromeric DNA. The COM had earlier given advice on the conduct of such studies.

ii. The Committee agreed that these studies had been satisfactorily conducted and that the data indicated a NOEL for aneuploidy of 1000 ng/ml (3.4 M) for benomyl and of 500 ng/ml (2.6 M) for carbendazim. However, it is difficult to define precise thresholds for activity from these data and the mathematical methods used for their analysis.

iii. The Committee also reviewed a recent publication (Kirsch-Volders study) which presented an evaluation of the dose response for the formation of centromere-positive micronuclei in isolated human lymphocytes incubated with carbendazim using FISH analysis. The Kirsch-Volders study used a different method of FISH analysis (ie a general centromere probe) to the investigations which had been undertaken at the request of PSD, but the results were very comparable to the data submitted to PSD with a similar NOEL identified.

iv. It was agreed that for practical purposes the NOELs identified above in paragraph (ii) could be used in conjunction with appropriate safety factors for risk assessment purposes.

v. The Committee felt that the Kirsch-Volders data indicated that a threshold of detectable activity existed for aneuploidy for carbendazim in the test system used. It was recommended that in this regard the dose-response data for the studies submitted to PSD for both benomyl and carbendazim should be further analysed by an independent statistician with a view to identifying the confidence intervals for the reported NOELs and also to provide information regarding optimum number of cells and replicate cultures that could be recommended in any further

studies.

Test strategies and evaluation

2.25 The Committee continued to provide advice on test strategies and interpretation of genotoxicity tests particularly in the context 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 also commented on the preliminary results of a Japanese interlaboratory collaborative study on the use of the mouse lymphoma assay as a potential screening test for aneugenic chemicals. The Committee agreed that there was a need for independent validation before the mouse lymphoma assay could be accepted as a test for aneugenicity. The Committee also considered thresholds for aneuploidy inducing chemicals as part of it evaluation of benomyl and carbendazim (see section 2.21 - 2.24 above) and commented on the health significance of chemical induced polyploidy whilst reviewing oxibendazole (see 2.20 section above).

2.26 The Committee provided advice on a wide range of specific topics as reported below, advised the Department of Health on research priorities in respect of mutagenicity testing and health implications and also commented on a document provided by the Office of Science and Technology (OST) regarding the EC Fifth Framework programme for research. A paper was also drafted for UK regulatory departments, which outlined some general principles for evaluating genotoxicity assays drawn from the Committees discussion of items during 1995.

Advice on research priorities

2.27 The Department of Health asked for advice on research priorities in respect of mutagenicity risk assessment of chemicals. The Committee agreed the following specific research priorities. No attempt was made to rank these in priority order but it was accepted that studies designed to improve knowledge of health implications of some of the end points measured in genotoxicity assays would be of particular importance to the Department of Health.

Methodology

a. Validation of methods for measuring induction of point mutation in rodents.

b. Validated methods for the study of the induction of aneuploidy in somatic and germ cells.

c. Improved metabolic systems using genetically engineered cells.

Health Implications

a. Provision of an understanding of the relationship between polyploidy and aneuploidy (and health significance of aneuploidy).

b. Provision of an understanding of the relative significance of breaks and exchanges in somatic cells seen in *in-vivo* rodent bioassays

c. Provision of an understanding of the relationship between DNA adducts and mutations with key compounds.

d. Provision of an understanding of dose response relationships with specific mutagens and identification of any thresholds.

e. Establishment of a databank of samples from chemically exposed individuals with information on exposure and

biomarkers of early changes and with epidemiological follow-up.

f. Improved risk assessment and assessment of inter-individual human susceptibility.

In-vivo gene mutation assays using transgenic animal models

2.28 At present there are no validated assays for routine investigation of tissues such as stomach, skin, and respiratory tract although it has been suggested in the literature that the use of transgenic animal models may provide such assays. The Committee agreed to undertake a more detailed review of the utility of *in vivo* mutagenicity testing using transgenic animal models when considering the most recent mutagenicity data on agaratine (see section 2.3(iii) above).

2.29 The Committee concluded that at present assays using transgenic animal models have not been validated for regulatory use and agreed that this was an important future research area. The assays had the potential to be of value with respect to investigation of activity at specific sites, often of initial contact eg gastrointestinal tract, skin, respiratory tract rather than as general screens for *in-vivo* activity. Further work on optimising protocols for the examination of these specific sites was needed. This should be followed by some inter-laboratory validation with selected compounds being tested coded in order to facilitate regulatory acceptance of data generated by these methods for specific tissues.

SHE cell transformation assay

2.30 The COM had considered cell transformation assays including the modified SHE method, in 1994 and had agreed that transformation assays using Syrian Hamster cells were not yet ready for routine use, but warranted further work on both validation and understanding of the underlying mechanisms. The Committee considered a draft proposal for an OECD guideline for cell transformation using the modified SHE method. A number of supporting publications and papers claiming a predictive correlation between cell transformation in this test system and carcinogenicity were also reviewed.

2.31 The Committee agreed that there were no mechanistic data available which gave an insight into the relationship between cell transformation in the SHE assay and the carcinogenic process. Although apparently high correlations had been reported in trials using a range of animal carcinogens including non-genotoxic carcinogens, only a very limited number of laboratories were involved and little value could be attributed to these results in the absence of appropriate supporting mechanistic data.

2.32 The COM concluded that it was not possible to support the proposed OECD guideline for the reasons given below;

i. The current draft of the guideline was poorly referenced and the text was vague and could apply to several different methods.

ii. The mechanisms underlying the changes observed in SHE cells were unknown.

iii. There were no objective criteria to define the end point which is subjective.

iv. Data from a validation exercise involving a number laboratories testing compounds blind should be undertaken before consideration could be given to the proposed draft OECD guideline.

In-vitro Micronucleus test

2.33 The Committee considered the progress in the development of an *in vitro* micronucleus test in 1994 where it was agreed there would be significant advantages in terms of costs compared to metaphase analysis if the *in vitro* micronucleus test could be adequately validated as a general screening assay. The Committee concluded that an inter-laboratory collaborative study was required. Results from a collaborative exercise involving 4 laboratories from the pharmaceutical sector and 52 chemicals tested in both the *in vitro* micronucleus test and by metaphase analysis were now available in a prepublication report. Members were asked to consider whether there were now enough supporting data for the COM to consider the use of this method for regulatory purposes, and for the development of an OECD guideline.

2.34 The Committee concluded.

The draft paper by Miller B et al (*Mutation Research, in press*) on the comparative evaluation of the *in-vitro* micronucleus test and the *in-vitro* chromosome aberration test using 52 chemicals tested in 4 pharmaceutical laboratories provided valuable information which suggested that the *in-vitro* micronucleus test could identify clastogenic compounds but no definite conclusions regarding the validity of the assay could be drawn. In particular, relevant information on chemical structure was not available, a number of different protocols were used and the criteria for defining a positive response were not consistent between the laboratories. The Committee agreed consideration of these issues was necessary in order to make conclusions regarding the adequacy of this assay. The Committee concluded that issues regarding test protocol optimisation should be considered as the next step to developing a harmonised protocol and that it would be most appropriate if the European Environmental Mutagenicity Society (EEMS) were to take this forward.

Use of Historical control data in mutagenicity studies

2.35 The Committee considered the significance to be placed on the use of historic control data in genotoxicity assays submitted for regulatory purposes and particularly on the use of historic data to overrule an apparent positive result based on the concurrent controls, in for example, an *in vitro* metaphase analysis for clastogenicity.

2.36 The Committee agreed that for the time being, it could only recommend the use of historical control data as a quality assurance check.

Joint meeting of COM and COC on the significance of low level exposures to DNA adduct inducing chemicals.

