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

1991 Annual Report

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1991

ANNUAL REPORT

OF THE

COMMITTEES ON

TOXICITY
MUTAGENICITY
CARCINOGENICITY

OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

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Foreword by the Chief Medical Officer

In keeping with the Government's policy to make the work of Expert Advisory Committees more accessible to the public, we publish for the first time the Annual Report of three of the principal Advisory Committees of the Department of Health. These Committees are concerned with various aspects of chemical safety; they are: 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).

Each Committee is comprised of independent experts drawn from diverse backgrounds, such as Academia, Industry and independent consultancies. Members are appointed by me for a three year term in the first instance. Membership is appraised every three years in the light of advances in the science relevant to the safety evaluation of chemicals, to ensure that an appropriate breadth of coverage is maintained.

I am grateful for the work of these Committees and the excellent quality of the toxicological advice provided. Their advice is highly regarded, not only by the Department of Health, but also by the other Government Departments which seek the opinion of these Committees. The Committees provide a central resource of outstanding independent toxicological expertise.

I look forward to continuing to work with these Committees in the interests of the health of the nation.

DR K C CALMAN

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About the Committees

This is the first 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 the report is to provide the toxicological background to the committees' decisions for the concerned professional.

The COT, COC and COM are independent advisory committees whose members 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.

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 the Chairman so deems, members whose outside interests may be considered to be too close to the topic under discussion can be excluded from discussion and from decision making.

The usual way in which committee reviews are conducted is that the appropriate 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 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.

The main task of the COT since its inception has been to advise Ministers on the safety-in-use of food additives. Until recently the COT has classified food additives under review into one of the following groups:

Group A: Substances that the available evidence suggests are acceptable for use in food.

Group B: Substances that on the available evidence may be regarded as provisionally acceptable for use in food, but about which further information must be made available within a specified time for review.

Group C: Substances for which the available evidence suggests possible toxicity and which ought not to be permitted for use in food, until adequate evidence of safety has been provided to establish their acceptability.

Group D: Substances for which the available information indicates definite or probable toxicity and which ought not to be permitted in food.

Group E: Substances for which inadequate or no toxicological data are available and on which it is not possible to express an opinion as to their acceptability for use in food.

Since 1990 the Committee has given its advice in numerical rather than descriptive form, allocating Acceptable Daily Intakes (ADIs) where possible for food additives. Details of this change, which brought it into line with the way most other national and international bodies express their advice on food additives, have been given elsewhere*. The ADI is 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'. ADIs are usually quoted as a specified intake in milligrams per kilograms body weight (mg/kg bw). The ADI can be either unqualified or temporary, and in many ways these two classifications are similar in philosophy to the Group A and Group B classifications used in the past by the COT. For those additives which would previously have been classified into Groups C, D or E, it would not be possible to set an ADI. The annual report of the COT for 1991 makes reference to both the old and new ways in which the COT has given its advice on the safety-in-use of food additives.

* Rubery ED, Barlow SM and Steadman JH (1990). Criteria for setting quantitative estimates of acceptable intakes of chemicals in food in the UK. Food Additives and Contaminants Volume 7, no. 3, pp 287-302.

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

Professor P Turner
(Chairman)
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Preface

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment was created in 1978 to replace the Toxicity Subcommittee of the Committee on Medical Aspects of Food Policy. The establishment of the COT as an independent advisory committee in its own right reflected the growing importance of toxicology as a science and the increasing recognition by Government that chemicals in food, in consumer products such as cosmetics and in the environment should be subject to expert toxicological review.

Since its inception the COT has been the source of advice to UK Government on the safety-in-use of food additives and on the potential adverse effects of food chemical contaminants. In order to provide this advice the COT has the necessary expertise among its membership to advise on all the general aspects of the toxicity of a chemical ie metabolism and pharmacokinetics, clinical biochemistry, pathology and effects on the reproductive system and the foetus, and also on more specialised toxicological disciplines such as immunotoxicity and neurotoxicity. For specialist advice on mutagenicity and carcinogenicity it seeks the views, when necessary, of the Committee on Mutagenicity and the Committee on Carcinogenicity.

New chemicals are continually being synthesised. In addition, chemicals occur in food due to contamination of the environment in which it is produced or as part of the intrinsic biochemical make-up of plants ie natural toxins. As new and better analytical methods are developed, the detection limits for these chemicals are being improved. It is now possible to detect chemicals present in food at a concentration of one part per million or less. The COT faces new and interesting challenges in assimilating the advances being made throughout the broad field af toxicology into the advice it gives. I found chairing the committee to be a valuable and rewarding experience and I am sure the committee will continue to meet new challenges, under the chairmanship of Professor H Frank Woods, with the same distinction it has shown in the past.

PAUL TURNER

Additives in Infant Formulae and Follow-on Formulae – COT advice on Annex V.1. and V.2. to proposed EC Directive on certain food additives

1.1 In September 1990 the European Commission issued a proposal for a Council directive on certain food additives (Working Document III/9049/90). Annex V.1. and Annex V.2. listed additives which would be permitted in infant formulae and follow-on formulae, respectively, once the directive was agreed. The directive will then be implemented into UK Regulations. All the additives listed in Annex V.1. and most of those listed in Annex V.2. had previously been considered by the COT, either for the COT Report on Infant Formulae (Appendix A to the Food Standards Committee Report on Infant Formulae 1981), or for the recent Review of the Use of Additives in Foods Specially Prepared for Infants and Young Children (see paragraph 1.40). L(+)-Lactic acid and citric acid (Annex V.2.) had been considered to be acceptable for use in weaning foods in 1983, although they are not included in the final 'Report of the Review of Additives Used in Foods Specially Prepared for Infants and Young Children' since they are no longer required by UK Industry. The COT was asked for an opinion on the rest of the additives in Annex V.2. in order to assist MAFF officials in negotiations in Brussels. The following additives for use in follow-on formulae were therefore considered in February 1991:

Guar gum

1.2 Guar gum was considered by the COT recently as an additive for general food use. It was classified into Group A as published in the 1992 Food Advisory Committee 'Report of the Review of the Emulsifiers and Stabilisers in Food Regulations 1980' (HMSO, London, 1992). Based on the extensive data available during the above review, the COT concluded that the use of guar gum in follow-on formulae at a level of up to 1g/l was acceptable.

Carrageenan

1.3 Carrageenan was also recently considered by the COT as an additive for general food use. It was classified into Group B with a request for further investigation of the extent of absorption of food-grade carrageenan, particularly by the immature gut, and of any possible immunological consequences associated with uptake by the gut-associated lymphoid tissues (see Food Advisory Committee 'Report of the Review of the Emulsifiers and Stabilisers in Food Regulations 1980'). Also, the COT recommended during its Review of Additives Used in Foods Specially Prepared for Infants and Young Children that carrageenan should not be used in infant formulae. In considering the proposed use of carrageenan in follow-on formulae, the COT concluded that carrageenan should not be used in any foods specially prepared for infants and young children until its request for further studies had been received and evaluated.

Lactic acid producing cultures

1.4 Annex V.2. of the EC proposal states that 'for the manufacture of acidified milks, lactic acid producing cultures may be used'. The COT considered that more information was required on the process and microorganisms involved in the production of acidified milks using lactic acid producing cultures before their safety could be judged.

Ascorbyl palmitate – review of general food use, use in infant formulae and use in weaning foods

1.5 Ascorbyl palmitate was reviewed by the predecessor committee of the COT, the Toxicity Sub-committee of the Committee on Medical Aspects of Chemicals in Food and the Environment, for the Food Additives and Contaminants Committee (FACC)'Report on the Antioxidant in Food Regulations 1966 and 1974' and classified in Group A. The COT reviewed ascorbyl palmitate again in February 1991. The consideration of its use in infant formulae and weaning foods was part of the review of additives in foods specially prepared for infants and young children (see paragraph 1.40). The 1974 review of the general food use of ascorbyl palmitate was updated at the same time.

1.6 Limited toxicity data were available on ascorbyl palmitate itself – a nine month and two year rat study showing no adverse effects. It was not mutagenic in ***in vitro*** studies. Studies on the effects of ascorbyl palmitate on tumour cells and on cells in culture were also considered. Based on the chemical structure of ascorbyl palmitate and the fact that it has equivalent vitamin C activity to ascorbic acid, the COT considered that it would be hydrolysed ***in vivo*** to ascorbic acid (Vitamin C, itself classified in Group A) and palmitic acid (which is a common component of a normal diet). It was reconfirmed as acceptable for general food use.

1.7 No ADI was set for ascorbyl palmitate at this stage. It was considered more appropriate to set a group ADI covering all sources of ascorbic acid when further data regarding the use of ascorbic acid as a flour treatment agent is presented to the COT. With regard to the use of ascorbyl palmitate in infant formulae, the COT considered its use as an antioxidant at levels of up to 1mg/100ml of infant formula to be acceptable. The COT also considered ascorbyl palmitate acceptable for use in weaning foods.

Astaxanthin in farmed fish

1.8 Synthetic astaxanthin is a carotenoid pigment added to the feed of farmed salmonid fish (salmon and trout) to give their flesh the characteristic pink colour which occurs naturally in their wild counterparts as a result of a diet rich in astaxanthin-containing crustacea. In 1982 the Committee approved its limited use for this purpose and classified astaxanthin in Group A for use at levels of up to 100mg/kg in the feed of farmed fish. In May 1990 the COT was asked to reconsider astaxanthin, following its earlier decision to withdraw the ADI for the synthetic pigment canthaxanthin, which is an alternative additive for use in the feed of farmed fish. The COT concluded in 1990 that it was satisfied as to the safety-in-use of astaxanthin at the existing levels of intake but asked for further details of some of the toxicity studies which had been performed on astaxanthin and for intakes of astaxanthin by the UK public to be monitored, with a report back within one year.

