



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

London: HMSO

© Crown copyright 1993

Applications for reproduction should be made to HMSO

First published 19.93

ISBN 0 11 321604 1

If you require any information about the references used in the preparation of this report please write to the committee's administrative secretary at the following address:-

*The Department of Health  
Room 679D  
Skipton House  
80 London Road  
Elephant and Castle  
London SE1 6LW*

# Contents

	<i>Paragraph No</i>
<i>About the Committees</i>	
<i>Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment</i>	
Aspartame	1.1
General Principles for Setting Acceptable Daily Intakes	1.2
Butylated Hydroxyanisole (BHA) – Review of Further Studies and Setting of an ADI	1.3 – 1.13
Novel Fat for Use in Confectionery	1.14
Comfrey	1.15 – 1.18
Dimethyldicarbonate	1.19 – 1.23
Guidelines for the Safety Assessment of Microbial Enzyme Preparations used in Food	1.24
Iodine Toxicity in Adults and Young Children	1.25 – 1.31
Propylene Carbonate	1.32 – 1.35
Thiamphenicol	1.36
Food Surveillance Papers	1.37 – 1.38
Topics Under Review	1.39
Membership	
Members' Interests	

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

Agaritine	2.1 – 2.3
Alitame	2.4
Dimethyl Dicarbonate	2.5 – 2.6
Gallates Used as Antioxidants in Food	2.7
Imidocarb	2.8 – 2.9
Omethoate	2.10 – 2.11
Dimethoate	2.12
2-Phenylphenol and its Sodium Salts	2.13 – 2.14
Aniline	2.15 – 2.16
1,3-Butadiene	2.17 – 2.18
DNA Gyrase Inhibitors	2.19
Mutagenicity Testing Strategies on New Substances: Further Advice to HSE	2.20
Classification of Chemicals on Basis of Mutagenic Properties	2.21
Mouse Spot Test	2.22 – 2.25
Generic Issues When Considering Natural Toxins	2.26
JointCOM/COC Symposium on Peroxisome Proliferators	2.27 – 2.29
Membership	
Members' Interests	
New Chairman for the COM	

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

Agaritine	3.1 – 3.3
Acrylamide	3.4 – 3.5
Epidemiology of Chlorinated Drinking Water and Cancer	3.6 – 3.7

	<i>Paragraph No</i>
<b>Avoparcin</b>	3.8 – 3.9
<b>Enrofloxacin</b>	3.10 – 3.11
<b>Imidocarb</b>	3.12
<b>Toltrazuril</b>	3.13 – 3.15
<b>Further Epidemiology on Breast Implants</b>	3.16
<b>1,3-Butadiene</b>	3.17 – 3.18
<b>DNA Gyrase Inhibitors</b>	3.19
<b>Setting Air Quality Guidelines</b>	3.20 – 3.24
<b>Generic Issues when Considering Natural Toxins</b>	3.25
<b>Consideration of Large Bioassays on N-Nitroso Compounds</b>	3.26 – 3.28
<b>Topics Under Review</b>	3.29
<b>Membership</b>	
<b>Members' Interests</b>	
<i>Terms of Reference</i>	<i>Annex 1</i>
<i>Glossary of Terms</i>	<i>Annex 2</i>
<i>Other Publications Produced by these Committees</i>	<i>Annex 3</i>



## 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.

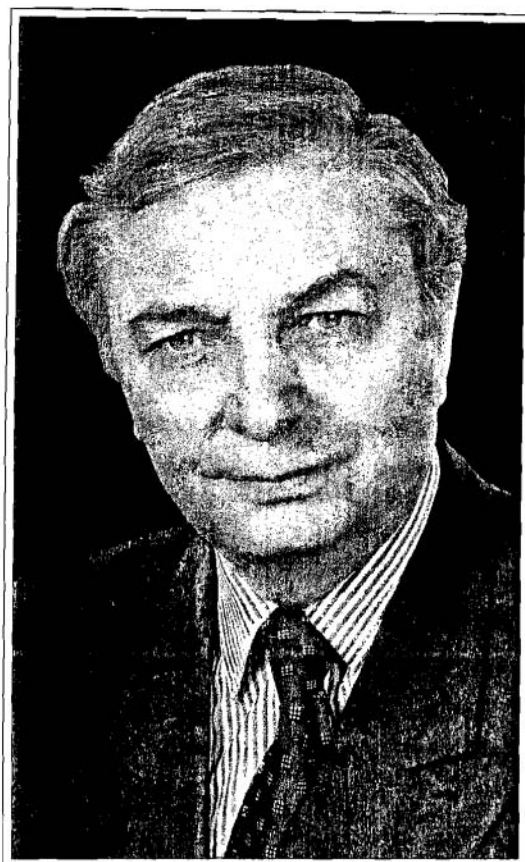
The annual report of the COT for 1992 makes reference to both the old and new ways in which the COT has given its advice on the safety-in-use of food additives.