2.37 The symposium was held on 30 May 1996 at Skipton House, Elephant and Castle, London, UK and was attended by members of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) and the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM), invited speakers, and delegates from Government Departments and research institutes. The purpose of the symposium was to bring together experts in the field of molecular epidemiology, mutagenicity and carcinogenicty in order to evaluate the position when low levels of DNA adducts found in biological samples and thus to consider if any significance could be placed on such data with respect to carcinogenic and genetic risks attributable to low level chemical exposures. The symposium considered presentations on methodology, and background levels of DNA damage detected in humans in the morning session and studies in animals, biological monitoring and epidemiological aspects in the afternoon.

2.38 Briefly, the measurement of DNA adducts provide data on both exposure and the nature of the chemical to which exposure has occurred but there are many gaps in our knowledge regarding the relationship between DNA adducts and the production of tumours and mutations leading to congenital abnormalities. Overall, the symposium concluded that DNA adducts were a useful indicator of exposure, ie biomarkers, but that currently adduct formation alone could not be used to undertake risk assessments.

2.39 The symposium concluded that in assessing DNA adduct studies, questions to be asked relating the validity of the methods include: were the measurements valid; what was the responsible chemical or other cause; was the measured adduct level a good marker of the internal dose of mutagen/carcinogen; is the tissue where the adduct was measured an appropriate one for the objective of the study ie is a surrogate tissue a good marker for target tissues

2.40 In assessing the significance to be placed on DNA adduct data, questions which needed to be considered included: the persistence of DNA adducts measured and the biological significance of low levels of adducts found in studies using animals or in biological samples obtained from humans, and how to relate the adduct determinations with the results of epidemiology studies of cancer or birth defects.

1996 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. University of Wales Swansea

Members

Professor J Ashby BSc PhD CChem FRCS
Expert in Mutagens. Zeneca Central Toxicology Laboratory

Professor R L Carter CBE MA DM DSc FRCPath FFPM
Consultant Histopathologist. Childrens Department, Royal Marsden Hospital, Sutton

J Cole BSc DPhil
MRC Cell Mutation Unit, University of Sussex

Professor C Cooper BSc PhD DSc
Head of Molecular Carcinogenesis Section. Institute of Cancer Research, Haddow Laboratories

Professor D S Davies BSc PhD CChem FRCS FRCPath MRCP
Section of Bio-Chemical Pharmacology. Hammersmith Hospital

Professor H J Evans CBE PhD DSc(Hon) CBiol FIBiol FRCPE FRCSE FRSE
Professor of Genetics. MRC Human Genetics Unit, Western General Hospital

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

D J Tweats BSc PhD CBiol FIBiol FRCPath
Head of Pre Safety Sciences. Glaxo Wellcome Research & Development Ltd

S Venitt BSc PhD
Institute of Cancer Research, Haddow Laboratories

Secretariat

R J Fielder BSc PhD Dip RCPath (Scientific)
K N Mistry (Administrative)

J M Battershill BSc MSc

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

	Personal Interest		Non-Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Prof J M Parry (Chairman)	Albright and Wilson Astra British Telecom Compass Catering Glaxo Jib Insurance National Power Powergen Smith Kline Beecham	Share Holder Grant Share Holder Share Holder Grant Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Smith Kline Beecham Welsh Water	Grant Grant Studentship Grant
Prof J Ashby	Zenceca	Employee,Salary, Share option	NONE	NONE
Prof R L Carter	British Airways Johnson Matthey Marks and Spencer plc Morrison(Wm) Supermarkets Powergen RTZ Corp Thames Water plc Unilever	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE
Dr J Cole	NONE	NONE	NONE	NONE
Prof C Cooper	NONE	NONE	NONE	NONE
Prof D S Davis	ICI plc ML Laboratories plc Schering Plough, USA Zeneca plc	Share Holder Non Executive Director and Share Holder Consultantancy Share Holder	Astra Draco Boehringer Mannheim Bristol Myers Squibb Genentech Glaxo Wellcome Hoechst Marion Roussel Jassen Kali Chemie Pharma Lilly Research E Merck Merck, Sharpe &Dohme ML Laboratories plc Parke Davis Pharmacia Upjohn Pfizer Prodesfarma Rhone-Poulenc Rorer Roche Sanofi Winthrop Servier Smith Kline Beecham Solway Health Care TAP Holdings Upjohn Research Europe Zeneca	Fellowship & Research Research Support for Meeting Advisor Advisor Research & Studentship Research Advisor Research Research Meeting Support Research Fellowship & Research Research & Support For Meeting Research Research Research Advisor Research Research Research & Support for meetings Research Meeting Support Royalties Research

				Research
Prof H J Evans	British Petoleum	Share Holder	NONE	NONE
	British Telecom	Share Holder		
	Croda	Share Holder		
	ICI plc	Share Holder		
	Merck,Sharpe & Dohme	Consultant		
	National Grid	Share Holder		
	RTZ	Share Holder		
	Scottish Power	Share Holder		
	Southern Electric	Share Holder		
	Y Water	Share Holder		
	W Water	Share Holder		
	Zeneca	Share Holder		
Prof R F Newbold	NONE	NONE	NONE	NONE
Dr D J Tweats	Glaxo Wellcome	Salary Employee	NONE	NONE
		Share Option Holder		
		Share Holder		
	Halifax	Share Holder		
Dr S Venitt	Abbey National plc	Share Holder	NONE	NONE

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

Professor R L Carter CBE (Chairman) MA DM DSc FRCPath FFPM

Preface

The COC evaluates chemicals for their human carcinogenic potential at the request of the Department of Health and other Government Departments. A large part of this years work has been taken up with preparing statements for the Department of Health on two issues: the occurrence of three cases of acute leukaemia in children at a school in Camelford, North Devon, and the publication of an epidemiological report claiming an association between the synthetic sweetener aspartame and the occurrence of brain tumours. I am grateful to the Members of the Committee for the considerable effort made in drafting these statements, which are reproduced in full in this report. The Committee also provided advice to Government Departments on a wide range of other topics. The Department of Health requested advice on diesel exhaust with particular reference to new animal studies which provided information on the likely mode of carcinogenic action in experimental animals. The Department also sought a view from the Committee in respect of the carcinogenicity of trichloroethylene and tetrachloroethylene, and advice regarding certain man made mineral fibres (rock and slag wool, glass wool and continuous filaments). The Committee reached final conclusions in potency ranking for 25 polycyclic aromatic hydrocarbons for which MAFF and DoE had requested guidance.

On a more general issue, the Committee provided advice to the Department on research priorities in relation to carcinogenicity evaluation and risk assessment.

Aspartame

3.1 The Committee was asked by the Department of Health to review and comment on the recent publication by Olney JW, Farber NB, Spitznagel E and Robins LN (1996) Journal of Neuropathology and Experimental Neurology, 55, (11), 1115-1123 entitled Increasing brain tumour rates: Is there a link to aspartame? This substance has been used as an artificial sweetening agent in the USA since 1981 and was introduced in the UK in 1983. The authors argued that aspartame might be associated with the increased incidence of brain tumours documented in the USA between 1975-1992. The following conclusions were reached.

i. The Committee agreed that the methods used in the paper by Olney et al were inappropriate and that the presentation of data was often selective, inadequate and misleading. Changes in the histopathology and classification of brain tumours during the study period were inadequately considered, and disparate tumour types were sometimes arbitrarily grouped together. The evaluation of time trends for the incidence of brain tumours was flawed, and data for the mean annual incidence of certain brain tumours according to age were inconsistent with the hypothesis that consumption of aspartame was responsible for any increase in incidence of these tumours. No information regarding the likely consumption of aspartame was included in the results section of the paper, other than the date when the material was first marketed. The Committee agreed that the findings provided no evidence of the proposed biphasic increase in the incidence of either all brain tumours or selected tumour types in the USA during the 1980s, although Members noted that the data were consistent with an increase in the ascertainment of brain tumours during the study period (1975-1992).

ii. The Committee was aware that there was evidence of an increased incidence of brain tumours in a number of countries, including the UK, over recent decades. Improvements in diagnostic procedures were likely to be in part responsible for these observed trends. Certain exogenous factors have been proposed as possible aetiological agents, but the evidence remains weak.

iii. The Committee noted that the animal carcinogenicity data had already been fully reviewed by the COT. References in Olneys paper to these previous studies were found to be misleading in several respects. Allusions to recent work describing the *in-vitro* nitrosation of aspartame were also misleading, and it was agreed that any potential for aspartame to undergo nitrosation under physiological conditions *in-vivo* was low.

iv. The Committee concluded that the data published by Olney et al did not raise any concerns with regard to the use of aspartame in the United Kingdom.