1.9 Consequently, in May 1991 the COT was asked to review all the available data on astaxanthin. These data indicated that astaxanthin was absorbed to a lesser extent and eliminated more rapidly than canthaxanthin, which suggests

that astaxanthin is much less likely to accumulate in the eye, the target organ for canthaxanthin. The Committee noted that the acute and sub-chronic toxicity of astaxanthin is low. A No Observed Adverse Effect Level (NOAEL) of 234mg/kg bw/day was reported in a 90-day rat study. A fertility study in the rat indicated an NOAEL for toxic effects on the foetus and neonate (reduced survival and weight gain) of 100mg/kg bw/day and in a rat teratology study there was a NOAEL of 200mg/kg for depression of maternal weight gain and reduced neonatal organ weights. A rabbit teratology study was considered to be inadequate due to poor methodology and high resorption rates in the control group. An Ames test and *in vivo* mouse micronucleus test were negative.

1.10 Estimated intakes of astaxanthin had changed very little between May 1990 and May 1991. Average intakes were reported to range from 0.054-0.144mg/kg bw/day, with an extreme intake of 0.71mg/kg/day.

1.11 The COT concluded that astaxanthin should retain its Group A classification as an additive to the feed for farmed fish only, to a maximum level of 100mg/kg of the complete feedingstuff. The Committee asked to be informed if intake increased or if Industry requested any additional usages for astaxanthin. In either event a further review of the safety data would be necessary.

Chymosin from genetically modified *Escherichia coli* K12 in cheese manufacture

1.12 The enzyme chymosin is traditionally obtained from calf stomach and is used in the production of cheese. Recently, due to increased demand, alternative sources have been developed. In 1991 the COT considered a chymosin derived from a genetically modified strain of *E.coli*. Previously, two other chymosin preparations from genetically modified microorganims had been assessed by the Committee and given provisional approval.

1.13 The manufacturers submitted data on many aspects relating to the safety-in-use of their product including production techniques, specification (including DNA content), mutagenicity studies and oral toxicity studies. The COT considered that minor effects seen in the oral toxicity studies, such as increased adrenal weight in the animals dosed with the chymosin preparation, were coincidental findings and not due to the test preparation. Particular attention was paid to the presence of small quantities of DNA in the preparation. The Committee was satisfied that the production techniques degraded the DNA and that the remaining short, partially depurinated strands of less than 200 base pairs would not consitute a hazard. The mutagenicity data were referred to the COM (see paragraphs 2.1-2.2 below) which considered that they showed that the preparation did not have genotoxic activity but which requested that some confirmatory tests be undertaken. Specific aspects of the submission, such as the genetic modification procedures, were also considered by the Advisory Committee on Novel Foods and Processes (ACNFP).

1.14 The chymosin derived from genetically modified E. *coli* was considered provisionally acceptable for use in cheese manufacture, subject to the provision

of further mutagenicity data and further review once the guidelines on microbial enzyme preparations had been finalised (see paragraphs 1.20-1.22 below).

Di-2-Ethylhexyl Adipate (DEHA) in PVC cling films

1.15 The COT had previously discussed DEHA, a plasticiser used in PVC cling films, in 1989 as part of a review which formed an annex to the Steering Group on Food Surveillance report No. 30 – 'Plasticisers: Continuing Surveillance'. In this report the COT welcomed the reduction in estimated dietary intakes of DEHA from 16 to 8.2 mg/person/day that had been achieved since the previous review in 1987. However, it also reaffirmed its belief that it would be prudent, as with any other contaminant in food, for intakes to be further reduced. The Committee also reiterated its previous view that reductions in DEHA intake should not be achieved by substituting it with compound(s) of unknown toxicity.

1.16 In July 1991 the COT was informed that the COM had reconsidered an earlier decision to ask for a dominant lethal study on DEHA using the intraperitioneal route and had decided that there was now no reason to maintain this request (see paragraphs 2.3-2.6). In light of this decision the COT was asked to review the safety data on DEHA with the aim of setting a tolerable daily intake (TDI) for this substance.

1.17 In setting a TDI, the COT was aware that there is limited evidence that DEHA increases the incidence of liver tumours when given to mice over their lifetime. The Committee was also aware that DEHA had no effect on the incidence of liver tumours in a rat carcinogenicity study and that mutagenicity studies have shown that DEHA is not genotoxic. As it is not considered to be a genotoxic carcinogen, it was assumed that a threshold exists for any carcinogenic effect. Therefore the Committee concluded that it was appropriate to set a TDI for DEHA, by applying a suitable safety factor to the lowest No Observed Adverse Effect Level (NOAEL) in animal studies, in the usual way. The COT set a TDI $_{1992}$of 0 - 0.3 mg/kg bw/day, based on NOAEL of 28 mg/kg bw/day for minor adverse effects on the rat fetus in a teratology study and a safety factor of 100. This figure also gives a safety margin of nearly 3000 between the TDI and the lowest dose causing liver tumours in mice.

Guidelines for the safety assessment of microbial enzyme preparations used in food

1.18 Microbial enzyme preparations were last reviewed by the COT in 1982 in the Report of the Food Additives and Contaminants Committee (FACC, the predecessor to the Food Advisory Committee) on the Review of Enzyme Preparations. The COT recommended that in order to obtain a Group A classification, microbial enzymes should be tested in a 90 day feeding study and in non-specific screening tests. The latter requirement was based on concern that unknown toxins might be produced by the microbial organisms used in the fermentation process. The COT discussed potential screening tests and the

rationale for the assessment of microbial enzymes on several occasions since 1982.

1.19 During 1991, the COT considered guidelines for the assessment of microbial enzymes at several meetings. The Committee has now agreed guidelines which set out the potential adverse effects following ingestion of residues of enzyme preparations in food and the data now required to assess the safety-in-use of microbial enzyme preparations. The finalised guidelines will be made publicly available in due course. The essential elements are:

A. A decision tree which divides enzyme preparations into appropriate classes on the basis of source microorgansim used to manufacture the enzyme preparation. The decision tree outlines the safety data requirements for each class of enzyme preparation under the following seven categories:

(i) Identity, Use, Stability and Specification

(ii) Quality Assurance data

(iii) Purification data

(iv) Antibiotic resistance data

(v) Toxicity data (including a requirement for a 90 day study in a rodent species and mutagenicity tests)

(vi) Intake data

(vii) Additional data for immobilised enzymes.

B. A system for ensuring high standards of quality control for microbial enzyme preparations (ie assuring the consistency of enzyme preparations), as follows:

(i) A specification for each enzyme preparation based on identity, use and quality assurance data from an appropriate number of pre-production batches.

(ii) A quality assurance record, produced on a continuing basis.

(iii) Full details of the production process and process controls.

In addition, the COT has recommended that manufacturers adopt standardised systems of quality assurance such as certification under BS 5750 (or ISO 9000).

1.20 The COT has agreed that, in view of the increased requirements for safety data and quality assurance data, there is no need for development work on non-specific screening tests to continue. The guidelines will be used as the basis for the safety assessment of new microbial enzyme preparations and of those currently classified as provisionally acceptable for use in food (Group B). It is considered that the guidelines will be of use to the Commission of the European Communities' Scientific Committee for Food, which has also been engaged in developing strategies for the safety assessment of microbial enzyme preparations used in food.

Ohmic heating: a novel food sterilisation process

1.21 In December 1990 the COT was asked by the Advisory Committee on Novel Foods and Processes (ACNFP) to consider the toxicological aspects of a novel food sterilisation process called ohmic heating. Although the process is already in commercial use, details were submitted to the ACNFP for evaluation under the voluntary notification scheme for novel foods and processes. During the ohmic heating process a continuous stream of food is passed through a tube containing a series of electrodes. A voltage is applied between the electrodes and the food is sterilised by the heat generated in it due to its electrical resistance. This method of heating enables the sterilisation of liquid-based foods without overcooking the liquid phase.

1.22 There are no reports of adverse effects to humans from exposure to foods currently on the market heated by this process. The COT considered two issues in detail. The first was the possibility that metal ions from the platinum/iridium coated electrodes might leach into the food and the second was that free radicals might be formed when foods, such as fats, were heated in this way. The Committee was, however, reassured by data from the company making the submission which showed that no electrolytic effects occurred which might lead to the formation of free radicals, or result in toxic platinum or iridium complexes leaching into the food. The Committee considered that any trace amounts of platinum and iridum which may be present in food as a result of ohmic heating did not represent a hazard to health.

1.23 The conclusions of the COT were reported to the ACNFP which, after considering the microbiological and nutritional aspects of food treated by ohmic heating, recommended clearance of the process subject to several clearly defined conditions. Full details of the ACNFP's assessment will be available in the 1991 annual report of that committee.

Sulphur dioxide and other sulphiting agents

1.24 The COT reviewed the toxicity of sulphur dioxide (E220) and other inorganic sulphites (listed below) at its February and March meetings in 1991. These substances are used as preservatives in a wide variety of foods.

Sodium sulphite E221
Sodium bisulphite E222
Sodium metabisulphite E223
Potassium metabisulphite E224
Calcium sulphite E226
Calcium bisulphite E227

1.25 One of the main purposes of the review was to provide MAFF officials with appropriate advice for use in discussions on the Commission of the European Communities' proposal for a Council Directive on Food Additives other than Colours or Sweeteners. At the request of the Food Advisory Committee (FAC), the COT also considered the proposed use of these preservatives in dealcoholised wine and the use of sulphur dioxide and sodium metabisulphite as flour treatment agents.

