



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

1992
Annual Report



1992

ANNUAL REPORT
OF THE
COMMITTEES ON
TOXICITY
MUTAGENICITY
CARCINOGENICITY
OF CHEMICALS IN FOOD,
CONSUMER PRODUCTS AND THE
ENVIRONMENT

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About the Committees

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

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

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

For the first time, this report publishes the commercial interests of committee members. Members are required to declare any commercial interests on appointment and, again, during meetings if a topic arises in which they have an interest. If the Chairman so deems, members whose outside interest 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 relevant secretariat critically assesses all the relevant data and prepares papers for the Committee. These normally consist of appendices giving detailed summaries of the studies reviewed – methodology and results – and a covering paper in which the available data are briefly summarised, the most important points highlighted and recommendations presented for discussion by the Committee. Although original study reports are not routinely circulated to members, they are made available on request, and are circulated if the study is particularly complex. Definitive summaries are necessary because documentation on any one chemical can amount to many hundreds of pages.

The Committees 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.

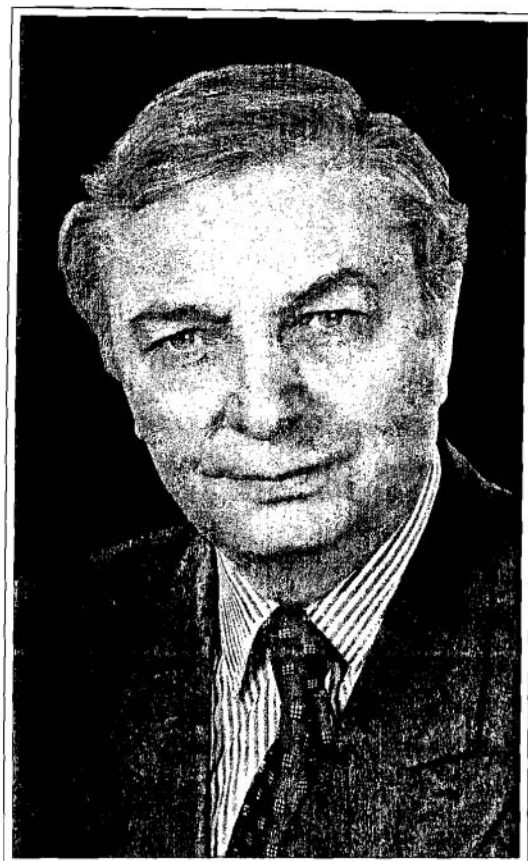
Since 1990 the Committee has given its advice in numerical rather than descriptive form, allocating Acceptable Daily Intakes (ADIs) where possible for food additives. Details of this change, which brought it into line with the way most other national and international bodies express their advice on food 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 kilogram bodyweight (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 1992 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

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Preface

As the newly appointed Chairman of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, I found Professor Paul Turner's legacy to be a knowledgeable, industrious committee, well versed in the assessment of the risk to man posed by exposure to chemicals. The committee's work is very dependent upon the quality and mode of presentation of toxicological information and as a "new boy" I have found both the administration and information to be of the highest quality.

The COT is a source of advice to Government Departments on the toxicity of chemical contaminants in food and the safety of food additives. During the year the committee examined topics which present a wide range of toxicological problems and a number of aspects of safety-in-use. There is the important role of reviewing the safety of food additives already in use together with the consideration of new compounds. This report contains examples of both.

The committee uses data derived from well-established toxicological methodology. Over the past twelve months we have had the opportunity to consider the methodology relating to the allocation of an Acceptable Daily Intake (ADI) and have faced the challenge presented by the need to review certain herbal substances for which the quantity and quality of toxicological data is less than that usually available for new chemicals. These issues have fully tested the committee members and demonstrated the value of having a wide range of expertise within the membership.

FRANK WOODS

Aspartame

1.1 In **1992** the COT completed a review of the artificial sweetener aspartame. The COT's advice was issued in July **1992** in the form of the following statement (paragraphs i to xiii). A list of references used in the review can be obtained from the committee's secretariat.

Introduction

- (i) We last fully reviewed aspartame as part of the Food Additives and Contaminants Committee's review of sweeteners in food in **1982**, when we classified it as acceptable for use in food. We also recommended that, after allowing for new food manufacturing practices to develop and the market to **stabilise** following the implementation of new regulations and within five years of implementation, information should be collected so that intake levels in the general population and in special groups could be measured.
- (ii) Since **1982** we have reviewed issues relevant to the **safety** of aspartame as they have arisen in the scientific literature. None of these reviews caused us to alter our advice that aspartame was safe-in-use. In **1989** the Food Advisory Committee considered the results of surveys carried out by MAFF on the intakes of sweeteners by the general and diabetic populations and asked us for advice on the potential risk to health of the levels of intake of aspartame reported. In view of the large amount of data on aspartame which has been published in the scientific literature since **1982**, we took the opportunity to conduct a full review of this sweetener. In keeping with our current policy, we have decided to formulate our advice in terms of an acceptable daily intake (ADI) for aspartame. We have also taken the opportunity to review the safety of the degradation product of aspartame, a diketopiperazine derivative (DKP).

Animal studies reported since 1982

- (iii) We have reviewed the available animal studies on aspartame which have been published in the scientific literature since our last review in **1982**. These studies have mainly investigated the potential effects of high doses of aspartame on brain neurotransmitters and on behaviour. We conclude that adverse neurochemical or neurobehavioural effects have only been seen in animal studies using exposures to aspartame which far exceed extreme intake figures for the UK. At current intake levels no neurotoxic effects have been observed or would be expected. Overall, the results of animal studies conducted since our previous review give no cause for **concern**.

Human data

- (iv) Metabolism studies on aspartame in four species of animals and in humans indicate that aspartame is metabolised to its component moieties methanol, **aspartic** acid and phenylalanine, with metabolism taking place largely in the gastrointestinal tract. The amino acids **aspartic** acid and phenylalanine are normal components of dietary protein and methanol is a low level constituent of common foods. The amount of methanol contributed to the diet by aspartame is very low compared with that from other foods, eg one pint of orange juice would provide more methanol to a three year old child than a 40 milligram per kilogram bodyweight (**mg/kg bw**) bolus dose of

aspartame. Unchanged aspartame is not likely to be present in the systemic circulation even at high levels of human dietary intake. We have reviewed the results of the extensive tolerance and pharmacokinetic studies on aspartame in humans. The available data indicate no adverse effects in various human subpopulations following exposure to aspartame at doses up to 75 mg/kg bw/day for 24 weeks or up to 135 mg/kg bw/day for a shorter period. Pharmacokinetic studies have established that both large acute bolus doses and repeated doses of aspartame result in plasma levels of its components which are considered safe.

- (v) We are aware of anecdotal reports linking aspartame with a variety of adverse effects such as headache, seizures, and behavioural difficulties in children. In the US, reports on adverse reactions associated with aspartame ingestion are collected by the US Food and Drug Administration via a toll-free telephone number and reported quarterly. These quarterly reports are made available to the Department of Health. Occasional consumer complaints of adverse reactions in the UK received by Nutrasweet are also made available to the Department. We have reviewed these reports. Most of the frequently reported symptoms were mild and are common background symptoms in the general population. The most common adverse symptom reported was headache, which accounted for approximately 20% of all complaints in the US. Two studies have investigated the relationship between aspartame and headache. Both were double blind, cross over studies. The results differed markedly from each other. In one study 40 subjects, who had previously complained about the effects of aspartame containing products, were hospitalised and entered into a six day study programme. The conclusions from this study were that the incidence of headache from aspartame and placebo was equivalent. In the second study migraine sufferers, who considered that they were adversely affected by aspartame, were recruited by newspaper adverts. Only 11 of 25 subjects recruited finished the study. Because of the high drop-out rate, the conclusions of this study are necessarily tentative. However, it was considered that the ingestion of aspartame by migraine sufferers may cause a significant increase in the frequency of migraine in some subjects. More studies are needed to confirm the impression gained from this latter study that aspartame, in common with some other food substances, may need to be avoided by a sub-group of migraine sufferers. Claims that aspartame causes other adverse effects, such as behavioural effects or seizures, have not been substantiated in controlled studies in humans. In fact, as indicated in a recent report, researchers trying to carry out such studies can encounter difficulties in recruiting sufficient individuals with a history of adverse reactions to aspartame to make the studies viable.

Phenylketonuria

- (vi) Sufferers of the inherited metabolic disease, phenylketonuria (PKU), are unable to metabolise phenylalanine effectively, leading to the accumulation of potentially toxic levels. The disease is due to a genetic defect in which an autosomal recessive gene is inherited from both parents. The fetus of mothers with PKU and the young child with PKU are particularly at risk since the excessively high phenylalanine levels at this time can result in

delayed development and mental retardation. These patients are treated by placing them on a phenylalanine restricted diet. There is evidence that dietary control into adult life is indicated for some patients with PKU. PKU sufferers are therefore advised to avoid consumption of aspartame, as this is a source of phenylalanine. We *recommend* that all food products containing aspartame are clearly labelled to indicate the presence of phenylalanine from this source, so that those suffering from PKU can avoid consuming these products. This would be in addition to the statutory ingredient label already required for aspartame.

- (vii) People who inherit one of the genes for PKU (termed PKU heterozygotes) have a somewhat reduced ability to metabolise dietary phenylalanine. The tolerance and pharmacokinetic studies cited in paragraph iv include studies in these individuals and the results indicate that, like normal individuals, they are not at risk of adverse effects from aspartame at intakes up to the acceptable daily intake (ADI, see below).

Fetal toxicity due to phenylalanine

- (viii) Since maternal hyperphenylalaninaemia is associated with mental retardation in the fetus, high maternal plasma levels of phenylalanine are hazardous to the fetus. In the US, the National Collaboration Study for Maternal Phenylketonuria has recommended that during pregnancy blood phenylalanine levels should not exceed 380 $\mu\text{mol/l}$. Recently, it has been shown that the birthweights and head circumferences of infants born to a group of PKU women were inversely related to the maternal phenylalanine concentrations around the time of conception. However, at plasma phenylalanine concentrations below 300-400 $\mu\text{mol/l}$, these parameters were within the normal range. Thus although these data could be taken to suggest that there is no threshold for subtle adverse effects in the fetus from high maternal phenylalanine concentrations, we consider there must be an effective threshold, particularly since hypophenylalaninaemia is also harmful to the fetus. Studies in PKU heterozygotes indicate that after high bolus doses of aspartame (10-20 mg/kg bw) or even following repeated doses of 10 mg/kg bw/day aspartame given every hour for eight hours, plasma phenylalanine concentrations do not exceed 200 $\mu\text{mol/l}$. Thus we are satisfied that fetotoxic effects due to phenylalanine would not result from ingestion of aspartame by normal individuals or by PKU heterozygotes at the ADI (see paragraph xi).

Validity of early studies on aspartame

- (ix) Aspartame was first submitted for approval in the UK by G D Searle and Co in 1974. In 1974, a task force of the US Food and Drug Administration (FDA), investigating Searle laboratory practices, questioned the quality of the data in certain aspartame studies. During 1977 and 1978, FDA inspectors investigated three of these studies in detail and a further 10 studies were investigated by an independent US organisation, the Universities Associated for Research and Education in Pathology (UAREP). These investigations concluded that although there were discrepancies in the conduct of some of these studies, they did not invalidate the studies or their conclusions. Aspartame was subsequently permitted for use in the US. A subsequent investigation of the FDA's approval process followed for aspartame by the US General Accounting Office concluded that the process was satisfactory.

- (x) Since 1982 questions have been asked on a number of occasions about the decision to accept the early animal studies on aspartame and while our review was in progress we received a submission on this. It is agreed that by current standards there were flaws in the conduct of some of the early animal toxicity studies on aspartame. However, we accept the conclusions of the detailed FDA and UAREP investigations, ie that the flaws do not invalidate the conclusions of the studies and that they can be accepted as part of the safety evaluation of aspartame. It **should be** noted that in forming our view on the safety-in-use of aspartame, we took into account not only the standard animal toxicity studies but also the known metabolic profile in humans (paragraph iv) and the many human studies on aspartame.