Advice on three paediatric leukaemia cases in Camelford, North Cornwall.

3.2 The Department of Health requested advice on a possible association between the occurrence of leukaemia in three children who attended the same class at Sir James Smith School in Camelford, North Cornwall, and the Lowermoor water contamination incident in July 1988. The Committee reviewed the available toxicological and epidemiological literature on the water pollutants present in Camelford drinking water during the Lowermoor incident and sought advice from the National Radiological Protection Board (NRPB) in respect of the significance of some recent data regarding uranium levels found in one aluminium mains distribution pipe in the Camelford area. The COC statement was issued by the Director of Public Health for Cornwall and Isles of Scilly Health Authority, St Austell on the 22 October 1996 and is reproduced in full below. A separate annex is also included which contains summaries reached by COC and NRPB for individual pollutants identified during the Lowermoor incident.

Statement Regarding 3 Cases of Childhood Leukaemia Identified In Camelford, North Cornwall

Introduction

3.3 The Department of Health asked the Committee for advice on a possible association between the recent reports of 3 children with acute leukaemia in Camelford and the consumption of chemicals that polluted drinking water in that area after the Lowermoor incident of 1988. The Committee agreed to approach this question first by considering the general clinical and epidemiological data in respect of these cases together with the reports of the Lowermoor Incident, and then reviewing available epidemiological and toxicological information on the chemical contaminants known to have been present.

The General Clinical and Epidemiological Problem

3.4 The Committee was informed that acute leukaemia had been diagnosed between Autumn 1995 and May 1996 in three children aged between 13-14 years at the time of diagnosis, who attended the same class of Sir James Smith School in Camelford. Two of them had acute lymphoblastic leukaemia; the third child, who has now died, had acute myeloid leukaemia. All three patients were living in Camelford in 1988 when the water pollution incident occurred; they were then aged between 5-6 years. The patients in Camelford were unusual in that most paediatric leukaemias occur within the first decade of life, acute lymphoblastic leukaemia showing a peak incidence between the ages of 2-5 years. Certain differences between acute lymphoblastic leukaemia and acute myeloblastic leukaemia were noted but the Committee agreed that, for the purposes of this evaluation, the three leukaemic patients could be regarded as a single group.

The Lowermoor Incident

3.5 The Committee considered information regarding the Lowermoor Incident which occurred in early July 1988. Heavy but transient contamination of drinking water in the Camelford area had followed the accidental discharge of 20 tonnes of concentrated aluminium sulphate solution into the water reservoir at the Lowermoor treatment works. Two reports by the Lowermoor Incident Health Advisory Group (LIHAG), published in 1989 and 1991 provided detailed documentation of the incident.

3.6 LIHAG concluded that:

> "As a result of the Lowermoor incident, it is likely that for a period of up to three days consumers were supplied with acidic water of pH as low as 3.9-5.0. An aluminium content of up to 620,000 g/l and a sulphate concentration of up to 4,500,000 g/l were recorded. It is possible that in some parts of the [distribution] system gross contamination persisted for longer, but there is no clear evidence to this effect. The presence of acidic water in household plumbing systems resulted in levels of copper or zinc up to 9,000 g/l being recorded in water from cold taps , and up to 22,500 g/l from hot taps (depending on the plumbing in individual systems). High levels of lead would be expected in water from systems where this plumbing metal was used. There was some evidence of this, with concentrations of up to 350 g/l recorded in cold water samples and up to 460 g/l in hot water samples. However, lead plumbing is not widely used in this area. After two or three days the pH was restored to normal (7.5-9.5) in most parts of the system, as were levels of sulphate, copper and zinc. The level of aluminium fell below 1000 g/l in this second phase of the incident, but the European Community (EC) Maximum Admissible Concentration (MAC) of 200 g/l was only achieved throughout the system after a period of intensive cleaning and flushing [between October 1988 and March 1989; during this period less than 4% of samples exceed the MAC]. After the pH of the water had been restored to normal, a substantial proportion of the aluminium remaining in the distribution system would have been in an insoluble form."

3.7 The Committee also noted that the process of intensive cleaning and flushing disturbed the coal tar pitch lining used in some water mains, resulting in occasional and transient elevations in the concentration of fluoranthene in water.

3.8 The Committee commented that there was little information about the actual consumption of polluted water by people living within the Camelford distribution area; but contemporary anecdotal reports had indicated that many residents described the water as undrinkable during the first 2-3 days after the incident. Members considered that only a low proportion of the ingested metallic pollutants in this incident would be absorbed from the gastrointestinal tract and commented that the absorption of these materials in 5-6 year old children, the age of the three leukaemic patients at the

time of the episode, would be expected to be similar to adults. It was, however, noted that the bioavailability of the individual metals could have been modified as a result of their occurrence as part of a complex mixture and the lowered pH of the contaminated water.

Requirements for chemical induction of the three paediatric leukaemias in Camelford

3.9 The Committee noted that the aetiology of paediatric leukaemias is poorly understood, but there is no current evidence that chemical carcinogens are implicated. The Committee agreed that if a chemical pollutant in the drinking water was indeed involved in the three cases of acute leukaemia in Camelford, it would have to fulfil certain criteria: in particular it would have to be absorbed from the alimentary tract and, following a high exposure over a short time span (2-3 days), it would have to exert its leukaemogenic effects after a latent period of approximately 8 years. Such a chemical would have to be a highly potent or persistent genotoxic substance comparable to the few substances that have been shown to be carcinogenic in animals following a single dose.

3.10 The Committee reviewed the available epidemiological and toxicological data on aluminium, copper, zinc, lead and fluoranthene. There was no evidence that any of these chemicals met the criteria outlined in this statement.

Conclusion : Epidemiological and toxicological evidence

3.11 The Committee agreed that there was no epidemiological or toxicological evidence to support the hypothesis that short term exposure to the chemical contaminants identified in drinking water in the Camelford area following the Lowermoor Incident in 1988, was involved in the aetiology of the three cases of childhood leukaemia under investigation. A brief summary of the Committees consideration of each of 5 contaminants is annexed to this statement.

3.12 The Committee agreed to keep this aspect of the Lowermoor incident under review.

Recommendations for further work

3.13 The Committee considered the additional research proposed by the experts in childhood cancer who are advising the Director of Public Health (DPH) for Cornwall and Isles of Scilly Health Authority. Three projects were strongly endorsed;

i. A study of cancer incidence (particularly for leukaemias) in the resident population since 1988.

ii. A geographical analysis, from 1984, of the incidence of leukaemias, pre-leukaemias and related conditions, and childhood cancers, in all people up to age 24 who still live in the Camelford area.

iii. A study of the medical records of children from the 1988 cohort who still live in the area and are aged 8-16. The study would aim to identify any unusual frequency of any rare medical events; it would be important to select an appropriate control group.

3.14 Members concluded that it was unlikely that information relevant to the Lowermoor incident would emerge from a current ecological study of water quality and leukaemia.