1.26 The COT concluded that sulphur dioxide and inorganic sulphites did not induce any significant systemic toxicity in a number of species of laboratory animals. The main effect following ingestion was a localised irritation of the stomach. The COT agreed a no observed adverse effect level for this effect of 70 mg sulphur dioxide equivalents/kg bw/day based on short term feeding studies in the rat and a multigeneration reproduction study in the rat. In settting an ADI, the Committee considered that a safety factor of 20 would be appropriate since the critical toxic effect was a localised effect and the only potential differences between the rat and man would be in the sensitivity of the stomach. The toxicological data indicated there was no variation in the sensitivity of different species of laboratory animals to sulphur dioxide and inorganic sulphites. The COT therefore set a full ADI_{1991} of 0-3.5mg sulphur dioxide equivalents/kg bw/day. In reaching its overall conclusion the COT sought the advice of the COM in respect of the mutagenicity data on sulphur dioxide and sulphites (see paras 2.12-2.13 below). The COM considered the available data and concluded that the use of sulphiting agents in food did not give rise to concern regarding the risk of heritable effects in humans.

1.27 The COT recommended that, provided that overall intakes did not regularly exceed the ADI, the current permitted uses of sulphur dioxide and inorganic sulphites as preservatives were acceptable, their use in dealcoholised wine was acceptable and that the use of sulphur dioxide and sodium metabisulphite as flour treatment agents was also acceptable.

1.28 However, the Committee was aware of the occurence of respiratory hyperreactivity following ingestion of red wine containing sulphur dioxide or inorganic sulphites in a number of individuals with asthma and chronic bronchitis. The COT recommended that the presence of added sulphites in food should be stated on food product labels and that consideration be given to making health advice available to asthmatics and individuals with chronic bronchitis.

Thiabendazole used as a preservative

1.29 Thiabendazole (TBZ) is a permitted preservative used to prevent the growth of moulds on the skin of citrus fruit and bananas. It is also used as a fungicide in agriculture and in human and veterinary medicine as an anthelmintic. The COT reviewed TBZ as part of a general review of additives classified in Group B.

1.30 A large number of toxicity studies have been carried out on TBZ but many of them, although largely satisfactory, are rather old and were not conducted to present day standards. The COT was therefore pleased to learn that the manufacturers of TBZ are updating the toxicological data at the request of the US Environmental Protection Agency. The COT reviewed both the older studies and those new studies which had been reported as of May 1991.

1.31 The COT decided that the critical adverse effect of TBZ was on the development of the fetus ie this effect occurs at lower doses of TBZ than those

which cause other adverse effects. In the mouse, at sufficiently high doses, TBZ can cause deformities in the unborn pups if given to pregnant mice at the critical stage of pregnancy. The highest dose which is without effect (the No Observed Adverse Effect Level or NOAEL) is 120 mg/kg bw/day. In the rat and rabbit, no teratogenic effects are seen but TBZ is fetotoxic ie it can impair fetal growth and viability in these species. A NOAEL of 10 mg/kg bw/day was identified in the rat and a NOAEL of 24 mg/kg bw/day in the rabbit. None of the acute, short-term and chronic toxicity studies which were reviewed indicated a NOAEL lower than 10 mg/kg bw/day.

1.32 The Committee therefore recommended a temporary $ADI_{1991-1994}$ of 0-0.05 mg/kg bw/day for TBZ. This was based on the NOAEL of 10 mg/kg bw/day in the rat and a safety factor of 200, which incorporates a factor of 2 for the temporary nature of the ADI. The COT recommended that a temporary rather than a full ADI be set out because it did not wish to finalise its advice until the results of all the new toxicity studies now being carried out on TBZ have been reviewed. It is anticipated that these will be available for review in 1994.

Jointmeeting with the Advisory Committee on Novel Foods and Processes

1.33 A joint meeting was held in November 1991 between the COT and the Advisory Committee on Novel Foods and Processes (ACNFP) to consider approaches of the two Committees to the assessment of novel foods.

1.34 The meeting was divided into four sessions: history of safe use; the concept of 'equivalence' or 'sufficient similarity'; toxicological requirements; and standards for assessment. The two Committees agreed that the ultimate aim of the advisory process was to ensure that the nation's diet was safe, adequate and nutritious and that novel foods should be at least as safe as comparable existing foods.

1.35 The main conclusions of the meeting were as follows:

(i) The initial step in the assessment of a novel food should be to compare it with an appropriate existing food; taking into account its composition, place in the diet and any particular cooking requirements. It was important to consider the novel food in the context of the whole diet.

(ii) Phytochemical data and information on traditional usage might be important. A history of safe use might also be a factor to be considered, although care had to be exercised in interpreting the appropriateness of the history of safe use in the context of consumption by the UK population.

(iii) Novel foods derived from genetically modified sources should be assessed in a similar manner to those produced by conventional techniques such as plant breeding.

1.36 Members from both Committees agreed that the meeting had been most valuable in developing common ground for the assessment of novel foods.

Review of additives in foods specially prepared for infants and young children

1.38 During 1991 the COT completed a review of these additives, conducted at the request of the Food Advisory Committee (FAC). The report of the review has been published as an annex to the FAC Report on the Review of Additives in Foods specially prepared for Infants and Young Children (FdAC/REP/12, HMSO, London, 1992).

Food Surveillance Papers

1.39 The Steering Group on Chemical Aspects of Food Surveillance is a government advisory committee which keeps under review the possibilities of chemical contamination of any part of the national food supply. It has a number of Working Parties which carry out specialist parts of its programme of work. These Working Parties periodically report on their work and the reports, which are published as Food Surveillance Papers, usually carry a consideration of the results of the work by the COT, which advises on the significance to public health of the results reported and recommends future work programmes.

1.40 During 1991 the COT considered three Working Party reports: the report of the Working Party on Organic Environmental Contaminants in Food on Dioxins in Food (Food Surveillance Paper no. 31, HMSO, London, 1992); the first supplementary report of the Working Party on Nitrate, Nitrite and N-Nitroso Compounds in Food (Food Surveillance Paper no. 32, HMSO, London, 1992) and the report of the Working Party on Veterinary Residues in Animal Products 1986 to 1990 (Food Surveillance Paper no. 33, HMSO, London, 1992). The COT's advice is included in these Food Surveillance Papers as an appendix.

Topics still under review

1.41 The following topics, which were discussed by the COT at meetings held in 1991, are still under review:

Alitame
Aspartame
Azodicarbonamide
Gellan gum
Food products derived from lupins
Mineral hydrocarbon waxes
Oxidised polyethylene waxes
Oxygen in breadmaking

1.42 The outcomes of these reviews will be published in due course.

1991 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

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Mrs D Davey (Administrative)

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Mr J M Battershill BSc MSc
Miss S O'Hagan BSc
H A Walton BSc DPhil.

New Chairman for the COT

The Department of Health has announced the appointment of a new chairman for the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT).

The vacancy will be created by the retirement of the current chairman, Professor P Turner BSc, MD, FRCP, FFPM, HonMRPharmS, HonFIBiol. Professor Turner's long and valuable service has spanned almost **17** years, first as chairman of the COT's predecessor committee, the Toxicity Sub-committee of the Committee on Medical Aspects of Chemicals in Food and then, since **1978,** as chairman of the COT.

The Chairmanship of the Committee will pass to Professor H F Woods, BSc, BM, BCh, MRCP, DPhil, FRCP (Lond), FFPM, FRCP (Edin). He is currently the Head of the Department of Medicine and Pharmacology, Royal Hallamshire Hospital, Sheffield and Dean, University of Sheffield Medical School, Faculty of Medicine and Dentistry.

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

Professor B A Bridges
(Chairman)
BSc PhD CBiol FIBiol

Preface

When mutations (inherited alterations) occur in human cells their consequences are often far-reaching, including genetic disease and cancer. The field of genetic toxicology is concerned with the identification of agents that can induce mutations (mutagens) and the assessment of their hazard to humans. One of the roles of the Committee on Mutagenicity is to recommend approaches that should be used when evaluating the mutagenic potential of chemicals in the UK; advice is given both on methodology and on the overall strategy of testing. The Committee also evaluates experimental data on specific chemicals when requested by the Department of Health or other Government Departments. It is now accepted that many chemical carcinogens act by a mechanism involving mutations and there is a clear interface with the Committee on Carcinogenicity which is facilitated by cross-membership and occasional joint meetings on important topics of mutual interest.

It is the view of the Committee on Mutagenicity that almost all compounds with mutagenic potential can be identified by the use of **2** or, at most, **3** well conducted in *vitro* tests. There is thus no need for the use of animal tests in the general screening of chemicals for mutagenicity. Unfortunately substances giving positive results in such tests include a significant proportion which are inactive in animals and present a negligible hazard to humans. It is in this area that animal tests for mutagenicity must still be employed, and some of the tests currently available are not regarded as properly validated. This unsatisfactory situation should be ameliorated by the use of advanced methods such as transgenic mice and DNA technology over the next few years.

An increasing amount of time is needed for interaction with international bodies such as OECD and the EC where the pressure to harmonize testing and regulatory strategies is revealing the limitations of the current international consensus. The Committee's policy is to require the minimum number of tests needed to obtain a clear answer, with the minimum usage of animals, and with an emphasis on well designed studies and reproducible results.

BRYN BRIDGES

Chymosin from genetically modified *Escherichia coli K12*

2.1 The COT requested advice from the COM on the mutagenicity data provided on this commercial chymosin obtained from a genetically modified *E coli* source, to support its use in the manufacture of cheese. In addition to this specific consideration, the COT was preparing guidelines on the testing of enzymes, and was seeking advice on whether there was anything to be gained from testing such enzyme preparations in a desalted/concentrated form as well as the commercial preparation, having due consideration to the loss of potentially genotoxic low molecular weight components during the desalting/concentrating procedures.

2.2 The Committee reached the following conclusions:

(i) Negative results were obtained when chymosin from genetically modified E *coli* **K12** was tested in *in vitro* assays for gene mutation in Salmonella and in Chinese hamster ovary (CHO) cells and in a metaphase assay for chromosome damage. The mammalian cell assays were, however, limited by the low concentrations of test compound that could be used, the insensitive protocols employed and the failure to confirm the results in an independent experiment.