Acceptable Daily Intake

- (xi) With one exception, the available standard animal toxicity studies on aspartame indicate a no observable adverse effect level of 4 g/kg bw/day. Application of the usual safety factor of 100 would lead to an ADI of 40 mg/kg bw/day aspartame. In the rabbit, which was used as a second species in teratology studies, administration of 4 g/kg bw/day could not be achieved but the available metabolism and toxicological data on aspartame is sufficient for this not to be of concern. Tolerance studies in humans in which 40 mg/kg bw aspartame has been administered as a bolus dose show that the resultant blood concentrations of aspartame components do not give any cause for concern. Therefore, taking the animal and human data together, we recommend an ADI₁₉₉₂ of 0-40 mg/kg bw/day for aspartame.

Diketopiperazine

- (xii) On storage and particularly in aqueous solution, aspartame breaks down to a diketopiperazine derivative (DKP; aspartyl phenylalanine diketopiperazine) by hydrolysis and cyclisation. A variety of cyclic dipeptide derivatives can be found in many protein-rich foods and many of these dipeptides contain phenylalanine. In pharmacokinetic studies in humans, DKP has been detected in some subjects during the placebo phase, indicating that it is a naturally occurring dietary and/or endogenous substance.
- (xiii) Substantial animal toxicity data presented on DKP at the time of last review give no cause for concern. Metabolic studies in humans, both normal subjects and PKU heterozygotes, indicate that DKP is poorly absorbed after oral administration and that which is absorbed is excreted unchanged in the urine. Since DKP performs no technological function in food and is essentially equivalent to a contaminant, we consider it appropriate to set a tolerable daily intake (TDI) rather than an ADI for this substance. We recommend a TDI₁₉₉₂ of 0-7.5 mg/kg bw/day for DKP.

General Principles for Setting Acceptable Daily Intakes

1.2 As explained on page 8, the COT has been allocating acceptable daily intakes (ADIs) for additives reviewed since 1990. ADIs are set by selecting the maximum amount of an additive which causes no ill effects in animal studies (the no observed adverse effect level) and then dividing by a safety factor to derive an acceptable

daily intake for man. An arbitrary safety factor of 100 is usually used. This is derived from a safety margin of 10 to allow for uncertainties in extrapolating from animals to man (inter-species variation) and another safety margin of 10 to allow for variations in the human population (inter-human variation). In some cases, there is information available on the likely variations between animals and man or among humans. In common with various international scientific organisations, the Committee has been considering ways in which this information could be used to estimate safety factors more accurately and, at the September meeting, it heard a presentation on this subject from Dr Renwick of the University of Southampton. On occasions, for logical reasons, the Committee has already used safety factors other than 100. For example, the Committee considered that a safety factor of 20 was appropriate for the preservative sulphur dioxide since the critical toxic effect (stomach irritation) was a localised effect and the only potential differences between rat and man would be in the sensitivity of the stomach (see 1991 Annual Report). In setting an ADI for the intense sweetener cyclamate in 1990, the percentage of cyclamate converted to the toxic metabolite cyclohexylamine in humans was taken into account. The ADI for the antioxidant butylated hydroxyanisole (BHA) (see below) provides an example where the appropriate safety factors were considered in particular detail. The Committee intends to continue, wherever possible, to select safety factors on a more scientific basis.

Butylated Hydroxyanisole (BHA) - Review of Further Studies and Setting of an ADI

1.3 The use of BHA as a food additive was last reviewed by the COT in 1987 and 1988. At that time, BHA was confirmed as provisionally acceptable for use in food (Group B) and the COT requested a 2-3 week study in rats involving sequential observations which would focus on the time course and nature of any early changes in the oesophagus and forestomach. Studies performed in response to this request were considered by the COT in March 1992. The COT also reviewed the relevant literature published from 1987-1991 and set an ADI for BHA.

1.4 The COT confirmed the 1987 conclusion that BHA was a rodent forestomach carcinogen. BHA was not considered to be a carcinogen at any other site. Although two recent poorly-conducted studies had shown that BHA caused adenomatous hyperplasia and adenomas in the lung of the Japanese house musk shrew and liver cell carcinomas in the fish *Rivulus ocellatus marmoratus*, the Committee considered these findings to be outweighed by the lack of any effect in these tissues in better conducted, long-term studies in more common laboratory species. The 1988 COM conclusion that BHA may be regarded as non-genotoxic at the concentrations likely to result from its use as an antioxidant in food also remained unchanged. Two separate studies which failed to find any DNA adducts in the BHA-treated rat forestomach provided further reassurance that BHA was non-genotoxic.

1.5 The new two week studies which were carried out in response to the request by the COT provided information on very early BHA induced effects in the rat forestomach at a range of doses (0.05-2.0% BHA in the diet). However, the mechanism of action of BHA remains unknown. The Committee noted that the development of hyperplasia and inflammatory changes appeared to be related

but concluded that there was insufficient information to judge whether the inflammatory response caused the hyperplasia. Similarly, the Committee considered that it was not clear at this stage whether tert-butylquinone (TBQ) was the BHA metabolite responsible for the forestomach damage as had been proposed in the literature. There appeared, however, to be adequate protective mechanisms present in the forestomach to prevent the accumulation of TBQ at lower doses of BHA.

1.6 The new studies provide the first evidence that BHA can cause minimal to slight hyperkeratosis in the rat oesophagus. However, there was no hyperplasia nor change in the labelling index at any dose. Another study found an increase in labelling index in oesophageal cells from BHA treated rats compared with pair-fed controls (rats given the same low level of food as taken by BHA treated rats). The Committee was not convinced that this increase was of concern since the labelling index in the pair-fed controls was unphysiologically low and dramatic short-term changes in labelling index can be caused by limited mechanical effects.

1.7 It was not possible to state categorically whether the minimal hyperkeratosis in the oesophagus found in the new studies was a transient protective effector a response which could develop further if feeding was continued for longer than two weeks. The Committee was, however, reassured by the fact that the oesophageal effect in rats was less severe than in the forestomach (probably due to reduced time of contact). In addition, detailed electron microscopy of the oesophagus in the dog given high doses of BHA did not reveal any adverse effects and no oesophageal tumours had been found in earlier long-term studies in various species.

Selection of no observed adverse effect level

1.8 Man does not have a forestomach. The Committee therefore considered selecting a no observed effect level (NOAEL) on the basis of effects on the oesophagus. However, it decided that the new studies were of too short a duration to use as the basis for an ADI and that other longer term studies which entailed an examination of the oesophagus were not as detailed or had not been specifically designed to select a NOAEL. On balance, the Committee considered that the well-established NOAEL from long-term studies of effects of BHA on the forestomach epithelium should be used. The Committee considered that the oesophageal squamous epithelium and the forestomach squamous epithelium were likely to react similarly to the same level and duration of tissue exposure to BHA. The Committee was aware that the duration of exposure would be much shorter in the human oesophagus than in the rat forestomach but took this into account in selecting an appropriate safety factor. In summary, the Committee recommended that:

- (i) the effect used to determine the NOAEL should be forestomach hyperplasia and
- (ii) the NOAEL should be 50 mg/kg bodyweight/day taken from a two year study in rats.

1.9 The Committee noted that peak blood concentrations of BHA in man were higher than in the rat in relation to the BHA dose. In addition, BHA metabolites increased in the urine with time in man which could indicate accumulation of BHA

(there was no other evidence for accumulation). The Committee concluded that this difference in blood concentrations between rats and man was not of concern since the action of BHA on the forestomach was the direct effect of BHA in the ingested food and the effects caused by the BHA in the bloodstream were few and minor. Therefore, the differences in blood concentrations did not need to be taken into account when setting the ADI.

Selection of safety factors

1.10 The Committee did not consider that an additional safety factor was required for the severity of the effect. The dose causing forestomach tumours was 10 fold higher than the NOAEL based on forestomach hyperplasia.

1.11 The Committee considered in detail the likely retention time of food containing BHA in the rat forestomach compared with transit times of food through the mouth, pharynx and oesophagus in humans. Although it was not possible to define the variations in kinetics precisely, it was clear that the transit time over the squamous epithelium in humans was only about a minute compared with a storage time of several hours in the rat. Thus, man would be expected to have a much briefer exposure to the same concentration of BHA in the food. This difference was likely to be much greater than any variations between man and the rat in tissue reactivity. In addition, unlike the rat, the human oesophagus secretes mucus which provides extra protection. The Committee therefore considered that an inter-species safety factor to allow for the possibility that man was more sensitive than the rat was unnecessary.

1.12 The Committee also considered the possible inter-human variations in oesophageal transit time and in chewing behaviour (eg chewing gum). There was no information on human variation in the sensitivity of the squamous epithelium to BHA. Overall, the Committee considered that the usual inter-human safety factor of 10 would be appropriate.

Recommendation

1.13 The Committee set a full ADI₁₉₉₂ for BHA of 0.5 mg/kg bodyweight/day based on a NOAEL of 50 mg/kg bodyweight/day, an inter-species safety factor of one and an inter-human safety factor of 10. The Committee noted that, in addition to these safety factors, there are extra safety margins due to the facts that (i) forestomach tumours in rats only occur at BHA concentrations 10 fold greater than the NOAEL and (ii) man is likely to be less susceptible to BHA than the rat because food takes only a minute to pass over the squamous epithelium in man but is stored in contact with the squamous epithelium for several hours in rats.

Novel Fat for Use in Confectionery

1.14 The Advisory Committee on Novel Foods and Processes (ACNFP) sought the advice of the COT on particular aspects of the toxicological data submitted in support of the use of a novel fat in confectionery. The COT considered the data at its meetings in July and November and was able to reach conclusions, which have been forwarded to the ACNFP. That Committee will be including this advice in its overall evaluation of this product, which is not yet complete.

Comfrey

1.15 In 1991 an *ad hoc* Department of Health/Ministry of Agriculture, Fisheries and Food working group published a report on dietary supplements and health foods. This report identified a number of herbal ingredients which used to be added to medicines but which have now been withdrawn by the Medicines Control Agency on safety grounds. The working group was concerned that some of these substances could still be sold as foods. Consequently, the COT was asked to review the safety-in-use of a number of herbal substances and to advise on their continued availability.

1.16 Much of the COT's work in the past has been to review food additives. Normally, an extensive, standard toxicological data set is available for each additive. However, such data are rarely available for most herbal substances. Although the Committee considers that it would be desirable to have more information on these substances before formulating its advice, it has acknowledged that it is impractical simply to request further studies as it is not possible to identify a specific company to finance and carry out the studies. Therefore, the Committee has formulated its advice as best it can on the basis of the available but, in most cases, limited data.

1.17 The first herbal substance to be considered by the COT was comfrey. This plant has a long history of use in herbal medicine and is often consumed in the form of a tea, as an alternative to traditional beverages such as tea and coffee, or as a vegetable. The concern about the consumption of comfrey products centres on substances in comfrey known as pyrrolizidine alkaloids. The COT's advice on comfrey was given in the form of the following statement:

Introduction

- (i) Following the publication of the Report of the Working Group on Dietary Supplements and Health Foods in 1991 (1), we were asked to review the safety-in-use of certain herbal substances. The Working Group was concerned that the Medicines Control Agency (MCA) had withdrawn product licences from preparations containing certain herbs. However, preparations containing these herbs can still be sold as foods if no direct medicinal claims are made on the packaging. The Working Group recommended that "urgent steps be taken to review the safety and use of these herbal substances", a recommendation that was subsequently endorsed by both Ministry of Agriculture, Fisheries and Food (MAFF) and Department of Health (DH) Ministers. Comfrey is the first of these substances to be reviewed.

Pyrrolizidine Alkaloids

- (ii) The available data indicate that the toxic effects of comfrey are due to its pyrrolizidine alkaloid (PA) constituents. These alkaloids belong to a family of over 180 compounds and occur in more than eight plant families. Their toxicity to animals, in particular livestock, is well known (2). The alkaloids themselves are not toxic but are metabolised in the liver to pyrrolic derivatives which are reactive alkylating agents. The target organ for these metabolites is the liver, but the lungs may also be damaged.