3.15 The following information was annexed to the COC Statement on 3 cases of leukaemia in Camelford

Consideration of the individual pollutants

Aluminium

3.15.1 Aluminium sulphate was the major pollutant in Camelfords contaminated drinking water. Available epidemiological studies refer exclusively to cancer risks in the industrial context of aluminium production, where the main route of exposure is by long term inhalation of fumes emitted during the manufacture of certain types of electrodes. The Committee

considered five papers selected by the Secretariat from a large database of studies because they recorded limited evidence of leukaemias in the exposed work force. Evidence linking industrial exposure to aluminium and leukaemias had previously been regarded as inadequate by the International Agency for Research on Cancer in 1987. The Committee agreed that there was no consistent evidence for an increased risk of leukaemias and lymphohaematopoietic disorders in these five industrial studies, and that they were not relevant to the conditions obtaining in Camelford. The Committee noted that no neoplastic effects had been reported in relation to the longstanding and widespread exposure to aluminium compounds, for example, in medicaments such as antacids. The Committee agreed that there was no evidence that aluminium salts were carcinogenic. Adequately conducted tests for mutagenicity were negative.

Copper and Zinc

3.15.2 Only limited information was available. The Committee found no evidence for carcinogenic or genotoxic effects in animals.

Lead

3.15.3 Evidence for carcinogenic effects in human populations, based on occupational exposure mainly to vapourised lead, was considered inadequate and to have no bearing on the events in Camelford. The Committee noted that certain soluble lead salts (acetate, subacetate, phosphate) were carcinogenic in lifetime studies in rats and mice but did not induce leukaemias. Evidence for mutagenic activity was almost entirely negative.

Fluoranthene

3.15.4 The Committee had recently evaluated the available data on fluoranthene as part of a separate review concerned with ranking the carcinogenic potency of polycyclic aromatic hydrocarbons (PAHs). It was agreed that the small amount of carcinogenicity data available for fluoranthene was inadequate, but the compound had given negative results in well conducted in vivo tests for genotoxic activity. The Committee had included fluoranthene in the group of PAHs for which there were no current concerns with respect to carcinogenic hazard.

Other potential contaminants

3.15.5 Members were aware of recent analytical work which reported that deposits of trace metals, including uranium, had built up on a galvanised steel service pipe in the Lowermoor area. The investigators had speculated that the acidic water present during the incident might have mobilised the uranium that had been deposited. No data on uranium levels in the local drinking water were available in 1988/89 and the only information which subsequently became known to members of the Committee were chemical measurements undertaken in August 1996 which found levels of uranium to be below the limit of detection (ie <0.2 g/l). The Committee noted that the majority of service pipes within the Lowermoor area are made of plastic, which are not prone to accumulate such residues. Health effects due to radiation do not fall within the Committees remit, and an independent opinion was therefore sought from the National Radiological Protection Board (NRPB) with respect to the potential radiological hazards associated with drinking water which might have transiently contained increased amounts of uranium.

3.15.6 The NRPB noted that it was not possible to estimate the likely levels of uranium in drinking water from the published data on levels of uranium residue in a steel pipe. Radiation levels in water from the Lowermoor reservoir measured routinely between 1989 to 1995 consistently fell below half the values at which further analysis is recommended by the World Health Organisation. From the information available, NRPB concluded that levels of uranium in water from the Lowermoor reservoir were most unlikely to present a significant radiation dose or subsequent risk to health.

Carcinogenicity of diesel exhaust: Update from 1990

3.16 In 1990, the COC concluded that diesel exhaust, inhaled by rats at high concentrations over long periods of time, induced lung tumours. (A similar contemporary study in hamsters was negative). In addition epidemiological investigations provided some evidence that certain prolonged occupational exposures to diesel exhaust were associated with increased mortality from lung cancer.

3.17 Before considering the most recent data it is appropriate to point out that inhaled diesel exhaust is an extremely complex material. Diesel fuels are mixtures of normal, branched and cyclic alkanes, aromatics and small amounts of alkenes obtained from the middle distillate gas oil fraction in petroleum separation. Diesel exhaust consists of gases (oxygen, nitrogen, carbon monoxide, carbon dioxide) together with small amounts of carbon particles, a wide variety of inorganic compounds (such as sulphate and nitrogen oxides) and organic compounds including known carcinogens such as benzene and certain polycyclic aromatic hydrocarbons (PAHs and nitro-PAHs) which are present in both the gaseous and particulate phase. The final composition of inhaled diesel exhaust is determined by many factors such as the characteristics of the fuel (quality, composition, ignition behaviour, density, viscosity and sulphur content) the age of engine and its running cycle all of which influence the emission of pollutants.

3.18 Recently, the Committee was asked by the Department of Health to evaluate results from new inhalation experiments published from the Inhalation Toxicology Research Institute (ITRI) in the US and from the Fraunhofer Institute in Germany. In these studies, rats were exposed either to high dose levels of diesel exhaust, or to an aerosol of carbon black composed of inert carbonaceous particles containing minimal amounts of surface-adsorbed organic chemicals such as PAHs, or to an aerosol of titanium dioxide. In addition, the Fraunhofer Institute investigated the carcinogenic dose response in rats inhaling diesel exhaust, and compared long term effects of inhaling diesel exhaust, carbon black and titanium dioxide in mice. The main objectives of the work were to clarify the carcinogenic effects of diesel exhaust observed in, and apparently confined to, the rat. Were the lung tumours specifically associated with the diesel particles (or with substances adsorbed onto their surfaces)? or were they the consequence of a more general phenomenum of particulate overload ? This is an important consideration when assessing the animal data and evaluating the potential risks to humans.

3.19 The Committee agreed that the comparative studies using diesel exhaust, carbon black or titanium dioxide in rats had been undertaken to a satisfactory standard. A greater mortality (mainly in male rats) and reduction in body weight gain was seen at high doses. The non-neoplastic morphological changes in the lung were similar in rats exposed to diesel exhaust or carbon black or titanium dioxide: increased numbers of alveolar macrophages, chronic inflammation, reactive hyperplasia of bronchiolar and alveolar epithelium and focal fibrosis. A clear carcinogenic effect with respect to lung tumours was shown with both diesel exhaust and carbon black in the ITRI study. Increased numbers of lung tumours were also demonstrated in rats exposed to diesel exhaust, carbon black and titanium dioxide in the Fraunhofer study.

3.20 The Committee noted that the observed carcinogenic effects of diesel exhaust, carbon black or titanium dioxide in rats occurred only at high dose levels where there was an accumulation of particulate material in the lung, an observation consistent with overloading of the normal clearance mechanisms for the removal of particles from the lung. The presence of adsorbed chemical carcinogens on the diesel particles did not appear to be important as increases in tumours were demonstrated in the experiments using carbon black or titanium dioxide. Furthermore, the dose response experiments reported by the Fraunhofer Institute showed no increase in lung tumours in rats breathing the lowest dose of diesel exhaust; clearance of diesel particles from lungs in this group of animals was not impaired. A similar absence of lung tumours has been reported in other investigations of diesel exhaust in rats, inhaled at dose levels which were considered to have no effect on the local clearance of particles. No lung tumours were induced in mice inhaling high concentrations of diesel exhaust, carbon black or titanium dioxide for 2 years.

3.21 The Committee agreed that the carcinogenic effect of inhaled diesel exhaust in rats resulted from progressive overloading of the clearance mechanisms in the lung and the subsequent local accumulation of particle aggregates. This was a general response following exposure to all three types of particles examined, rather than a specific response to diesel exhaust.

3.22 The Committee noted that both laboratories had looked for DNA adducts in lungs from rats chronically exposed either to diesel exhaust or to carbon black and to the other particulates that had also been tested for carcinogenicity. There was no convincing evidence that chemical mutagens adsorbed to diesel exhaust induced novel DNA adducts in the lungs from any exposed group.