(ii) Negative results were obtained in an *in vivo* bone marrow assay, using metaphase analysis, but the value of these data were again limited by the very low concentration of chymosin that could be administered in the formulation used.

(iii) Although the Committee saw no reason to prohibit the use of this chymosin preparation in cheese manufacture, it requested that both the CHO gene mutation assay and the *in vitro* metaphase assay be repeated using currently recommended protocols and with the enzyme in desalinated form.

(iv) The Committee felt that, in general, it would be advisable to test any similar enzyme preparations in a desalinated form.

Di-2-ethylhexyl adipate (DEHA)

2.3 DEHA is an adipic acid ester based PVC plasticizer that is widely used in PVC wrappings. The compound has been undergoing review since it was first referred to the Committee for advice on mutagenic potential by the COT in **1986;** further mutagenicity data were requested at that time.

2.4 Additional data, namely *in vitro* cytogenetics assays were considered by the Committee in **1990;** these were negative. However the Committee had also requested additional data to ascertain whether the published report of a positive dominant lethal assay, using the intraperitioneal route, was reproducible. Industry had suggested that negative data in a one generation reproductive toxicity study at high dose levels provided reassurance regarding the absence of significant dominant lethal effects. The Committee's view was that such a study could not be regarded as a surrogate for a dominant lethal test, and that the

fertility study did not provide any evidence that could be used to negate the one positive dominant lethal assay. These views were conveyed to industry.

2.5 In **1991** the Committee considered another request from industry regarding the need for a further dominant lethal assay in the light of the recently adopted revised COM guidelines. It was argued that DEHA had no structural alerts, had consistently given negative results in numerous *in vitro* assays, and also in an *in vivo* micronucleus test. There was now much evidence to support the view that all compounds active in germ cell assays would also be detected in a bone marrow assay and this was the basis of the revised strategy of testing now recommended by the COM. The Committee also noted that the dominant lethal assay that had given the result of concern had used extremely high dose levels by the intraperitoneal route, much above the limit dose level in the present guidelines.

2.6 The Committee concluded that in the light of the negative *in vitro* and *in vivo* micronucleus test data, and in view of the fact that the experiment used what would now be considered as unacceptably high exposure levels, the results were of questionable relevance to lower oral exposure. There was therefore no reason to maintain the request for a dominant lethal assay.

Erythrosine

2.7 The COM was asked by the COT Secretariat to review the mutagenicity of this compound. This was prompted by the recent FDA withdrawal of all listings of erythrosine for use in foods, cosmetics and drugs, based on the facts that thyroid tumours were induced in animals at high doses and that a genotoxic mechanism could not be ruled out. This was in contrast to the views expressed by the COM in **1986** and more recently by the CEC's Scientific Committee for Food and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), namely, that erythrosine was not genotoxic *in vivo* and that a hormonal mechanism was involved in the induction of the thyroid tumours in animals. This meant that provided that exposures were kept below the acceptable level calculated from the NOAEL for the initial non-carcinogenic thyroid effects erythrosine was safe for use in food.

2.8 The Committee thus considered an update of the available data on erythrosine, including all the information considered by the FDA. Most of these data, including all the *in vivo* mutagenicity studies, had been considered by the COM in **1986;** there were however a number of additional *in vitro* studies. The following conclusions were reached.

(i) *In vitro* data now provided somewhat more evidence that erythrosine had some limited mutagenic potential. However the compound was probably not mutagenic *in vivo* and the available data did not support a genotoxic mechanism for the induction of tumours in rodents.

(ii) The Committee was able therefore to reaffirm their earlier conclusions that the lack of genotoxic activity of erythrosine was consistent with the hypothesis that a non-genotoxic (hormonal) mechanism was involved in the production of the benign thyroid tumours seen in male rats fed high

dose levels of erythrosine in the diet. It was relevant that a very plausible alternative hypothesis existed for the induction of the tumours. [This was based on tumour induction by a hormonal mechanism secondary to its effects on thyroid function.]

Type I caramel

2.9 Food use of Type I Caramels (plain or spirit caramels) is essentially limited to the coloration of spirits such as whisky or rum. Extreme consumer intakes from such use were estimated to be in the range 3.5 - 7 mg/kg bw/day. The Committee considered a package of mutagenicity data provided by the British Caramel Manufacturers Association on representative samples of Type I caramels. Negative results were obtained in bacterial assays for gene mutation using *Salmonella typhimurium* but the substance gave positive results in two other *in vitro* assays, namely the mouse lymphoma assay and a metaphase assay in CHO cells in the absence of an exogenous metabolic activation system. Negative results were obtained in an *in vivo* bone marrow micronucleus test.

2.10 The Committee believed that further *in vivo* data were needed to provide adequate safety assurance, although it saw no reason for Type I Caramels not to continue in the current very limited food use in the interim. It was accepted that the potential risk was likely to be trivial compared to the alcohol content of spirits but for the compound to be allowed as a permitted additive to foodstuffs adequate evidence of safety was needed. Identification of the most appropriate *in vivo* studies presented some difficulty since the *in vitro* activity was due to the direct activity of the substance rather than from any active metabolite. It was felt that in the first instance information regarding the inherent DNA reactivity of Caramel I by use of an *in vitro* unscheduled DNA synthesis (UDS) assay should be obtained and that a cell system should be used that did not contain significant metabolic capability which would rapidly deactivate the caramel. It was thus recommended that cells other than hepatocytes (eg CHO or HeLa cells) be used, without the addition of an exogenous metabolic activation system. This information would be valuable in the design of the further *in vivo* assays needed.

2.11 The Committee therefore concluded that a non-hepatocyte UDS assay using autoradiography should be conducted *in vitro* on batch(es) of material known to be active in the clastogenicity assay, in'order to gain more information regarding the mechanism of the *in vitro* clastogenicity of Type I caramels. Depending on the results of this study it might be possible to suggest further *in vivo* tests in the gut but lack of validated protocols would make testing very difficult.

Sulphur dioxide and other sulphiting agents

2.12 Sulphiting agents have been used as preservatives in food for many years. The COT carried out a comprehensive review of this area in 1991 (see paragraphs 1.24 to 1.28). As part of this review the advice of COM was requested on the mutagenicity of sulphur dioxide and sulphiting agents.

2.13 The Committee considered the extensive amount of data available in the literature on the mutagenicity of these compounds and reached the following conclusions:

(i) Bisulphite has been shown to be mutagenic in certain strains of *Salmonella typhimurium* when tested under acidic conditions (pH 4-6) but not at neutral pH. There is only very limited evidence of mutagenic potential in other microorganisms, restricted to conditions of acidic pH and very high concentrations of bisulphite. Negative results were obtained in studies for gene mutation in mammalian cells (**V-79** cells). There was, however, some evidence of clastogenicity and of the production of sister chromatid exchanges (SCEs) in Syrian Hamster Embryo (SHE) cells and also for the induction of cell transformation in these cells.

(ii) Negative results were obtained in *in vivo* bone marrow assays for chromosome aberrations, micronuclei and SCEs even under extreme conditions in animals made deficient in sulphite oxidase, the detoxication enzyme for sulphite and bisulphite.

(iii) Negative results were obtained using *in vivo* germ cell assays (the dominant lethal assay, the heritable translocation test, and studies on oocytes) with the compound being given at high dose levels using parenteral routes.

(iv) Although bisulphite and related compounds can, under certain conditions (acidic pH, relatively high concentration) produce mutations *in vitro,* these compounds are rapidly converted to sulphite *in vivo* and there is no evidence that activity can be expressed *in vivo.*

(v) The use of sulphiting agents in food does not give rise to concern regarding the risk of heritable effects in humans.

Prioritization of microcomponents of the diet for further toxicity testing or surveillance – mycotoxins as an example

2.14 In collaboration with the COC, consideration has been given to establishing a system for advising MAFF on priority setting (for further toxicity testing or surveillance) for chemical contaminants on the basis of concern for their mutagenic and carcinogenic potential and the extent of exposure in the diet. As an example the Committees have looked at a group of mycotoxins for which there were some data on occurrence in the diet and some concern about their mutagenic and carcinogenic potential. This topic, and the conclusions reached, are summarised in the report of the COC (see paragraphs 3.22 to 3.40).

Ranking of mutagens for priority setting purposes

2.15 As part of an ongoing programme of work to consider methods of ranking chemicals with mutagenic and carcinogenic potential for priority setting the Committee considered the work of the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) Committee I on a

mutation index. Professor Ashby outlined the work of the group over the last 5 years. It had been established to compare the sensitivity of short-term tests for identifying carcinogens and ranking them in order of potency. Chemicals from the US National Toxicology Program (NTP) data base had been evaluated and the information was now computerized. A difficulty was the number of tests considered by the group (over 90). The different tests were treated equally since it was considered that there was insufficient information on which to base any weighting. No distinction was made between *in vitro* and *in vivo* assays. The data obtained from each test were weighted for potency, reproducibility and averaged within *in vitro* and *in vivo* groupings, and finally merged to give an overall agent score.

2.16 The COM had many concerns about this approach and the assumptions made (eg assuming that all tests were equivalent with no distinction even between *in vitro* and *in vivo* data), and believed that any ranking based on the overall scores obtained needed to be viewed with much caution. It was felt that expert judgement based on consideration of the inherent reactivity of the compound or metabolites (along the lines of the model compound proposed by Professor Ashby and described in the recently revised COC guidelines on the testing of chemicals for carcinogenicity – see paragraph 3.1) was a preferable approach.