Toxicity data

- (iii) Some PAs such as retrorsine, monocrotaline and lasiocarpine have been studied in detail. Liver damage due to these alkaloids has been recorded in several animal species, although some species appear to be more resistant than others (3). The liver lesions have been characterised, particularly in laboratory animals. In rats, high doses of these alkaloids cause confluent haemorrhagic necrosis in the liver which is often followed by changes in the central and sub-lobular veins of the liver lobules, such as subintimal oedema, necrosis, fibrin deposits, thrombosis and occlusion of the lumen (4, 5). Chronic liver damage observed in rats and other laboratory species includes parenchymal damage with prominent megalocytosis, ductular proliferation, fibrosis, nodular hyperplasia and thickening of the central veins. In some animals this may progress to cirrhosis and hepatocellular carcinoma (4, 5, 6).
- (iv) Many PAs, including those listed above, have produced positive results in vitro mutagenicity tests and therefore have the potential to damage genetic material. Carcinogenicity studies on lasiocarpine, monocrotaline and retrorsine report an increased incidence of liver tumours, as do studies with the leaves and flowers of PA-containing plants such as coltsfoot (*Tussilago farfara*) and thread-leaf groundsel (*Senecio longilobus*) (3). Heliotrine has been demonstrated to be teratogenic in the rat (7).

Human data

- (v) There is much evidence of PA toxicity in humans. Several cases of human veno-occlusive disease associated with the consumption of plants containing these alkaloids have been recorded, particularly in third world countries where consumption of grain contaminated with seeds of the *Senecio*, *Crotalaria* and *Heliotropium* genera have caused large outbreaks of the disease, eg South Africa 1920, Soviet Union 1930's & 40's, India 1973 & 1975, Afghanistan 1974 (8, 9, 10, 11, 12). In Jamaica, many cases of the disease have been diagnosed in people who regularly consumed bush teas made from plants from the *Crotalaria* genus (13).
- (vi) In humans, acute veno-occlusive disease is similar to Budd-Chiari syndrome, ie thrombosis of hepatic veins leading to liver enlargement, portal hypertension and ascites. It can cause rapid death or it may progress to a sub-acute form of the disease. Often patients with the acute or the sub-acute disease appear to make a full recovery then after a latent period of several years develop the chronic form of the disease. Individuals ingesting small amounts of PAs over a long period of time may also develop the chronic form of the disease, which proceeds from fibrosis to cirrhosis of the liver (3). No data are available on the incidence of liver cancer in individuals who have consumed large amounts of these alkaloids.

Comfrey

- (vii) Several PAs have been identified in the species of comfrey most commonly consumed and used in herbal preparations in the UK, namely common comfrey (*Symphytum officinale*) and Russian comfrey (*Symphytum x uplandicum* Nym). Thirteen alkaloids have been reported to be present in

common comfrey, including the highly toxic alkaloid lasiocarpine. Nine alkaloids have been identified in Russian comfrey. Many of these alkaloids are in fact isomers and the reliability of some of these "identifications" has been questioned (14). From the MAFF data¹ it would appear that only a small proportion of the alkaloids reported to be present in comfrey were in fact detected in the comfrey preparations examined, ie lycopsamine, acetyl lycopsamine, symphytine and their isomers intermidine, acetyl intermidine and symlandine. Echimidine and its isomers were present in only some of the preparations. Lasiocarpine was not detected in any of the comfrey preparations tested. The levels of alkaloids present in comfrey may vary depending on the age or part of the plant and the season of growth. In general, comfrey root tends to have a higher alkaloid content than the leaf (2).

Toxicity data

- (viii) The toxicity of the alkaloids found in the above species of comfrey has not been studied in any detail. The rat intra-peritoneal (ip) LD50 values reported for symphytine and echimidine are similar to those of monocrotaline and heliotrine (2). However, there are little acute or chronic toxicity data available on any of the comfrey alkaloids. A carcinogenicity study on symphytine in rats has been reported. Liver tumours occurred in four out of 20 rats which received symphytine ip 13 mg/kg bw twice weekly for four weeks, then once a week for 52 weeks. Three haemangioendothelial sarcomas and a liver cell adenoma were identified. No liver tumours were reported in 20 control rats who received ip injections of sodium chloride (0.1 ml/100 mg bw). However, this study was not performed to current standards of testing and only 30% of the test animals survived until autopsy at day 650 (15).
- (ix) Symphytine has been shown to be positive in an Ames test with TA 100 and in the induction of the 8-azaguanine resistant mutation in V79 Chinese hamster cells (16). Echimidine and echinatine, an alkaloid also reported to be present in comfrey, have been shown to be positive in *in vitro* mutagenicity tests, though again the tests were performed to different standards from those accepted today (3).
- (x) Comfrey root and leaf have been tested for carcinogenicity. In a study by Hirono *et al*, both comfrey root and leaf were fed at different levels in the diet of groups of 19-30 ACI rats (leaf 8%, 16% & 33%, root up to 4%). A control group of 129 rats received only basal diet. Liver tumours (hepatocellular adenomas) were induced in all comfrey-treated groups. The incidence of liver tumours was higher in the groups fed a diet containing comfrey root than in those fed comfrey leaf. Haemangioendothelial sarcomas in the liver were reported in three experimental groups: in 1/21 rats receiving comfrey leaf at the 16% level in the diet, in 4/24 rats receiving 1% root for 275 days, then basal diet and 0.5% root alternately at three-week intervals, and in 9/30 rats receiving 0.5% root throughout the experimental period. The incidence of

1. Details of these data, which show the levels of pyrrolizidine alkaloids in various comfrey preparations, can be obtained from: MAFF, Food Safety Directorate, Chemical Safety of Food Division, Room 429D, Ergon House, c/o Nobel House, 17 Smith Square, London SW1P 3JR.

haemangioendothelial sarcomas in the liver in this latter group of animals is particularly striking: tumours were reported in 9/13 animals which survived beyond 590 days. The data suggest that long-term exposure to low levels of comfrey root may induce malignant tumours in the liver of rats. No liver tumours were recorded in the control group (16).

Human case reports

- (xi) Four case reports of human veno-occlusive disease associated with the consumption of comfrey or comfrey-containing products are reported in the literature. Two of the cases occurred in the United States, one in the UK, the other in New Zealand. In the American cases veno-occlusive disease was diagnosed in two middle-aged women who had been consuming both comfrey teas and tablets (17, 18). In the UK case a 13 year old boy with Crohn's disease had been treated with comfrey root and comfrey tea (19). In the final case a 23 year old man died from veno-occlusive disease and subsequent liver failure following the consumption of steamed comfrey leaves (4-5 leaves/day) for 1-2 weeks (20).

Conclusions

- (xii) Our concern over comfrey is focused on its PA content. Several of these alkaloids are known to be highly toxic in animals and the consumption of certain plants containing these alkaloids causes liver damage in both animals and humans. Although the quality and the quantity of toxicity data available on comfrey and its constituent alkaloids is limited, we consider that the data are sufficient to warrant action in the interests of public safety. We are particularly concerned by the results of the carcinogenicity study on comfrey, discussed in paragraph x, and consider that although this study was not performed to the current standards, its results cannot be ignored. Limited testing of the alkaloid, **symphytine**, suggests that it is potentially mutagenic and that it may induce liver tumours in the rat. Such results are not surprising given the toxicity profile of other members of this alkaloid family. We recognise that the four case reports of human veno-occlusive disease are isolated and anecdotal and we cannot be completely certain of a causal link with comfrey ingestion. Nevertheless, veno-occlusive disease is a rare condition and is often associated with the consumption of plants and seeds containing PAs. Thus we consider it probable that comfrey products were implicated in these cases.
- (xiii) Although the data available on comfrey and its alkaloids are limited, we consider that further toxicological testing, in the absence of a standard comfrey preparation with a clearly defined alkaloid content, would provide results that would be of limited use in toxicological evaluations. Vagaries of cultivation and the effect of other factors on alkaloid concentrations make the production of standard comfrey preparations difficult. Comfrey preparations have been used for many years but we remain concerned about the possible short-term effects of extreme comfrey consumption. Also, the possibility of long-term effects from comfrey consumption cannot be excluded.
- (xiv) Comfrey may be purchased as tablet and capsule preparations or in the form of a tea or dried plant for infusion. Comfrey tinctures and dried

comfrey root are also freely available. We understand that in many instances comfrey is cultivated at home and used in home-made teas and infusions. The results from the MAFF study indicate that certain preparations, eg tablets and capsules, have a very high alkaloid content (4–5 g/kg), as does the dried root (up to 8 g/kg), whilst teas have relatively low alkaloid content (less than 0.1 g/kg), with only a small proportion, at most 10%, of the alkaloids being extracted into the tea liquor. The level of alkaloids in comfrey leaf samples was below 0.06 g/kg and infusions of the leaves were found to contain alkaloid levels of less than 0.008 g/kg. Comfrey tinctures had an alkaloid content of less than 0.12 g/kg.

- (xv) In view of the risk of toxicity associated with the consumption of high levels of PAs we recommend that concentrated forms of comfrey such as capsules and tablets should no longer be available. We also believe that the presentation of comfrey, as a food, in the form of tablets and capsules could be misconstrued by consumers as a medicine, resulting in inappropriate use. In addition, the physical form of tablets gives greater opportunity for excessive intake. The ingestion of comfrey root and infusions prepared from the root may also result in a very high alkaloid intake and should therefore be avoided. Although comfrey leaves contain lower levels of PAs than the root, we recommend that ingestion of leaves should also be avoided.
- (xvi) Comfrey teas and tinctures contain relatively low levels of PAs and these preparations may continue to be available to the public. However, this recommendation should not be construed as an endorsement of these products and we recommend that the public should be warned of the potential dangers associated with the consumption of comfrey and products containing comfrey. This advice also applies to home-grown comfrey and preparations made from it.

Recommendations

(xvii) In *summary*, we therefore recommend that:

- fa*) the public should be warned of the potential dangers associated with the consumption of comfrey and products containing comfrey. This advice applies equally to commercial and home-grown comfrey and preparations made from it.
- (b)* concentrated forms of comfrey such as tablets and capsules should no longer be available.
- (c)* the public should be advised against the ingestion of comfrey root and leaves, and of teas and infusions made from comfrey root.
- (d)* comfrey teas and tinctures may continue to be available to the public. However, this recommendation should not be construed as an endorsement of these products.

1.18 The COT's advice was subsequently endorsed by the Food Advisory Committee. DH and MAFF Ministers accepted the committees' advice and action was taken to implement it.

Dimethyldicarbonate

1.19 Dimethyldicarbonate (DMDC) is a cold sterilisation agent for use in soft drinks and low alcohol wines. It is reported to have a broad antimicrobial range of action and breaks down rapidly whilst preserving the drink contents. On contact with water, DMDC decomposes to form carbon dioxide and methanol. However, in complex mixtures such as fruit juices, a range of reaction products is formed, eg dimethyl carbonate, methyl ethyl carbonate, methyl carbamate and N-carbomethoxy derivatives of amino acids.

1.20 DMDC was first considered by the COT in 1987. At the time the Committee reviewed a number of toxicity studies on DMDC-treated beverages and was reassured that there were no significant adverse toxicological effects. However, the Committee did raise two points which centred on the levels of the reaction products found in DMDC-treated beverages, in particular, the levels of methanol and methyl carbamate. The COT asked to see data on the levels of methanol formed in a DMDC-treated beverage and on the natural methanol content of beverages, such as fruit juices, for comparative purposes.

1.21 Methyl carbamate is structurally related to ethyl carbamate (urethane), a known animal carcinogen. As part of its review of DMDC the Committee considered the results of a recent study carried out by the National Toxicology Programme (NTP) in the USA which indicated that methyl carbamate was carcinogenic in Fischer 344 rats. The COT sought the expert opinion of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) as to whether methyl carbamate was potentially genotoxic and requested data on the levels of methyl carbamate found in DMDC-treated beverages.