3.23 The Committee agreed on the following conclusions:

i. The carcinogenic effects of diesel exhaust observed in the lungs of rats exposed to high inhalation doses for 2 years were associated with overloading of physiological clearance mechanisms for the removal of particles from the lung. There was no convincing evidence of similar carcinogenic effects in mice and hamsters: the carcinogenicity of diesel exhaust thus appeared to be specific to the rat. Similar findings were reported in chronic inhalation studies in rats using high doses of carbon black and titanium dioxide. The Committee therefore agreed that the carcinogenicity of diesel exhaust in rats resulted from a general impairment of lung clearance associated with the accumulation of particles, and concluded that such an effect was not relevant to the assessment of risk to humans exposed to diesel exhaust.

ii. The Committee had noted in 1990 that the epidemiological findings indicated that exposure to diesel exhaust could exert a carcinogenic effect at occupational levels sustained over long periods. The new animal studies did not allow any conclusions to be made regarding the mechanisms of tumour induction by diesel particulates in humans.

Man made mineral fibres (MMMFs: Glass fibres, rock and Slag wool excluding Refractory Ceramic Fibres)

3.24 Man Made Mineral Fibres is a generic term denoting fibrous organic materials made primarily from rock, clay, slag or glass. They can be classified into 3 general groups: continuous filaments (eg glass filaments), insulation wools (glass, rock and slag wool) and refractory ceramic fibres (RCFs). Insulation wools are used in both industrial and domestic insulation, while RCFs are used only for more specialised industrial purposes. The Committee first provided advice to HSE and DoE on the MMMFs used in domestic insulation (glass, rock and slag wool) in 1986. The COC reviewed these materials again in 1994 following the publication of an IARC monograph on MMMFs, together with new results from inhalation bioassays in rats. The Committee noted in 1994 that there was little new epidemiology and agreed that further consideration would be appropriate when results of an additional follow-up from the IARC European multisite study became available.

3.25 This information has now been examined. It is based on detailed analysis of mortality data for workers employed in 12 factories from the year when production started until 1990. (The previous report, considered by the COC in 1994, had covered mortality only until 1983.) Members agreed that the new follow-up study had been well conducted: although no information about tobacco consumption had been reported for the cohort, the authors had considered other potentially confounding exposures such as asbestos, bitumen and formaldehyde. They excluded short term workers (employed for less than one year) from the analyses and, where possible, adjusted the data for local mortality rates and social class bias. Overall, there was less evidence that occupational exposure to rock/slag wool or glass wool in the early technological phase of the use of these materials showed a link with an increase in lung cancer compared to the previous IARC report. The Committee formulated conclusions with respect to lung cancer and mesothelioma for each of the fibre types examined.

Rock/Slag wool

i. The Committee agreed that the new data provided limited evidence that occupational exposure to rock/slag wool was associated with increased mortality from lung cancer. A significant trend with duration of employment was reported after short term workers had been excluded from the analyses. There was no association with time since first employment in the rock/slag wool industry. Additional investigations of a nested case-control group of rock/slag workers are planned by IARC to investigate the potential role of confounding variables, particularly tobacco smoking.

Glass wool

ii. The Committee agreed that there was very limited evidence that occupational exposure to glass wool was

associated with lung cancer. An increase in mortality from lung cancer was reported, but there was no evidence of a trend with time since first employment or duration of exposure when short term workers were excluded. The association reported for glass wool workers became non-significant when the data were adjusted for local factors (ie mortality rate).

Continuous filaments

iii. The Committee agreed that there was no evidence to associate occupational exposure to continuous filaments with lung cancer.

Mesothelioma

iv. Four new cases of mesothelioma were reported in the extended follow-up period, giving a total of five cases for the whole cohort. Although in excess of the expected number, the increase was not statistically significant. There was evidence to suggest that three of the affected workers had also been occupationally exposed to asbestos. The remaining two workers had been employed for less than one year at a rock/slag wool (RSW) plant and it was considered unlikely that these mesotheliomas were related to exposure to MMMFs. The IARC plan additonal investigations to evaluate the exposure histories of the these cases in more detail.

Overall conclusion

v. The Committee noted that data on important confounding factors such as tobacco smoking were not available and agreed that no definite conclusions could be drawn with respect to potential carcinogenic risks associated with occupational exposure to insulation wools from the report. The Committee reaffirmed its previous view that it would be prudent to act on the basis that sufficient exposure to these MMMFs in the production or user industries may increase the risk of lung cancer among the workforce.

Polycyclic aromatic hydrocarbons (PAHs)

3.26 Polycyclic aromatic hydrocarbons (PAHs) are a large group of highly lipophilic chemicals that are present ubiquitously in the environment as pollutants. Many of them are generated as byproducts of the combustion of organic material and they occur in particulate or vapour phases. Humans are widely exposed to low levels of mixed PAHs in air, food and drinking water. Higher levels of atmospheric exposure are encountered by workers employed in industries such as aluminium production, coal gasification, coke production and iron and steel founding. Cigarette smoke is also a major source of PAHs. The COM and COC were asked by DoE and MAFF for a scheme to evaluate and rank 25 selected PAHS which could be used as a basis for further monitoring and/or surveillance. It was originally intended in 1994 to use a ranking system based on toxic equivalency factors with benzo(a)pyrene as the standard comparator substance. The data were, however, inadequate for some of the listed PAHs and a simple 5 category system was devised, based on the following criteria.

i. There is a high level of concern about a carcinogenic hazard for humans because the compound is an *in vivo* mutagen and/or a multi-site carcinogen in more than one species.

ii. There is concern about a carcinogenic hazard for humans, but the data are incomplete or the mechanism is unclear.

iii. The compound is a non genotoxic carcinogen. (This category may contain compounds with an equal amount of evidence for carcinogenic hazard as compounds in categories A or B, but these are placed in a separate category because subsequent management may be different). In practice none of the 25 PAHs considered fell into this group

iv. The data are inadequate for assessment.

v. There is no concern about carcinogenic hazard, ie the compound is non-genotoxic and non-carcinogenic or the mechanism of carcinogenesis is not relevant to humans.

3.27 Sixteen of the 25 submitted PAHs were assessed jointly by COC and COM in 1994/95. The remainder - acenaphthalene, acenaphthene, benzo(b)fluorene, benzo(c) phenanthrene, cholanthrene, coronene, fluorene, perylene, and triphenylene - were considered 1995/96.

3.28 The final conclusions for the 25 PAHs are tabulated below.

Group	PAH
A	Dibenz(a.h)anthracene, Benzo(a)pyrene Benzo(a)anthracene
B	Anthanthrene Benzo(b)fluoranthene Benzo(k)fluoranthene Benzo(b)naph(2,1-d)thiophene Benzo(c)phenanthrene* Benzo(g,h,i)perylene Chrysene Cyclopenta(c,d)pyrene Cholanthrene* Indeno(1,2,3-c,d)pyrene
E	Anthracene Fluoranthene Phenanthrene Pyrene
D	Acenaphthalene Acenaphthene Benzo(b)fluorene Benzo(e)pyrene Coronene Fluorene Perylene Triphenylene

*For benzo(c)phenanthrene and cholanthrene allocation to group B was based on evidence of *in vivo* mutagenicity.
PAHs with no significant *in vivo* mutagenicity were assigned to category E.

Tetrachloroethylene

3.29 Tetrachloroethylene is a high production volume chemical used mainly as a chlorinated solvent (notably for dry cleaning) and as a chemical intermediate. It is widely dispersed in the food chain and, in particular, may contaminate fatty foods.