Mutagenic activity of chlorinated drinking-water

2.17 Concern about the potential mutagenicity of highly concentrated extracts of chlorinated drinking-water led to the COM reviewing this area, at the request of the Committee on Medical Aspects of Contamination of Air Soil and Water (CASW), in 1985. At that time there was clear evidence for the presence of mutagens in concentrated extracts of chlorinated drinking-water, with positive results being obtained in gene mutation assays in Salmonella and for clastogenicity in mammalian cells. Activity was reduced in the presence of serum and concentrates which were mutagenic *in vitro* did not produce chromosome damage in mouse bone marrow *in vivo*; it was therefore considered unlikely that the mutagenic compounds would reach the germ cells and a mutagenic risk to humans was unlikely. However a number of recommendations were made for further work to clarify the situation. This involved both the identification of the compounds responsible and investigating the ability of the mutagenic activity to be expressed *in vivo*, particularly in the gastrointestinal tract, using the nuclear anomaly assay. As a result the DOE contracted a substantial amount of work at the Water Research Centre (WRC) to investigate methods for highly concentrating extracts of chlorinated drinking-water containing the mutagenic activity, identifying the substances responsible and investigating *in vivo* activity. This work has now been completed, and was reviewed together with the data which had been published by other groups since this area had last been reviewed by the COM.

2.18 The following conclusions were reached:

(i) Concentrated extracts of treated drinking-water, prepared by adsorption on XAD resin at either pH 7 or pH 2 and eluted with acetone, accounted for the majority of the mutagenic activity seen *in vitro*.

(ii) Treatment of mice orally with these extracts, which represented a 100,000 fold concentration of the drinking-water, resulted in a slight increase in nuclear anomalies in the non-glandular stomach, using the pH 7 extract at the maximum tolerated dose. No effects were seen on other parts of the gastrointestinal tract or the liver and bone marrow

(iii) The compound 3-chloro-4-dichloromethyl-5-hydroxy-2-2 (5H) furanone (MX) has been shown to be a major component of the mutagenicity observed in concentrated water extracts. This has consistently been shown to be present in treated drinking-water, in amounts between approximately 1–90 ng/litre, in studies in several countries.

(iv) MX has given negative results in the *in vivo* bone marrow micronucleus test. Studies by two groups using the nuclear anomaly assay have shown that MX can produce an increase in nuclear anomalies in the glandular stomach (and the duodenum in one case) but only at very high dose levels approaching the LD_{50} value. No effects were seen at lower dose levels.

(v) The *in vivo* activity in the gastrointestinal tract is very much lower than might be expected from the very potent *in vitro* activity, presumably because of rapid detoxication in the gut by the enzyme glutathione transferase and other factors. This is supported by *in vitro* studies showing reduced activity in the presence of albumin or glutathione transferase.

(vi) The nature of the components responsible for the *in vitro* mutagenicity, other than MX, is not known, but work at WRC suggests that brominated analogues (BMXs) with similar properties to MX, may be important.

(vii) The results obtained, indicating marginal activity in the nuclear anomaly assay in the stomach only at toxic dose levels using highly concentrated (100,000 fold) extracts, or with doses of MX in the lethal range, suggest that treated drinking-water itself presents little risk in this regard.

(viii) No further studies on the mutagenic potential of these compounds are warranted.

Mutagenicity testing strategies for new substances

2.19 The Committee has provided advice to the Health and Safety Executive (HSE) on a strategy of testing of compounds for mutagenicity in the context of the EEC Notification Scheme on New Substances, a scheme for the safety testing of new chemical compounds. This was to support the HSE representative at CEC meetings in Brussels attempting to harmonise the response of member states to the need for more mutagenicity testing as tonnage triggers are reached (more safety testing when larger amounts are produced) or if positive results were obtained in initial studies.

2.20 The area of particular concern and controversy related to compounds that were apparently positive in an Ames test and negative in an *in vitro* cytogenetics test in the initial notification package (the base set tests). HSE had argued that the strategy recommended by the COM in their revised guidelines should be

adopted. Thus such compounds should be investigated *in vivo*, initially using a bone marrow assay for clastogenicity and, if this were negative, at least one additional assay in a different tissue; the second assay needed to be identified on a case by case basis. However other member states had argued that the first test to be carried out should be an additional *in vitro* assay, to investigate gene mutation in mammalian cells. Negative results would reduce the level of concern and delay the request for *in vivo* data. Furthermore they argued that such compounds were gene specific mutagens, and that an *in vivo* assay for clastogenicity would be inappropriate.

2.21 The HSE had requested that the COM provide justification for its strategy. This was based on the contention that, where compounds were apparently positive in the Ames test and negative in an *in vitro* cytogenetics test, this was likely to be due to problems with metabolic activation rather than an end-point specificity. The Committee considered in detail the published evidence for the absence of genetic specificity. A review of the **209** putative carcinogens examined by the International Agency for Research on Cancer (IARC) Supplement **6** working group indicated that **42** compounds were reported as positive in the Salmonella (Ames) assay. Of these **39** were positive in *in vitro* cytogenetics assays, one was equivocal and **2** were negative namely hydrazine (due probably to problems with metabolic activation) and vinylidene chloride (due to problems with volatility). In addition work by Dr. J. Cole at the MRC Cell Mutation Unit had shown that all gene mutagens examined in the same cell line for clastogenicity were also demonstrated to be clastogens. Recent data reviewed by Professor Bridges on germ cell mutagens had identified data on a total of 75 compounds; all of those that had been tested in the Salmonella assay were positive and where data were available for clastogenicity this (either *in vitro* or *in vivo)* was again positive. In addition extensive searches were carried out on **4** compounds that had been claimed by others to be specific gene mutagens. These compounds either had not been adequately tested for clastogenicity (bis (chloro-methyl)ether, BCME) or showed mutagenic activity that was very dependent on the metabolic system employed (sodium azide and N-butyl-N-(hydroxybutyl)nitrosamine). None of the data supported the existence of gene specific mutagens.

2.22 The published data thus supported the view that where compounds were apparently positive in the Ames test but negative in the *in vitro* cytogenetics test, this was most likely to be due to problems with metabolic activation or inadequacies in the test method, rather than end-point specificity.

2.23 It was clear that a major factor driving Germany and the Netherlands to different conclusions from the UK was the unpublished data generated under the new substances notification scheme. HSE had data on **20** compounds that were positive in the Salmonella assay and which had also been tested in an *in vitro* cytogenetics assay; **14** of these were negative in the latter test. These data are not available in the scientific literature and had not been peer reviewed. The Committee strongly felt that it would be unacceptable to alter the recommended strategy on the basis of such data. The strategy of following up a positive result in either of the initial mutagenicity assays by *in vivo* data from at least **2** assays before considering a compound negative *in vivo* represented a prudent approach.

The Committee was however willing to look at the full test reports from these unpublished data to see whether it would warrant any different conclusions being drawn.

2.24 The Committee agreed the following conclusions relating to the strategy of testing.

(i) It would not change its view on the necessity of *in vivo* testing when any *in vitro* test was positive and it was adamant that the first *in vivo* test should be in the bone marrow.

(ii) It would be interested in looking at the full German data and considering whether this provided scientific justification for the German view. However this would take considerable time.

Consideration of a request from industry that compounds positive in the Salmonella assay need not be subjected to further *in vitro* testing

2.25 Industry has requested advice from the COM as to whether any further *in vitro* testing can be justified after a substance has produced a clear positive result in a Salmonella (Ames) assay and, specifically, whether an *in vitro* cytogenetics assay is necessary.

2.26 As stated earlier it is the view of the Committee that specific gene mutagens are very rare (if indeed they exist). The knowledge that a compound produced chromosome damage *in vitro* in addition to gene mutation would thus be of little value in the design of the subsequent *in vivo* testing.

2.27 The Committee felt therefore that a strong case could be made out for not carrying out an *in vitro* cytogenetics assay in these circumstances. It was accepted however that this view was unlikely to be held throughout the EEC.

Advice on chemicals used at the BNFL Sellafield site and the Dounreay (UKAEA) Nuclear Establishment

2.28 The COM was asked in **1990** by the Secretariat of the Committee on Medical Aspects of Radiation in the Environment (COMARE) to provide advice on the mutagenicity of chemicals used at Sellafield and Dounreay, both currently and in the past. This was in order to fulfil one of the recommendations of the second report of COMARE, 'An investigation of the possible increased incidence of leukaemia in young people near Dounreay Nuclear Establishment' published in **1988.** Recommendation **5** was that a study be made of the chemicals used at both Dounreay and Sellafield, and in the immediate neighbourhood, identifying the time pattern of their use, the extent of worker exposure and the disposal routes employed.

2.29 In response to this recommendation UKAEA and BNFL have produced lists of chemicals used at present and in the past at either site, together with details of the manner in which they were discharged. In addition lists of chemicals used in the research and development area and which may give rise to concern regarding mutagenic and carcinogenic potential were provided.

2.30 The COM considered these substances and the other details provided at meetings in June 1990, March and May 1991. The following conclusions were reached:-

(i) The lists of process chemicals used currently at Sellafield and Dounreay as provided by BNFL/UKAEA do not give rise to concern regarding mutagenic potential. However, the Committee notes that in the past chemicals that do give rise to such concern were used, namely benzene, dichromates and hydrazine. The Committee also notes that the methods used to dispose of the stock of benzene over the period 1952-59 are unclear.

(ii) Regarding the chemicals handled by the R and D Section, clearly these include many compounds that have mutagenic and carcinogenic potential which should be handled accordingly.

Consideration of the possibility of chemicals inducing cancer in the offspring following paternal exposure

2.31 In addition to the specific consideration of the chemicals used at Sellafield and Dounreay (see above) the COM was also asked by the COMARE Secretariat to provide advice on whether there was any evidence that paternal exposure to chemicals could result in malignancies in the offspring. This request was prompted by the publication of the results of Professor M. Gardner's case-control study on leukaemia and lymphoma incidence in young people resident in West Cumbria [Gardner M.J., Snee M.P., Hall A.J., Powell C.A., Terrell J.D., (1990) British Medical Journal 300 (6722) 423–429]. This showed a statistical association between the recorded external radiation dose of men employed at Sellafield and the incidence of childhood leukaemia in their offspring. However the numbers involved were small and factors may well have been involved other than effects on the paternal germ cells eg internal radiation exposure, exposure via contaminated material being carried off-site etc. Further work is being carried out to help clarify the situation. However the study has raised much interest in the potential of paternal exposure to radiation or chemicals to induce malignancies in the offspring.