1.22 In 1992 further data were presented to the COT on these issues. The levels of methanol found in DMDC-treated beverages were judged not to represent a toxic hazard. The Committee was similarly reassured that the levels of methyl carbamate were very low. The COM advised that methyl carbamate is not genotoxic. Therefore, it can be assumed that a threshold exists for any carcinogenic effect. The COT considered that the safety factor between the intakes of methyl carbamate resulting from consumption of DMDC-treated beverages and the dose which was carcinogenic in the NTP study was sufficiently large for there to be no concern about the presence of methyl carbamate in DMDC-treated beverages.

1.23 The COT advised that, having considered the various data, there were no safety grounds on which to advise against the use of DMDC as a cold sterilisation agent at levels up to 250 mg/l.

Guidelines for the Safety Assessment of Microbial Enzyme Preparations used in Food

1.24 The COT agreed guidelines for the safety assessment of microbial enzymes in food in December 1991. The Committee agreed to undertake a wide consultation to seek the views of enzyme manufacturers, food manufacturers and processors, trade associations, consumers and research groups prior to

using the guidelines to assess individual submissions. A wide diversity of responses were received and a number of amendments to the guidelines were made. The Committee also agreed that the guidelines should be reviewed at some suitable period after they have been applied to a specific group of enzymes. The **finalised** guidelines are available from the Committee's administrative secretary and are in the process of being published in a scientific journal.

Iodine Toxicity in Adults and Young Children

1.25 The COT previously reviewed iodine concentrations in milk in 1989. At that time information was requested on:

- (a) the nutritionally required minimal intake; and
- (b) the toxicity of high levels of iodine.

1.26 During 1992 the COT considered the new intake data on iodine which had been gathered under the auspices of the Steering Group on Chemical Aspects of Food Surveillance.

1.27 The Committee on Medical Aspects of Food Policy (COMA) advises on the recommended dietary intakes of nutrients in the UK. In 1991 the COMA Panel on Dietary Reference Values published its recommendations for Dietary Reference Values for iodine. The Panel advised that 70 µg/day was the Lower Reference Nutrient Intake (LRNI) for adults since this intake in adults appeared to be the minimum necessary to prevent signs of goitre in a population. The Reference Nutrient Intake (RNI) was set at 140 µg/day.

1.28 New analytical data available from MAFF suggested an estimated daily mean intake of 190 µg/day for adults in the UK. This value is in excess of the recommended RNI but the COT considered that it gave little cause for concern because higher levels of iodine have been ingested with no toxic effects.

1.29 The COMA Panel set an LRNI of 40 µg/day for babies aged 0-12 months. It set an RNI of 50 µg/day for babies aged 0-3 months and of 60 µg/day for those aged 4-12 months.

1.30 The COT noted that when mean daily intakes in the UK were estimated from a quantitative seven-day diet record for the age group 6-12 months, the figure obtained was 204 µg/day (23 µg/kg bw), ie four times greater than the COMA recommended RNI. The Committee considered whether this level of intake might be toxic to the 6-12 month age group. It noted that in 1962 a study was undertaken to find the minimal effective dose against ¹³¹Iodine fallout. Fourteen children received 1000 µg iodine daily for four months. No toxic effects were encountered and the Committee considered that these data gave some reassurance about intakes of iodine by 6-12 month old infants.

1.31 The Committee was reassured by information from MAFF which indicated that iodine concentrations in milk appeared not to be increasing. It recommended that monitoring of milk should continue.

Propylene Carbonate

1.32 Propylene carbonate was found to leach into water from a material submitted for approval to the Department of the Environment Committee on Chemicals and Materials of Construction for Use in Public Water Supply and Swimming Pools (CCM). This Committee advises the Secretary of State for the Environment on the statutory approval of new chemicals and materials for use in contact with potable water. CCM requested appropriate toxicity data from the applicant and the COT was asked to assess these data and consider whether additional information should be requested.

1.33 Propylene carbonate was not carcinogenic to mouse skin following dermal application, and was not mutagenic in the Ames assay nor in an *in vitro* DNA repair test. However, equivocal results were obtained in a mouse micronucleus test. Exposure of pregnant female rats to a high dose of propylene carbonate was associated with slight increases in some skeletal variations and also maternal toxicity. No other reproductive toxicity studies were available. The Committee noted that the lowest observed adverse effect level, after administration (to rats) by gavage, for 90 days, was at least 10^5 times greater than the highest exposure predictable from drinking water in the first few days following initial contact with the material.

1.34 The Committee concluded that the dermal carcinogenicity study was inappropriate for the assessment of oral ingestion of propylene carbonate, but agreed that, given that the potential exposures were low and short term, there was sufficient reassurance from the toxicity data supplied to conclude that additional carcinogenicity testing was not warranted, if adequate mutagenicity studies were provided and showed no evidence of genotoxicity. In the light of the mutagenicity data available, the Committee concluded that *in vitro* tests for clastogenicity and mutagenicity in mammalian cells were required. Committee members also agreed that an additional teratology study (preferably in the rabbit) and a single generation reproductive toxicity study in a rodent species were required.

1.35 The Committee was also concerned about the stability of propylene carbonate in water and commented that the transient formation of propylene oxide from propylene carbonate could not be excluded.

Thiamphenicol

1.36 Thiamphenicol is a broad spectrum antibiotic, and is the active ingredient in some products licensed for veterinary medicinal use in several EC countries, but not in the UK. The Committee on Veterinary Medicinal Products (CVMP) is currently reviewing all of the veterinary medicines in use in the EC. The CVMP's Working Group on Safety of Residues had been unable to identify a safe level of exposure for chloramphenicol because it caused serious blood dyscrasia (including leukaemia and fatal aplastic anaemia) in a small number of people with no apparent no observed adverse effect level. They were concerned that, as the chemical structure of thiamphenicol was similar to that of chloramphenicol, it might have similar haematological properties. The COT was asked to advise UK delegates to the Working Group

on the potential of thiamphenicol to cause idiopathic blood dyscrasia in man. The COT commented on epidemiological evidence on the possible association of thiamphenicol exposure with aplastic anaemia and leukaemia, saying that there was no convincing evidence to suggest any causal effect. This advice allowed the UK delegation to the EC Committee to agree to the setting of a Maximum Residue Limit for residues in food resulting from the use of thiamphenicol in veterinary medicine.

Food Surveillance Papers

1.37 The Steering Group on Chemical Aspects of Food Surveillance is a committee which advises government and which keeps under review the possibility 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 may recommend future work.

1.38 During 1992 the COT considered three Working Party reports: the report of the Working Party on Pesticide Residues 1988-90 (Food Surveillance Paper No 34, HMSO, London, 1992); the third report of the Working Party on Naturally Occurring Toxicants in Food's Sub-group on Mycotoxins (Food Surveillance Paper No 36, HMSO, London, 1993) and a report of the Working Party on Inorganic Contaminants in Food: Aluminium in Food (HMSO, in press). The COT's advice is included in these Food Surveillance Papers as an appendix.

Topics Under Review

1.39 The following topics, which were discussed by the COT at meetings held in 1992, are under review:

- Agaratine
- Ascorbic acid (use as a flour treatment agent)
- Azodicarbonamide
- Ethylcarbamate
- Food products derived from lupins
- Gellan gum
- Mineral hydrocarbons
- Perchloroethylene
- Phyto-oestrogens
- Plasticisers
- Report of the Working Party on Naturally Occurring Toxicants in Food
- Vitamin A in liver

1992 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

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

DECLARATION OF INTERESTS DURING THE PERIOD OF THIS REPORT

Personal Interest			Non Personal Interest	
Member	Company	Interest	Company	Interest
Prof H F Woods (Chairman)	Cadbury Schweppes Edinburgh Invest Trust Foreign & Colonial Inv Trust Hanson Smith & Nephew	Share Holder	Wide range of national & international food & chemical companies	Dean of the University of Sheffield Medical School, Faculty of Medicine and Dentistry. Which has extensive activity in teaching and research in food science and technology and in topics related to and supported by, many companies in the food and chemical industry. Trustee of Hallamshire Therapeutic Research Trust Ltd, Harry Bottom Charitable Trust and Special Trustees for the former United Sheffield Hospitals
Prof V Beral	NONE	NONE	NONE	NONE
Prof A D Dayan	Welcome Foundation ML Laboratories Ortho Ltd Pfizer Ltd SmithKline Beecham Sterling Winthrop Ltd Syntex Ltd Wyeth Ltd	Pension Consultant Consultant Consultant Consultant Consultant Consultant	Glaxo	Research Fellowship
Dr J Dewdney	SmithKline Beecham	Employee	NONE	NONE
Prof G G Gibson	NONE	NONE	NONE	NONE
Dr G Hawsworth	NONE	NONE	ICI Glaxo Group Research Servier Research & Dev Pfizer Central Research SmithKline Beecham	Research Support
Dr I Kimber	ZENECA formerly ICI plc	Employee Share Holder	Unilever plc	Grant for Research
Dr D Prentice	Roche Holdings AG Syntex Ciba-Geigy Nestlé Sandoz Rhone-Poulenc Rorer Gensia TAP Pharmaceuticals Synthelabo (LERS) Wyeth-Ayerst Hoffman-La Roche	Share Holder Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant	NONE	NONE

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

DECLARATION OF INTERESTS DURING THE PERIOD OF THIS REPORT

Personal Interest			Non Personal Interest	
Member	Company	Interest	Company	Interest
Dr A G Renwick	Proctor & Gamble International Sweeteners Association	Consultant Consultant	Bayer Ciba-Geigy Warner Lambert Solvay-Duphar	Research Support
Mr F Sullivan	Rank Hovis McDougal Rothmans International Rhone Poulenc Rorer- France EC Community (DGV) Borax Ltd	Consultant Consultant Grant Occasional Fee Grant Occasional Fee	NONE	NONE
Dr A Thomas	NONE	NONE	NONE	NONE
Prof A M Tomkins	NONE	NONE	Glaxo	Research Funding
Prof D Walker	SmithKline Beecham British American Tobacco BP Nutrition	Consultant Consultant Consultant & Share Holder	Robens Institute, Univ of Surrey	Retaining Fee
Prof R Walker	Proctor & Gamble IDV Ltd Food Safety Advisory Centre Coffee Science Info Centre Cadbury Beverages Europe RHM	Consultant Consultant Consultant Consultant Consultant	Nestlé	Research Studentship

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

Professor B A Bridges
(Chairman)
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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.

Much of the Committee's time is taken up with consideration of specific substances which currently present problems for regulatory authorities within the UK. In addition to this, consideration is given to issues where guidance is sought by Departments, sometimes in response to pressure from other countries within the CEC, and sometimes on more general issues, for example the difficulties involved in the regulation of natural toxins.

An increasing amount of time is needed for interaction with international bodies such as OECD and the CEC where the pressure to harmonise testing and labelling 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

Agaritine

2.1 Naturally occurring hydrazines, such as agaritine, are found in edible mushrooms, and human consumption of the commercially grown edible mushroom *Agaricus bisporus* was increasing. Since certain synthetic hydrazines were known to have mutagenic and carcinogenic potential, the COM and COC considered these aspects of agaritine in detail.

2.2 The COM reached the following conclusions:

- (i) The chemical structures of agaritine and its putative metabolites (N-acetyl-4-(hydroxymethyl) phenylhydrazine and 4-(hydroxymethyl) benzene diazonium ion) gave rise to concern about mutagenic potential, a concern which was strengthened by the activity of agaritine and its metabolites in bacterial mutation assays.
- (ii) Although there was very little evidence of agaritine being mutagenic in mammalian cells there were no *in vivo* data and inadequate *in vitro* data to allow any firm conclusion to be reached about mutagenic potential in mammalian systems.
- (iii) Information from a bone marrow micronucleus test would provide reassurance and indicate a probable lack of heritable effect.
- (iv) In the COM's opinion, the concerns about agaritine's structure and mutagenic potential would give it a high priority for further consideration.