3.30 The COC evaluated the potential risk of tetrachloroethylene as a food contaminant in 1993. The Committee concluded that the compound was carcinogenic in rodents, inducing an increased incidence of hepatocellular adenomas and carcinomas in mice and renal adenomas and carcinomas in rats. The Committee considered the available information on mechanisms of tumour induction by tetrachloroethylene : it was agreed that the mechanisms were specific to the rodent liver and kidney, and that the resulting tumours did not give rise to concern for risks to human health. In addition, the Committee noted that residues of tetrachloroethylene measured in butter and lard were extremely low. The Committee also considered a number of epidemiological studies based on occupational groups. None of the reports dealt exclusively with workers exposed to tetrachloroethylene, and the exposure to mixtures of solvents were poorly defined. The Committee agreed that the available epidemiological data were inadequate to allow any definite conclusion to be reached on the relationship between occupational exposure to tetrachloroethylene and cancer in humans.

3.31 More recently, the International Agency for research into Cancer (IARC) published an update monograph (volume 63, 1995) which concluded that tetrachloroethylene was probably carcinogenic to humans, (group 2A) rather than a possible human carcinogen (group 2B), a change from the previous opinion which is at variance with the COCs conclusions from 1993. IARCs decision was based largely on the new epidemiological findings which suggested a link between tetrachloroethylene and increased incidence of cancers of the oesophagus and cervix uteri and non-Hodgkins lymphoma. The Department of Health therefore asked the COC to review the animal and human carcinogenicity data on this compound with particular reference to the recent epidemiology. The Committee endorsed its previous conclusion that tetrachloroethylene was carcinogenic in rodents. The weight of evidence suggested that tetrachloroethylene was not an *in vivo* genotoxin but Members were aware of recent positive results in a modified *in vitro* micronucleus test. The technique used was not a standard procedure and advice from COM was accordingly sought. The COMs conclusions are set out in section 2.14 of this report: it was agreed that tetrachloroethylene was not an *in-vivo* genotoxin but, for additional reassurance, it was desirable to see results of a well conducted *in-vivo* micronucleus assay using the rodent bone marrow.

3.32 The available epidemiological information consisted of mortality studies in cohorts of workers occupationally exposed to tetrachloroethylene and other solvents, case-control studies, and investigations involving exposure to drinking water containing tetrachloroethylene as a contaminant. Results from a further nested case-referent study were also available. The Committee drew the following conclusions.

i. The cohort studies predominantly considered occupational categories of workers who were exposed to a wide and often ill defined range of organic solvents and other chemicals - for example in chemical production, dry cleaning, degreasing, aircraft maintenance (including parachute repair) and in the graphics industry. Statistically significant increases in the Standardised Mortality Ratios were documented in a few reports for cancers of the oesophagus, cervix uteri and for non-Hodgkins lymphoma, but the analyses were based on very few cancer cases. No controls for multiple statistical tests were included. Small non statistically significant increases in the Standardised Incidence Ratio (SIR) for cancer of the cervix uteri and for non-Hodgkins lymphoma were documented in workers for whom biological monitoring data were available.

ii. The possibility of confounding - by alcohol and tobacco consumption, for cancer of the oesophagus or by sexual behaviour and/or herpes (HSV-2) or human papilloma virus infection for cancer of the cervix uteri - was not adequately addressed in any of the investigations. There was consistent evidence of a small elevation in risk of non Hodgkins lymphoma but it was not possible to draw any definite conclusions, particularly in view of the inadequacy of information on tetrachloroethylene exposure. The few analyses performed using individuals who were exposed to tetrachloroethylene alone did not confirm the claimed associations.

iii. There was thus no satisfactory epidemiological evidence to associate tetrachloroethylene exposure to cancer in the available cohort studies. No convincing evidence of an association between employment in the dry cleaning industry (ie presumed exposure to tetrachloroethylene) was documented in the nested case-referent study which specifically considered cancers of the liver and kidney.

iv. The case-control investigations examined a range of malignancies including cancer of liver/biliary tract, renal carcinoma, astrocytoma, and Hodgkins disease and non-Hodgkins lymphoma; but it was not possible to identify individuals exposed only to tetrachloroethylene in these studies. No convincing evidence of an association between exposure to tetrachloroethylene and cancer was documented in any of these accounts.

v. No conclusions can be drawn from the investigations which alleged an association between tetrachloroethylene contamination of drinking water and cancer, particularly as there was inadequate information regarding historical exposure to tetrachloroethylene in any of the epidemiological reports considered.

vi. The Committee endorsed its 1993 evaluation of the epidemiological studies on tetrachloroethylene, that the available data were inadequate to draw any definite conclusions between exposure to tetrachloroethylene and cancer in humans.

Trichloroethylene

3.33 Trichloroethylene is a high production volume chemical used mainly in metal cleaning or degreasing. Like tetrachloroethylene it is dispersed in the food chain. IARC examined trichloroethylene in 1987 and reported that there was limited evidence of carcinogenicity in animals. In a more recent evaluation of this compound, (volume 63, 1995), IARC concluded that there was now sufficient evidence of carcinogenicity in animals and limited evidence in humans to re-classify trichloroethylene as a category 2A carcinogen, (probably carcinogenic to humans). The issues raised by IARCs conclusion were similar to those encountered previously with tetrachloroethylene, and the Department of Health again requested the COC to review the data. The Committee accordingly considered the results from published animal carcinogenicity bioassays, investigations regarding tumourigenic mechanisms in animals and epidemiological studies. Data on mutagenicity were examined by the COM (See section 2.16 of this report).

Animal data

3.34 A number of animal carcinogenicity bioassays have been undertaken with trichloroethylene. Interpretation of some of them was vitiated by the use of trichloroethylene which contained epoxide stabilisers, a number of which are known to be genotoxic. Such studies were excluded, and the Committee only examined results from experiments in which highly purified, epoxide-free trichloroethylene was used. The following conclusions were reached.

i. Trichloroethylene has been tested for carcinogenicity in a number of bioassays in mice and rats using both oral and inhalation routes. It is difficult to assess the data since the early published reports do not present detailed accounts of the histopathology of lesions and some tissue diagnoses are unclear. The more recent NTP bioassays were poorly designed and suffered from a high intercurrent loss of animals due to toxicity and, in some instances, from poor animal husbandry.

ii. Trichloroethylene (amine stabilised) was carcinogenic in the male F344 rat, inducing a small number of renal tubular adenocarcinomas in a study where groups of 50 rats were dosed by gavage with 1000 mg/kg bw/day trichloroethylene for 5 days per week for 103 weeks. Small numbers of renal tubular adenomas were found at a dose level of 500 mg/kg bw/day. Small increases in the incidence of kidney tumours were also documented in bioassays using a number of other strains of male rat. Trichloroethylene was carcinogenic in male and female B6C3F1 mice, producing an increase in the incidence of hepatocellular adenomas and carcinomas in a study where groups of 50 mice were given daily doses by gavage of 1000 mg/kg bw for 5 days per week for 103 weeks. An increase in liver tumours was also documented in an inhalation study where groups of 90 B6C3F1 mice were exposed to 600 ppm trichloroethylene for 5 days per week for 78 weeks. An increase in the incidence of lung adenomas was claimed in female B6C3F1 and male Swiss mice exposed to up to 600 ppm trichloroethylene for 5 days per week for 78 weeks. The Committee considered that these lesions were not unequivocally tumours and could be regarded as localised irritant reactions in the lung.

iii. *in-vitro* studies indicate that trichloroethylene has some mutagenic potential but findings from *in-vivo* tests were equivocal. Negative results were obtained in the liver UDS and germ cell assays, and conflicting results were obtained in bone marrow assays. The COM recommended that an *in-vivo* bone marrow micronucleus test should be undertaken in male CD rats breathing trichloroethylene for 6 hours. If positive appropriate staining (to distinguish whole chromosomes and chromosome fragments) should be undertaken. Negative results would allow the conclusion to be drawn that trichloroethylene does not have mutagenic potential of significance for human health.