2.32 The COM considered the available data on chemicals from the published literature, covering both animal studies and from human exposure, relevant to this question at its meeting in June 1990. This involved the assessment of a considerable number of studies.

2.33 Results from a limited number of studies in laboratory animals did suggest that paternal exposure to certain mutagenic chemicals could result in malignancies in their first generation offspring. It was noted that limited data

from humans exposed to cytotoxic agents used therapeutically did not indicate such an effect. It was felt prudent however to assume that a chemical shown to be mutagenic in germ cells in laboratory animals had the potential to induce malignancies in the offspring.

2.34 The Committee gave further consideration to this issue, concentrating particularly on the mechanisms involved and how this related to the Sellafield data, at meetings in October 1990 and March 1991. Conclusions were reached regarding the mechanisms by which paternal exposure to relatively low levels of chemicals may result in malignancies in the F–1 generation and on further research work needed in this area. A summary of these conclusions was published as a written answer in Hansard on 16 July 1991 in response to a question from Dr. Cunningham.

2.35 The full conclusions reached by the Committee in this area are given below:

Evidence that paternal exposure to chemicals may result in malignancies in the offspring

(i) Radiation and chemical mutagens have been shown to produce the types of mutations at the gene and chromosome level that are known to be associated in humans with predisposition to the development of malignancies in offspring.

(ii) Only very limited data are available from animal studies on paternal exposure to mutagens and the development of tumours in offspring. These suggest that with ionizing radiation and certain chemicals paternal exposure results in the induction of malignancies in the offspring.

(iii) The data available on the effect of chemicals in humans do not allow any firm conclusions to be drawn. The limited data available on paternal exposure to cytotoxic agents used therapeutically have not indicated that there is any increased incidence of malignancies in the offspring of such patients.

(iv) It would be prudent to assume in principle that a mutagen capable of affecting both somatic cells and germ cells *in vivo,* has the potential to induce malignancies in offspring, following paternal exposure.

Environmental mechanisms by which paternal exposure to relatively low levels of chemicals may result in malignancies in the offspring

(v) Whether or not the excess childhood leukaemia reported at Sellafield is a consequence of paternal exposure (either to radiation or some other mutagen) the data are not readily reconcilable with what is known about the genetics of childhood leukaemias. The data are therefore worthy of further consideration.

(vi) If the predisposition to these leukaemias is a consequence of induced heritable mutations, then, both on theoretical grounds and from animal

experiments, one might, making certain assumptions, expect to see a higher level of congenital abnormalities in any population sufficiently mutagenized to show such a level of carcinogenic gene mutation; although it has not been properly investigated, we know of no evidence for this in the Sellafield area.

(vii) If the 6–7 fold excess incidence of childhood leukaemia reported among the offspring of male Sellafield workers is a consequence of induced inherited mutation, this would imply a germ line mutation frequency of at least 1 in 300. This is many orders of magnitude greater than most spontaneous mutation rates of single genes and considerably greater than the expected mutation frequency increases following exposure to low doses of radiation. The only classical mutations that could be expected to give rise to such large increases are chromosomal deletions which would be detectable cytologically. The majority of such deletions would however not be viable and those that were would also be associated with other, and often gross, phenotypic abnormalities. A genetic basis may be able to accommodate the results if there were a large number of genes (say 20–100) that could influence childhood leukaemias. If so, however, one would expect many of these to be general neoplastic genes and their effects would not be confined to childhood leukaemias. Nevertheless, even on such a model it is not possible to explain the apparently extremely low mutation doubling dose.

(viii) Extremely high frequencies of neoplasia among the offspring of male mice exposed to either radiation or the chemical carcinogens N-ethyl-N-nitrosourea (ENU) and, particularly, urethane have been reported by Nomura in a series of publications. Although these are mostly lung adenomas, leukaemias were apparently increased in some strains. In any case the genetic problem remains whatever the neoplastic endpoint. Nomura has argued that the mutations in his mouse model are unlikely to be chromosomal on the grounds that:

> *'(i)* urethane produces tumours but not translocations or dominant lethals and
>
> *(ii)* that tumours occurred no more frequently in mice with X-ray induced translocations than in those without translocation'.

However urethane is an established *in vivo* clastogen in the mouse and the latter argument is statistically invalid. On the other hand it should also be noted that in one of the two strains of mice studied by Nomura, no increased incidence of leukaemia was observed in progeny from irradiated spermatogonia, but a two-fold increase was seen in offspring from irradiated sperm and spermatids – a pattern which could imply an involvement of chromosomal mutations as opposed to more subtle gene alterations.

(ix) It is clearly important that a better understanding be gained of the mechanistic basis of tumour induction following paternal exposure. If the Sellafield cluster is an example of such an effect it is likely that there will be others resulting from chemical exposure; the increase in West Cumbria of leukaemia among the offspring of fathers working in the chemical, iron and steel, and agricultural industries for instance was just as great as that

found among the offspring of Sellafield workers. Chemicals capable of causing such an effect may not necessarily be recognised as conventional mutagens (although both ionizing radiation and urethane are). Moreover one may speculate about the possible involvement of other agents such as viruses. The Committee, however, would find it difficult to advise on these possibilities on the basis of current knowledge.

(x) The Committee therefore strongly recommends:

(a) That work be carried out in this country to confirm the observations of Nomura and to establish a similar experimental model that can be used for mechanistic studies.

(b) That in such work, the emphasis should not be exclusively upon ionizing radiation but should include chemicals, in particular urethane.

1991 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

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

Dr R L Carter
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Preface

The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment evaluates chemicals for human carcinogenic potential at the request of the Department of Health and other Government Departments. This evaluation utilises many sources of information, including epidemiology, structural chemistry, metabolic studies and short term mutagenicity tests, as well as the results of long term animal testing. Animal bioassays still form an important part of the appraisal, but the broad expertise and membership of the Committee enables the weight of all the evidence to be taken into account when making an assessment of carcinogenic potential. Cross-membership with the Committee on Mutagenicity, and the joint consideration of compounds with extensive short-term testing, enables the Committee to make full use of information about genotoxicity in its deliberations.

The Committee also considers generic issues which have previously included ranking of carcinogens, setting thresholds for non-genotoxic carcinogens and quantitative risk assessment. The Committee's Guidelines on the Evaluation of Chemicals for Carcinogenicity (published in 1991) give advice on the assessment of the carcinogenic potential of chemicals and cover topics such as chemical carcinogenesis and risk assessment. Joint scientific meetings are held with the Committee on Mutagenicity to discuss topics of common interest such as promotion in carcinogenesis and the role of peroxisome proliferation in tumour development.

RICHARD CARTER

Guidelines

3.1 The last set of guidelines drawn up by the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) was published in **1982.** They dealt in the main with the design, conduct and interpretation of long-term animal bioassays. Since these tests had become reasonably well standardised and the COC's considerations of potential chemical carcinogens included many other aspects of carcinogenicity, it seemed appropriate to produce a new edition which would address the overall evaluation of chemicals as potential human carcinogens in a more comprehensive fashion. The Guidelines for the Evaluation of Chemicals for Carcinogenicity [Department of Health. London: HMSO **1991** (Report on Health and Social Subjects 42)] were published in October **1991.**

3.2 Animal bioassays still form an important part of the text, but the Guidelines have been broadened to include an introductory chapter on general aspects of chemical carcinogenesis and further chapters on epidemiology, short-term predictive tests and approaches to risk assessment. It was not the COC's purpose to set out procedures which must be inflexibly followed, since other guidelines from appropriate regulatory authorities laid out in detail the recommended procedures for testing. The emphasis of these Guidelines had been deliberately directed to some of the problems that were encountered in appraising potential human carcinogens for regulatory purposes. Some of the issues considered were still controversial and reasonably-argued interim opinions sometimes had to stand in the place of definitive answers.

Summary

3.3 Chapter **1** gives some background information on general issues in chemical carcinogenesis. It briefly discusses mechanisms by which genotoxic and non-genotoxic substances may be involved in the development of tumours. The role of oncogenes and tumour-suppressor genes in molecular carcinogenesis is also described.

3.4 The contribution made by epidemiological studies to an overall assessment of carcinogenicity is dealt with in Chapter **2.** The relative merits and limitations of different types of epidemiological investigations are discussed.

3.5 Chapter **3** considers the major classes of chemical carcinogens with regard to the different mechanisms by which they exert their effects. The role of metabolism is discussed.

3.6 Chapter **4** covers the use of short-term predictive tests for screening for carcinogenic potential of chemicals (mutagenicity tests and cell transformation assays). Reference is made to the strategy for mutagenicity testing given in the Committee on Mutagenicity's 'Guidelines for the Testing of Chemicals for Mutagenicity' which is also relevant to predictive short-term testing for carcinogenicity.

3.7 The main points to be considered in designing a carcinogenicity bioassay are covered in Chapter **5,** and some of the difficulties which might be encountered

during the performance of such studies are discussed. Special problems associated with the carcinogenicity testing of certain classes of substances are reviewed.

3.8 The interpretation of results from carcinogenicity tests is covered in Chapter 6. Statistical methodology is not dealt with in detail, but reference is made to more specialized guidelines. Advice is given on the problems of interpreting the biological significance of results. A number of factors which can influence the interpretation of the results of a study (confounding factors) are examined. Mechanisms of carcinogenicity are discussed in the context of interpreting the relevance to humans of a carcinogenic response in animals.