2.3 Furthermore, it was felt that more understanding of the mammalian absorption, distribution, metabolism (and potential detoxification) of agaritine was needed before the Committees could make any real assessment of mutagenic and carcinogenic activity and its relevance to humans. There was insufficient concern arising from current information to warrant advising that extensive measures should be taken to reduce agaritine intake.

Alitame

2.4 Alitame, a new intense sweetener, had been considered by the COM in February 1990 when further data were requested, namely *in vitro* and *in vivo* cytogenetic studies on alitame, and data from bacterial mutagenicity assays on the β -isomer and alanine amide (decomposition products). A somewhat limited *in vitro* cytogenetics study, and a comprehensive bone marrow micronucleus test had been conducted on alitame and negative results were obtained in both cases. In addition, negative results were obtained when the β -isomer was tested in the Salmonella assay. However, no data had been provided on the other decomposition product, alanine amide, as had been requested. The Committee concluded that although the data presented indicated that alitame itself did not have any significant mutagenic potential, the request for information on the ability of the alanine amide to produce gene mutations using the Salmonella assay should be maintained.

Dimethyldicarbonate

2.5 The COT were considering the use of dimethyldicarbonate as a cold sterilisation agent for beverages, and had noted that this compound gave rise to small amounts of methyl carbamate as a decomposition product. Methyl carbamate was structurally very closely related to ethyl carbamate, a recognised genotoxic carcinogen. Methyl carbamate had been shown to produce tumours in F344 rats, but not in mice. The estimated safety margin between the dose producing tumours in rats and the likely intake from drinks was very large, but it was acknowledged that a safety margin approach could only be used if it was accepted that the tumours had arisen from a non-genotoxic mechanism. The advice of the COM had therefore been sought on the genotoxicity of methyl carbamate.

2.6 The Committee agreed the following conclusions:

- (i) Methyl carbamate had been extensively investigated for its ability to produce gene mutation and DNA damage in micro-organisms and mammalian cells *in vitro*. Negative results were consistently obtained. Negative results were also obtained in a limited assay to detect chromosome damage.
- (ii) The mutagenic potential and DNA damaging ability of methyl carbamate had been investigated *in vivo* in bone marrow, liver, kidney, lung macrophages and germ cells (dominant lethal assay). Again negative results were consistently obtained.
- (iii) These results were in marked contrast with those obtained with the very closely related ethyl carbamate. However, a rationale (Ashby J, *Mutation Research*, 1991, 260:307-8) for those differences was provided by the inability of the methyl compound to give rise to an epoxide metabolite.
- (iv) The fact that methyl carbamate had been found to be negative in *in vitro* and *in vivo* short-term tests indicated that any chemical carcinogenic effect of methyl carbamate suggested by the results in Fischer 344 rats does not arise from a genotoxic mechanism.

Gallates Used as Antioxidants in Food

2.7 Propyl gallate was one of a number of antioxidants reviewed by the COM in 1985/6. At that time the Committee was satisfied with the available data on propyl gallate, but asked for an *in vivo* bone marrow micronucleus test on gallic acid, a metabolite of propyl gallate, in view of some concern about one published paper on this compound. Since that time the Committee had considered in some detail the data on butylhydroxyanisole (BHA), another antioxidant, and had concluded that the clastogenic activity seen *in vitro* was due to the generation of hydrogen peroxide and was not relevant to conditions pertaining to the dietary use of BHA. The Committee emphasised that its earlier concerns over propyl gallate were not primarily due to the clastogenic activity of propyl gallate *in vitro* but to a study indicating that gallic acid might cause chromosomal aberrations *in vivo*. Although the study was poorly conducted, the COM believed it could not be ignored and wished to see a better conducted repeat study. Further studies with propyl gallate

confirmed the absence of activity in the bacterial assays and the presence of clastogenic activity *in vitro* in the absence of metabolic activation. There were no new *in vivo* studies on propyl gallate, but each of three independent earlier studies showed absence of activity in bone marrow. However, the Committee concluded that the original request for an *in vivo* micronucleus test using gallic acid should be maintained,

Imidocarb

2.8 Imidocarb is an antiparasitic drug used to treat babesiosis in cattle as a single subcutaneous injection equivalent to 2.4 mg/kg bodyweight. The residues of the drug are very persistent in the edible tissues of the treated cattle and these residues might be consumed by humans. There were indications that the compound might be carcinogenic (see entry in the report of the COC). The company which produced the drug had carried out mutagenicity testing in order to determine whether the compound was genotoxic and the results suggested the *in vitro* induction of polyploidy in the absence of aneuploidy. The Veterinary Products Committee (VPC) had asked the COM's advice on the relevance of the *in vitro* polyploidy to the safety of residues in meat and what further tests might resolve the question.

2.9 The COM were not convinced that the tests conducted so far ruled out the possibility of aneugenic potential. The VPC secretariat were advised to ask for further data.

Omethoate

2.10 The COM's advice on the mutagenicity of omethoate, an insecticide, was sought by the Sub-committee on Pesticides (formerly the Scientific Sub-committee) of the Advisory committee on Pesticides (ACP). The available mutagenicity data included *in vitro* studies which clearly indicated mutagenic potential. Negative results were obtained in *in vivo* studies, but a positive result was obtained in the mouse spot test. The Committee was asked to comment on the significance of this last result.

2.11 Members agreed the following conclusions:

- (i) Omethoate had been extensively investigated in *in vitro* mutagenicity assays. Positive results were obtained in Salmonella assays, in one assay for gene mutation in mammalian cells, and in assays for clastogenicity and also for SCE and UDS induction in mammalian cells. It clearly had mutagenic potential.
- (ii) It had also been extensively investigated *in vivo* using the oral route. Negative results were obtained, using acceptable protocols and doses up to those producing marked toxic effects in the whole animal, in the bone marrow (micronuclei induction and SCEs), liver (UDS) and germ cells (two dominant lethal assays in male mice). These negative results were consistent with the known metabolism and detoxification of omethoate. A positive result however was obtained in the mouse spot test.

- (iii) The weight of *in vivo* evidence was negative and there was no justification for recommending any change in the regulatory position.
- (iv) The positive mouse spot test could not however be completely discounted, as there was a remote possibility of a specific effect on the developing embryo. Consideration should be given to a further test for embryonic specific activity, the fetal micronucleus test being recommended.

Dimethoate

2.12 The mutagenicity of the insecticide, dimethoate, was also considered, since the closely related omethoate (see above) was a metabolite of this compound in plants and animals. The following conclusions were agreed regarding dimethoate:

- (i) Dimethoate gave positive results in the Salmonella assay using strain TA100. Negative results were obtained in a single mammalian cell assay for gene mutation in Chinese hamster ovary (CHO) cells. Positive results were, however, consistently obtained in assays for unscheduled DNA synthesis (UDS) and sister chromatid exchange (SCE) induction in mammalian cells *in vitro*. These studies indicated that dimethoate has mutagenic potential.
- (ii) Regarding *in vivo* data, dimethoate had given negative results in comprehensive bone marrow assays for clastogenicity using both the micronucleus test in mice and metaphase analysis in rats. Negative results were also obtained in a well conducted *in vivo* hepatocyte UDS assay. In all these studies dose levels were up to those producing significant toxicity in the animal. Several studies had also been carried out in germ cells, both dominant lethal assays and cytogenetic studies. Negative results were consistently obtained. A long term carcinogenicity bioassay had shown no evidence of carcinogenicity.
- (iii) Although *in vitro* studies indicated that dimethoate had mutagenic potential, *in vivo* assays in bone marrow, liver and germ cells indicated that this potential is not expressed *in vivo*. This could be due to protective effects of metabolism.

2-Phenylphenol and its Sodium Salts

2.13 This compound is used as a wood preservative and surface fungicide. There was renewed concern over the bladder tumours observed in male rats when they were treated with 2-phenylphenol at high dose levels. When the COM previously considered this compound in 1988, it had been felt that a genotoxic mechanism was most unlikely. Some additional mutagenicity studies had become available since that time and the Scientific Sub-committee on Pesticides had requested the advice of the COM on the significance of these further studies.

2.14 The Committee agreed the following conclusions after evaluating the more recent data:

- (i) Data from several assays to investigate the ability of 2-phenylphenol or its sodium salt to produce gene mutation in *Salmonella typhimurium* were consistently negative.
- (ii) Positive results were obtained in *in vitro* metaphase analysis studies in CHO cells. Mouse lymphoma assays in the presence of exogenous metabolic activation gave positive results in the form of small colonies. These results suggested that a metabolite of 2-phenylphenol had clastogenic potential. These findings conflicted with an earlier *in vitro* metaphase analysis in a single study.
- (iii) Negative results were obtained in bone marrow assays for clastogenicity *in vivo* and also in germ cells (dominant lethal assay), indicating that any possible clastogenic potential was not expressed in the whole mammal.
- (iv) Conflicting results appeared to be obtained in *in vivo* assays for effects on DNA in bladder epithelium, the target tissue of concern. The Committee therefore wished to see data from a more sensitive *in vivo* method to investigate adduct formation. There was insufficient evidence to recommend a departure from the use of the safety factor approach for regulation of this compound at the present time.

Aniline

2.15 HSE had asked for advice from the COM on the mutagenicity of aniline, an industrial chemical used in the rubber industry and in the manufacture of dyestuffs, drugs, and pesticides. This compound had been shown to produce tumours in the spleen but no other tissues in the rat, and was not a carcinogen in the mouse; leading to the possibility that the induction of tumours might be secondary to toxic effects.

2.16 The Committee agreed the following conclusions:

- (i) The chemical structure of aniline indicated that it was possible that it had mutagenic potential.
- (ii) Aniline had been investigated for its ability to induce gene mutations in bacteria. Negative results were obtained using standard conditions (exogenous metabolic activation using enzyme preparations from rodent livers), but positive results were seen in the presence of metabolic activation using plant preparations, and also norharman (a non-mutagenic constituent of tobacco tar which may enhance mutagenic activity in *Salmonella* by affecting the balance between metabolic activation and deactivation). The latter data indicated that aniline had mutagenic potential.
- (iii) Positive results were reported in studies to investigate gene mutation in mammalian cells using mouse lymphoma cells and also in studies to investigate clastogenicity in Chinese hamster cells.

- (iv) Aniline had also given positive results in bone marrow micronucleus tests in both the mouse (oral and intra-peritoneal administration) and the rat (oral administration) but only at extremely high dose levels. These data were not regarded as convincing evidence of clastogenicity *in vivo*, but further work would be needed to explain their significance.
- (v) From the available data, it was not possible to conclude that aniline was not genotoxic *in vivo*. In view of this, and because of concerns regarding closely related analogues, it would be prudent to assume that aniline was a genotoxic carcinogen.

1,3-Butadiene

2.17 As a result of an expansion of the Department of the Environment's urban air quality monitoring network, routine monitoring data will be generated in future on 1,3-butadiene. 1,3-Butadiene is a ubiquitous air pollutant produced in cigarette smoke, vehicle exhausts and incineration products from fossil fuels. The Department of Health therefore sought the advice of the COM and the COC on the possible long term health effects resulting from low level exposures to this compound.

2.18 The COM agreed the following conclusions:

- (i) The structure and metabolism of 1,3-butadiene would suggest that it had mutagenic potential; it was metabolised in rodents to epoxides with direct-acting mutagenic potential.
- (ii) 1,3-Butadiene produced gene mutation in *Salmonella typhimurium* strains TA1530 and TA1535 in the presence of an exogenous metabolic activation system. Positive results were also reported in the mouse lymphoma assay. No data were available from *in vitro* metaphase analysis for clastogenic potential, apart from some evidence of SCE in CHO cells.
- (iii) The mutagenic activity of 1,3-butadiene could be expressed in somatic cells *in vivo*. A clear positive result was obtained in a bone marrow micronucleus test in mice following exposure to 1,3-butadiene by inhalation. There was also some evidence of an increase in SCEs. Negative results were obtained in an *in vivo* assay to detect UDS in the liver, but the Committee recognised that such an assay was not the optimum method for investigating the mutagenic potential of 1,3-butadiene. There was some evidence of covalent binding to DNA in the liver in a study using a limited protocol.
- (iv) No data were available on the effects in germ cells, but it would be prudent to assume that 1,3-butadiene had the potential for producing heritable mutations.