iv. All the available information on carcinogenic mechanism(s) of trichloroethylene suggest that metabolites formed from trichloroethylene, rather than the parent compound, are involved. Toxicokinetic data indicate that the metabolism of trichloroethylene is qualitatively similar in all species tested, and also in humans. Quantitative differences have, however, been noted between mice and rats which may be important in assessing the different carcinogenic responses observed in the two species.

    a. With respect to liver tumours in mice, the available evidence indicates that these neoplasms probably arise as a consequence of peroxisome proliferation following the extensive hepatic oxidation of trichloroethylene to trichloroacetic and dichloroacetic acid. The data suggest that it is unlikely that trichloroethylene presents a hepatocarcinogenic hazard to humans.

    b. The Committee agreed that there was no evidence for a carcinogenic response in the lungs of mice inhaling trichloroethylene. The Committee considered that the lesions observed were not unequivocally tumours and could represent localised irritant reactions in the lung.

    c. With respect to kidney tumours in rats, it has been postulated that conjugation of trichloroethylene by glutathione and the subsequent metabolism of such conjugates in the kidney by the -lyase pathway to form reactive metabolites may be important. The available data do not, however, support this view and other metabolic pathways may be involved. The mechanism by which trichloroethylene induces kidney tumours in male rats thus remains unclear.

v. The Committee concluded that there were important limitations in current knowledge regarding the proposed mechanisms for some of the carcinogenic effects of trichloroethylene particularly in rat kidney. Available data suggest that a genotoxic mechanism is unlikely but results from the proposed *in-vivo* micronucleus test need to be examined before definite conclusions are reached. The Committee noted that no conclusions could be drawn with respect to the mechanism of the tumour induction by trichloroethylene in the rat kidney and it was not possible to discount the significance of these renal tumours to humans.

Epidemiological studies

3.35 The Committee agreed the following conclusions regarding the epidemiological data on trichloroethylene.

i. A number of different types of epidemiological investigation were available including cohort studies of workers occupationally exposed to trichloroethylene during its manufacture or use primarily as a degreasing agent, case-control studies, and investigations involving exposure to drinking water containing trichloroethylene. The quality of these reports varied considerably. Most emphasis had been placed on 3 cohort studies which were adequately undertaken and reported. No weight was attributed to drinking water and case control investigations particularly in view of the clear problems in obtaining satisfactory exposure data.

ii. Regarding liver cancer, there was limited evidence of an association in one cohort study which reported a statistically significant increase in SIR for this malignancy. No statistically significant association was documented in the other 2 satisfactorily conducted cohort studies or in the case-control and drinking water investigations. An analysis combining the data from the three cohort studies was undertaken by IARC which demonstrated a significant excess in cancers of the liver and biliary tract combined and a small non-significant excess in cancer of the liver. The Committee noted the difficulties in distinguishing primary and secondary liver cancer which were not addressed in all the reports.

iii. A small, statistically significant increase in the Standardised Incidence Ratio (SIR) for non-Hodgkin lymphomas (ICD codes 200-204) was documented in one cohort study for subjects in the >20 years since first exposure assessment group. No statistically significant association was documented in the other two satisfactorily completed cohort investigations. An analysis combining the data from these three cohort studies was undertaken by IARC which demonstrated a small excess in non-Hodgkins lymphomas. The majority of cancer patients identified in these investigations had been occupationally exposed to a wide variety of chemicals in addition to trichloroethylene.

iv. One limited study documented evidence of an association between trichloroethylene and kidney cancer amongst workers at a cardboard manufacturing plant in Germany. The workers at this plant reported prolonged heavy exposures to trichloroethylene, but no confirmatory monitoring data were available. The results were difficult to interpret because the investigation was set up following identification of a cluster of cases which were included in the study. A further recent investigation documented an increased prevalence of renal tubular damage in patients with kidney cancer who had been heavily exposed to trichloroethylene. It is not possible to derive definite conclusions from these investigations.

v. Overall, the epidemiological data are inadequate to draw definite conclusions in respect of cancer in humans although for cancer of the liver and biliary tract and non-Hodgkins lymphoma limited evidence of cancer was documented in a combined analysis of the 3 most informative cohort studies. No conclusions can be drawn with regard to the reports of kidney cancer in humans exposed to trichloroethylene.

3.36 There are considerable difficulties in deriving an overall conclusion with regard to trichloroethylene, particularly in view of the uncertainties regarding the mechanisms of the induction of kidney tumours in rats, the need for an additional *in-vivo* mutagenicity study and the limited epidemiological evidence for a carcinogenic effect in humans. At the present time trichloroethylene should be regarded as a potential human carcinogen.

Test Strategies and evaluation

3.37 The Committee provided advice on strategies in carcinogenicity for the ongoing discussions of the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The Committee also commented on the proposed guidelines for carcinogenic risk assessment drawn up by the EPA.

Advice on research priorities

3.38 The Department of Health asked for advice on future research relating to carcinogenicity evaluation and risk assessment. The Committee recommended the following topics, in which no order of priority is implied.

Methodology

i. Development and validation of short term tests for detecting both genotoxic and non-genotoxic chemical carcinogens using transgenic animals such as the p53 knock out mice.

ii. Development of short term animal tests for detecting early markers of carcinogenic activity by non genotoxic substances.

Risk Assessment

i. Establishment of baselines in defined environmental and/or occupational contexts for background levels of endogenous DNA damage.

ii. Establishment of a database of biological samples from individuals with documented exposure to known chemical carcinogens to identify biomarkers such as adducts and mutagens, combined with long-term epidemiological surveillance.

Cancer Registries

i. Maintenance and improvement of cancer registry data, particularly in terms of collation, accuracy and consistency, demographic analysis and dissemination.

General

i. Methods to clarify variations in human susceptibility to certain chemical carcinogens due to genetic polymorphism.

Issues under consideration

3.39 The utility of carcinogenicity testing in the mice for regulatory purposes.

1996 Membership of the Committee on Carcinogencity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor R L Carter CBE MA DM DSc FRCPath FFPM
Consultant Histopathologist. Childrens Department, Royal Marsden Hospital, Sutton

Members

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

Professor C E D Chilvers BSc(Econ) DSc Hon MFPHM
The Division of Public Health Medicine & Epidemiology. Queens Medical Centre, Nottingham

Professor C Cooper BSc PhD DSc
Head of Molecular Carcinogenesis Section, Institute of Cancer Research, Haddow Laboratories

Professor A D Dayan BSc MD FRCPath FFOM FFPM CBiol FIBiol
Director of DH Toxicology Laboratory, St Bartholomews 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. The Northern Yorkshire Cancer Registry and Information Service, University of Leeds

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 Biology Sciences, University of Wales, Swansea

I F H Purchase BVSc MRCVS PhD FRCPath CBiol FIBiol
Director. Zeneca Central Toxicology Laboratory

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

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

	Personal Interest		Non-Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Prof R L Carter (Chairman)	British Airways Johnson Matthey Marks and Spencer plc Morrison(Wm) Supermarkets Powergen RTZ Corp Thames Water plc Unilever	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE
Prof P G Blain	NONE	NONE	Unilever plc	Research Studentship
Prof C Chilvers	ICI plc Tomkins Seton Health care Morgan Crucible	Share Holder Share Holder Share Holder Share Holder	Share Holder Share Holder Share Holder Share Holder	Research Support
Prof C Cooper	NONE	NONE	NONE	NONE
Prof A D Dayan	British Petroleum Glaxo-Wellcome Schering Plough Smith Kline Beecham TI	Share Holder Pension Consultant Consultant Share Holder	NONE	NONE
Prof P B Farmer	British Gas National Power Powergen	Share Holder Share Holder Share Holder	BAT DOW Famous Pfizer SKB Wyeth Zeneca	Research Studentship Research studentship Research Support Research Support Collaborative - Studentship Research support Collaborative - Studentship
Prof D Forman	Halifax Woolwich	Share Holder Share Holder	NONE	NONE
Prof R F Newbold	NONE	NONE	NONE	NONE
Prof J M Parry (Chairman)	Albright and Wilson Astra British Telecom Compass Catering Glaxo Jib Insurance National Power Powergen Smith Kline Beecham	Share Holder Grant Share Holder Share Holder Grant Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Smith Kline Beecham Welsh Water	Grant Grant Studentship Grant
Dr I F H Purchase	Celltech ICI plc Zeneca	Share Holder Consultant Employee	NONE	NONE
Prof A G Renwick	International Sweeteners		Hoffmann-La Roche Unilever	Research Support Research Support

	Association	Consultant	Smith Kline Beecham	Research Support
Dr S Venitt	Abbey National plc	Share Holder	NONE	NONE
Prof G T Williams	Abbey National plc Norwich Union plc	Share Holder Share Holder	NONE	NONE

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 Chairmans 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 Chairmans 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 members 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.