3.9 Assessment of the hazards and risks from exposure to chemical carcinogens is dealt with in Chapter 7. It is the COC's view that threshold levels of exposure (below which there is no carcinogenic hazard) can be set for non-genotoxic carcinogens provided that their modes of action are understood. This approach is not appropriate for genotoxic carcinogens where it must be assumed that there is an increased risk at all levels of exposure so that no threshold level can be postulated. Current methods of quantitative risk assessment of exposures to non-threshold carcinogens are presented, and the COC's reasons for not using them on a routine basis are set out. The way in which the acceptability (or otherwise) of human exposure to chemical carcinogens is assessed by UK regulatory authorities is summarised with' particular reference to the role of the COC.

3.10 References and suggestions for further reading are given at the end of individual chapters. A glossary is also provided.

Benzene

3.11 The COC considered the carcinogenicity of benzene in order to advise the Health and Safety Executive (HSE) and the Department of the Environment (DOE) on the implications of occupational and environmental exposure to this compound.

3.12 The following conclusions were conveyed to the Health and Safety Commission's Advisory Committee on Toxic Substances (ACTS) and to DOE:

(i) Benzene is genotoxic and has been shown to induce leukaemia in humans. No threshold for carcinogenicity has been demonstrated and therefore it is not possible to set an entirely safe level with regard to carcinogenic effects. There is evidence of increased incidence rates for various leukaemias in occupational groups exposed to levels in the 10-20 ppm range.

(ii) Sampling and analytical techniques are adequate for measuring occupational exposures. The setting by HSC of a maximum exposure level (MEL) of 5 ppm placed a duty on employers to reduce levels to 'as low as reasonably practicable'. Adequate surveillance should be undertaken in order to ensure that this obligation is fulfilled. It is possible that people working in downstream occupations, such as petrol stations, could be exposed to levels close to the MEL. The MEL vaue of 5 ppm was relatively close to that at which effects on human health had been noted.

(iii) Information on non-occupational and environmental exposure levels was inadequate. Further data are required which take into account factors such as meteorological conditions, traffic flow, sources of environmental benzene etc. From the limited data seen by the COC, non-occupational/ environmental exposure to benzene appears to be 2 to 3 orders of magnitude below that of occupational exposure.

(iv) More information is required on other sources of benzene exposure such as food and cigarette smoking.

Polyurethane coated breast implants

3.13 The COC considered the carcinogenic risk of polyurethane-coated breast implants at the request of the Medical Devices Directorate of the Department of Health (MDD), in response to public concern over the safety of this particular type of breast implant and the results of recent studies which had shown that the polyurethane-coating (polyurethane foam) of breast implants has the potential to form 2,4-toluenediamine (2,4-TDA) by hydrolysis.

3.14 The COC reviewed the available data on the genotoxicity and carcinogenicity of 2,4-TDA. Although there was sound evidence of genotoxic effects *in vitro*, the compound had not been tested *in vivo*. The relevance of standard genotoxicity data to a situation in which the compound would be released within local tissues was also questioned. Long-term carcinogenicity tests indicated that 2,4-TDA might be carcinogenic in rodents, but the work was of poor quality.

3.15 Members then went on to consider the evidence for the possible release of 2,4-TDA from polyurethane-coated implants *in vitro* and in rodents. There were no data on the potential for breakdown of these implants in human. No epidemiological information was available on women with this type of breast implant.

3.16 The COC concluded:-

(i) 2,4-TDA should be regarded as a probable genotoxic carcinogen.

(ii) There was evidence that small amounts of 2,4-TDA (estimated 0.01 μg/g/day) could be released from polyurethane foam *in vitro.*

(iii) There was indirect evidence that implanted polyurethane foam broke down *in vivo* in rats, but the quantity and identity of the breakdown products had not been established. Their possible effects on local tissues were not known.

(iv) There was no information on the breakdown of implanted polyurethane foam in human tissues.

(v) There were no data on the possible release of harmful substances other than 2,4-TDA from implanted polyurethane foam, either *in vitro* or *in vivo*.

(vi) There was no direct evidence that 2,4-TDA was released from polyurethane-coated breast implants *in vivo* in women. If it were released a

carcinogenic risk could not be excluded, but it was not possible to estimate the size of any such risk.

3.17 The supply and use of these particular implants has been voluntarily withdrawn by the manufacturers.

Diesel exhaust

3.18 The COC considered the carcinogenicity of diesel exhaust in 1990.

3.19 In summary its conclusions were:-

> The main experimental studies demonstrate that lifetime exposure of rats to very high inhaled concentrations of whole diesel exhaust leads to an increased incidence of benign and malignant lung tumours. Epidemiological data indicates that sustained long-term exposure to diesel exhaust at high occupational levels is associated with an increased incidence of lung cancer. The possibility of a small increased risk of lung cancer due to general environmental exposure to diesel exhaust could not be excluded on the evidence currently available. It was not however possible to propose any health-based air quality guidelines on the basis of existing information. Insofar as the carcinogenic properties of diesel exhaust appear to be associated with the particulate component, it is recommended that the design, maintenance and operation of diesel engines should be such as to minimise particulate (ie smoke) emissions.

3.20 These conclusions were used by the Royal Commission on Environmental Pollution during their 1991 enquiry on emissions from heavy duty diesel vehicles which made various technical recommendations on control procedures.

Propoxur

3.21 At the request of the Veterinary Medicines Directorate the COC considered propoxur, an insecticide widely used in flea collars for domestic pets and (to a lesser extent) for treating wounds in food-producing animals. This latter use may give rise to residues of propoxur in meat. It is also used as a pesticide and may occur as residues in crops. The COC agreed that propoxur was a probable carcinogen, inducing dose-related urothelial hyperplasia, papillomas and carcinomas in male and female Wistar rats. No effect was seen below a dose of 1000 ppm in the diet. Carcinogenicity bioassays in hamsters and mice appeared to be negative but the tests were inadequately designed and/or reported.

3.22 It was agreed that propoxur was not genotoxic and the COC considered that the propoxur-related tumours would not develop at doses which did not cause hyperplasia. 200 ppm propoxur in the diet was the No Observed Adverse Effect Level (NOAEL) for hyperplasia in rats. This advice will be used during the regulatory procedure for licensing and determining conditions of use of propoxur-containing products.

Prioritization of microcomponents of the diet for further toxicity testing or surveillance – mycotoxins as an example

3.23 The COC and COM were asked by MAFF for advice on establishing a system for ranking chemicals present in food on the basis of mutagenic and carcinogenic potential. Both Committees expressed concern that they may be asked to assess and 'approve' chemicals without adequate data, but the need for some kind of ranking scheme was recognised.

3.24 As an initial exercise, the Committees were asked to assess the following mycotoxins for which there was some concern about possible genotoxic properties:

Ochratoxin A
Patulin
Alternaria toxins
Deoxynivalenol
Sterigmatocystin

Summary of Mutagenicity/Carcinogenicity Data and Levels in Foodstuffs

Ochratoxin A *(OA)*

3.25 Recent short term tests included a study using human lymphocytes *in vitro* which showed an increase in chromosome aberrations both with and without microsomal activation (S9). Positive results were also reported from *in vitro* studies of unscheduled DNA synthesis (UDS)in rat and mouse hepatocytes. A dose-related increase in sister chromatid exchange (SCE) was observed in Chinese hamster ovary cells *in vitro* after treatment with OA in the presence of S9. An *in vivo* test for DNA breaks in mouse spleen, liver and kidney cells indicated that DNA damage might be expressed *in vivo.* Although short-term testing was incomplete when compared to recommendations in COM guidelines, the COM concluded that OA was an *in vivo* mutagen.

3.26 Carcinogenicity studies in rats and mice have shown that OA induced renal adenocarcinomas at levels (70 mg/kg bodyweight/day and above) associated with nephrotoxicity. Incidence of hepatocellular adenocarcinomas was also increased in both male and female mice. The COC concluded that OA was carcinogenic to the kidney in two rodent species. Carcinogenic effects in the mouse liver were thought to be secondary to chronic hepatotoxicity. Epidemiology studies suggested an association between chronic dietary exposure to OA and Balkan nephropathy and urothelial cancer, but no definite causal link had been established.

3.27 Regarding intakes from food – fungal species capable of producing ochratoxin A cause contamination of cereals and porcine feedingstuffs (resulting in residues in pig tissues for human consumption). Data from a MAFF survery on contamination of cereals and pig kidney, combined with intake data from 2000 adults, gave the following intakes for extreme consumers:-
Maize (max levels 11 μg/kg) Intake from cornflour, cornflakes, cornmeal – 61 ng/kg bw/week, Barley (max level 45 μg/kg) Intake from food 46 ng/kg bw/

week, Oats (max level 4 μg/kg) intake from food 80 ng/kg bw/week and Pig kidney (max level 9 μg/kg), intake 50 ng/kg bw/week.

Sterigmatocystin

3.28 This compound clearly has mutagenic potential., It gave positive results in a range of *in vitro* assays for gene mutation (Salmonella, V79 cells) and induced UDS in hepatocytes. It was also positive in a single assay for clastogenicity *in vitro* (chromosome aberrations in human fibroblasts). Mutagenic potential was expressed *in vivo* with positive results in a bone marrow assay for SCE and DNA adduct formation in liver. COM concluded that sterigmatocystin was clearly an *in vivo* mutagen.

3.29 Sterigmatocystin has also been shown to be an animal carcinogen, inducing malignant liver tumours in rats at low dietary concentrations (0.15 mg/kg bodyweight/day and above), despite the use of a relatively short duration of dosing and small numbers of animals. There was also a probable carcinogenic effect in mouse lung. In addition both local (skin) and hepatic tumours were induced in rats following dermal application. There were no epidemiological studies on sterigmatocystin. Although the data were not of a high quality, the COC concluded that sterigmatocystin was an animal carcinogen and a potential carcinogen for humans.

3.30 Pratically no information is available on levels of sterigmatocystin in food in the UK. MAFF reports that this compound was detected by a non-quantitative method of analysis in 2/29 samples of maize and 1/2 samples of flake maize in 1980.

3.31 The detection limit was 10 μg/kg. No data were available on the actual levels present nor on contamination of other foodstuffs.