DNA Gyrase Inhibitors

2.19 Quinolone antibiotics exert their antibacterial effect by the inhibition of bacterial DNA gyrase, an essential enzyme in bacterial DNA replication. The inhibitory effect on topoisomerase II, the equivalent mammalian enzyme, is relatively small, so that bacterial growth is selectively suppressed. It had been suggested that long term carcinogenicity testing of new quinolones (all recently developed quinolones contain a fluorine atom and are known as fluoroquinolones) was unnecessary because there was a known pattern of mutagenic and carcinogenic activity for quinolones, with members of the class giving some positive results for mutagenicity *in vitro*, but consistent negative results *in vivo* and that quinolones tested in long term carcinogenicity studies were either negative or non-genotoxic carcinogens. This group of compounds was referred to the COM/COC by the Veterinary Products Committee (VPC) for general advice. The Committees considered the data available for several quinolones and the following conclusions were agreed as general advice on the testing of antibacterial quinolones:

- (i) If a compound gives negative results in adequately conducted *in vitro* tests in mammalian cells for gene mutation and clastogenicity and has a high relative inhibitory potency towards DNA gyrase compared to mammalian topoisomerase, it is unlikely to be genotoxic in mammalian systems.
- (ii) If a compound with a high relative inhibitory potency towards DNA gyrase compared to mammalian topoisomerase gives positive results in some *in vitro* mutagenicity assays, and this activity can be demonstrated to be entirely secondary to effects on topoisomerase II activity or due to artefacts which would not occur *in vivo*, eg metal chelation, then it is unlikely to be a genotoxic carcinogen. Negative results in an *in vivo* bone marrow assay would provide adequate reassurance in this respect.
- (iii) If it is not possible to attribute all the activity seen *in vitro* to such effects further work would be necessary which should be identified on a case-by-case basis. Further *in vivo* testing in addition to the bone marrow study might be needed to demonstrate that the activity seen *in vitro* is not expressed in the whole animal.
- (iv) Fluoroquinolones should be assessed for safety on the same basis as any other veterinary product. If they had been shown to be negative in standard mutagenicity tests, or to give positive results in *in vitro* mutagenicity tests due entirely to effects which could not be expressed in the whole animal, they should be treated as compounds which had given negative results in short term tests for genotoxicity. It was not possible to predict the potential tumour-inducing ability of members of this family of compounds.

Mutagenicity Testing Strategies on New Substances: Further Advice to HSE

2.20 This topic had been discussed by the Committee during 1991 in order to advise HSE for negotiations on testing of new chemical substances in Europe (see 1991 Annual Report). After a further meeting of the European working group final draft proposals were agreed. These were that the bacterial mutation assay should be carried out as the first test and if this was positive be followed up preferably by the mouse lymphoma assay (with a recommendation to measure colony size). If this were positive, or if it were negative but significant human exposure was anticipated, then this test would be followed up immediately by an *in vivo* bone marrow assay. If the mouse lymphoma assay were negative and there was no significant human exposure, then the request for the *in vivo* bone marrow assay could be delayed until a supply level of 10 tonnes per year was reached. It was agreed by the COM that this was a significant improvement on the previous proposed strategy, although it believed that the mouse lymphoma assay in this context was redundant. It was understood that the European Commission would include a note stating that the value of following a bacterial mutation positive with a mouse lymphoma test should be kept under review.

Classification of Chemicals on Basis of Mutagenic Properties

2.21 The EC Scheme for the classification and labelling of chemicals (Dangerous Substances Directive 67/548/EEC) recognised three categories with regard to mutagenicity. These were substances known to be mutagenic to man (Category 1), substances that should be regarded as if they were mutagenic to man (Category 2) and substances which caused concern for man owing to possible mutagenic effects (Category 3). Recently detailed advice on the interpretation of these criteria had been published in the Official Journal of the European Communities (OJ). For a compound to be considered a category 3 mutagen, *in vivo* data in somatic cells were needed. This was in line with the COM advice that for any meaningful hazard assessment *in vivo* data were necessary. However, the criteria for a category 2 mutagen had given rise to concern (in particular the criteria for a category 2 mutagen set out at 2c of the OJ publication – *in vivo* assays showing mutagenic effects in somatic cells of mammals, in combination with toxicokinetic methods, or other methodologies capable of demonstrating that the compound or relevant metabolite reaches the germ cells). The COM view was that there was a need to show that the compound (or metabolite) reached and *interacted with* DNA in germ cells for such classification. The present European criteria, however, would allow the demonstration that the compound reached the germ cells, together with information that it was mutagenic in somatic cells, for classification as a category 2 mutagen. This would remove any real distinction between a category 3 and a category 2 mutagen. The Committee agreed that the UK should press for the deletion of clause 2c.

Mouse Spot Test

2.22 The Committee was asked to advise on whether the statement on the mouse spot test given in the COM guidelines [Guidelines for the testing of chemicals for mutagenicity, Department of Health, London: HMSO 1989 (Report on Health and Social Subjects 35)] still held. The statement was:

"The available results [for the mouse spot test] from a number of laboratories are fairly encouraging. However, the test has several disadvantages. Conflicting results have been obtained from different laboratories, and further inter-laboratory validation is desirable. The specific strains of mice needed are not widely available – at least 300 offspring must be scored at each dose level – and considerable expertise is required to correctly score the spots. The method is little used in the UK."

2.23 The question as to whether views on this test should be revised was prompted specifically by the discussion on omethoate (see above); but also by concern at a number of apparently anomalous results reported from this test and the reliance being put on this assay by the German authorities in their strategy for mutagenicity testing.

2.24 During the 1992 discussion there were many reservations voiced about the mouse spot test, including:

- (i) The database, particularly for negative compounds, was too small for adequate validation.
- (ii) The end-point measured was highly subjective.
- (iii) Much work would be needed to show clearly that the spots measured were specific for a heritable effect on DNA, rather than a non-specific effect on gene expression.

2.25 The Committee felt that the limitations noted in the COM guidelines were still valid and the test had been inadequately validated. However it was felt that results from a well conducted, positive mouse spot test should not be ignored when making an overall assessment of a compound's mutagenicity.

Generic Issues when Considering Natural Toxins

2.26 The question of advising on and possible regulation of natural toxins is extremely complex, particularly when they are constituents of well established foods. The problem is further complicated by the lack of adequate toxicity data on these compounds and little or no information on interactions between dietary factors. The COM and COC separately discussed some general issues relating to this problem and made the following points:

- (i) The COM was concerned about the implications of the intake of abnormal doses of natural toxins from concentrated extracts of plants for example, or new varieties of plant which might have much higher levels of toxic substances than the traditional strains. Extracts were of particular concern and could relatively easily be tested for mutagenic potential.

- (ii) The COC agreed that the first area for consideration should be concentrates and extracts, such as herbal medicines where there was an enormous potential for over exposure by users of such products. The Committee felt that practical steps should also be taken to ensure the toxicological safety of novel foods and new cultivars.

Joint COM/COC Symposium on Peroxisome Proliferators

2.27 A joint scientific meeting was held on 13 January 1992. Peroxisome proliferation in hepatocytes was of interest because many of the compounds which cause this effect (which include various types of chemical known collectively as peroxisome proliferators) in rats and mice have been shown to cause liver tumours in these species. The possible relevance of this effect to human cancer needed to be clarified. This meeting gave members an opportunity to hear about recent advances in understanding of the mechanisms involved in the tumour induction from researchers in the field and discuss the relevance of the information to the regulation of chemical carcinogens.

2.28 The following presentations were given:

Toxicological significance of peroxisome proliferation: an overview – Dr T Gray, Sterling Winthrop Research.

Mechanisms of peroxisome proliferator-induced hepatocarcinogenicity – Dr B Lake, BIBRA Toxicology International.

Mechanisms of peroxisome proliferation and species differences – Dr C Elcombe, ICI Central Toxicology Laboratory.

Peroxisome proliferator activated receptor (PPAR): A receptor that mediates peroxisome proliferator action? – Dr S Green, ICI Central Toxicology Laboratory.

2.29 The presentations were followed by discussion and the following general conclusions were drawn:

Possible mechanisms of action of peroxisome proliferators: There were several possible explanations for the tumour promoting action of peroxisome proliferators. These included sustained oxidative stress caused by active oxygen species leaving the peroxisomes and causing oxidative damage to cellular components. However this did not seem to be wholly responsible for the carcinogenicity seen in rodent liver and there was evidence that increased and sustained cell replication was an important factor in allowing previous DNA damage to be expressed.

Species differences in susceptibility to peroxisome proliferators: Rats and mice are extremely responsive to the effects of peroxisome proliferators, while hamsters are less responsive and guinea-pigs, dogs and marmosets are essentially unresponsive. Humans appear to be relatively unresponsive, although no definite conclusion could yet be drawn on whether the hepatocarcinogenic effect was relevant to humans. It was unclear at present whether PPAR occurs in an active form in humans.

Suggestions for further research arising from the meeting:

- (a) Establishment of dose-response relationships for peroxisome proliferation and cell replication.
- (b) Evaluation of other potential biomarkers as a measure of rodent hepatocarcinogenicity.
- (c) Further studies on species differences in response to peroxisome proliferators, especially in terms of effects on cell replication.
- (d) Studies to investigate whether all the effects of peroxisome proliferators, including carcinogenesis, are mediated through the PPAR.

1992 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

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MEMBERS

Professor J Ashby BSc PhD CChem FRCS

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

DECLARATION OF INTERESTS DURING THE PERIOD OF THIS REPORT

Personal Interest

Non Personal Interest

Member	Company	Interest	Company	Interest
Prof B A Bridges (Chairman)	Rank Xerox Rhône Mérieux UPSA Laboratories Glaxo Group	Consultant Consultant Consultant Consultant	Unilever Boots	Student Support Student Support
Prof J Ashby	ZENECA formerly ICI plc	Employee	NONE	NONE
Dr RL Carter	ICI plc	Share Holder	NONE	NONE
Dr C Cooper	NONE	NONE	NONE	NONE
Prof DS Davies	ICI plc ML Laboratories plc Clinical & Biochemical Pharmacology Consultancy Ltd ini	Share Holder Non Executive Director Director	Abbott Astra/Draco Astra Boots Dompe Glaxo Hoechst ML Laboratories PLC ML Laboratories PLC Pfizer Proctor & Gamble Rhône Poulenc Rorer Roche Products Wellcome Foundation Daiichi Pharmaceuticals Otsuka Merck Sharp & Dohme SmithKline Beecham	Research Fellowship Research Research Research Research Research Fellowship Research Research Research Research Fellowship Research Research Consultancy Research Research Fellowship Research Fellowship Research
Prof H J Evans	Merck, Sharpe & Dohme (Astra Sweden) ICI plc Fisons Croda	Consultant Share Holder Share Holder Share Holder	Boehringer Mannheim Pharmacia ICI plc Wellcome	Capital Equipment Fellowships Studentships Running Expenses
Dr M Fox	Sun Alliance Barclays Bank Burmah Oils Hanson plc Midland Bank Royal Insurance British Oxygen Co Allied Lyons BP Greenhall Whitley General Accident Bass plc BAT Joseph Holt Nat West Burton Wood Wiggins Teape	Share Holder of these Companies	NONE	NONE
Prof RF Newbold	NONE	NONE	NONE	NONE

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

DECLARATION OF INTERESTS DURING THE PERIOD OF THIS REPORT

Personal Interest

Non Personal Interest

member	Company	Interest	Company	Interest
Prof J M Parry	Glaxo Group Research Fisons Pharmaceuticals SmithKline Beecham BAT British Petroleum Compass Catering British Telecom South Wales Electricity JIB Insurance	Consultancy & Grant Consultant Consultant Consultant Share Holder Share Holder Share Holder Share Holder Share Holder	Amersham International Pfizer SmithKline Beecham	Grant Studentship Studentship
Dr S Venitt	NONE	NONE	NONE	NONE
Dr R M Winter	NONE	NONE	NONE	NONE



New Chairman for the COM

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

The vacancy will be created by the resignation of the current chairman, Professor Bryn Bridges, who has taken over the chairmanship of the Committee on the Medical Aspects of Radiation in the Environment. Professor Bridges has been a member of departmental committees considering mutagenicity since **1972** and chairman of COM since **1983**.