Glossary of Terms

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

ACUTE MYELOBLASTIC LEUKAEMIA See leukaemia.

ACUTE TOXICITY STUDY A short toxicity study in which only one dose of the substance under investigation is administered.

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.

ADENOCARCINOMA A malignant tumour arising from the epithelia (qv) (see tumour).

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.

AGLYCONE The non-sugar portion of a glycoside.

AGONIST RESPONSE The ability of a chemical (alone or in a mixture) to mimic the actions of another chemical following interaction with a cellular receptor.

ALKALOIDS A diverse group of nitrogen-containing substances produced by plants which may have potent effects on body function.

ALKYLATING AGENTS Chemicals which leave an alkyl group covalently bound to biologically important molecules such as proteins and DNA (see adduct). Many alkylating agents are mutagenic, carcinogenic and immunosuppressive.

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

ANDROGENISATION Formation of male sex hormones.

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.

ANTHELMINTIC A chemical used to destroy parasitic worms.

ANTAGONIST RESPONSE The ability of a chemical (when present in a mixture) to partially and/or completely reverse the action of another chemical following interaction with a cellular receptor.

ANTIMICROBIAL The ability to destroy microbes.

BIOMARKER A readily measurable biological concentration or similar quantity which acts as a surrogate for a biological effect.

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.

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.

CENTROMERE The characteristic region of each chromosome with which the spindle filament becomes associated during cell division. When a chromosome has replicated it consists of two chromatids joined together at the centromere.

CENTROMERE POSITIVE MICRONUCLEI See Micronuclei.

CHROMOSOME PROBE A length of DNA, tagged for ease of identification (in this case with a fluorescent marker) which hybridises with its complementary region of a chromosome.

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.

CONFOUNDING VARIABLE (Synonym - confounder) A factor that distorts the apparent magnitude of the effect of a study factor on risk. Such a factor is a determinant of the outcome of interest and is unequally distributed among the exposed and the unexposed; it must be controlled for in order to obtain an undistorted estimate of a given effect.

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.

CYTOGENETIC Concerning chromosomes, their origin, structure and function.

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.

ERYTHEMA Reddening of the skin due to congestion of blood.

EPIDEMIOLOGY Study of the distribution and, in some instances, the causal factors of disease in communities and populations. Originally confined to infectious diseases - epidemics - but now increasingly applied to non-infectious conditions such as cancer.

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).

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.

FISH (FLUORESCENCE IN-SITU HYBRIDISATION) A technique which allows individual chromosomes and their centromeres (qv) to be visualised in cells.

FOLLICULAR PHASE A stage during the ovulation process.

GAVAGE Administration of a liquid via a stomach tube, commonly used as a dosing method in toxicity studies.

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

GENETICALLY MODIFIED ORGANISM An organism which has had genetic material from another species inserted into its cells.

GERM CELL Reproductive cell eg spermatid.

GLANDULAR STOMACH The stomach in rodents consists of two separate regions - the fore stomach and the glandular stomach. The glandular stomach is the only area directly comparable to human situations.

GUT MICROFLORA The colony of micro-organisms found in the gastrointestinal tract.

HAEMATOPOIETIC Concerning the production of formed elements - red cells, white cells and platelets - in the circulating blood.

HAEMORRHAGIC Adjective from haemorrhage - bleeding.

HALIDES Binary compounds containing anions of the elements fluorine, chlorine, bromine, iodine or astatine.

HEPATOCELLULAR Relating to the cells of the liver.

HODGKINS DISEASE See Non-Hodgkins - Lymphoma.

HYDRAZINES A class of organic bases derived from hydrazine - H2N.NH2.

HYDROLYSIS The breakdown of a chemical by water into simpler products.

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

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.

JOULE Unit of energy.

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).

Acute Myeloblastic Leukaemia (AML); a form of acute leukaemia associated with the presence of myeloblast cells in the blood stream. Myeloblasts are normally found in the blood-forming tissue of the bone marrow. Acute Lymphoblastic Leukaemia (ALL); a form of acute leukaemia associated with the presence of lymphoblast cells in the blood stream and blood making organs. Lymphoblasts are a form of abnormal cell.

LIPIDS Fats, substances containing a fatty acid and soluble in alcohols or ether, but insoluble in water.

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 ADMISSIBLE CONCENTRATION The upper limit stipulated by the Council of the European Communities in Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption.

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.

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.

MID-CYCLE PEAKS OF LH AND FSH The pulsatile release of luteinising hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary during certain stages of the menstrual cycle.

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 fetus are measured.

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.

NON-GENOTOXIC See carcinogens.

NON-HODGKINS 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.

OESTROGEN ACTIVITY Hormonal activity of the female steroid hormone oestrogen or its analogues.

OSTEOPOROSIS Reduction in amount of bone mass due to increased resorption (bone is normal) which can lead to fractures after minimal trauma.

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.

PERI-RENAL Adjacent to the kidneys.

PLASTICISER A substance which increases the flexibility of certain plastics.

POLYMER A very large molecule comprising a chain of many similar or identical molecular sub units (monomers) joined together (polymerized). An example is the polymer glycogen, formed from linked molecules of the monomer glucose.

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.

PREVALENCE The number of cases of a disease that are present in a population at one point in time.

PUVA therapy A treatment used to treat the skin disease psoriasis in which patients consume a psoralen (another word for the natural plant constituents furocoumarins) and are then exposed to UVA radiation.

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.

RENAL Relating to the kidney.

SOLVENT EXTRACTS Separation of constituents of a mixture by means of selective solubility of constituents in various solvents.

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

SPERMATIDS Cells formed following, or by, meiosis (cell division which halves the number of chromosomes) in the male gonads. They undergo a process of maturation without further division to produce spermatozoa (sperm).

STABILISER A type of food additive which maintains the uniform dispersion of two or more immiscible substances.

T CELLS Cellular component of the immune system concerned with inactivation of foreign bodies.

TDI Tolerable daily intake.

TERATOGEN A substance which, when administered to a pregnant woman or animal, can cause congenital abnormalities (deformities) in the baby or offspring.

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).

TRANSGENIC ANIMAL MODELS Animals which have extra (exogenous) fragments of DNA incorporated into their genomes. This may include reporter genes to assess in-vivo effects such as mutagenicity in transgenic mice containing a recoverable bacterial gene (lacZ or *lac* I). Other transgenic animals may have alterations of specific genes believed to be involved in disease processes (eg cancer). For example strains of mice have been bred which carry an inactivated copy of the p53 tumour suppressor gene (qv) -, or an activated form of the ras oncogene which may enhance their susceptibility of

these mice to certain types of carcinogenic chemicals.

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 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.

UVA Ultraviolet light of a particular range of wavelengths (315 to 400 nm).

XENOBIOTIC A chemical foreign to the biologic system.

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.

- 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