Patulin

3.32 Negative results were obtained in a single Salmonella assay for mutagenicity. Results in assays for DNA damage in micro-organisms and mammalian cells were conflicting: it induced SCEs, but was negative in an UDS assay. Tests for clastogenicity were positive *in vitro* (chromosome aberrations in Chinese hamster V79 cells) and in the bone marrow of Chinese hamsters *in vivo*. The COM concluded that patulin, although not completely tested, was an *in vivo* mutagen.

3.33 A number of carcinogenicity bioassays by oral administration (one in mice and two in different strains of rat) have been carried out, all gave negative results but were inadequate for any definite conclusions to be drawn as to the carcinogenicity of patulin. In another study rats were given subcutaneous injections of patulin and devloped local fibrosarcomas, however this was a very limited study.

3.34 Patulin is mainly found as a contaminant in apple and grape juice, with possibly over 40% of apple juices being contaminated. MAFF have estimated intakes from fruit juices for extreme consumers as follows:
Apple juice (56 μg/kg max level) 1.7 μg/kg bw/week.
Grape juice (8 μg/kg max level) 0.07 μg/kg bw/week.
Total intake 1.7 μg/kg bw/week.

Alternaria toxins (consisting of tenuazonic acid, alternariol, alternariol monomethyl ether [AME], and altertoxins I, II and III)

3.35 Only limited information on mutagenicity was available. There was evidence of mutagenic acitivity for AME and altertoxins I, II and III in the Salmonella gene mutation assay and for AME in gene mutation assays in Chinese hamster V79 cells. No data were available on clastogenicity or from any *in vivo* tests. No long term animal bioassays to assess carcinogenicityhave been reported.

3.36 Species of *Alternaria* have been found frequently in fruit and vegetables but no surveillance data are available from MAFF. The COC pointed out that some altemaria toxins could be present in mg/kg amounts (compared to the μg/kg amounts of the other mycotoxins).

Deoxynivalenol (DON)

3.37 Limited data were available on mutagenicity. DON was negative in the Salmonella gene mutation assay and in a gene mutation assay in Chinese hamster V79 cells. There was some evidence of clastogenicity from a limited cytogenetic study in V79 cells but this was inadequately reported. The chemical structure shows alerts for potential genotoxicity in the form of an acrylamide grouping, which would be consistent with clastogenic activity, and an epoxide grouping. No short term *in vivo* mutagenicity tests were reported. There were no bioassay data to enable an assessment of carcinogenicity.

3.38 DON is reported to be a common contaminant of cereal and cereal products. Levels in UK cereals (1980-1982)were generally below 100 μg/kg and frequently not detected, but a few wheat samples had levels in the range 100-500 μg/kg. Higher levels have been measured in imported cereals, particularly from North America. Levels detected in final products were 20-240 μg/kg (cornflour) and 30-100 μg/kg (bran-based breakfast cereal).

Overall conclusions by COM and COC

3.39 Two of the mycotoxins (sterigmatocystin and ochratoxin A) were *in vivo* mutagens and multispecies animal carcinogens. Patulin was an *in vivo* mutagen (clastogen) but no adequate carcinogenicity data were available. Several of the altemaria toxins had been shown to have mutagenic potential from *in vitro* studies, but no short term *in vivo* mutagenicity tests were reported. Deoxynivalenol did not induce gene mutation *in vitro* but gave some evidence of clastogenicity, no *in vivo* data from mutagenicity tests were available.

3.40 Estimates for dietary intakes by extreme consumers have been made only for ochratoxin A and patulin. Limited information was available on levels of deoxynivalenol and altemaria toxins in certain foodstuffs. No quantitative data were available for sterigmatocystin.

3.41 The COC recommended that the emphasis with all the compounds should be on obtaining better monitoring/exposure data.

In summary:

Mycotoxin	Mutagenicity in vitro	Mutagenicity in vivo	Carcinogenicity	Priority
OA	+	+	+	High
Sterigmatocystin	+	+	+	High
Patulin	+	+	limited data	Moderate
Alternaria toxins	±	no data	no data	Lower
DON	±	no data	no data	Lower

The effects of dietary restriction on carcinogenesis in rats

3.42 The COC were asked to comment on a study of the effects of different types of dietary restriction (limiting time of access to the diet, reducing the amount of diet fed, or feeding a low energy diet) on the incidence of spontaneous tumours in untreated rats. Members concluded that the result of this study provided no fundamentally new insight into the effects of dietary restriction, although effects were reported on the incidences of a wider range of tumours than had been seen in the previous studies. The results confirmed earlier observations that rats on restricted diets lived longer and had a lower overall incidence of spontaneous tumours in addition to lower incidences of many individual types of tumours, as compared with rats given free access to high energy diets. Recommendations were made for some further analyses of the data which might usefully be made.

Presentations by Professor John Ashby

3.43 Professor Ashby gave two presentations to the COC at its July meeting:-

(a) A scheme for classifying carcinogens. This had been propose by a group of 17 authors (including himself) in a paper in 'Regulatory Toxicology and Pharmacology' (12: 270-295 (1990)). An International Agency for Research on Cancer (IARC) working group had met in June 1991 to discuss similar approaches to evaluating carcinogenic risks to man. While IARC would still carefully consider the *strength* of the evidence, they would now take account of mechanisms in their overall conclusion. The COC welcomed this modification of IARC's classification and looked forward to the publication of the new criteria, which would also list what IARC regarded as *strong* evidence for a mechanism relevant to man.

(b) The use of transgenic mice as an *in vivo* mutagenesis assay. These systems allow a wide variety of mutations in a *lac* gene to be recovered from a variety of tissues and may therefore allow studies of intermediate steps in carcinogenesis. This presentation was also given to the COM.

Topics still under review

3.44 Dithiocarbamates were discussed by the COC at meetings held in 1991, and are still under review. The outcome of this review will be published in due course.

1991 Membership of the committee on carcinogenicity of chemicals in food, consumer products and the environment

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Terms of Reference

To advise at the request of:

Department of Health and Social Security
Ministry of Agriculture, Fisheries and Food
Department of the Environment
Department of Trade and Industry
Department of Transport
Department of Energy
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.

Glossary of Terms

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').

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'.

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

ANTHELMINTIC A substance used in the treatment of worm infections.

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 casual 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').

CELLS IN CULTURE Cells which have been isolated from animals and grown in the laboratory.

CHROMOSOMAL ABERRATION Deviation from the normal structure of chromosomes (qv) (see clastogen).

CHRONIC TOXICITY STUDY A study in which repeated daily doses of the compound under test are administered for a substantial length of time eg one year or more.

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.

CYTOGENETIC Concerning chromosomes, their origin, structure and function.

DELETION Usually a chromosome aberration in which a proportion of a chromosome is lost.

DIFFERENTIATION The process by which cells develop into particular types of cells and become organised into a mature tissue, if this does not happen or is reversed the cells are unstructured.

DNA (DEOXYRIBOSENUCLEIC 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).

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

ELECTROLYTIC EFFECT The decomposition of a substance caused by an electrical current.

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.

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

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

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

FIBROSARCOMA A malignant tumour arising from connective tissue (see 'tumour').

FREE RADICAL An unstable, highly reactive molecule which is capable of reacting with cellular proteins and DNA giving rise to adverse effects.

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.

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

GUT ASSOCIATED LYMPHOID TISSUE An area of the gut wall containing immune cells which will produce an immune response to foreign matter.

HEPATOCARCINOGEN A chemical, or other agent or factor, causing cancer of the liver.

HEPATOCYTE The principal cell type in the liver, possessing many metabolising enzymes (see 'metabolic activation').

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 constitutents.

INTERPERITONEAL 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.

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).

LYMPHOCYTE Type of white blood cell.

LYMPHOID TISSUE Tissue which produces lymphocytes (qv), it is widely distributed in the body eg spleen, lymph nodes.

LYMPHOMA Malignant tumours arising from lymphoid tissues (qv). 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').

MALIGNANCY See 'tumour'.

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

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

METASTASIS The process whereby malignant cells become detached from the primary tumour mass, disseminate (mainly in the blood stream or in lymph vessels) and 'seed out' in distant sites where they form secondary or metastatic tumours. Such tumours tend to develop at specific sites and their anatomical distribution is often characteristic; it is non-random. The capacity to metastasise is the single most important feature of malignant tumours (see tumour).

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.

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 alteration 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 or 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'.

ONCOGENE The name given to activated forms of proto-oncogenes (qv).

PAPILLOMA A benign tumour arising from the epithelia (qv) (see 'tumour').

PHENOTYPE The observable physical, biochemical and physiological characteristics of a cell, tissue, organ or individual, as determined by its genes and the environment in which it develops.

PLASTICISER A substance which increases the flexibility of certain plastics.

PRESERVATIVE A substance which prolongs the shelflife of foodstuffs by protecting them against deterioration caused by micro-organisms.

PROTO-ONCOGENE A group of normal cellular genes, highly conserved, which are concerned with the control of cellular proliferation and differentiation (qv). They can be activated in various ways to form which are closely associated with one or more steps in carcinogenesis. Mechanisms of activation include point mutations which alter the structure of the proto-oncogene, or changes in the regulatory regions which alter the level of expression. Activating agents include chemicals and viruses. The process of proto-oncogene activation is thought to play an important part at several stages in the development of tumours.

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

RESPIRATORY HYPER-REACTIVITY A greater than normal response of the respiratory system to an external stimulus.

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

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').

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.

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 cell acquires the ability to divide indefinitely in culture without undergoing senescence (aging and death). 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 (qv) 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).

TRANSLOCATION The transfer of a region of one chromosome to another chromosome.

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 (qv) of cells. Many tumour suppressor genes probably exist, deletion or suppression of which appears to be a critical event in tumour development.

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

XAD RESIN An absorbent amberlite resin used to selectively remove and concentrate compounds from solutions.

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