The chairmanship of the Committee will pass to Professor J M Parry, Professor of Genetics, University College, Swansea.

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

Dr R L Carter
(Chairman)
MA DM DSc *FRCPath*



Preface

The Committee on the Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) has considered a wide range of topics in the past year. Evaluations of specific chemicals, such as acrylamide and various veterinary products, were made. The Committee maintained a watching brief on the hazards associated with oral snuff, and members welcomed UK regulations (following the adoption of a European Directive) whereby certain types of tobacco for oral use, including oral snuff, would be banned as from 1 January 1993. Other, more general issues, have also been addressed. In particular, the Committee's advice was sought on the assessment of carcinogens in atmospheric air. Once identified, some of these compounds can be removed, but others cannot be eliminated so readily. It is essential that some kind of evaluation based on the relative hazards of such substances should be made which can be used in establishing priorities for action in terms of control.

Knowledge of the molecular aspects of carcinogenesis has greatly increased in recent years, and new members with particular expertise in this field have been appointed to the Committee. Working links between the COC and the Committee on Mutagenicity remain close, with four individuals serving on both Committees. Scientific meetings for the two Committees are held annually, and the subject for 1993 is mutations of the p53 gene – the most common cancer-related genetic abnormality known to occur in human cancers at the gene level.

RICHARD CARTER

Agaritine

3.1 Agaritine was considered by the COM and COC (see COM section of report for background and COM conclusions).

3.2 The COC's conclusions on agaritine were as follows:

- (i) Although the chemical structure of agaritine and its mutagenic activity in bacterial mutation tests gave cause for concern about its potential genotoxicity, there were no adequate data which indicated whether or not the substance was genotoxic *in vivo*.
- (ii) The stabilised forms of agaritine's putative metabolites showed evidence of carcinogenicity in long term testing in Swiss mice, although the studies were of unusual design. N-acetyl-4-(hydroxymethyl) phenylhydrazine induced increased incidences of tumours of the lung and blood vessels: 4-(hydroxymethyl) benzene diazonium ion tetrafluoroborate induced an increase in adenocarcinomas of the glandular stomach. While there was no evidence that agaritine itself was carcinogenic in mice, it had not been adequately tested for carcinogenicity; further long-term testing would be necessary to confirm the apparent lack of carcinogenicity.
- (iii) Data available from short term mutagenicity testing of mushroom (*Agaricus bisporus*) extracts showed that mutagenic activity was not correlated to agaritine content and a long term carcinogenicity bioassay of mushrooms in mice induced tumours at an unusual site (skeletal osteomas and osteosarcomas) that were not induced by agaritine metabolites. These results suggested that mushrooms might contain mutagenic and carcinogenic activities which were due to constituents other than agaritine.

3.3 Both COM and COC agreed that more understanding of the mammalian absorption, distribution, metabolism (and potential detoxification) of agaritine was needed before the two Committees could make any real assessment of mutagenic and carcinogenic activity and its relevance to humans. There was insufficient concern arising from current information to warrant advising that extensive measures should be taken to reduce agaritine intake.

Acrylamide

3.4 In 1988 the COC concluded that the level of acrylamide monomer present in commercial polyacrylamide used in the treatment of drinking water should be reduced to the lowest practical level because of concerns over safety. Acrylamide monomer had been shown to be an *in vivo* mutagen, and there was also limited evidence in 1988 of a carcinogenic effect in rodents. In response to this advice, DoE altered approvals for use of polyacrylamide in drinking water treatment, decreasing by a factor of 4 the theoretical levels of the monomer in drinking water.

3.5 In 1992 the results from a further long term carcinogenicity study in rats were available, together with two occupational studies in humans. The Committee considered this additional data and came to the following overall conclusions:

- (i) No causal relationship between acrylamide exposure and all cause or site-specific cancer mortality was evident in two occupational investigations. These studies were limited by the small numbers of subjects and sparse exposure data.
- (ii) The COC endorsed the conclusions reached by the COM in 1988 that acrylamide should be regarded as an *in vivo* mutagen capable of producing heritable effects in germ cells. The demonstration of mutagenicity in somatic cells *in vivo* was consistent with a genotoxic mechanism of carcinogenicity.
- (iii) Two bioassays had been carried out in rats. The first study provided limited evidence of carcinogenic effects (largely benign tumours in the thyroid and mammary glands and therefore possibly secondary to hormonal effects). The second study showed evidence of tumour induction in treated animals, mainly benign neoplasms of the thyroid and mammary gland, together with mesotheliomas of the tunica vaginalis. Evaluation of the incidence of glial tumours in acrylamide-treated animals was not possible because of inadequate pathological examination.
- (iv) Although no long-term carcinogenicity studies had been carried out in the mouse, acrylamide was shown to be an effective initiator of skin tumours in Sencar mice and lung adenomas in A/J mice. There was a clear dose response and acrylamide was effective by a number of routes.
- (v) The COC concluded that the level of acrylamide monomer present in commercial polyacrylamide used in the treatment of drinking water should be reduced to the lowest practicable level. Furthermore the Committee expressed reservations about this use of polyacrylamide, which could lead to the presence of acrylamide monomer in drinking water, and requested information on the possibility of replacements for polyacrylamide being developed.
- (vi) The Committee asked for details of the present level of detection for acrylamide monomer in drinking water; whether it would be possible to develop a more sensitive analytical method; and whether the polymers could degrade to monomer in water.

Epidemiology of Chlorinated Drinking Water and Cancer

3.6 In 1986, the DH Committee on the Medical Aspects of the Contamination of Air, Soil and Water (CASW) reviewed the animal carcinogenicity, mutagenicity and cancer epidemiology of chlorinated drinking water. CASW advised at that time that there was no sound reason to conclude that the consumption of the by-products of chlorination in drinking-water, treated and chlorinated according to current practices, increased the risk of cancer in humans; no reason to change current good practice of chlorination, no requirement for further epidemiological studies and no need for long term carcinogenicity bioassays on concentrated extracts of chlorinated water. Concentrated extracts of chlorinated drinking water were known to be mutagenic *in vitro* and further work was carried out to clarify the relevance of this finding. The results of this research had been presented to the COM in 1991 (see 1991 Annual Report) who concluded that chlorinated

Department of Health

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CORRECTION

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The following paragraph was omitted.

3.7 Since the review by CASW in 1986, several new epidemiological investigations had been conducted on the possible association between cancers at various sites and consumption of chlorinated drinking water. A meta-analysis of the available epidemiological studies had also been published (Morris RD *et al* Chlorination, chlorination by-products and cancer: a meta-analysis. *Amer J Public Health* **82(7)** 955 - 963 (1992)), which suggested an association between chlorinated drinking water and bladder and rectal cancer. After consideration of the more recent publications, together with the meta-analysis, the Committee concluded that the 1986 conclusions were adequately founded and that information from subsequent investigations did not alter those conclusions. The Committee could not recommend that further epidemiological studies should be undertaken in the UK at the present time, unless a population could be found with a distinctive exposure to chlorinated drinking water.

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drinking water itself presented little risk on direct contact with the gastrointestinal epithelium.

Avoparcin

3.8 Avoparcin is a glycopeptide antibiotic used as a veterinary feed additive (growth promoter). It is licensed throughout the EC for use in chickens, turkeys, cattle, pigs and lambs. There was evidence that avoparcin was poorly absorbed, so residues in meat and milk would be negligible, but operators could be exposed to avoparcin when handling feed, or via residues in animal excreta. The safety of the product had been questioned in European discussions due to the existence of an allegedly positive carcinogenicity study. The COC was asked to review three long-term bioassays in laboratory rodents and advise on the carcinogenicity of avoparcin and any possible risk to humans. This advice would be used in European discussions over the continued licensing of avoparcin as a veterinary product.

3.9 The Committee came to the following conclusions:

- (i) Despite the limitations of the mutagenicity testing of avoparcin there were adequate data to indicate that the compound did not have mutagenic potential.
- (ii) The long term carcinogenicity bioassay in the rat was of less than adequate design, but appeared to give a negative result. The Committee accepted the results of the independent histopathological review of the first long term bioassay in the mouse, which showed that hepatocellular neoplasms had been initially over diagnosed and that the bioassay was negative. A second study in the same strain of mouse confirmed that avoparcin showed no carcinogenic activity in the mouse.
- (iii) The COC found no convincing evidence of the carcinogenicity of avoparcin in these three long term studies in rodents.

Enrofloxacin

3.10 Enrofloxacin is a quinolone antibiotic (see DNA gyrase inhibitors section in COM entry) with a potential use in veterinary medicine. There was concern over the safety of DNA gyrase inhibitors in general, because their action depended on the inhibition of bacterial DNA gyrase. Enrofloxacin gave positive results in some inutagenicity tests *in vitro*. Long term carcinogenicity bioassays had therefore been carried out. Enrofloxacin was not carcinogenic in an adequately conducted study in the mouse. In rats, the compound appeared to induce an increase in the incidence in subendocardial mesenchymal tumours in male animals at all test doses (770, 2000, and 6000 ppm in the diet) and in females at the top dose. The COC was asked for its opinion on the carcinogenicity of enrofloxacin and the significance of the tumours induced in treated rats.

3.11 The COC advised that no conclusion could be reached on the rat bioassay because of the lack of adequately documented pathology results. Members

recommended that the VPC should seek a re-examination of all the histopathological findings.

Imidocarb

3.12 This veterinary drug was assessed by both the COM and the COC at the request of the VPC – see COM entry for background and COM conclusion. The COC considered the carcinogenicity of imidocarb, since there was some doubt as to whether it should be considered as a carcinogen. Only one poor quality study in rats was available. The Committee's advice to the VPC was as follows:

- (i) There was equivocal evidence that imidocarb was carcinogenic in rats. Levels of residues of the drug remaining in edible tissues were an important factor in the safety assessment of this drug.
- (ii) The data from the carcinogenicity study needed to be presented more clearly and re-analysed before any conclusion could be reached as to its carcinogenicity. The Committee would reconsider the topic when this had been done and when the results of the studies requested by the COM were available.

Toltrazuril

3.13 Toltrazuril is a new veterinary drug designed to be included in the feed and drinking water of chickens to prevent coccidiosis. The residues found in the tissues of treated chickens were mainly the sulphone metabolite. Exposure would be expected to be greater for operators than for consumers. The company had examined both the parent drug and its metabolites in a series of short-term mutagenicity tests, and conducted carcinogenicity bioassays in rats and mice. The study in mice gave negative results, but there was an increased incidence of endometrial adenocarcinomas in rats fed the highest dose of toltrazuril.

3.14, The Committee considered the data and came to the following conclusions:

- (i) There was no evidence that toltrazuril was genotoxic.
- (ii) A theoretically plausible non-genotoxic mechanism had been suggested for the carcinogenic effect in rats, attributing the increased incidence of endometrial adenocarcinomas at high dose levels of the compound to hormonal imbalance. Available data on hormone levels did not, however, provide sufficient supporting evidence.
- (iii) Although the Committee had reservations about the validation of the hormonal mechanism for the tumour-inducing action of toltrazuril, there was an apparent threshold effect in rats at 20 ppm in the diet.

3.15 This advice would be used during the regulatory procedure for licensing and determining conditions of use of toltrazuril-containing products.

Further Epidemiology on Breast Implants

3.16 The Committee considered a newly published epidemiological study on breast implants in women and the associated risk of breast cancer, reference: Berkel H, Birdsell DC, Jenkins H (1992). Breast augmentation: a risk factor for breast cancer? *New England J Med* 326 (25): 1649-1653. (See 1991 Annual Report for earlier considerations of breast implants.) The Committee concluded that the study provided qualified reassurance that silicone breast implants were not associated with breast cancer, but information from larger numbers of women with adequate follow-up would be needed before firm conclusions could be drawn.