---

\* 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

*Professor H F Woods  
(Chairman)  
BSc MB BCh MRCP  
D Phil FRCP(Lon) FFPAM  
FRCP(Edin)*



## 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 ADI<sub>1992</sub> 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 TDI<sub>1992</sub> 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 ADI<sub>1992</sub> 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*, *Crotolaria* 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 *Crotolaria* 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 data<sup>1</sup> 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. Varieties 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.





















































































**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<sup>+/−</sup>). 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  $\beta$ -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:

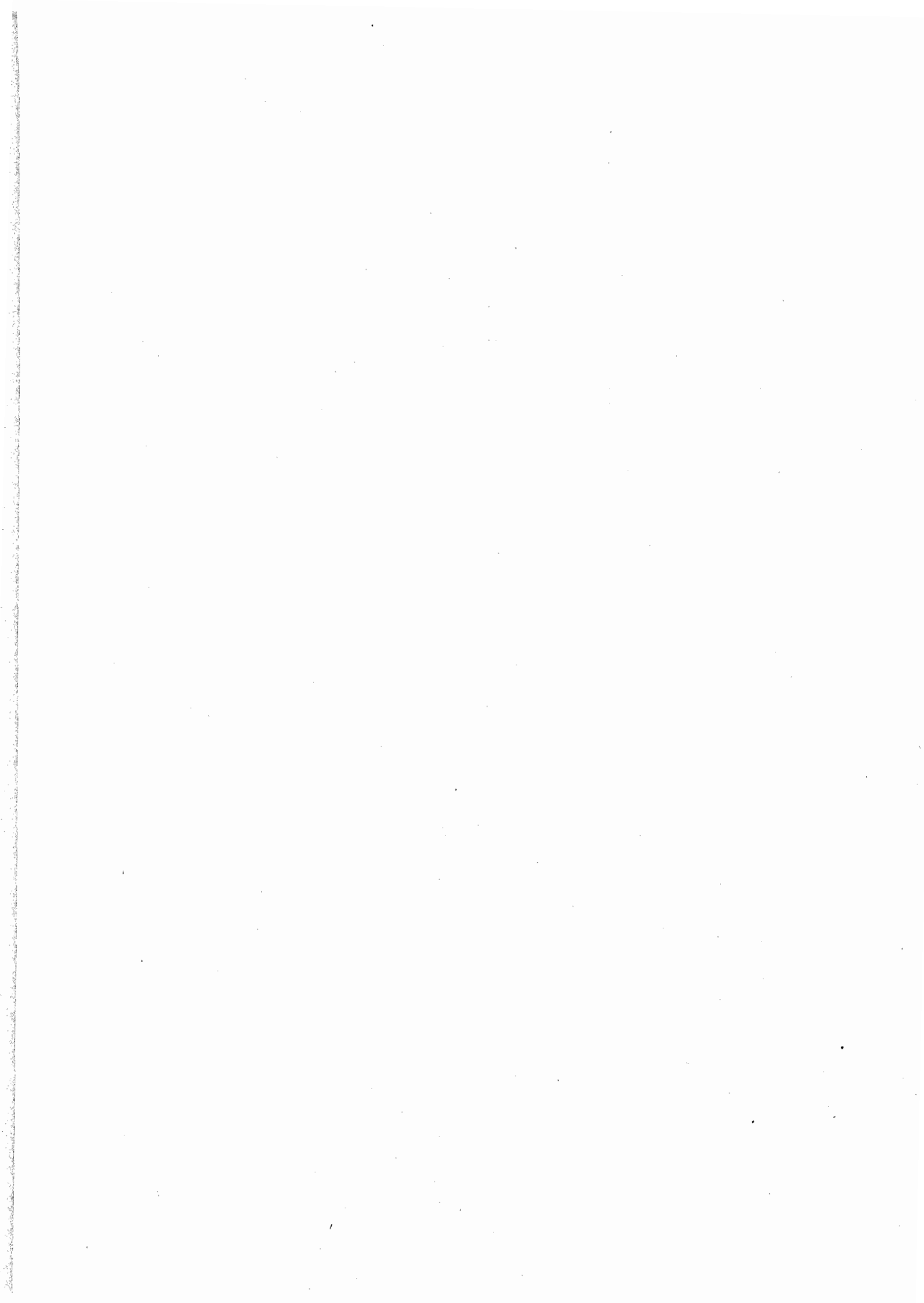
Tumours arising from epithelia (qv): *benign* – adenomas, papillomas; *malignant* – adenocarcinomas, papillary carcinomas.

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.
- Guidelines for the Evaluation of Chemicals for Carcinogenicity DH Report on Health and Social Subjects 42 HMSO ISBN 0 11 321453 7 Price £7.30.
- Guidelines for the Testing of Chemicals for Mutagenicity DH Report on Health and Social Subjects 35 HMSO ISBN 0 11 321222 4 Price £6.80.
- Guidelines for the Preparation of Summaries of Data on Chemicals in Food, Consumer Products and the Environment submitted to DHSS DHSS Report on Health and Social Subjects 30 HMSO ISBN 0 11 321063 9 Price £2.70.





**HMSO publications are available from:**

**HMSO Publications Centre**

(Mail, fax and telephone orders only)  
PO Box 276, London, SW8 5DT  
Telephone orders 071-873 9090  
General enquiries 071-873 0011  
(queuing system in operation for both numbers)  
Fax orders 071-873 8200

**HMSO Bookshops**

49 High Holborn, London, WC1V 6HB  
(counter service only)  
071-873 0011 Fax 071-873 8200  
258 Broad Street, Birmingham, B1 2HE  
021-643 3740 Fax 021-643 6510  
33 Wine Street, Bristol, BS1 2BQ  
0272 264306 Fax 0272 294515  
9-21 Princess Street, Manchester, M60 8AS  
061-834 7201 Fax 061-833 0634  
16 Arthur Street, Belfast, BT1 4GD  
0232 238451 Fax 0232 235401  
71 Lothian Road, Edinburgh, EH3 9AZ  
031-228 4181 Fax 031-229 2734

**HMSO's Accredited Agents**

(see Yellow Pages)

and through good booksellers

**£11.70 net**

ISBN 0-11-321604-1



9 780113 216048