1,3-Butadiene

3.17 This air pollutant was considered by both COM and COC – see COM section for background and COM conclusions.

3.18 The COC agreed with the COM's conclusion that 1,3-butadiene was an *in vitro* and *in vivo* mutagen. The Committee came to the following further conclusions:

- (i) Animal bioassay data indicated that 1,3-butadiene was tumourigenic in both the rat and the mouse. In inhalation studies in the Sprague-Dawley rat the compound induced follicular thyroid adenomas and Leydig cell adenomas. 1,3-butadiene was a potent multi-tissue carcinogen in the B6C3F1 mouse by the inhalation route at concentrations of 20 ppm and above.
- (ii) Evidence from mortality studies in workers at one butadiene manufacturing site and at several styrene-butadiene rubber sites suggested that exposure to 1,3-butadiene was associated with an increased incidence of malignancies of the haematopoietic/lymphopoietic system. Further data were needed, particularly on exposure levels, before conclusions could be drawn regarding a definite causal relationship.
- (iii) 1,3-butadiene should be regarded as a potent genotoxic animal carcinogen and a probable human carcinogen.

DNA Gyrase Inhibitors

3.19 COM and COC considered this group of compounds and offered joint general advice to the VPC on the safety testing of antibacterial quinolones, a group of compounds which owe their antibacterial activity to DNA gyrase inhibition. The background and joint advice can be found under the COM entry.

Setting Air Quality Guidelines

3.20 The Expert Panel on Air Quality Standards (EPAQS), established by the

Department of the Environment in 1992, requested general advice from COC on the setting of standards for carcinogenic air pollutants. Such compounds were ubiquitous in air and could not be avoided. Actual standards would be recommended by the Expert Panel on a compound-by-compound basis.

3.21 The Committee agreed that for non-genotoxic carcinogens where the mechanisms of carcinogenesis were adequately understood, standards could be based on the threshold for the primary effect, in the same way as for other toxicological endpoints.

3.22 The Committee emphasised that the no-safe-level approach was the scientifically correct approach for genotoxic carcinogens: but, since pollutants could not be totally removed from the air, nor completely avoided, a pragmatic approach to the setting of air quality standards for genotoxic carcinogens was appropriate. It was agreed that a sensible approach might involve the application of further safety factors to a primary standard at which the risks were agreed to be low (such as occupational exposure limits or a level decided upon from examination of the scientific literature). In each case careful consideration of all the available data would be needed.

3.23 It was recognised that it would not always be possible to set standards based on health criteria, since there might not be adequate data available. However, a health input should be included wherever possible.

3.24 In addition, a presentation was made to the Committee by Dr Derwent from the Air Quality Division of the Department of the Environment on air quality monitoring in the UK.

Generic Issues when Considering Natural Toxins

3.25 Both the COM and COC had discussions on this topic. A summary of the discussion and specific conclusions from both Committees is included in the COM entry.

Consideration of Large Bioassays on N-Nitroso Compounds

3.26 The Committee considered the reports of extensive studies carried out on the dose-response relationship in rats of two carcinogenic nitrosamines (Swenberg JA, Hoel DG, Magee PN. *Cancer Res* **51**: 6409 – 6414, 1991; Peto R, Gray R, Brantom P, Grasso P. *ibid* **51**: 6415 – 6451; Peto R, Gray R, Brantom P, Grasso P. *ibid* **51**: 6452 – 6469; Gray R, Peto R, Brantom P, Grasso P. *ibid* **51**: 6470 – 6491).

3.27 The experiments involved unusually large numbers of animals and more extensive dose range than had been used previously. In discussion the Committee commented that it was disappointing that even such extensive experiments had not greatly clarified dose-response mechanisms.

3.28 The following conclusions were drawn:

- (i) When considering the dose-response relationship in chemical carcinogenesis it was important to take the spontaneous rate of tumour development into account.
- (ii) Even with very large bioassays several different mathematical models fitted the data equally well and gave rise to very different extrapolations for the magnitude of response at lower doses. Therefore, it was very unlikely that experimental results would ever provide sufficient data to support the use of one particular mathematical model above others.
- (iii) The data from these bioassays supported adopting linear extrapolation as the prudent approach for estimating carcinogenic response at lower doses, in circumstances where there was a spontaneous incidence of the tumour of interest.
- (iv) There was no reason to alter the design of conventional carcinogenicity tests. It might be informative to extend a study to the natural life span if there was interest in the dose response of a particular tumour. Dosing with the test substance from an earlier age than conventional might also increase the sensitivity of the bioassay, but would cause problems with the use of the maximum tolerated dose (MTD).

Topics Under Review

3.29 The following topics, which were discussed by the COC at meetings held in 1992, are under review:

Captan

Dithiocarbamates in latex products

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (commonly known as dioxin)

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

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Dr R L Carter (Chairman)	ICI plc	Share Holder	NONE	NONE
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 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
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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 Describes a disease of rapid onset, severe symptoms and brief duration.

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

ADENOMA (See 'tumour').

ADI Acceptable daily intake, defines 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'.

A/J MICE A strain of mice with a high spontaneous rate of lung tumours which can be greatly enhanced by relatively short-term treatment with carcinogens.

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

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

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

ANEUGENIC Inducing aneuploidy (qv).

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

ANTIMICROBIAL The ability to destroy microbes.

ANTIOXIDANT A compound which is capable of delaying or preventing the development in food of rancidity or other flavour deterioration due to oxidation. May be used as a general term for an ingredient which prevents oxidation.

ASCITES The accumulation of fluid in the peritoneal cavity causing abdominal swelling.

BABESIOSIS Redwater disease of cattle, caused by the parasite *Babesia* spp.

BASAL DIET Normal diet without any of the test material added.

β -ISOMER Isomers are two or more chemical compounds with the same molecular formula but having different properties owing to a different arrangement of atoms within the molecule. The β -isomer of alitame is formed when the compound degrades and the atoms within the molecule are rearranged.

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

BOLUS DOSE When all the test substance is administered at the same time, ie as a single dose.

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

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

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

CHELATION The removal of metallic ions from solution by formation of a complex with an organic molecule.

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

CIRRHOSIS A condition in which the liver responds to injury or death of some of its cells by producing interlacing strands of fibrous tissue between which groups of regenerating cells can be found.

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.

CROHN'S DISEASE A condition in which segments of the alimentary tract become inflamed, thickened and ulcerated.

CULTIVAR A plant variety developed from the naturally occurring species by breeding and cultivation.

DIETARY REFERENCE VALUE (DRV) A term used to cover LRNI (qv), RNI (qv) and safe intake.

DNA (DEOXYRIBOSENUCLEICACID) 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 two 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 three elements: a pentose sugar, a phosphate group and a nitrogenous base derived from either purine (adenine, guanine) or pyrimidine (cytosine, thymine).

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

ELECTRON MICROSCOPY A type of microscopy with higher magnification than is possible with the ordinary light microscope.

ENDOMETRIAL Relating to the lining of the uterus.

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

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

FIBROSIS Thickening and scarring of connective tissue.

FOLLICULAR THYROID ADENOMA Benign tumour (qv) of the follicular cells of the thyroid gland.

FORESTOMACH (See 'glandular stomach').

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

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

GERM CELL Reproductive cell, eg spermatid.

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

GLIAL Pertaining to glia (or neuroglia), a group of specialised cells which are found in the brain and spinal cord. They are concerned with the support and nutrition of nerve cells.

GLYCOPEPTIDE A compound formed by the conjugation of a **peptide** (short chain of amino acids) and carbohydrate molecules.

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

HAEMORRHAGIC NECROSIS The death of some or all of the cells in an organ or tissue associated with or resulting from blood loss.

HEPATOCARCINOGENICITY Ability to induce liver tumours.

HEPATOCELLULAR Relating to the cells of the liver.

HYDRAZINES A class of organic bases derived from hydrazine – $\text{H}_2\text{N.NH}_2$.

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

HYPERKERATOSIS A thickening of the tough keratin layer on the surface of the squamous epithelium (see also 'squamous epithelia').

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

INTRA-PERITONEAL (IP) Refers to the dosage route where a chemical is injected directly into the peritoneal cavity of the abdomen.

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.

ISOMERS (See ' **β -isomer**').

LABELLING INDEX A measure of the frequency of cell division.

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

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

LEYDIG CELL ADENOMA Benign tumour (qv) of the cells interspersed between the seminiferous tubules of the testis.

LINEARITY The assumption that the size of an effect is directly proportional to the size of the dose.

LOWER REFERENCE NUTRIENT INTAKE (LRNI) The amount of a nutrient that is enough for only the few people in a group who have low needs.

LYMPHOPOIETIC A term applied to the body tissues where normal lymphocytes, a variety of white blood cell, are formed.

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

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

MEGALOCYTE An enlarged cell.

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

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

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

METAPHASE Stage of cell division (mitosis and meiosis) during which the chromosomes are arranged on the equator of the nuclear spindle (the collection of microtubule filaments which are responsible for the movement of chromosomes during cell division). As the chromosomes are most easily examined in metaphase,

cells are arrested at this stage for microscopical examination for chromosome aberrations (qv) – known as metaphase analysis.

MONOMER A chemical compound made up of a single molecule (as opposed to a polymer which is built up by the repeated union of many monomer molecules).

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

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

MUTATION A permanent change in the amount or structure of the genetic material in an organism which can result in a change in the characteristics of the organism. The 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 of sexually reproducing organisms may be transmitted to the offspring, whereas a mutation that occurs in somatic cells may be transferred only to descendent daughter cells.

MYCOTOXIN Toxic compound produced by a fungus.

NON-GENOTOXIC See 'carcinogens'.

OEDEMA Excessive accumulation of fluid in body tissues.

OSTEOMAS AND OSTEOSARCOMAS Benign and malignant tumours (qv) of the bone.

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

PEROXISOMES Subcellar cytoplasmic particles bound by a single membrane. Probably present in all mammalian cells, but seemingly more abundant in liver and kidney. They contain the enzymes of a β -oxidation system, the function of which is not entirely clear, but which generates hydrogen peroxide. This is normally detoxified by the peroxisomal enzyme, catalase. In some species hepatic and, to a lesser extent, renal peroxisomes may undergo proliferation in response to certain physiological conditions or as a result of treatment with various

chemicals ('PEROXISOME PROLIFERATORS'). The rodent liver and kidney is particularly susceptible to these effects whereas primates, including humans, appear to be more resistant.

PLASTICISER A substance which increases the flexibility of certain plastics.

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

PORTAL HYPERTENSION A state in which the pressure within the hepatic portal vein is increased, causing enlargement of the spleen and accumulation of fluid in the peritoneal cavity.

RECEPTOR Part of a cell which specifically combines with an agent.

REFERENCE NUTRIENT INTAKE (RNI) An amount of the nutrient that is enough, or more than enough, for most (usually at least 97%) of people in a group. If the average intake of a group is at the RNI, then the risk of deficiency in the group is very small.

SENCAR MICE A strain of mice particularly sensitive to skin application of carcinogens, developing skin tumours in 2-3 months.

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

SQUAMOUS EPITHELIA A type of epithelium consisting of square-shaped cells. Examples are the skin and the lining of the oesophagus (see also 'epithelia').

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.

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

TUMOUR (Synonym – neoplasm) A mass of abnormal, disorganised cells, arising from pre-existing tissue, which are characterised by excessive and

unco-ordinated proliferation and by abnormal differentiation. 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. 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 recognisable 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; 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.

TUNICA VAGINALIS Membrane covering the testis and epididymis.

Other Publications Produced by these Committees

- 1991 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321529 0 Price £9.50.
- Guidelines for the Testing of Chemicals for Toxicity DHSS Report on Health and Social Subjects 27 HMSO ISBN 0 11 320815 4 Price £4.30.
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